# SCREENING FOR PREECLAMPSIA IN TWIN PREGNANCIES

**PhD Thesis**


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**ABBREVIATIONS**

| | |
|---|---|
| ACOG | American College of Obstetricians and Gynaecologists |
| APS | Antiphospholipid syndrome |
| ART | Assisted Reproductive Technology |
| AUROC | Area under the receiver operating characteristic curves |
| BMI | Body mass index |
| CRL | Crown-rump length |
| DC | Dichorionic twin pregnancy |
| DCDA | Dichorionic, diamniotic |
| DIC | Disseminated intravascular coagulation |
| HELLP | Haemolysis, elevated liver enzymes, low platelets |
| GH | Gestational hypertension |
| ISSHP | International society for the study of hypertension in pregnancy |
| IQR | Interquartile range |
| IVF | In vitro fertilization |
| MC | Monochorionic twin pregnancy |
| MCDA | Monochorionic, diamniotic twin pregnancy |
| MCMA | Monochorionic, monoamniotic twin pregnancy |
| NICE | National Institute for Health and Clinical Excellence |
| NHS | National Health System |
| PE | Preeclampsia |
| PLGF | Placental growth factor |
| ROC | Receiver operating characteristic |
| sEng | Soluble Endoglin |
| sFlt-1 | Soluble Fms-like tyrosine kinase-1 |
| SLE | Systemic lupus erythematosus |
| SPREE | Screening ProgRamme for prE-Eclampsia |
| TGF | Transforming growth factor |
| UK | United Kingdom |
| VEGF | Vascular Endothelial Growth Factor |
| WHO | World health organisation |

# 1. INTRODUCTION

# 1.1. PREECLAMPSIA

## 1.1.1 Background

Preeclampsia, which is one of the leading causes of maternal and perinatal morbidity and mortality,[1] is a global health problem and great efforts have been made worldwide to develop methods for prediction and prevention of PE. In singleton pregnancies, the incidence of PE is 2–3%.[2]

In the last few years there is an increasing number of twin pregnancies due to the increase in maternal age and the improvement in assisted reproductive technology. Overall, twin gestations constitute 2-5% of all pregnancies, and they have an increased risk of almost every pregnancy complication, including GH and PE, gestational diabetes and thromboembolism.[3] In twin pregnancy, the incidence of PE is about 9%.[4-13] Consequently, the relative risk of PE for twin compared to singleton pregnancies is about 3. However, twins are delivered at an earlier gestational age than singletons and consequently comparison of the overall rates of PE between twin and singleton pregnancies underestimates the relative risk of preterm-PE in twins which is 9-times higher.[P1]

## 1.1.2 Definition of PE

Preeclampsia is a multisystem syndrome developing during the second half of pregnancy. The most commonly used definition of PE is from the International Society for the Study of Hypertension in Pregnancy (ISSHP): characterised by maternal hypertension and either proteinuria or maternal organ dysfunction or uteroplacental dysfunction.[14]

Revised definition of PE (ISSHP, 2014).[14]
The revised definition of PE, according to the ISSHP is develoment of hypertension after 20 weeks gestation and the coexistence of one or more of the following new-onset conditions:

1. Proteinuria (spot urine protein/creatinine >30 mg/ mmol [0.3 mg/mg] or >300 mg/day or at least 1 g/L [‘2 + ’] on dipstick testing)
2. Other maternal organ dysfunction:
   - renal insufficiency (creatinine >90 umol/L; 1.02 mg/dL)
   - liver involvement (elevated transaminases – at least twice the upper limit of normal ± right upper quadrant or epigastric abdominal pain)
   - neurological complications (examples include eclampsia, altered mental status, blindness, stroke, or more commonly hyperreflexia when accompanied by clonus, severe headaches when accompanied by hyperreflexia, persistent visual scotomata)
   - haematological complications (thrombocytopenia – platelet count below 150,000/dL, DIC, haemolysis)
3. Uteroplacental dysfunction
   - fetal growth restriction

However, at the time of the study for this Thesis the traditional definition of PE was widely used, which requires the development of both hypertension and proteinuria.[15] Hypertension was defined as systolic blood pressure of ≥140 mm Hg and/or diastolic blood pressure of ≥ 90 mmHg on two occasions four hours apart developing after 20 weeks’ gestation in previously normotensive women. Proteinuria is the presence of ≥300 mg of protein in a 24-hour collection of urine or urinary protein to creatinine ratio of ≥30 mg/mmol or two readings of at least ++ on dipstick analysis of a midstream or catheter urine specimen.[15]

## 1.1.3 Pathogenesis of PE

The pathophysiology of PE is still not fully understood therefore there is a wide-range of theories.

### 1.1.3.1 Placental dysfunction

One of the most important elements of the pathogenesis of PE lies in impaired placentation. Uterine arteries provide the main blood supply to the uterus, which branch

to form the arcuate, radial, basal and spiral arteries. In pregnancy, the blastocyst implants into the maternal endometrium and the outer layer develops into trophoblast, which invade and transform the spiral arteries. Essentially, trophoblasts replace the endothelial lining and destroy the musculoelastic tissue in the walls of the spiral arteries so that they are converted from narrow muscular vessels into large non-muscular channels thereby increasing maternal blood flow to the placenta. An impaired trophoblastic invasion of spiral arteries can cause increased blood flow and damage the placenta, resulting in placental hypoxia leading to oxidative stress. In this process trophoblast-derived factors are released into the maternal bloodstream and cause widespread endothelial dysfunction; the clinical manifestations of endothelial dysfunction include hypertension, proteinuria or multi organ dysfunction.[16-18]

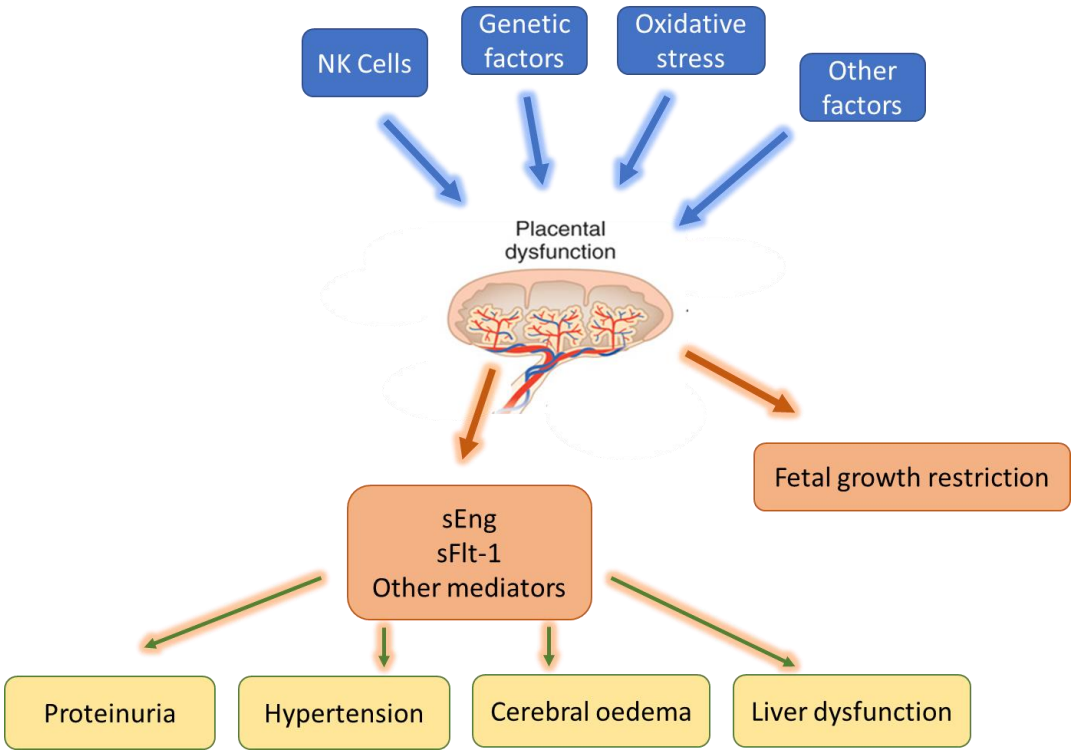


**Figure 1.1** Pathogenesis of PE.[18]

### Angiogenic factors

According to the angiogenic balance theory there is imbalance in the angiogenic proteins, inflammatory cytokines and other immune-modulating molecules in pregnancies with PE. These factors are thought to cause angiogenic imbalance which results in maternal endothelial dysfunction and systemic inflammatory reaction.[18-20]

