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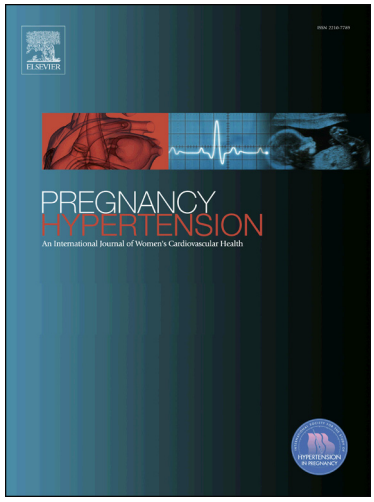

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Postpartum Evaluation of Cardiovascular Disease Risk for Women with Pregnancies Complicated by Hypertension

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Abstract

Objectives: Postpartum stratification of cardiovascular risk for women with pregnancies complicated by pre-eclampsia is challenging. Our aim was to identify potential clinical and biomarker predictors of future cardiovascular risk at six weeks postpartum in women with hypertensive pregnancies.

Study design: Prospective longitudinal cohort

Main outcome measures: Ten year- Framingham cardiovascular risk scores were calculated for 477 women (94 with gestational hypertension, 288 with pre-eclampsia, 30 with superimposed pre-eclampsia, 51 with chronic hypertension, 14 women with uncomplicated pregnancies). B-type natriuretic peptide (BNP), neutrophil gelatinase–associated lipocalin (NGAL) and placental growth factor (PlGF) were quantified at six weeks postpartum.

Results: Framingham cardiovascular risk scores were not higher in women with pregnancies complicated by pre-eclampsia than healthy controls, nor were scores higher in women with pre-existing chronic hypertension complicated with superimposed pre-eclampsia compared with those without superimposed pre-eclampsia. Women with gestational hypertension had higher risk scores than women with pre-eclampsia and healthy controls. Established risk factors of cardiovascular disease including diastolic blood pressure and previously diagnosed chronic hypertension were associated with higher scores, and African ethnicity, parity and estimated glomerular filtration rate also were independently associated with higher Framingham risk scores at six weeks postpartum. PlGF, BNP and NGAL concentrations were not associated with Framingham cardiovascular risk scores after adjustment for independent variables.

Conclusions: A history of pre-eclampsia or superimposed pre-eclampsia in most recent pregnancy was not associated with elevated Framingham risk score at six weeks postpartum. Established clinical predictors may enable risk stratification at six weeks postpartum, which are not enhanced by the biomarkers included in this study.

Key words (**5-7 MeSH terms**): cardiovascular disease; blood pressure; pregnancy-induced hypertension; pre-eclampsia; postpartum; biomarkers; endothelium.

Introduction

Women with a history of hypertension in pregnancy are at increased risk of future cardiovascular disease (CVD) events, [1] and National Institute for Health and Clinical Excellence (NICE) and the American Heart Association recommend preventative strategies for CVD in these women. [2,3] Given that 10% of women have hypertension in pregnancy, implementation of these recommendations may be best targeted if improved identification of those at greatest risk was possible.

It is undetermined whether the association between pre-eclampsia and CVD is secondary to underlying common risk factors, such as dyslipidaemia and obesity, or if pre-eclampsia is an independent factor causing vascular damage. Regardless of the aetiology, it is possible that biomarkers of persistent inflammation and endothelial dysfunction, detected after pregnancy[4] could be used as predictors of future CVD enabling enhanced stratification of those at greatest risk.

The Framingham cardiovascular risk scoring system (FCVRS) is used to assess risk of future cardiovascular disease. It is an established and validated algorithm which enables inclusion of individuals younger than 40 years and was chosen for assessment of risk in this study.[5] Placental growth factor (PlGF) is an angiogenic protein which is synthesized by endothelial cells promoting angiogenesis and atherosclerotic intimal thickening in non-pregnant individuals.[6] Abnormal concentrations of PlGF in women with history of hypertension in pregnancy could be associated with endothelial dysfunction and contribute to atherosclerotic plaque formation and increased risk for CVD.[7] B-type natriuretic peptide (BNP) is released with cardiac ventricular strain, and reflects left ventricular function.[8] BNP concentrations are observed to be higher at time of diagnosis in women with pre-eclampsia compared with normotensive controls,[9] and could be used to identify women with ongoing CVD risk. Plasma neutrophil gelatinase–associated lipocalin NGAL is a marker of inflammation, which has been demonstrated to be elevated in pregnant women with pre-eclampsia[10] , and in middle-aged women with chronic hypertension compared to normotensive controls.[11] Postpartum concentrations of PlGF, BNP and NGAL and their role in the prediction of future CVD are yet to be elucidated.

The aim of this exploratory study was to assess the relationship between a history of hypertension in pregnancy with CVD assessment by FCVRS at six weeks postpartum, and its association with demographics, inflammatory and endothelial biomarkers in order to determine the potential for this approach for stratification of risk to guide future implementation of prophylactic strategies.

