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The correlation between maternal serum sST2, IL-33 and NT-proBNP concentrations and occurrence of pre-eclampsia in twin pregnancies: A longitudinal study

Qianqian Xiang MD[1,2,3] | Yang Chen MD[1,2,3] | Xunke Gu MD[1,2,3] | Yike Yang MD[1,2,3] | Yan Wang MD[1,2,3] | Yangyu Zhao MD, PhD[1,2,3]

[1]Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China

[2]National Clinical Research Center of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China

[3]National Center for Healthcare Quality Management in Obstetrics, Beijing, China

Correspondence
Yangyu ZHAO, Department of Obstetrics and Gynecology, Peking University Third Hospital, No. 49 Huayuan North Road, Haidian District, Beijing 100191, China.
Email: zhaoyangyu@bjmu.edu.cn

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Abstract

The primary objective of this study was to determine the longitudinal profile of serum sST2 (soluble suppression of tumorigenicity 2), IL-33 (interleukin-33) and NT-proBNP (N-terminal pro-brain natriuretic peptide) concentrations in twin pregnancies with pre-eclampsia (PE) and those normotensive twins. The secondary objective was to test whether the change of serum sST2,IL-33 and NT-proBNP is related to PE in twin pregnancies. This is a longitudinal nested case–control study and all 156 dichorionic (DC) pregnancies were from a prospective cohort of twin pregnancies who received antenatal care and gave two live births at Peking University Third Hospital between October 2017 and September 2020. Four to five milliliters of peripheral blood of each pregnant woman were collected during the following three intervals: (1) 6–11^{+6} weeks; (2) 24–27^{+6} weeks; (3) 28–31^{+6} weeks. We found that sST2 and NT-proBNP levels increased as pregnancy progressed in normotensive twin pregnancies and further increased in PE group, while no differences were found in IL-33 levels throughout pregnancy. Then the correlation of biomarker levels with the occurrence of PE was assessed. Our results indicated that combining maternal serum sST2 and NT-proBNP levels yielded the highest predictive value on the occurrence of PE significantly higher than the predictive value of any markers alone. Interestingly, the predictive value of second trimester (AUC = 0.876, 95%CI 0.824–0.928, LR−0.338, LR+7.67, $p < 0.001$)was higher than that of early-third trimester (AUC = 0.832, 95%CI 0.769–0.896, LR−0.29, LR+3.845, $p < 0.001$). Serum sST2 and NT-proBNP concentrations during second and early-third trimester were associated with the occurrence of PE in twin pregnancies.

KEYWORDS
cardiac function marker, IL-33, NT-proBNP, pre-eclampsia, sST2, twin pregnancy