PlGF is one of the members of the VEGF family. It is mainly expressed in the placenta and has pro-angiogenic effects, it mediates increased endothelial vascular permeability cells and angiogenesis.[20,21] sFlt-1 is also protein produced by the placental syncytiotrophoblast. It has an anti-angiogenic effect by inhibiting VEGF and PlGF activity. VEGF is important for maintaining endothelial function in fenestrated endothelium especially in the brain, liver and kidneys. High levels of sFlt-1 cause endothelial dysfunction and vasoconstriction by interfering with VEGF function.[20] sEng is an anti-angiogenic protein produced by the placenta and appears to be another important mediator of PE. This protein causes amplified vascular permeability and hypertension by suppressing the signalling cascade of TGF[20]. Many studies described elevation in the level of sFlt and sEng and decrease in the level of PlGF prior the clinical manifestations of PE. Furthermore, increase in the sFlt-1/PlGF ratio has also been shown to be useful in the prediction of PE[21].

#### 1.1.3.2 Immunological factors

In the immune incompatibility theory immunological and genetic differences between mother and fetus contribute to the development of PE. The theory states that an immunologic event early in pregnancy activates a maladaptation of the maternal immune system to the fetal trophoblastic tissue. [19]

## 1.1.4 Consequences of PE

The severity of PE ranges from a mild disorder with transient hypertension near the end of pregnancy, to a life-threatening disorder with seizures (eclampsia) or HELLP syndrome. PE is one of the leading causes of maternal and perinatal morbidity and mortality. PE accounts for at least 11% of maternal deaths per annum worldwide.[22,23]

Pregnant women with PE may develop hepatic, renal, brain and haematological abnormalities, as well as placental dysfunction as a result of inadequate blood supply through the damaged placenta. Rare but serious complications include eclampsia (seizures superimposed on the syndrome of preeclampsia); stroke; haemolysis; elevated liver enzymes and low platelets. The main complications for the fetus and neonate relate to FGR and preterm birth, with consequent risk of perinatal mortality.

In addition to the immediate effects on mother and neonate, there is increasing evidence that there may be long term adverse cardiovascular effects as well. Studies have shown that women who develop PE, compared to those that do not develop PE, have higher lifetime risk of cardiovascular disease, including hypertension, ischemic heart disease, stroke and death.[24,25] Furthermore children exposed to PE before birth have greater risk to develop cerebral palsy. Similarly, children and young adults exposed to PE have higher blood pressure, body mass index and increased risk for cardiovascular disease in adulthood.[26,27]

### 1.1.5 Screening for PE

Identification of pregnancies at high-risk of developing PE is beneficial because therapeutic interventions in such pregnancies, including prophylactic use of aspirin, closer surveillance and earlier delivery can reduce the incidence of the disease and / or its associated maternal and perinatal complications. The established method of assessing the risk for development of PE is to identify risk factors from maternal demographic characteristics and medical history; in the presence of such factors the patient is classified as high-risk and in their absence as low-risk. For example, in the UK, according to guidelines by the NICE women should be considered to be at high-risk of developing PE if they have any one high-risk factor or any two moderate-risk factors.[28] The high-risk factors are history of hypertensive disease in previous pregnancy, chronic kidney disease, autoimmune disease, diabetes mellitus or chronic hypertension and the moderate-risk factors are first pregnancy, age >40 years, inter-pregnancy interval >10 years, BMI at first visit of >35 kg/m2 or family history of PE. The advantage of this approach is that it is

simple to perform but the disadvantages are first, poor performance of predicting PE[29,30] and second, no quantification of individual patient-specific risks.

We proposed a new approach which allows estimation of patient-specific risks of delivery with PE before any specified gestational age by maternal demographic characteristics and medical history with biomarkers. The competing-risks approach is based on a survival-time model for the gestational age at delivery with PE.[29,31,32] Each woman has a personalized distribution of gestational age at delivery with PE, and the risk of delivery with PE before a specified gestational age, assuming no other cause of delivery, is given by the area under the probability density curve. In this approach, it is assumed that, if the pregnancy was to continue indefinitely, all women would develop PE, and whether they do so or not before a specified gestational age depends on competition between delivery before and after development of PE. The risk of delivery with PE before a specified gestational age, assuming no other cause delivery, is given by the area under the probability density curve (Figure1.2 and Figure 1.3).[29]

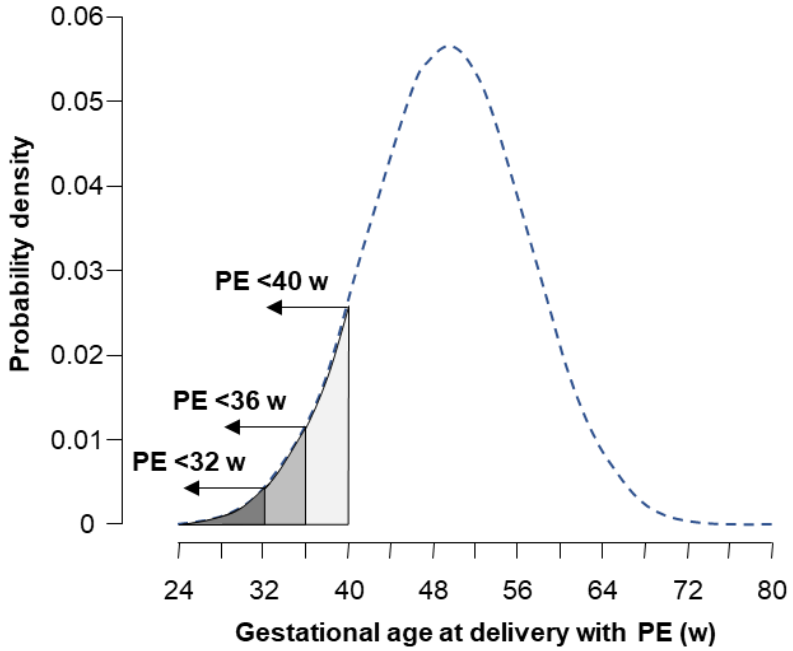


**Figure 1.2.** Personalized distribution of gestational age of delivery with PE. The risk of delivery with PE <32, <36 and <40 weeks' gestation is shown is the shaded area under the probability density. [29]

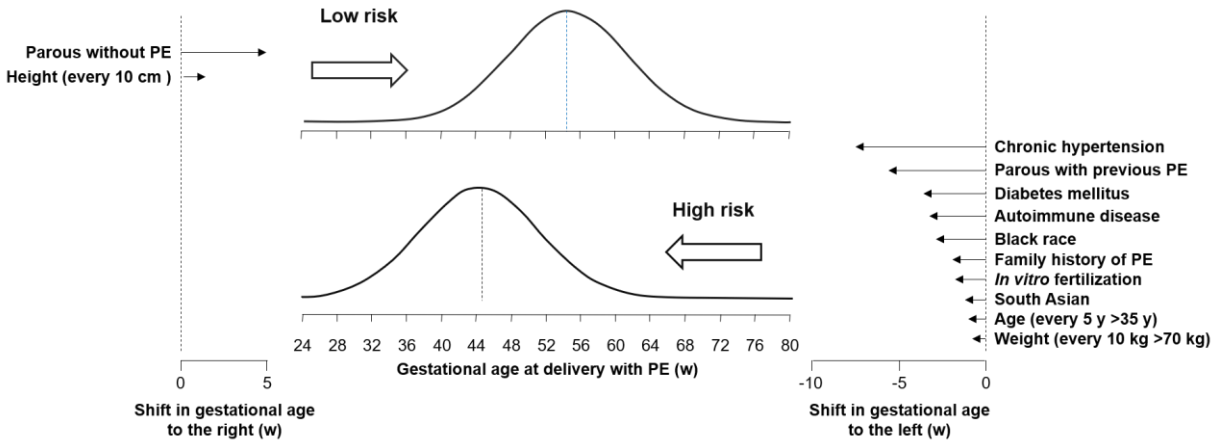


**Figure 1.3.** Prior distribution of gestational age of delivery with PE in a low-risk and a high-risk risk pregnancy and the effect of maternal factors in shifting the distribution to the left or right. [29]