Methods

All women with a history of hypertension in pregnancy and healthy controls were invited to a six-week postpartum follow-up visit at the Maternity Unit, Nottingham City Hospital, United Kingdom. Participants were recruited from November 2009 to December 2013. Women with previous diagnosis of chronic kidney disease, chronic liver disease and cardiovascular disease were excluded. All participants gave written informed consent, and the study was approved by Derby Research and Ethics Committee (ref 10/H0401/75) and Nottingham University Hospitals Research and Development department (ref 10OB002). Hypertension in pregnancy was classified according to International Society of Study of Hypertension in Pregnancy (ISSHP) guidelines.[12] Demographics, systolic and diastolic blood pressure during pregnancy, previous and current body mass index (BMI), pregnancy characteristics, fetal birth weight and outcomes were recorded. Birthweights were assessed by customized birthweight percentile charts (www.gestation.net/birthweight centiles/centile online.htm).[13] Three measurements of blood pressure were conducted in the right arm in the supine position by trained healthcare professionals. Random zero mercury sphygmomanometers were used and cuff sizes were chosen accordingly to participant's arm circumference. Mean values between second and third measurements were used in the analysis. Plasma samples were taken and stored immediately at -80°C.

Framingham 10-year cardiovascular risk scores were calculated using the following variables: age, HDL cholesterol, total cholesterol, systolic blood pressure, use of anti-hypertensive drugs, smoking status and diabetes history.[5] Age only adds risk to the model above 30 years in this predicting score. A standardized age of 30 years was imputed for women younger than 30 years.

Biomarker analysis

Standard biochemical assays were undertaken according to the procedures of Nottingham University Hospitals NHS Trust. Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula. Estimated glomerular filtration rate (eGFR) was calculated by the CKD-EPI equation. Serum and urinary creatinine were measured through a Beckman AU analyser using the IDMA-traceable kinetic

colour test with Jaffe method to compensate for protein interference. Coefficient of variations were 1.7% for total cholesterol, 7.0% for high-density (HDL) cholesterol, 2.1% for triglycerides, 2.2% for creatinine.

Total urinary protein was quantified using Beckman AU clinical chemistry analysers using a colorimetric test method.

Quantitative measurements of plasma BNP, NGAL and PlGF concentrations were undertaken without awareness of clinical information using a standard commercially available assay (Cardiorenal Triage Assay, Alere Inc., San Diego, CA, USA). Plasma samples were available for 107 women, 49 women with history of pre-eclampsia, 30 with previous gestational hypertension, 13 women with chronic hypertension, 10 that experienced superimposed pre-eclampsia and 5 women with a previous healthy pregnancy. The assay uses fluorescently labelled recombinant murine monoclonal antibodies and assesses BNP, NGAL and PlGF specifically and quantitatively, in the range of 5 to 5000 pg/ mL, 15 to 1300 ng/ mL and 12 to 3000 pg/ mL, respectively, in approximately 15 minutes. BNP and PlGF concentrations lower than the inferior limit of detection were reported as 5 pg/ mL and 12 pg/ mL, respectively. The total precision (coefficient of variation) on plasma controls at concentrations of BNP 78 pg/ mL, NGAL 98 ng/ mL and PlGF 85 pg/mL are 9.2%, 12.5% and 12.8%, respectively. Test procedures and information are based on the manufacturer's package insert generated before the study.

Statistical analysis

Normality of distribution was explored using a Q-Q plot and logarithmic transformations were performed when appropriate. Demographic data are presented as medians (interquartile range) or frequencies (percentages). Categorical variables were compared with the use of the λ^2 test. One-way analysis of variance (ANOVA) and Kruskal–Wallis were used to test the differences between groups normally distributed and non-normally distributed continuous data respectively, with Mann– Whitney post hoc pair-wise test was used for further analysis. Pearson correlation coefficients were calculated to assess associations between biomarkers and dependent variables, including individual components of the Framingham Cardiovascular Risk Score and total score. For multiple hypotheses testing, Bonferroni's correction was used. To assess association between independent variables and increasing Framingham Cardiovascular Risk Score, multivariable linear regression using backward selection was performed.

Variables associated with a significance level of 0.20 at univariate linear regression were included in the model after highly collinear variables were substituted. Variables included in the algorithm for calculation of the Framingham Cardiovascular Risk Score were not included in the model. A probability value of α 0.05 was determined to be statistically significant. Analysis was conducted with Stata software (version 14.0; StataCorp, College Station).

Results:

Four hundred and seventy seven women were studied at a median time of 7.1 weeks (IQR 6.3-8.3) after delivery. Participants were classified according to prior hypertension in pregnancy diagnosis: 94 (20%) women had gestational hypertension, 288 (60%) women had pre-eclampsia, 30 (6%) women had chronic hypertension with superimposed pre-eclampsia, 51 (11%) women had chronic hypertension without superimposed pre-eclampsia, and 14 (3%) healthy control women with uncomplicated pregnancies. Demographic data, medical history and clinical parameters at postpartum visit are displayed in table 1 according to hypertension group.