1 | INTRODUCTION

Pre-eclampsia (PE) is a life-threatening pregnancy complication associated with the aggradation of fetal and maternal morbidity and mortality. Serum soluble fms-like tyrosine kinase-1(sFlt-1)/placental growth factor (PIGF) ratio has been developed to estimate the risk of PE in singleton pregnancies, based on the hypothesis that the imbalanced production of pro- and anti-angiogenic factors is involved in the pathogenesis of PE. However, studies of this risk prediction model showed inconsistent results in twin pregnancies.[1] The risk of developing PE in twin pregnancies is two- to three-fold higher than in singleton pregnancies that may appear in atypical ways, possibly due to the larger placental mass and use of assisted reproductive technology (ART).[2] It is essential to find new screening biomarkers in twin pregnancies with the increasing incidence of PE. In recent decades, pregnancy has been hypothesized as a cardiovascular stress test revealing the biomarker profile in pre-eclamptic pregnancies may thus be an early reflection of the biochemical imbalance in cardiovascular disease (CVD) at advanced age. Compared with uncomplicated singleton pregnancy, maternal cardiac function and hemodynamic changes appear to be more pronounced in twin pregnancies, presumably in order to supply the higher uteroplacental demand.[3] A longitudinal study of twin pregnancies found that the maternal preload reserve decreased after the second trimester, making these pregnant women more vulnerable to fluid overload.[4] V. GIORGIONE et al.[5] found that twin pregnancies complicated by hyper-tensive disorders of pregnancy exhibited more severe changes in maternal cardiovascular function. Therefore, serum biomarkers which reflect the early changes of cardiac dysfunction may indicate the development of hypertensive disorders in twin pregnancies. NT-proBNP is a cardiac hormone secreted by ventricular myocytes and the rise of NT-proBNP can reflect the degree of compliance change of cardiomyocytes and evaluate the reserve function of the heart. Paula Lafuente-Ganuza et al.[6] found that sFlt-1/PIGF combined with NT-proBNP could more accurately predict PE in singleton pregnancies. Additionally, sST2 is a novel marker of myocardial cell secretion, which can predict the occurrence of adverse cardiovascular events. In the heart, ischaemia reperfusion injury and infarction are moderated by IL-33 which protects cardiomyocytes from apoptosis, and the benefit is partially abolished by sST2 that acts as a decoy receptor for IL-33 and is thought to inhibit IL-33 function.[7] Eda Gokdemir I et al.[8] found lower serum levels of IL-33 in the PE group compared to controls in singleton pregnancies. Romero R et al.[9] showed that maternal serum sST2 concentrations are elevated 6 weeks prior to the diagnosis of PE and the prediction efficiency for pre-term PE of sST2 is comparable to that of sflt-1/PIGF in singleton pregnancies. These CVD biomarkers showed prospects in identifying PE among singleton pregnancies. However, few studies have focused on twin pregnancies. This study aimed to explore the longitudinal changes during pregnancy of three serum cardiac function markers including NT-proBNP, sST2 and IL-33, and their association with the occurrence of PE in twin pregnancies.

2 | MATERIALS AND METHODS

2.1 | Subject

This is a longitudinal nested case–control study and all subjects were from a prospective cohort of twin pregnancies who received antenatal care and gave two live births at Peking University Third Hospital, China between October 2017 and September 2020. They were followed up until delivery and their pregnancy outcomes were recorded. All women provided written informed consent during their first antenatal examination. Four to five milliliters of peripheral blood was collected from each pregnant woman during the following three intervals: (1) 6–11^{+6} weeks, (2) 24–27^{+6} weeks, (3) 28–31^{+6} weeks. The exclusion criteria included: (1) patients with chronic hypertension and serious heart disease before pregnancy; (2) other serious medical complications (such as autoimmune disease, hyperthyroidism, liver failure, chronic kidney disease or renal failure); (3) monochorionic (MC) twin pregnancies, abortion or delivery before 28 weeks; (4) patients with incomplete serum samples. The diagnostic criteria of PE is new-onset hypertension (systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥90 mmHg on at least two occasions 4 h apart developing after 20 weeks' gestation in a previously normotensive woman) accompanied by proteinuria or other signs of severe PE, as previously described by American College of Obstetricians and Gynecologists (ACOG).[10] The total cohort comprised of 774 twin pregnant women, including 502 dichorionic (DC) pregnancies and 108 women of them were diagnosed with PE. Thirty women whose serum samples were incomplete were excluded. Finally, 78 dichorionic twin pregnancies with PE were defined as the PE group. The control cases were chosen randomly from the same cohort with a ratio of 1:1.

2.2 | Sample collection and detection of NT-proBNP, sST2 and IL-33 by ELISA

The blood samples collected by venipuncture were stored in tubes containing EDTA and the serum was separated by centrifugation. Then, the samples were stored at −80°C. Specific ELISA was used for the determination of maternal serum concentrations of NT-proBNP, sST2 and IL-33(Mercodia, Shanghai Kexing Biological Technology, China). The limit of detection for the NT-proBNP method was 1.0 pg/ml and the sensitivities of the assays for sST2 and IL-33 were 10 pg/ml and 2.5 pg/ml, respectively. The intra- and inter-assay precision was <10% and <15%, respectively.