The effect of variables from maternal factors and biomarkers is to modify the distribution of gestational age at delivery with PE so that, in pregnancies at low risk for PE, the gestational age distribution is shifted to the right with the implication that, in most pregnancies, delivery will actually occur before development of PE. In high-risk pregnancies, the distribution is shifted to the left and the smaller the mean gestational age the higher the risk for PE.

# 1.2 TWIN PREGNANCIES

## 1.2.1 Incidence of twin pregnancies

Twin pregnancies account for about 2% of all pregnancies but the incidence is increasing mainly due to delayed childbirth (advanced maternal age at conception) and the increasing number of IVF conceptions with the use of assisted reproduction techniques.[33] The rate of twin birth increased by almost 70% between 1980 and 2006 reaching .[34] The incidence was generally stable between 2006 and 2014, however since then birth statistics have shown a small decrease reaching the level of 3.2% by 2018.[35]

One fourth of twin pregnancies are MC and three fourths are DC. In DCDA twin pregnancies, the fetuses are separated by a thick layer of fused chorionic membranes showing the lambda sign, whereas in MCDA twins there are only two thin amniotic layers separating the twins the T-sign (Figure 1.4.).[36] It is best to determine chorionicity by ultrasound in the first trimester, because after 14 weeks the accuracy becomes much poorer. If it is difficult or too late to determine correctly the chorionicity, it is safer to classify the pregnancy as monochorionic.

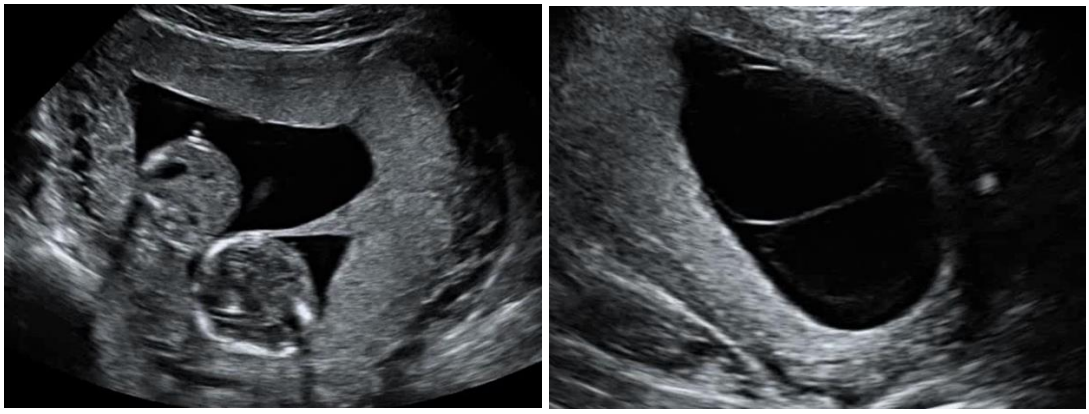

**Figure 1.4.** Ultrasound findings of lambda sign in a DC twin pregnancy (left) and T-sign in a MC twin pregnancy (right) at 12 weeks' gestation. [36]

## 1.2.2 Incidence of PE in twins

In singleton pregnancies the rate of PE is 2-3%; in 25-30% of cases of PE delivery occurs at <37 weeks' gestation (preterm-PE) and in 70-75% delivery is at term. In twin pregnancies, the rate of PE is higher than in singletons. In 10 studies reporting on between 256 and 9,998 twin pregnancies the overall rate of PE was 9.5% (2,069 of 21,817).[4-13] Consequently, the relative risk of PE for twin compared to singleton pregnancies is about 3.

We have previously examined 2,219 twin pregnancies to estimate the incidence of PE in twins and compared it to that in 93,297 singletons.[P2] The rate of PE in singletons was 2.3% (2,162 of 93,297), in DC twin pregnancies it was 8.1% (145 of 1,789) and in MC twins it was 6.0% (26 of 430); compared to singletons, the relative risk of total PE was

3.5 for DC twins and 2.6 for MC twins. The median gestational age at delivery was 40.0 weeks for singletons, 37.0 for DC twins and 35.4 for MC twins (Figure 1.5). Delivery at <37 weeks' gestation occurred in 5.5% of singletons, 46.5% of DC twins and 91.4% of MC twins. The rate of preterm-PE was 0.6%, 5.5%, 5.8% for singletons, DC twins and MC twins, respectively; compared to singletons, the relative risk of preterm-PE was 8.7 for DC twins and 9.1 for MC twins.[P2] This study has therefore established that first, in twin pregnancies, compared to singleton pregnancies, the overall rate of PE is about 3-times higher, and second, the rate of preterm-PE is 9-times higher. The underestimate of the relative risk of PE in twins, by comparison with singletons, when reporting the total rate of PE from 24 to 42 weeks' gestation is the mere consequence of the lower gestational age at delivery in twin than singleton pregnancies.

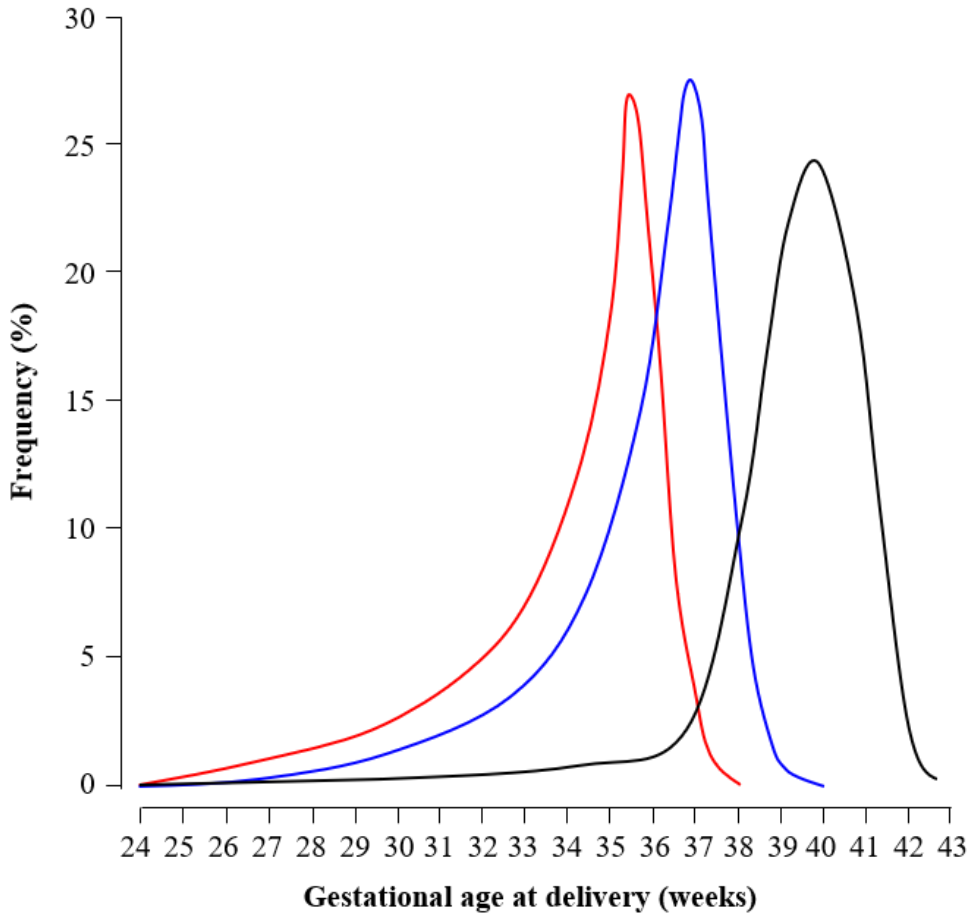


**Figure 1.5.** Frequency of gestational age at delivery in singletons (black line), DC twins (blue lines) and MC twins (red lines). [P2]