There was no difference in maternal age or time after delivery visit between groups. The majority of women were of European white ethnicity in all groups, and there were no significant differences in proportion of women of African or Asiatic ethnicities between groups.

Median systolic and diastolic blood pressures at study visit were higher in women with previous superimposed pre-eclampsia, chronic hypertension, pre-eclampsia and gestational hypertension when compared to healthy controls. Systolic and diastolic blood pressure also were higher in women with chronic hypertension with and without superimposed pre-eclampsia and in women with gestational hypertension when compared to women with pre-eclampsia without pre-existing disease.

There were no significant differences between groups in liver enzymes, triglycerides, total cholesterol and HDL concentrations (table 2). Serum creatinine concentrations were higher in women with prior gestational hypertension, chronic hypertension with and without superimposed pre-eclampsia than controls or women with pre-eclampsia Protein to creatinine ratio on random urine samples (PCR) was comparable between all groups at the postpartum visit. Forty (14%) women with pre-eclampsia, three (10%) women with superimposed pre-eclampsia and four (8%) women with chronic hypertension had persistent proteinuria at 6 weeks post-partum, but there were no statistical significant differences in proportions between groups.

Pregnancy outcomes are detailed in table 3. Gestational age at delivery was lower in women with superimposed pre-eclampsia compared to healthy controls, women with gestational hypertension, pre-

eclampsia and women with chronic hypertension without superimposed pre-eclampsia. Gestational age at delivery also was lower in women with pre-eclampsia compared to women with gestational hypertension and chronic hypertension without superimposed pre-eclampsia. The proportion of infants with birth weight lower than the 3rd customized centile for gestational age was higher in women with superimposed pre-eclampsia compared to all of the other groups.

Framingham 10-years cardiovascular risk score (FCVRS)

Median FCVRS was low in all groups (1.5% (95% confidence intervals 0.9, 2.0) (Figure 1), i.e. 1.5% chance of developing cardiovascular disease in the next 10 years). Women with chronic hypertension without superimposed pre-eclampsia had the highest median risk score, 2.8% (2.0, 3.0) compared with women with chronic hypertension with superimposed pre-eclampsia (1.7% (1.0, 2.0), and women with gestational hypertension (1.6% (1.0, 2.4)) (P<0.001), or women with pre-eclampsia (1.2% (1.0, 2.0)) or controls (1.0% (1.0, 1.5)). Women with chronic hypertension with superimposed pre-eclampsia also had higher median risk when compared to women with pre-eclampsia and healthy controls. Women with gestational hypertension had higher median scores than controls and women with pre-eclampsia. Since the sample did not present scores higher enough to be stratified into the conventional risk categories of FCVRS (<10%: low risk; 10-20%: moderate risk; >20%: high risk), [12] we defined a ≥ 5% risk threshold and categorized women to a <5% lower risk category and ≥5% higher risk category. There were 16 (17%), 34 (12%), four (13%) and eight (16%) women with gestational hypertension, pre-eclampsia and chronic hypertensive with and without superimposed pre-eclampsia in the higher risk category for FCVRS. None of the healthy control participants had a FCVRS higher than 5%. There was no relationship between FCVRS and gestation at delivery for women with pre-eclampsia or the whole cohort.

Evaluation of association between biomarkers and cardiovascular risk.

There were no significant differences in plasma BNP, NGAL or PlGF concentrations between groups. Due to censored values, BNP and PlGF measurements were categorized according to three different

thresholds (table 4): ≤5 pg/ mL, 5.1 – 9.9 pg/ mL and ≥10 pg/ mL for BNP, and ≤12 pg/ mL, 12.1 – 17.9 pg/ mL and ≥18 pg/ mL for PlGF and there were no significant differences between groups.

Correlation coefficients found between FCVRS, biomarkers and known cardiovascular risk factors are shown in table 4. The FCVRS demonstrated positive correlations with study visit diastolic blood pressure (*R* 0.597, *P*<0.001), systolic blood pressure at first antenatal visit (*R* 0.345, *P*<0.001), diastolic blood pressure at first antenatal visit (*R* 0.366, *P*<0.001), BMI at first antenatal visit (*R* 0.2014, *P*<0.001), BMI postpartum (*R* 0.200, *P*<0.001), uric acid concentration postpartum (*R* 0.092, *P*0.06), triglycerides (*R* 0.167, *P*<0.001) and LDL cholesterol postpartum (*R*0.139, *P*0.05). A negative correlation was found between FCVRS and eGFR (*R* -0.255, *P*<0.001). NGAL, BNP or PlGF concentrations were not correlated with any of the known cardiovascular risk factors evaluated.

Factors associated with FCVRS by univariate linear regressions are shown in Table 6. At multivariate linear regression analysis, diastolic blood pressure, parity, African ethnicity and eGFR were independently associated with FCVRS. None of the biomarkers, single or in combination, contributed to the model performance (Table 7). The post hoc analysis indicated a statistical power of 0.80 to detect a hypothesized incremental effect size on R-squared of 0.05 at the alpha level of 0.05 after adding each biomarker of interest to the final regression.