2.3 | Statistical analysis

Continuous variables were summarized as mean ± SD (standard deviation) or median (25th, 75th percentile). Categorical variables were presented as counts (%). For two-group comparison, parametric

(2-sample *t*-test) and non-parametric tests (Mann–Whitney *U* test) for continuous variables, and the χ^2 test or Fisher exact test for nominal variables were adopted. Correlation between two continuous variables was determined using Spearman's rank correlation. Log_{10}-transformed data were used where the data were not normally distributed. The data collected in this study contain longitudinal measurements from the same individuals belonging to the two groups, so repeated measures ANOVA was used with a Bonferroni post hoc test to assess the change of serum sST2,IL-33 and NT-proBNP during pregnancy. The predictive power of biomarker levels concerning the occurrence of PE were analyzed by univariate logistic regression analysis adjusted for maternal age, pre-BMI, prenatal weight gain, blood sampling time, primigravida or not, conception by IVF/ICSI and MAP in the first trimester. The true-positive and false-positive rates for the diagnosis of PE were estimated from ROC, and the diagnostic powers of the biomarker levels were assessed from the respective area under the curve (AUC). Cut-off values for maternal serum sST2, NT-proBNP and IL-33 levels were calculated using Youden's index. The positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR) and negative likelihood ratio (−LR) values were used to assess model performance. Statistics analysis was conducted using SPSS version 26(IBM, Armonk, NY, USA).In all analyses, two-tailed $p \leq 0.05$ were considered to indicate statistical significance and have been adjusted for multiple testing.

3 | RESULTS

This longitudinal nested case–control study included DC twin pregnancies in the following groups: (1) PE group ($n = 78$) (2) control group ($n = 78$). The demographic and clinical characteristics of the study groups are displayed in Table 1.There were no statistical differences between the PE and control group in maternal age, prenatal weight gain, blood sampling time, primigravida or not and conception by IVF (in-vitro fertilization) or ICSI (intracytoplasmic sperm injection). About the kidney function, there were no statistical differences between the PE and no-PE patients in the level of serum creatinine in first trimester(55.80 ± 11.42 vs52.98 ± 8.40 umol/L, $p = 0.125$).The pre-BMI (body mass index) of PE group was significantly higher than control group ($p<0.001$). As expected, pre-eclamptic women had significantly higher mean arterial pressures (MAP) in the first trimester compared to the controls (all $p<0.001$). The median gestational age at delivery and birthweight were lower in PE group than those in the controls ($p<0.001$ both). In PE group, 83.33% (65/78) women delivered before 37 weeks of gestation, 15.38% (12/78) women delivered before 34 weeks of gestation, and 51.28% (40/78) women had severe PE. None of the subjects had pregestational diabetes mellitus and there were no statistical differences between the PE and no-PE patients in presence of gestational diabetes mellitus (30.77(24/78) versus 20.51(16/78), $p = 0.142$). Adverse obstetric outcomes including a higher percentage of low birthweight (LBW) and small for gestational age (SGA) were apparently found in women with pre-eclampsia. In PE group, 66.03% (103/156) delivered neonates whose birthweights were below 2500 g (LBW), while the percentage was lower in the control group (32.05%). There were no statistical differences between the PE and no-PE patients in small for gestational age (SGA) ($P = 0.097$).

TABLE 1 Demographic characteristics and pregnant outcomes of the enrolled women

Parameter	No pre-eclampsia $N = 78$	Pre-eclampsia $N = 78$	*P* value
Maternal age (years)	32.62 ± 4.21	33.01 ± 3.27	0.511
Primiparous (%)	70(89.74)	65(83.33)	0.241
IVF/ICSI(%)	61(78.21)	59(75.64)	0.572
Pre-BMI (kg/m^2)	21.28 ± 2.42	23.61 ± 3.32*	<0.001
Weight gain (kg)	17.46 ± 4.39	17.68 ± 4.97	0.763
SBP-T1(mmHg)	110(103,116)	118(110,123)*	<0.001
DBP-T1(mmHg)	67(62,72)	74(69,79)*	<0.001
MAP-T1(mmHg)	81(77,86)	89(83,92)*	<0.001
Scr (umo/L)	52.98 ± 8.40	55.80 ± 11.42	0.125
Blood sampling time (gestational weeks)			
T1 (first trimester)	8(7, 10)	9(7, 11)	0.207
T2 (second trimester)	24(24, 25)	24(24, 25)	0.721
T3 (third-early trimester)	29(28, 30)	29(28, 30.25)	0.43
Delivery pregnancy weeks	37(37,37)	35(34,36)*	<0.001
GDM(%)	20.51(16/78)	30.77(24/78)	0.142
NBW	2667 ± 302	2284 ± 441*	<0.001
LBW (%)	50 (32.05)	103 (66.03)*	<0.001
SGA(%)	6 (3.84)	13 (8.33)	0.097