## 1.2.3 Competing risks model for PE in twins

In a study of 1,789 DC and 430 MC twin pregnancies and 93,297 singleton pregnancies a survival-time model for the gestational age at delivery with PE was developed from variables of maternal characteristics and history. [P2] In singleton pregnancies comprising women of Caucasian racial origin, weight of 69 kg at 12 weeks' gestation, height of 164 cm, nulliparous, with spontaneous conception, no family history of PE and no history of diabetes mellitus, systemic lupus erythematosus or antiphospholipid syndrome, the mean of the Gaussian distribution of gestational age at delivery with PE was 55 weeks (Figure 1.6).

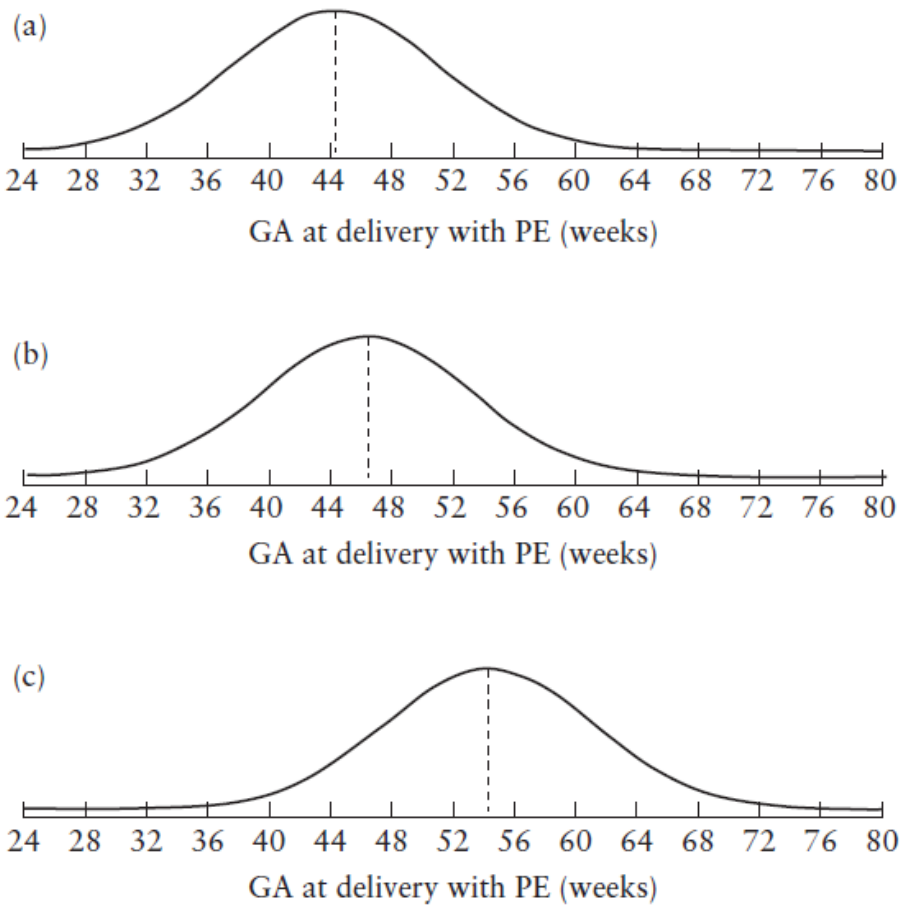


**Figure 1.6.** Distribution of gestational age (GA) at delivery with pre-eclampsia (PE) for monochorionic twin (a), dichorionic twin (b) and singleton (c) pregnancies. Dashed line shows mean GA. [P2]

In twin pregnancies with the same characteristics as singleton pregnancies the distribution of gestational age of delivery with PE was shifted to the left in a uniform way by 8 weeks in the case of DC and 10 weeks in case of MC twins. For a reference population with the above characteristics the estimated risk of PE at <37 weeks' gestation was 0.6% for singletons, 9.0% for DC twins and 14.2% for MC twins; the respective values for PE at <42 weeks were 3.6%, 27.0% and 36.5%.

A limitation of this study was that the performance of screening by a model derived and tested using the same dataset is overestimated and we suggested the necessity for external validation on independent data from different sources. [P2]

# 2 OBJECTIVES OF THE THESIS

The objectives of this thesis are:

1. To examine the predictive performance of the competing risks model in screening for PE in twins in the training dataset for development of the model and an independent validation dataset.

2. To modify the previously proposed competing risk model using the original training dataset and to examine the predictive performance of the new model in screening for PE with delivery <34 weeks (early-PE), <37 weeks (preterm-PE) and delivery at any gestation (all-PE) in twins in an independent validation dataset.

3. To demonstrate the application of the new model in screening in a mixed population of singleton and twin pregnancies.

# 3. METHODS

## 3.1. Study populations

Three datasets were used for this study. First, 2,219 twin pregnancies (training dataset) that were examined at King's College Hospital and Medway Maritime Hospital, UK, between January 2006 and December 2015. [P2] Second, 2,999 twin pregnancies (validation dataset) that were examined in five hospitals in England, one hospital in Bulgaria, and one hospital in Spain. [P3] Third, 16,747 singleton pregnancies from the Screening ProgRamme for prE-Eclampsia study.[30] The study was approved by the NHS Research Ethics Committee in England and the Hospital Ethics Committees of the participating hospitals in Bulgaria and Spain.

The data for these studies were derived from prospective screening for adverse obstetric outcomes in women attending for their routine hospital visit at $11^{+0}$ - $13^{+6}$ weeks' gestation. In this visit we recorded maternal demographic characteristics and medical history, measurement of maternal weight and height. Ultrasound examination was carried out first, to determine if the fetuses were alive and had any major abnormalities, second to estimate gestational age from the measurement of fetal crown-rump length[37] (in twin pregnancies the measurement from the larger twin was used), and third, determine chorionicity in twin pregnancies by examining the inter-twin membrane at its junction with the placenta.[36]

Patient characteristics included maternal age, racial origin, method of conception, smoking during pregnancy, history of chronic hypertension, diabetes mellitus, SLE or APS, family history of PE in the mother of the patient and obstetric history including parity, previous pregnancy with PE, gestational age at delivery and birth weight of the neonate in the last pregnancy and interval in years between birth of the last child and estimated date of conception of the current pregnancy.

## 3.2. Inclusion criteria

The inclusion criteria for this study on screening for PE were twin pregnancy with delivery of phenotypically normal live birth or stillbirth at >24 weeks' gestation. We

excluded pregnancies with aneuploidies and major fetal abnormalities, those ending in termination, miscarriage or fetal death before 24 weeks and those with an interval of more than three days between death of one fetus and live birth of the second twin.

## 3.3. Outcome measures

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy associated hypertension were examined to determine if the condition was PE as defined by the ISSHP.[15]

## 3.4. Statistical analyses

Patient-specific risks of delivery with PE at <34, <37 and $<41^{+3}$ weeks' gestation were calculated using the competing risks model based on maternal characteristics and medical history.[2] We assessed the performance of screening for early-PE, preterm-PE and all-PE in twins in training and validation datasets. The number of affected cases was too small to provide separate results for DC and MC twins.