Discussion

Framingham cardiovascular 10 year risk scores (FCVRS), for future cardiovascular disease, were not higher in women at six weeks postpartum if their pregnancy was complicated by pre-eclampsia compared with healthy controls, nor were scores higher in women with pre-existing chronic hypertension with pregnancies complicated by superimposed pre-eclampsia compared with those without superimposed pre-eclampsia. Established predictors of cardiovascular risk including diastolic blood pressure, and previously diagnosed chronic hypertension were associated with higher FCVRS at six weeks postpartum. Women with gestational hypertension had higher FCVRS than women with pre-eclampsia without pre-existing disease and healthy controls. Other risk factors for cardiovascular disease in the general population including African ethnicity, parity and reduced renal function also were independently associated with higher FCVRS in this postpartum population. However, endothelial or inflammatory biomarkers tested did not contribute to risk stratification by FCVRS.

Meta-analyses of observational studies have demonstrated a two-fold increase in the risk for CVD later in life in women with previous pre-eclampsia compared to those normotensive pregnancies.[1,14,15] However, at six week postpartum there were no differences in FCVRS between women pregnancies complicated by pre-eclampsia and normotensive women. Other studies have used FCVRS for CVD risk stratification in postpartum women with previous hypertensive disorders of pregnancy and reported marginally higher risk scores in women with early-onset pre-eclampsia[16] and women with previous pre-eclampsia women with persistent hypertension[17] compared to women with uncomplicated pregnancies. Increased cardiovascular risk in women with pre-eclampsia is strongly associated with severity of disease, including need for preterm delivery, and has been estimated to be two-fold higher in those with severe compared with mild disease.[15] The cohort included in the present study predominantly had milder presentations of pre-eclampsia and it is possible that the low FCVRS risk scores calculated in women with pregnancies complicated by pre-eclampsia in the present study reflects a lower risk group. However, in keeping with our findings, others have recently reported that women with gestational hypertension have a greater risk of future cardiovascular disease then women

with pre-eclampsia [18] which might reflect underlying chronic hypertension, particularly in multiparous women.

Alternatively, the FCVRS model, generated from an older population of men and women (age 30 to 74 years), may not be directly comparable to a younger female population. Furthermore, age contributes significantly to the predicting equation and thus risk scores for CVD in this cohort may be underestimated. Absolute predictive performance of the FCVRS in this population, and the contribution of pre-eclampsia severity remains to be confirmed in a longitudinal cohort, and generation of tailored algorithms for postpartum women is likely to be needed.

Women with chronic hypertension with or without superimposed pre-eclampsia are frequently excluded from large prospective studies evaluating cardiovascular events postpartum. However, in a recent cohort study reported cardiovascular outcomes over 50 years after pregnancy in over 14,000 women including those with pre-existing hypertension superimposed pre-eclampsia in women with chronic hypertension was associated with a nearly six-fold increase in cardiovascular events, compared with a four-fold increase for women with chronic hypertension without superimposed pre-eclampsia.[19] In contrast, in the present study women with superimposed pre-eclampsia did not have a higher FCVRS compared with women with chronic hypertension, which provides further evidence for the need for the generation of a specific postpartum cardiovascular risk score as pregnancy complications in isolation appear to be insufficient to identify those at risk, although this finding may be limited by small numbers in this group.

Blood pressure and African ethnicity are well-established risk factors for cardiovascular disease in the general population,[20] but has not previously been studied in postpartum women, and were also identified as independent predictors in this cohort. The FCVRS does not include ethnicity which is likely to reflect the white European population used to develop the algorithm. In the present study, primigravid women in the index pregnancy had significantly lower FCVRS than parous women, but it is not clear whether this reflects differences in maternal age. However, others also have reported parity to be independently associated with increased risk of death from ischaemic heart disease.[21] Similarly the negative association of renal function with CVD risk is well recognised in the general population, and

some studies have shown predictive algorithms including eGFR with better performance than the FCVRS alone.[23,24] However, the association between eGFR and FCVRS has not previously been described in a cohort of postpartum women with hypertensive pregnancy, and requires validation in a larger prospective cohort.

Other established and potentially modifiable risk factors including total cholesterol and LDL were not different between women with different hypertensive disorders of pregnancy, in contrast to the findings of Verbeek *et al* who reported a less favourable lipid profile in women with early onset pre-eclampsia compared to those with late onset or gestational hypertension.[24] However, the small number of women with early onset pre-eclampsia in this cohort prohibited a meaningful comparison with later onset disease.

Natriuretic peptides including BNP and NT-pro-BNP which are correlated with left ventricular function, have been demonstrated to predict mortality due to cardiovascular disease in older populations,[8]and several studies have reported raised concentrations of natriuretic peptides at time of diagnosis of pre-eclampsia and associations with future cardiovascular complications proposed. [9] However, there are conflicting studies of natriuretic peptide concentrations in postpartum studies with some reporting elevated NT-pro-BNP concentrations in women with previous pre-eclampsia at three to six months postpartum compared to women with non-complicated pregnancies[25] and others reporting no difference in BNP concentrations between women with previous pre-eclampsia and healthy controls at one to four weeks postpartum.[26,27] Postpartum comparison of BNP concentrations between women with pre-eclampsia and pre-existing chronic hypertension has not previously been reported, and no differences were identified in this study. The absence of any association between BNP concentration and future CVD risk suggests that this marker is unlikely to have any useful predictive value in clinical practice.