Values are given as mean ± standard deviation, median (interquartile range) or count (percentage).
Abbreviation: IVF, in-vitro fertilization; ICSI, intracytoplasmic sperm injection; Pre-BMI, pre-pregnancy body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; Scr, serum creatinine; T1, first trimester; T2, second trimester; T3, early-third trimester; GDM, gestational diabetes mellitus; NBW, newborn birth weight; LBW, low birth weight infant; SGA, small for gestational age.
*PE vs. no-PE $P<0.05$.

IL-33, sST2 and NT-proBNP were then measured in serum samples taken from the included 156 DC pregnancies during three intervals that were showed in Figure 1. The gestational weeks of blood sampling were comparable between the two groups. In the first trimester, there were no differences in IL-33,sST2 and NT-proBNP levels between the two groups. About the second trimester and early-third trimester, maternal serum levels of sST2 and NT-proBNP were significantly higher in PE group ($P<0.001$). However, no differences were found about IL-33 between the two groups.

The longitudinal analysis was showed in Figure 2. Despite a raised median value in the pre-eclamptic women there were no significant

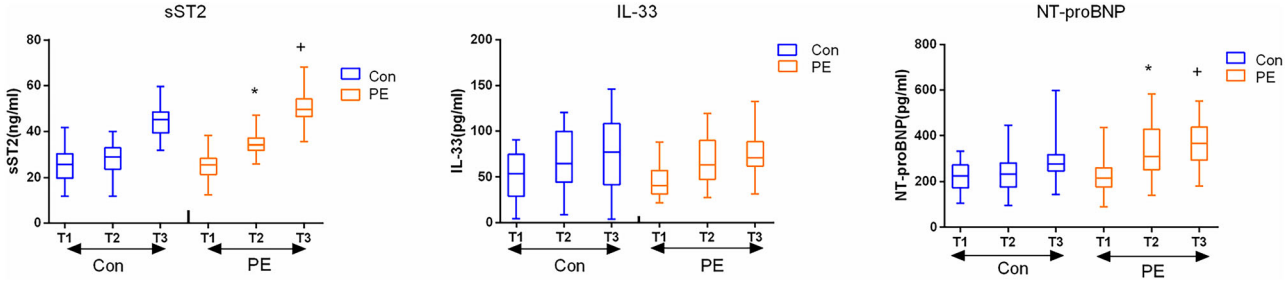

FIGURE 1 Circulating sST2, IL-33 and NT-proBNP in PE and no-PE group. sST2,IL-33 and NT-proBNP were detected by ELISA in the serum from women with a diagnosis of PE, matched controls who had a normal pregnancy. Blood samples were taken in each trimester of pregnancy (T1, T2, T3). T1: first trimester, T2: second trimester, T3: early-third trimester. *PE versus no-PE in T2 $P<0.001$, $^{+}$PE versus no-PE in T3 $P<0.001$