We examined the predictive performance of the model by first, the ability of the model to discriminate between the PE and no PE groups using the AUROC curve (this indicates perfect discrimination if the value is 1 and no discrimination beyond chance if the value is 0.5) and second, calibration, which assesses agreement between predicted risks and outcomes. Calibration was assessed visually through a series of figures showing the observed incidence against that predicted from risk for PE <34, <37 and $<41^{+3}$ weeks' gestation. The plots were produced by grouping the data into bins according to risk. The observed incidence in each group was then plotted against the incidence predicted by the model (i.e. the mean risks within each group).

The risks produced from our competing risks model are for delivery with PE before a specific gestation assuming no other cause for delivery. Because other cause deliveries are effectively censored observations, the actual incidence of PE would be expected to be

lower than predicted. Consequently, we applied survival analysis (Kaplan Meier) to estimate incidence of delivery with PE treating deliveries from other causes as censored observations.

3.4.1. Model development

Using data on 120,492 singleton pregnancies we developed a parametric survival model in which the distribution of gestational age at delivery with PE has a Gaussian distribution with a mean determined from maternal characteristics and a constant standard deviation.[2] We extended this model using data on the 2,219 pregnancies in the training data set by including effects for DC and MC twins. [P3] Using this model, the prior distribution of the gestational age at delivery with PE is the same as that in a singleton pregnancy with the same maternal characteristics but, with the mean reduced by 8 weeks in DC twins and 10 weeks in MC twins. In the new model we developed an alternative extension of the singleton model for twins by including the singleton prior mean as a covariate in a parametric survival model. The relationship between the singleton prior mean and gestational age at delivery with PE was examined by first treating the prior mean as a factor with levels determined by deciles (10 groups of equal size). Effects plots showed a linear relationship for both DC and MC twins. We therefore fitted a model with a constant slope but different intercepts for DC and MC twins in the training dataset and tested the model on the validation data set.

3.4.2. Choice of gestational ages for risk assessment

The model we have adopted gives risks of delivery with PE before a specified gestational age assuming no other cause delivery. For singleton pregnancies we focused on risks of delivery with PE at <34, <37 and $<41^{+3}$ weeks gestation.[38] In singleton pregnancies 12% reach $41^{+3}$ weeks' gestation, but in the case of twins <0.1% reach $41^{+3}$ weeks; consequently, in the case of twins the risks of PE with delivery $<41^{+3}$ are hypothetical and unrealistically high. Therefore, in twin pregnancies it is more appropriate to use a risk of delivery with PE at <39 weeks with 2.7% (95% CI 2.1 to 3.5%) of those in the training data set and 1.4% (95% CI 1.0 to 1.9%) of those in the validation data set reaching 39 weeks' gestation before delivery.

### 3.4.3. Risk calibration

Calibration was assessed visually by plotting the observed incidence against that predicted risk for PE <34, <37 and <39 weeks' gestation. The plots were produced by grouping the data into bins according to risk. The observed incidence in each group was then plotted against the incidence predicted by the model (i.e. the mean risks within each group). The risks produced from our competing risks model are for delivery with PE before a specific gestation assuming no other cause for delivery. Because other cause deliveries are effectively censored observations, the actual incidence of PE would be expected to be lower than predicted. Consequently, we applied survival analysis (Kaplan Meier) to estimate incidence of delivery with PE treating deliveries from other causes as censored observations. Statistical assessment of calibration of the fitted survival model was undertaken with calibration-in-the-large and calibration slope with correction for censoring. The calibration of the previous model and the new model, both fitted to the training data set, are compared on the validation data set.

### 3.4.4. Screening performance in a mixed population of twin and singleton pregnancies

Performance of screening in a mixed population of twin and singleton pregnancies was examined using a stratified analysis of the population of twins described above with the singleton population of 17,747 pregnancies from the SPREE study. The strata weights for the detection rates are proportional to the incidence rates in the twins and singletons in the mixed population. Those for the false positive rate are proportional to 1 – incidence, and those for screen positive rate are proportional to the proportions of twins and singletons.

The statistical software package R was used for data analyses. The package pROC was used for the ROC curve analysis and the package survival was used for survival analysis.[39-42]

# 4. RESULTS

## Characteristics of the two datasets

Maternal and pregnancy characteristics in the training and validation datasets are provided in Table 4.1. [P4] In the validation dataset, compared to the training dataset, the median maternal age was higher, but the median weight and body mass index were lower, the incidences of conception by *in vitro* fertilization, chronic hypertension and nulliparity were higher and the incidences of diabetes mellitus, cigarette smoking and family history of PE were lower. The incidence of early-PE, preterm-PE and all-PE in the two datasets was similar.

**Table 4.1.** Maternal and pregnancy characteristics in the training and validation datasets for twin pregnancies.

| **Variables** | **Training set (n=2,219)** | **Validation set (n=2,999)** | **p-value** |
|---|---|---|---|
| Maternal age in years, median (IQR) | 32.9 (28.7, 36.3) | 33.7 (30.1, 36.9) | <0.00001 |
| Maternal weight in kg, median (IQR) | 68.0 (60.0, 79.0) | 66.0 (58.8, 76.0) | <0.00001 |
| Maternal height in cm, median (IQR) | 165 (160, 170) | 165 (161. 170) | 0.739 |
| Body mass index in kg/m$^2$, median (IQR) | 24.9 (22.3, 28.6) | 23.9 (21.6, 27.7) | <0.00001 |
| Gestational age in weeks, median (IQR) | 12.9 (12.5, 13.3) | 12.6 (12.1, 13.1) | <0.00001 |
| Racial origin, n (%) | | | <0.00001 |
| White | 1,710 (77.1) | 2,627 (87.6) | |
| Black | 353 (15.9) | 240 (8.0) | |
| South Asian | 80 (3.6) | 78 (2.7) | |
| East Asian | 33 (1.5) | 20 (0.7) | |
| Mixed | 43 (1.9) | 34 (1.2) | |
| Conception, n (%) | | | <0.00001 |
| Natural | 1,547 (69.7) | 1,619 (54.0) | |
| Assisted by use of ovulation drugs | 55 (2.5) | 63 (2.1) | |
| *In vitro* fertilization | 617 (27.8) | 1,317 (43.9) | |
| Medical history | | | |
| Chronic hypertension, n (%) | 30 (1.4) | 57 (1.9) | <0.00001 |
| Diabetes mellitus, n (%) | 23 (1.0) | 17 (0.6) | <0.00001 |
| SLE/APS, n (%) | 4 (0.2) | 12 (0.4) | 0.243 |
| Cigarette smokers, n (%) | 203 (9.1) | 190 (6.3) | <0.001 |
| Family history of preeclampsia, n ( %) | 97 (4.4) | 35 (1.2) | <0.00001 |
| Parity, n (%) | | | <0.00001 |
| Nulliparous | 1,184 (53.4) | 1,877 (62.6) | |
| Parous with no previous PE | 967 (43.6) | 1,095 (36.5) | |
| Parous with previous PE | 68 (3.1) | 27 (0.9) | |
| Chorionicity, n (%) | | | 0.103 |
| Dichorionic | 1,789 (80.6) | 2,472 (82.4) | |
| Monochorionic | 430 (19.4) | 527 (17.6) | |
| Preeclampsia, n (%) | | | |
| Total | 171 (7.7) | 215 (7.2) | 0.497 |
| Delivery <37 weeks | 124 (5.6) | 167 (5.6) | 1 |
| Delivery <34 weeks | 41 (1.9) | 43 (1.4) | 0.288 |

**Performance of screening for PE in the two datasets**

The ROC curves for the performance of screening for early-PE, preterm-PE and all-PE in the two datasets and their combination are shown in Figure 4.1. The two datasets had similar AUROC curves for early-PE (training dataset 0.670, 95% CI 0.593, 0.747; validation dataset 0.677, 95% CI 0.594, 0.760), preterm-PE (training dataset 0.666, 95% CI 0.617, 0.715; validation dataset 0.652, 95% CI 0.609, 0.694), and all-PE (training dataset 0.656, 95% CI 0.615, 0.697; validation dataset 0.644, 95% CI 0.606, 0.682).