Women with previous pre-eclampsia are reported to have altered acute inflammatory responses at several months to years after delivery,[28] particularly in those with early onset pre-eclampsia,[24] which has been proposed to contribute to future cardiovascular disease. Small studies have described elevated plasma and serum NGAL in women with pre-eclampsia compared to healthy controls,[29] and

in older women with hypertension,[11] in keeping with increased inflammation. To our knowledge, plasma NGAL concentrations have not been explored in postpartum women. Plasma NGAL concentrations were similar in all women with hypertensive disorders of pregnancy and was not associated with FCVRS. This finding is comparable to a report of urinary NGAL concentrations which were elevated in pregnant women with severe pre-eclampsia compared with women with chronic hypertension and healthy controls but differences had resolved by three to eight weeks postpartum,[30] although urinary and plasma concentrations may not be directly comparable.

Angiogenic imbalance, including elevated or decreased PlGF has been demonstrated to lead to endothelial damage *in vitro*.[6] A case control study from the Nurses' Health study suggested that elevated PlGF concentrations were predictive of coronary heart disease in asymptomatic women more than 10 years after testing. The findings remained significant even after adjustment for comorbid conditions such as hypertension and dyslipidaemia (RR 2.78; 95% CI 1.2-6.6),[7] whereas Akhter et al observed elevated sFlt-1:PLGF ratios a year after delivery in women with pre-eclampsia compared to controls. Furthermore, high sFlt-1:PLGF ratios were positively associated with carotid artery intima: media thickness ratio, a marker or arterial aging.[31] In the present study more than 60% of participants had PlGF concentrations below the detection threshold of 12 pg/ mL, in keeping with the findings of other reports of PlGF concentrations between 1-12 months postpartum.[32-34] In this study comparable PlGF concentrations were found between women with different hypertensive disorders of pregnancy and controls. Conversely, Noori *et al* found significantly higher serum PlGF concentrations at 12 weeks postpartum in women with previous pre-eclampsia or gestational hypertension compared with women with normotensive pregnancies, [34] which may reflect differences in plasma and serum concentrations postpartum. In agreement with our findings, Gaugler-Senden *et al* reported similar concentrations of angiogenic factors, including PlGF in 16 women almost ten years after early onset severe pre-eclampsia compared to 20 women with previous uncomplicated pregnancy.[35] Thus it is possible that persistent angiogenic balance after pregnancy does not contribute to future development of cardiovascular disease.

An important limitation of our study may be inadequate power to detect small differences in biomarker concentrations, predictive of future risk. However, in order to have clinical utility, the predictive value of additional biomarkers would need to be greater than pre-existing demographic risk factors, and none of those studied enhanced predictive performance of traditional risk factors.

Conclusion

Six weeks after delivery is an opportunistic time to assess cardiovascular risk for women with hypertensive disorders of pregnancy. African ethnicity, parity and reduced eGFR were identified to be independently associated with higher cardiovascular risk scores, in addition to diastolic blood pressure and a diagnosis of chronic hypertension, but biomarkers assessed did not contribute to risk stratification. Future prospective cohort studies to refine and validate a modified FCVRS for use in this population derived from relevant individual patient data with targeted biomarkers are needed.

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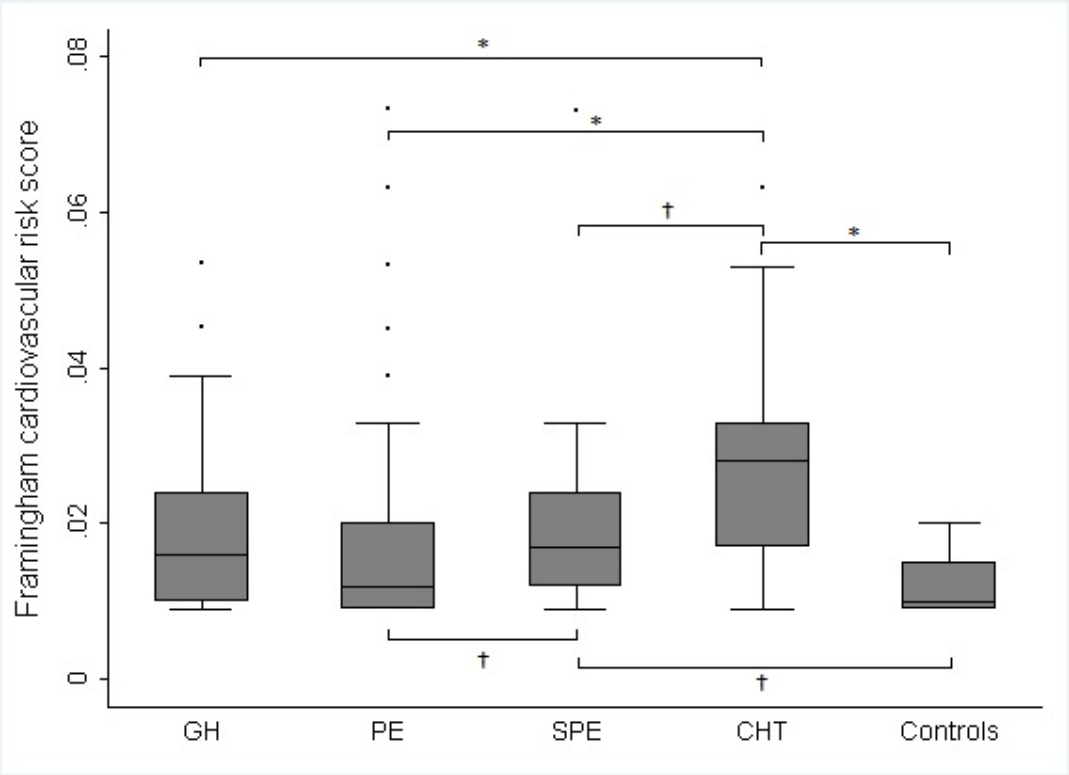