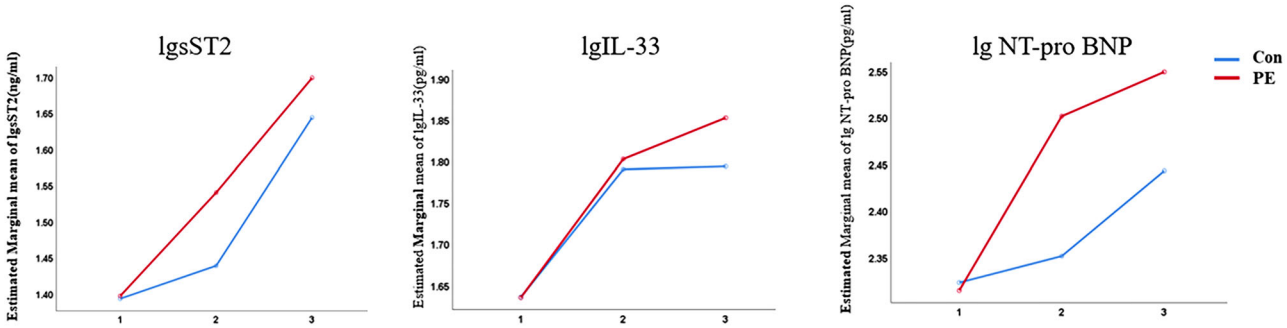

FIGURE 2 Longitudinal profiles of serum sST2,IL-33 and NT-proBNP concentrations. Maternal serum concentration of sST2 IL-33 and NT-proBNP (log, base 10, thereof) in women with no-PE pregnancy (bule line) and those who subsequently developed PE (red line). The horizontal axis represents time: 1 first trimester, 2 second trimester, 3 early-third trimester. The vertical axis represents the estimated marginal mean of sST2, IL-33 and NT-proBNP controlling time, group and the interaction of time and group factors

differences of IL-33 levels between the two groups throughout pregnancy (F (1.805, 138.992) = 1.988, P = 0.145). On the contrary, this study found that sST2 and NT-proBNP levels increased as pregnancy progressed in normal twin pregnancies and further increased in PE group (all $P<0.001$). There was a significant increase in sST2 in the early-third trimester of control compared to the first (mean difference 1.78, 95%CI 1.65–1.93 ng/ml, $P<0.001$) and second (mean difference 1.60, 95%CI 1.52–1.69 ng/ml, $P<0.001$) trimesters. Interestingly, the mean difference of sST2 levels were maximal of PE compared to the controls in the second trimester (1.26, 95%CI 1.19–1.29 ng/ml, $P<0.001$) throughout pregnancy. Similar results can be obtained in the analysis of NT-proBNP in second trimester (mean difference 1.41, 95%CI 1.26–1.59 pg/ml, $P<0.001$).

Results of multivariate logistic regression and ROC curve analysis are summarized in Figure 3 and Tables 2–3. Increased serum sST2 (≥30.7 ng/ml) and NT-proBNP (≥282.2 pg/ml) were found to be independently associated with the occurrence of PE (OR: 8.13; 95%CI: 3.33–19.84; $P < 0.001$, OR: 7.20; 95%CI: 3.14–16.47; $P < 0.001$). A sST2 level cut-off at 30.7 ng/ml had a LR+ of 2.13 for the PE (AUC = 0.802, 95%CI 0.735–0.869, $P < 0.001$) as well as NT-proBNP level with a cut-off at 282.2 pg/ml had a LR+ of 3.47 for the outcome of PE (AUC = 0.768, 95%CI 0.695–0.841, $P < 0.001$). Similarly, increased serum sST2 (≥51.0 ng/ml) and NT-proBNP(≥341.3 pg/ml) were found to be independently associated with the occurrence of PE in early-third trimesters (OR: 4.81; 95%CI: 1.92–12.06; $P = 0.001$, OR: 4.22; 95%CI: 1.88–9.50; $P < 0.001$). Combining maternal serum sST2 and NT-proBNP levels improved the predictive value of PE and the predictive value of second trimester (AUC = 0.876, 95%CI 0.824–0.928, LR−0.338, LR+7.67, $P < 0.001$) was higher than that of early-third trimester (AUC = 0.832, 95%CI 0.769–0.896, LR−0.29, LR+3.845, $P < 0.001$).

4 | DISCUSSION

This is a longitudinal study to explore the correlation between the changes of cardiovascular biomarkers including NT-proBNP, sST2 as well as IL-33 and the occurrence of PE in twin pregnancies. Our results showed that maternal serum sST2 levels increased as pregnancy progressed in normal twin pregnancies and further increased in pre-eclamptic women, before the onset of clinical symptoms. Ingrid Granne' study[11] found sST2 levels were significantly higher in the third trimester of PE compared to normal pregnancies, but not in second trimester. Concentrations of sST2 were relatively constant until 30 weeks of gestation in singleton pregnancies, after which they increased steadily until the time of delivery due to changes in cytokine