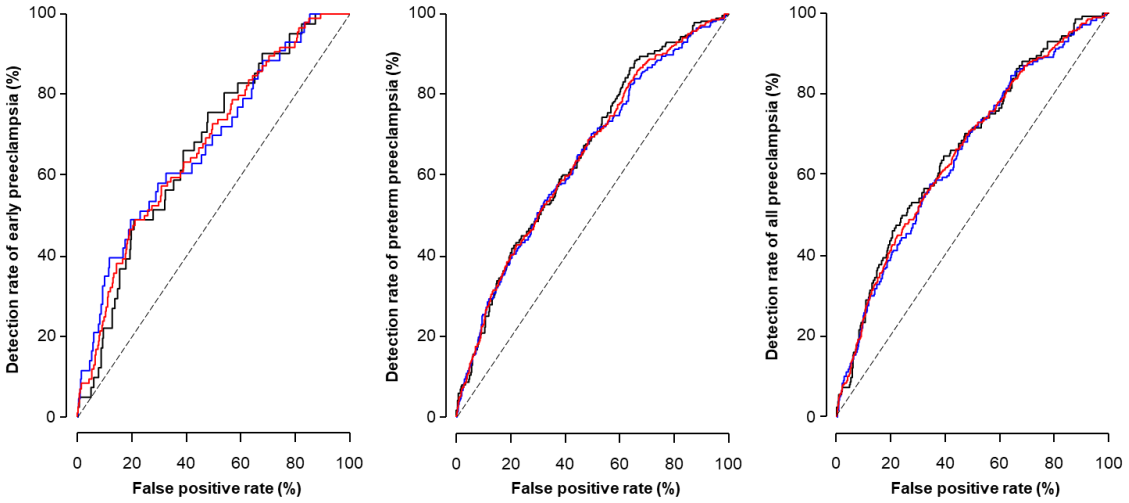


**Figure 4.1**. Receiver operating characteristic plots of screening for early-PE, preterm-PE and all-PE in the training dataset (black line), validation dataset (blue line) and the combination of the two datasets (red line). [P3]

Calibration plots of the predictive performance of the competing risks model for early-PE, preterm-PE and all-PE in the two datasets are shown in Figures 4.2 to 4.4. In these figures the diagonal grey line is the line of perfect agreement. The overall mean risk is shown by the vertical interrupted line and the overall incidence by the horizontal interrupted line. The histograms show the distribution of risks in pregnancies with PE (red) and those without PE (grey).

In both the training sets and validation datasets there was a general tendency for overestimation of risks, which was most marked for early-PE.

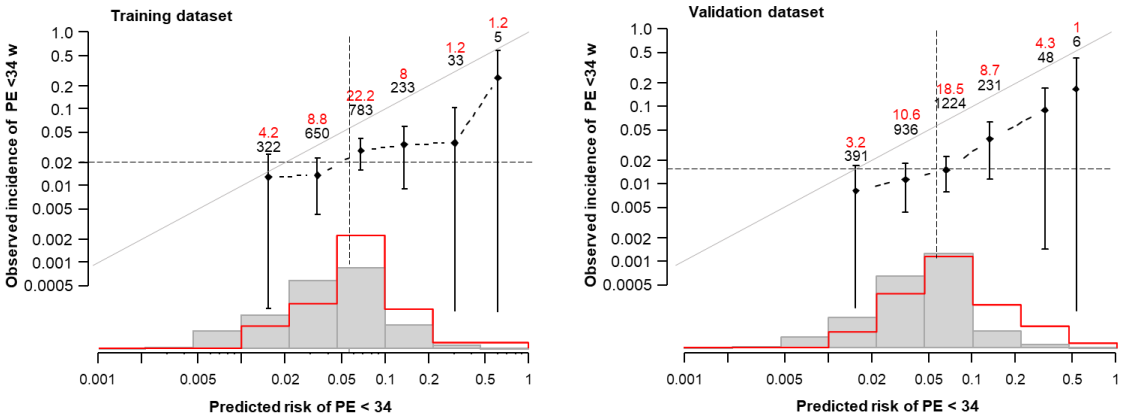


**Figure 4.2**. Calibration plots for screening using the competing risks model for prediction of early-PE in the two datasets after adjustment for the effect of censoring due to births from causes other than PE. [P3]

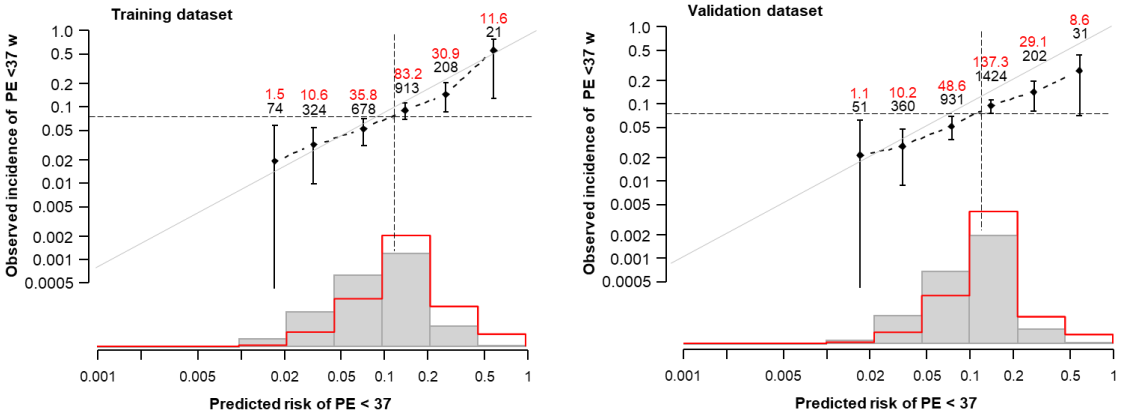


**Figure 4.3**. Calibration plots for screening using the competing risks model for prediction of preterm-PE in the two datasets after adjustment for the effect of censoring due to births from causes other than PE. [P3]

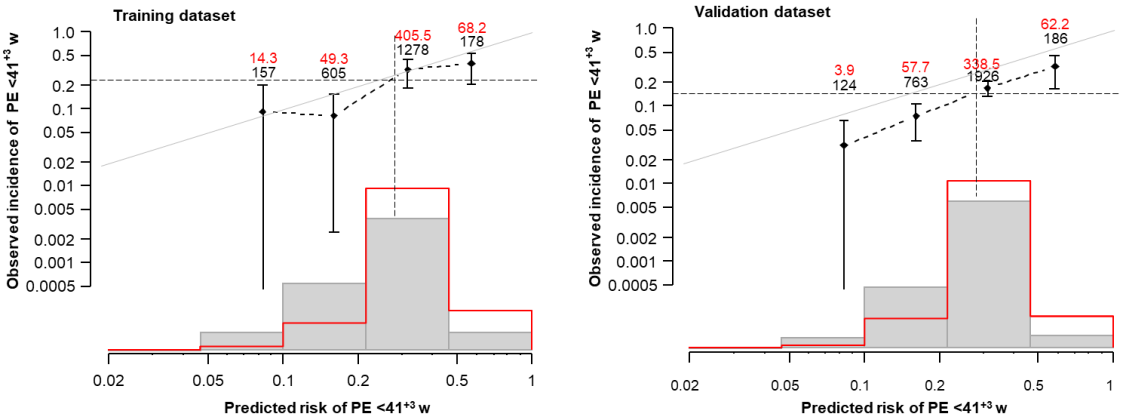


**Figure 4.4.** Calibration plots for screening using the competing risks model for prediction of all-PE in the two datasets after adjustment for the effect of censoring due to births from causes other than PE. [P3]

**Model development**

Estimates for the effect of twins (DC and MC grouped together) on the gestational age at delivery with PE grouped according to deciles of the mean of the Gaussian distribution for gestational age at the time of delivery with PE in singletons are shown in Figure 4.5.