Table1. Descriptive characteristics of study participants at postpartum visit divided by diagnostic group

Characteristic	Gestational Hypertension n=94	Pre-eclampsia n=288	Superimposed Pre-eclampsia n=30	Chronic Hypertension n=51	Controls n=14	*P* value
Age, y	30 (26-35)	29.0 (24-33)	29.0 (24-33)	32.0 (28-36)	31.5 (28-33)	0.06
Time postpartum visit, wk	6.92 (6.1-7.7)	7.14 (6.3-8.6)	7.79 (6.9-8.4) ¥	6.43 (6.0-8.4) #, **	7.36 (6.1-8.1)	0.02
Body Mass Index, kg/m²	29.9 (25.7-34.1) *	28.2 (24.7-32.6) †, ¥	33.1 (27.0-39.3) *, †	30.1 (25.6-34.9) †, #	24.3 (22.6-26.7)	<0.001
Systolic blood pressure, mmHg	124.0 (120-132)*	120.0 (110-130) †, ϕ	130.0 (120-140) *, §, ¥	134.0 (124-144) *, §, ϕ	110.0 (100-118)	<0.001
Diastolic blood pressure, mmHg	80.0 (74-88) *	78.0 (70-82) *, ¥	84.5 (76-92) *, §	88.0 (82-94) *, §, ϕ	68.0 (58-74)	<0.001
Primiparous ¶	35 (21)	203 (70)	20 (67)	21 (41) ‡, §, ||, ϕ	11 (79)	0.002
***Ethnicity* ¶**						
White	85 (90)	237 (82)	27 (90)	45 (88)	11(79)	0.40
Black	5 (5)	29 (10)	3 (10)	2 (4)	0 (0)	0.37
Asian	3 (3)	16 (6)	0	0	1 (7)	0.23
Other	1 (1) †	6 (2) †	0	4 (8) #	2 (14)	0.02
Other disease						
Smoking ¶	13 (14)	17 (7)	4 (10)	4 (8)	1 (7)	0.34
Alcohol use ¶	32 (34)	60 (21) ¥	9 (30)	19 (37)	2 (14)	0.02

Pre-gestational DM ¶	0	9 (3)	1 (3)	0	0	0.35
Anti-hypertensive use						
Nil	87 (93)	250 (87)	15 (50) †, §, ϕ	22 (43) *, §, **, ϕ	14 (100)	< 0.001
1 drug	7(7)	33 (13)	12 (31)	23 (45)	0	
2 drugs	0	0	1 (3)	5 (10)	0	
≥ 3 drugs	0	0	1 (3)	1 (2)	0	

Values are median (IQR) or ¶frequency (percentage). DM indicates diabetes mellitus; GH, gestational hypertension; PE, pre-eclampsia; SPE, superimposed pre-eclampsia; CHT, chronic hypertension.

* $P<0.001$ when compared to control group.

† $P<0.05$when compared to control group.

ϕ $P<0.001$ when compared to GH group.

¥ $P<0.05$ when compared to GH group.

§ $P<0.001$ when compared to PE group.

# $P<0.05$ when compared to PE group.

|| $P<0.001$ when compared to SPE group.