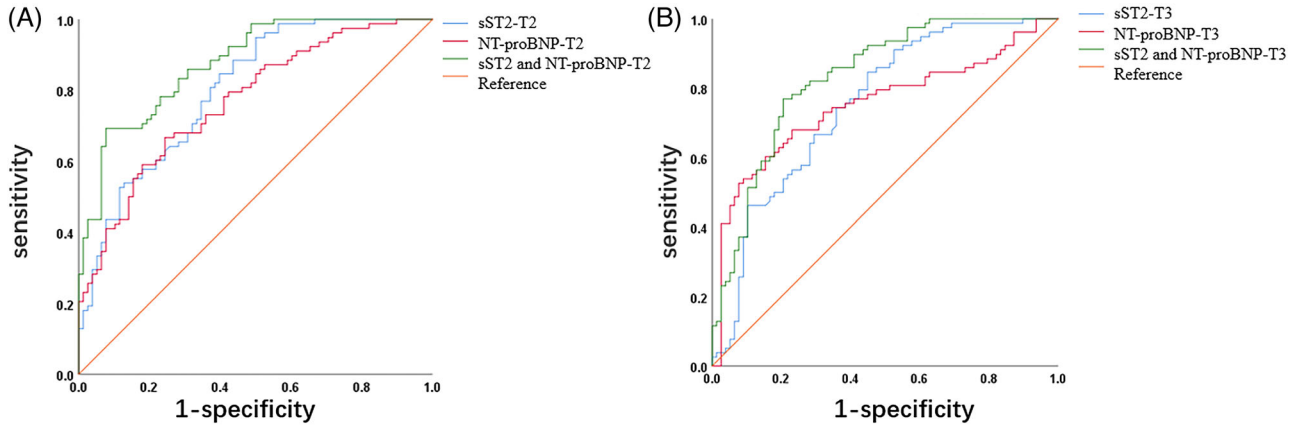

FIGURE 3 ROC curves showing the predictive value of serum sST2 and NT-proBNP in pre-eclampsia (PE). Predictive value of serum biomarkers alone and in a combination of serum sST2 and NT-proBNP on the occurrence of PE. A: second trimester(T2): (sST2: AUC = 0.802; NT-proBNP: AUC = 0.768; serum sST2 and NT-proBNP: AUC = 0.876). B: early-third trimester(T3): (sST2: AUC = 0.752; NT-proBNP: AUC = 0.753; serum sST2 and NT-proBNP: AUC = 0.832)

TABLE 2 Multivariate regression analyses showing the association between sST2, NT-proBNP and PE in twin pregnancies

Parameter	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
sST2-T2 ≥ 30.7 ng/ml	8.21(3.82–17.91)	<0.001	8.13(3.33–19.84)	<0.001
sST2-T3 ≥ 51.0 ng/ml	6.42(3.00–13.71)	<0.001	4.81(1.92–12.06)	0.001
NT-proBNP-T2 ≥ 282.2 pg/ml	8.40(4.03–17.50)	<0.001	7.20(3.14–16.47)	<0.001
NT-proBNP-T3 ≥ 341.3 pg/ml	4.77(2.43–9.38)	<0.001	4.22(1.88–9.50)	0.001

Maternal age, pre-BMI, prenatal weight gain, blood sampling time, primigravida or not, conception by IVF/ICSI and MAP in the first trimester were adjusted.
Abbreviations: PE, pre-eclampsia; sST2, soluble suppression of tumorigenicity 2; NT-proBNP, N-terminal pro B-type natriuretic peptide; OR (95% CI), odds ratio (95% confidence interval).

TABLE 3 Sensitivity, specificity, negative and positive predictive value, likelihood ratios, and area under the curve (AUC) values of determined sST2 and NT-proBNP cut-off levels in patients with PE

Parameter	Cut-off	Sensitivity	Specificity	NPV	PPV	LR-	LR+	AUC (95% CI)	*P* value
sST2-T2	30.7 ng/ml	0.846	0.603	0.797	0.68	0.255	2.13	0.802(95%CI 0.735–0.869)	<0.001
NT-proBNP-T2	282.2 pg/ml	0.667	0.808	0.798	0.776	0.412	3.47	0.768(95%CI 0.695–0.841)	<0.001
sST2 and NT-proBNP(T2)		0.692	0.910	0.747	0.885	0.338	7.67	0.876(95%CI 0.824–0.928)	<0.001
sST2-T3	51.0 ng/ml	0.846	0.551	0.778	0.647	0.281	1.88	0.753(95%CI 0.677–0.830)	0.001
NT-proBNP-T3	341.3 pg/ml	0.679	0.692	0.683	0.679	0.453	2.20	0.752(95%CI 0.673–0.831)	0.001
sST2 and NT-proBNP(T3)		0.769	0.795	0.775	0.789	0.29	3.85	0.832(95%CI 0.769–0.896)	<0.001