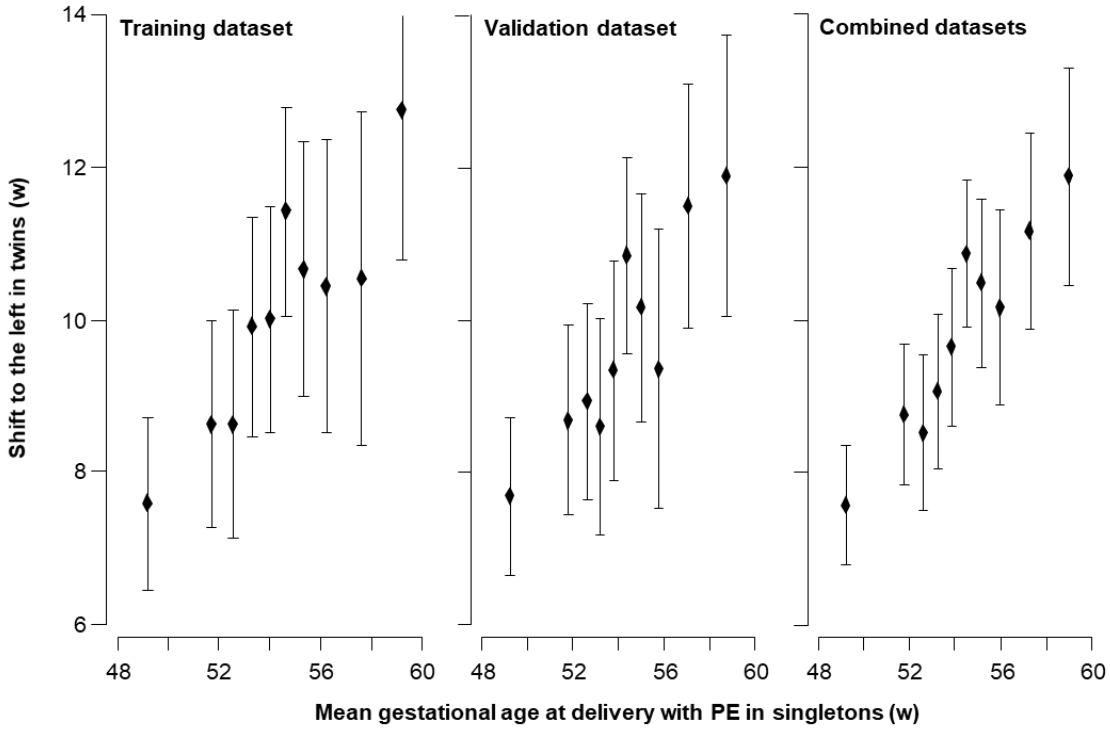


**Figure 4.5.** Estimates with 95% confidence intervals for the effect of twins on the gestational age at the time of delivery with PE grouped according to deciles of the mean of the Gaussian distribution for gestational age at the time of delivery with PE in singletons. [P4]

The effect of twins in reducing gestational age at delivery with PE is not uniform, but the effect increases with increasing singleton prior mean. On the basis of this, a model in which the effect of twins depends linearly on the singleton prior mean with a common slope but different intercepts for DC and MC twins was fitted to the training dataset. Table 4.2 shows the coefficients of the regression model fitted to the training dataset alone and the training and validation datasets combined. The fitted regression lines for DC and MC twins with 95% confidence intervals are shown in Figure 4.6. The regression lines have

the same slope but different intercepts; MC twins delivered with PE an estimated 1.48 weeks (95% CI 0.51 to 2.46 weeks) weeks earlier than DC twins (p = 0.0028).

**Table 4.2:** Fitted regression model for dichorionic and monochorionic twin pregnancies. The singleton mean is obtained from reference 2.

| | Value (95% confidence interval) | p |
|---|---|---|
| **Training Data** | | |
| Singleton mean | 0.487 (0.3588, 0.6158) | <0.00001 |
| Dichorionic | 17.268 (10.634, 23.902) | <0.00001 |
| Monochorionic | 15.783 (8.989, 22.578) | <0.00001 |
| Standard deviation | 4.5058 (4.0073, 5.0663) | |
| **Combined Data** | | |
| Singleton mean | 0.492 (0.4036, 0.5811) | <0.00001 |
| Dichorionic | 17.115 (12.532, 21.698) | <0.00001 |
| Monochorionic | 15.768 (11.059, 20.477) | <0.00001 |
| Standard deviation | 4.6019 (4.2557, 4.9761) | |

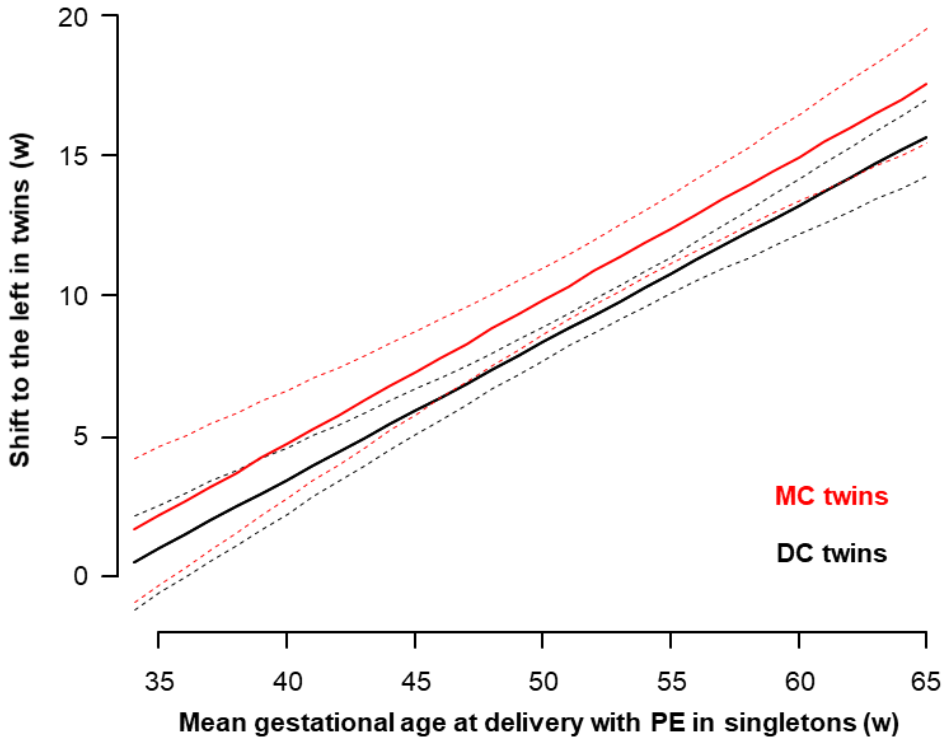


**Figure 4.6.** Relationship between the effect of DC and MC twin pregnancies in reducing the gestational age at delivery with PE and prior mean of gestational age at delivery with PE in singleton pregnancies. [P4]

**Risk calibration**

Calibration intercept and slope statistics for the predictive performance of early-PE, preterm-PE and all-PE for the previous model and the new model are given in Table 4.3. The corresponding calibration plots showing predictive performance for early-PE and preterm-PE are shown in Figures 4.7 and 4.8. With the new model, the observed incidence of early-PE and preterm-PE is close to that predicted and is substantially better than the previous model. Calibration of the 39 week risks, when used for prediction of PE at any gestation is also satisfactory.