** $P<0.05$ when compared to SPE group.

Table 2. Laboratory parameters of study participants at postpartum visit divided by diagnostic group

Characteristic	Gestational Hypertension n=94	Pre-eclampsia n=288	Superimposed Pre-eclampsia n=30	Chronic Hypertension n=51	Controls n=14	*P* value
Total cholesterol, mmol/L	5.20 (4.5-5.9)	5.20 (4.7-5.8)	5.00 (4.5-5.9)	5.30 (4.8-5.9)	5.60 (5.2-6.2)	0.41
HDL cholesterol, mmol/L	2.04 (1.7-3.0)	2.04 (1.7-3.0)	1.88 (1.7-3.6)	1.95 (1.7-3.3)	2.35 (1.6-3.0)	0.68
LDL cholesterol, mmol/L	2.94 (2.0-3.8)	2.68 (1.8-3.6)	2.92 (1.8-3.6)	2.54 (1.8-3.5)	2.27 (1.7-3.8)	0.49
Triglycerides, mmol/L	1.50 (0.9-1.9)	1.30 (0.9-1.9)	1.65 (1.1-2.2)	1.30 (0.9-1.8)	1.20 (0.8-1.6)	0.13
Alanine Transaminase, U/L	27.0 (18-45)	24.0 (18-39)	24.0 (18-48)	26.0 (16-41)	24.0 (21-39)	0.87
Creatinine, mmol/L	77.0 (71-85) *	75.0 (68-81)	78.0 (71-86)	78.0, (71-87)	72.0 (66-78)	0.01
eGFR, ml/min/1.73m²	109 (101-114)	111 (105-117)	108 (104-116)	107 , (101-112)	111 (106-114)	0.004
eGFR < 90 ml/min/1.73m² ¶	4 (4)	6 (2)	2 (7)	5 (10)	0	0.06
PCR, mg/mmol	7.0 (6-10)	9.0 (7-15)	9.5 (7-17)	8.0 (6-12)	8.0 (6-10)	<0.001
Proteinuria ≥ 30 mg/dL ¶	0	40 (14)	3 (10)	4 (8)	0	<0.001

Values are median (IQR) or ¶frequency (percentage). HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; PCR, urinary protein to creatinine ratio; GH, gestational hypertension; PE, pre-eclampsia; SPE, superimposed pre-eclampsia; CHT, chronic hypertension.

Table 3. Pregnancy outcomes of study participants at postpartum visit divided by diagnostic group

Characteristic	Gestational Hypertension n=94	Pre-eclampsia n=288	Superimposed Pre-eclampsia n=30	Chronic Hypertension n=51	Controls n=14	*P* value
Maternal Outcomes						
Highest systolic blood pressure, mmHg	155.0 (150-164) *	160.0 (150-173) *, ¥	161.0 (158-173) *, ¥	159.0 (149-166) *	131.5 (124-138)	<0.001
Highest diastolic blood pressure, mmHg	100.0 (93-104) *	100.5 (95-108) *	105.0 (101-112) *, ¥	99.0 (94-106) *	79.0 (74-83)	<0.001
Highest PCR, mg/mmol	18.0 (13-23)	106.0 (53-298) ϕ	69.0 (50-131) ϕ, #	17.0 (13-22) §, ||	N/A	<0.001
Gestational diabetes ¶	1 (1)	9 (3)	1 (3)	0	0	0.61
HELLP syndrome ¶	N/A	5 (2)	3 (10)	N/A	N/A	0.04
Mode of delivery ¶						
Vaginal	41 (44)	117 (40)	13 (43)	29 (57)	8 (57)	0.22
Assisted vaginal	23 (24)	59 (21)	6 (20)	8 (16)	0	0.24

Emergency Caesarean section	10 (11)	57 (20) ¥	9 (30) ¥	5 (10) **	3 (21.5)	0.04
Elective Caesarean section	20 (21)	55 (19)	2 (7)	9 (17)	2 (21.5)	0.46
Neonatal Outcomes						
Stillbirth ¶	0	4 (1.4)	1 (3)	0	0	0.55
Gestation at delivery, wk	39.8 (39-41)	38.6 (37-40) ¥	37.7 (34-39) †, ϕ, #	39.0 (38-40) ¥, #, **	39.3 (39-40)	<0.001
Preterm <37 wk	6 (6)	82 (28) ϕ, †	12 (40) ϕ, †	5 (10) #, **	0	<0.001
Preterm <34 wk	0	29 (10) ϕ	6 (20) ϕ	2 (4) **	0	<0.001
Birth Weight, g	3230 (3000-3590)	2975 (2330-3400) ϕ	2470 (2050-3270) ¥	3285 (2860-3600) #, **	3280 (3130-3410)	<0.001
SGA <10th customised birth weight centile ¶	19 (20)	84 (29) †	13 (43) ¥, †	12 (24)	0	0.01
SGA <3rd customised birth weight centile ¶	9 (10)	53 (18) ¥	11 (37) ϕ, †, #	6 (12) **	0	0.004

Neonatal unit admission ¶	6 (6)	51 (18) ¥	7 (23) ¥	2 (4) ¥, #, **	0	0.001

Values are median (IQR) or ¶frequency (percentage). PCR indicates urinary protein to creatinine ratio; HELLP, haemolysis, elevated liver enzymes and low platelets, SGA, small for gestational age; SCBU, special care baby unit; GH, gestational hypertension; PE, pre-eclampsia; SPE, superimposed pre-eclampsia; CHT, chronic hypertension; N/A, non-applicable.

* $P<0.001$ when compared to control group.

† $P<0.05$ when compared to control group.