Abbreviations: PE, pre-eclampsia; sST2, soluble suppression of tumorigenicity 2; NT-proBNP, N-terminal pro B-type natriuretic peptide; AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; LR−, negative likelihood ratio; LR+, positive likelihood ratio.

concentrations and in the volume of maternal circulation.[9] However, in this study we found different pattern in DC pregnancies compared with the singleton pregnancies revealed in other studies. The mean difference of sST2 levels were maximal of PE compared to the control in the second trimester throughout pregnancy. Sasmaya PH et al. found that mean sST2 level in the PE group (85.89 ng/ml) was significantly higher than the control group mean (38.3 ng/ml) during the third trimester (30–38 weeks) in singleton pregnancies.[12] The median of sST2 in the PE group in our study was lower than in the previous study focused on singleton pregnancies that further explained the

different pathogenesis of PE in twin pregnancies. Additionally, Stampalija T et al.[13] found that serum sST2 concentration in singleton pregnant women with PE was higher than that in normal pregnant women, especially in preterm and severe PE. Increased endothelial cell production of sST2 were found in patients with diastolic cardiac dysfunction due to volume overload.[14] Twin pregnancies have 10–20% augmentation of serum volume and 20% higher cardiac output than singleton pregnancies. Thus, the upward trend of sST2 levels occurred in the advanced gestational weeks. Moreover, twin pregnancies have larger placental mass which is one source of sST2. Our study found the mean difference of sST2 levels were maximal of PE compared to the controls in the second trimester throughout pregnancy, So that sST2 showed prospect in identifying PE among twin pregnancies.

Here we can find no differences in circulating IL-33 between PE group and controls at any stage of gestation. The similar findings in singleton pregnancies were showed in previous study.[11] Maternal cardiac function and hemodynamic changes appear to be more pronounced in twin pregnancies, presumably in order to supply the higher uteroplacental demand. IL-33 is a member of IL-1 family, originally identified as a potent driver of T helper type 2(Th2) immune response that play a significant protective role in heart and despite IL-33 having atherogenic protection and may prevent obesity and type 2 diabetes by regulating lipid metabolism, on the other hand, IL-33 appears to drive endothelial inflammation.[15]Hong Chen et al[16] detected that IL-33 expression was decreased significantly in placenta from severe PE patients and in an in vitro PE model treated with sodium nitroprusside (SNP) that demonstrated that the reduction of IL-33 production was connected with the reduced functional capability of trophoblast cells, thus inducing placental insufficiency that has been linked to the development of PE. Evidence suggests that macrophage-derived IL-33 plays a vital role in the proliferation of trophoblast cells and decidual stromal cells.[17] Several studies[18,19]have proven that the disorganization and dysregulation of trophoblast cells, which contribute to poor placentation, are a major underlying mechanism of PE development. To what extent these functions are relevant to the pathogenesis of PE remains to be elucidated.

This study also found that serum NT-proBNP levels were relatively constant during normal pregnancy before third trimester but significantly increased in cases of PE in second and early-third trimester compared with controls throughout pregnancy that were independent factors affecting the incidence of PE in DC pregnancies. As expected, our results showed that serum NT-proBNP levels behaved differently in twin pregnancies compared with singleton pregnancies whose NT-proBNP levels do not change during normal pregnancy.[20] Similar results were obtained in Takashi Yamada's study on twins, they indicated that women with twin pregnancies were likely to exhibit increased serum NT-proBNP levels in the late stage of pregnancy. The greater degree of blood volume expansion occurring in twin pregnancies than singletons is considered to be responsible for this phenomenon.[21] As we know, NT-proBNP is accepted as a more stable and reliable factor for measurement than BNP itself, becoming a convenient and objective diagnostic marker of cardiac dysfunction in adults.[22] Recently, Verlohren et al. assessed the role of NT-proBNP for predicting PE,[23] consistent with our findings, high levels of NT-proBNP were related to the incidence of PE. Junus K et al. found in singleton pregnancy that women with PE have higher NT-proBNP serum levels compared with controls of similar gestational age ($p < 0.001$).[24A] longitudinal cohort study[25] showed that the addition of NT-proBNP assessment improves the short-term prediction of delivery as a result of PE compared with sFlt-1/PlGF ratio alone (AUC = 0.845; 95%CI: 0.787–0.896). All of the previous studies focused on the singleton pregnancies,but our results indicate that NT-proBNP could be used as a predictive biomarker of PE in DC pregnancies. Further research will be needed in the future. Therefore, we focused on these three cardiac biomarkers to find new screening biomarkers in twin pregnancies with the increasing incidence of PE.

In singleton pregnancies, the risk factors for PE are well established, and a combined first-trimester prediction model has been shown to adequately predict preterm disease. Furthermore, intervention with low-dose aspirin at 150 mg/day in those identified as high-risk reduces the rate of preterm pre-eclampsia by 62%.[26]But these significant advances in the prediction and prevention of PE in singleton pregnancies have not yet translated to twin pregnancies. PE may often occur more in twin pregnancies than in singleton pregnancies due to mild vascular endothelial cell dysfunction (relatively low sFlt-1/PlGF ratio) and the larger pregnancy-related burden on the maternal cardiovascular system in twin pregnancies compared with that in singleton pregnancies. In 2017, the Fetal Medicine Foundation (FMF) proposed that the competing-risks model used for the prediction of pre-eclampsia in singletons could perhaps be adapted for risk assessment in twins.[27] However, validation studies have demonstrated that the use of an adapted singleton competing-risks model of maternal characteristics and history has poor calibration with overestimation of risk when applied to twin pregnancies.[28,29] PE is a result of excess uteroplacental demands overwhelming the normal cardiovascular adaptations of pregnancy which in turn results in endothelial dysfunction and the clinical manifestations of disease.[30] Lisheng Liu et al found[31]that serum ST2 served as diagnostic biomarkers for gestational hypertension(GH) and PE in singleton pregnancy, and the AUCs were 0.734 for GH and 0.816 for PE, respectively. Regardless of the role of other controlled factors that modify serum NT-proBNP and sST2, since sST2 levels were not significantly influenced by age and BMI as well as NT-proBNP levels do not change during pregnancy in healthy women,[20] and its transplacental transfer seems negligible. So that the objective of this study was to longitudinally evaluate maternal cardiac markers in DC pregnancies and our results revealed that sST2 as well as NT-proBNP may be promising predictor of PE in twin pregnancies. Furthermore, given the high rate of PE in twin pregnancies and the lack of an adequate screening model, the benefit of screening prior to intervention within the population is uncertain. Therefore, this study aimed to explore the association of longitudinal changes during pregnancy between serum cardiac function markers NT-proBNP, sST2 as well as IL-33 and PE to guide the prediction of PE in twin pregnancies as well as to allow for lifestyle modification, close monitoring and guide long-term CVD management after delivery.

The strength of the study lies in the longitudinal study design that is helpful to reflect the dynamic change process of disease. Additionally, this study focused on the different profiles of serum sST2,IL-33 and NT-proBNP concentrations in relation to gestational age in twin pregnancies with PE. But our study was a single-center, nested case–control study so it is significant for prospective evaluation of cardiac function markers of twin pregnancies in future.

5 | CONCLUSION

This study found that serum sST2 and NT-proBNP concentrations during second or early-third trimester were associated with the onset of PE in twin pregnancies. In short, maternal sST2 and NT-proBNP are expected to be potential predictor of PE in twin pregnancies.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Qianqian Xiang contributed to the project development, data collection and analysis, manuscript writing and editing. Yangyu Zhao contributed to the conception of the study and contributed significantly to analysis and manuscript preparation. Yan Wang, Yang Chen, Xunke Gu and Yike Yang helped perform the analysis with constructive discussions.

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