ϕ $P<0.001$ when compared to GH group.

¥ $P<0.05$ when compared to GH group.

§ $P<0.001$ when compared to PE group.

# $P<0.05$ when compared to PE group.

|| $P<0.001$ when compared to SPE group.

** $P<0.05$ when compared to SPE group.

Table 4. Biomarkers levels of study participants at 6-weeks postpartum visit divided by group

	Gestational Hypertension	Pre-eclampsia	Superimposed Pre-eclampsia	Chronic Hypertension	Controls	
Biomarker	n=30	n=49	n=10	n=13	n=5	*P* value
NGAL, ng/ mL	35.0 (31-38)	34.5 (28-44)	28.0 (24-47)	33.0 (29-36)	34.0 (27-35)	0.82
BNP, pg/mL	8.1 (5-12)	6.2 (5-9)	9.0 (5-15)	7.4 (5-11)	9.3 (5-11)	0.45
BNP categories ¶						0.82
≤ 5 pg/ mL	10 (33.3)	21 (40)	1 (14)	3 (23)	2 (40)	
5.1 – 9.9 pg/ mL	10 (33.3)	19 (37)	3 (43)	5 (38)	1 (20)	
≥ 10 pg/ mL	10 (33.3)	12 (23)	3 (43)	5 (38)	2 (40)	
PlGF,pg/mL	12.1 (12-14)	12.0 (12-14)	12.0 (12-13)	12.0 (12-14)	14.4 (12-15)	0.66
PlGF categories ¶						0.42
≤ 12 pg/ mL	15 (50)	34 (65)	5 (72)	9 (69)	2 (40)	
12.1-17.9 pg/ mL	13 (43)	12 (23)	1 (14)	2 (15.5)	2 (40)	
≥ 18 pg/ mL	2 (7)	6 (11)	1 (14)	2 (15.5)	1 (20)	

Values are median (IQR) or ¶frequency (percentage). NGAL indicates neutrophil gelatinase–associated lipocalin; BNP, B-type natriuretic peptide; PlGF, placental growth factor; GH, gestational hypertension; PE, pre-eclampsia; SPE, superimposed pre-eclampsia; CHT, chronic hypertension.

Table 5. Spearman's correlation coefficients for association between demographics or biomarkers with the 10-year Framingham cardiovascular risk score at postpartum visit

Variable	Spearman's *R* value	*P* value
Diastolic blood pressure	0.597	<0.001*
Systolic blood pressure at booking visit for pregnancy	0.345	<0.001*
Diastolic blood pressure at booking visit for pregnancy	0.366	<0.001*
Body mass index	0.200	<0.001*
Body mass index at booking visit for pregnancy	0.214	<0.001*
Creatinine	0.113	0.02
eGFR	-0.255	<0.001*

Alanine transaminase	0.039	0.42
Uric acid	0.092	0.06
Triglycerides	0.167	<0.001
LDL cholesterol	0.139	0.005
BNP †	0.214	0.04
NGAL ‡	0.162	0.12
PlGF †	-0.038	0.71

eGFR indicates estimated glomerular filtration rate; LDL, low-density lipoprotein; BNP, brain natriuretic peptide; NGAL, neutrophil gelatinase-associated lipocalin, PlGF, placental growth factor.

* statistical significance after Bonferroni correction.

Sample variation: † n=96; ‡ n=91; § n=326.

Table 6. Univariate and multivariate analysis of risk variables associated with 10-years Framingham cardiovascular risk score at postpartum visit

	Univariate				*Multivariate*			
Risk Factor	OR	SE	95% CI	*P*	OR	SE	95% CI	< 0.001
Diastolic blood pressure	1.0005	0.00004	1.0005-1.0006	< 0.001	1.0005	0.00005	1.0004-1.0006	0.004
Primiparity	0.994	0.001	0.992-0.996	<0.001	0.997	0.001	0.995-0.999	0.001
African ethnicity*	1.006	0.002	1.002-1.01	0.001	1.007	0.002	1.003-1.01	0.004
eGFR	0.9998	0.00005	0.9997-0.99993	<0.001	0.9998	0.00005	0.9997-0.9999	0.061
Family history of IHD	1.004	0.002	1.0005-1.007	0.024	1.003	0.001	0.9999-1.005	< 0.001

eGFR indicates estimated glomerular filtration rate; IHD, ischemic heart disease.

* compared to white European ethnicity.

Adjusted *R*-squared of model is 0.33, *P*<0.001

Highlights

- At six weeks postpartum Framingham cardiovascular risk scores are not higher in women with pregnancies complicated by pre-eclampsia compared with healthy controls, nor in women with pregnancies complicated by superimposed pre-eclampsia compared with women with chronic hypertension
- Women with gestational hypertension have higher Framingham cardiovascular risk scores than women with pre-eclampsia and healthy controls at six weeks postpartum
- B-type natriuretic peptide (BNP), neutrophil gelatinase–associated lipocalin (NGAL) and placental growth factor (PlGF) concentrations at 6 weeks after delivery are not associated with Framingham cardiovascular risk scores