**Table 4.3.** Risk calibration in the validation data set. For a perfectly calibrated model the intercept should be 0 and the calibration slope should be 1.0.

| Condition | Model | Calibration intercept | Calibration slope |
|---|---|---|---|
| Early-PE (<34 weeks) | Previous | -1.244 (-1.544, -0.944) | 0.746 (0.308, 1.184) |
| | New | -0.353 (-0.641, -0.066) | 0.891 (0.433, 1.349) |
| Preterm-PE (<37 weeks) | Previous | -0.464 (-0.629, -0.300) | 0.771 (0.553, 0.988) |
| | New | -0.100 (-0.274, 0.074) | 0.941 (0.655, 1.228) |
| All-PE (<39 weeks) | Previous | -0.293 (-0.538, -0.047) | 0.802 (0.578, 1.026) |
| | New | -0.263 (-0.486, -0.039) | 1.096 (0.693, 1.500) |

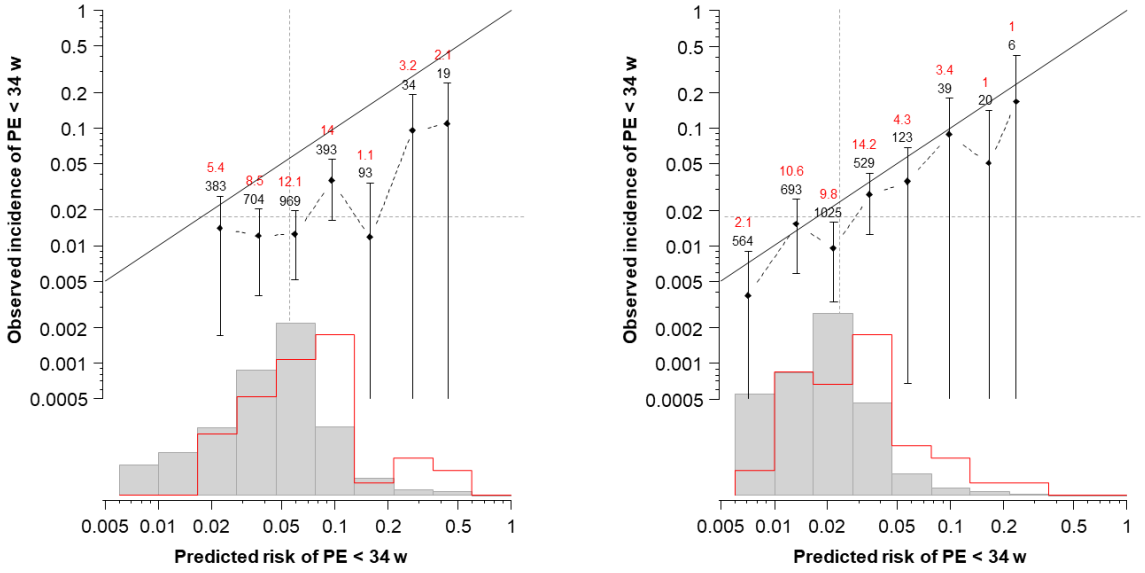


**Figure 4.7**. Calibration plots for screening using the competing risks model for prediction of early-PE in the validation dataset according to the previous (left) and new model (right), after adjustment for the effect of censoring due to births from causes other than PE. [P4]

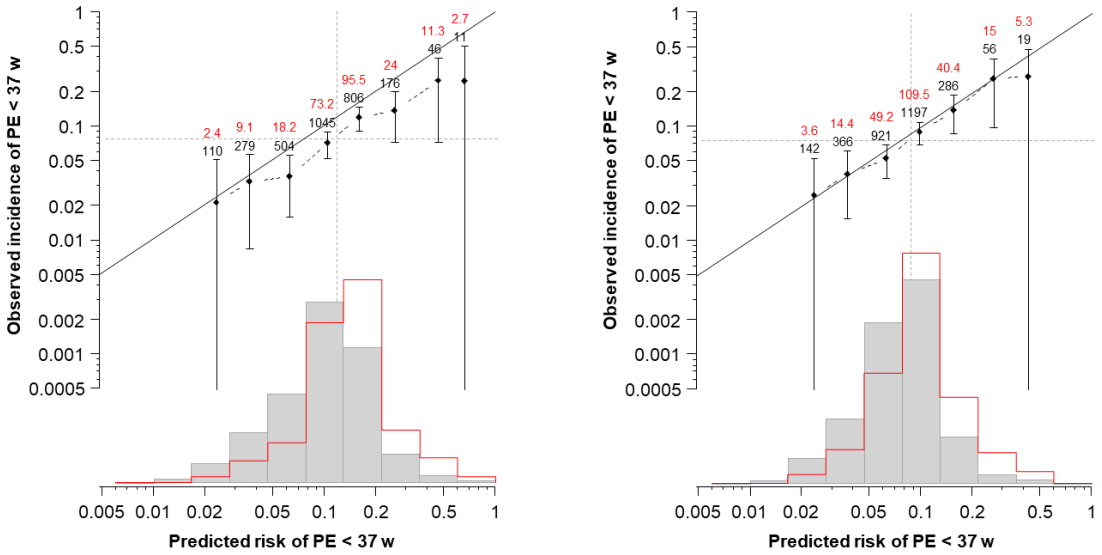


**Figure 4.8.** Calibration plots for screening using the competing risks model for prediction of preterm-PE in the validation dataset according to the previous (left) and new model (right), after adjustment for the effect of censoring due to births from causes other than PE. [P4]

## Performance of screening

ROC curves for twins, singletons and for a mixed population comprising 98% singletons and 2% twins are shown in Figure 4.9.

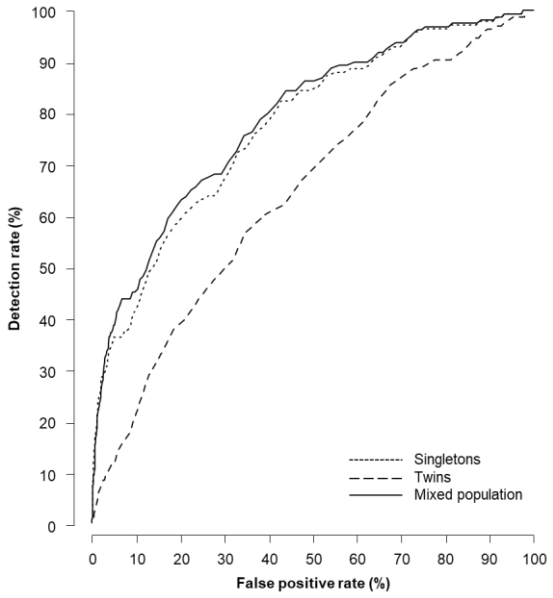


**Figure 4.9**. Receiver operating characteristic curves for preterm-PE in singletons, twins and a mixed population comprising of 98% of singleton and 2% of twin pregnancies. [P4]

The AUROC for the mixed population is 0.790 (95% CI 0.755 - 0.826) compared to 0.775 (95% CI: 0.735-0.815) for singletons and 0.647 (95% CI: 0.604-0.690) for twins and the performance of screening in the mixed population is superior to that in the sub-populations comprising the mixture. This happens because twins are at higher risk than singletons and whether a pregnancy is a singleton or twin is informative improving screening performance over that achieved in singletons. To illustrate this, consider screening for PE <37 with a screen positive rate of 10%. In the mixed population a cut-off of 1 in 60 gives an overall screen positive rate of 10% (8.2% for singletons and 100% for twins) with an overall detection rate of 45%, including 38% for singletons and 100% for twins. In contrast, for singletons, a risk cut-off of 1 in 70 gives a screen positive rate of 10% with a detection rate of 41% and for twins, a risk cut-off of 1 in 7 gives a screen positive rate of 10% and a detection rate of only 19%.

# 5. DISCUSSION

In the first study we found that in both the training and validation datasets the incidence of early-PE and peterm-PE in twin pregnancies was substantially higher than in our previous studies in singleton pregnancies. [P3] The findings on predictive performance of the competing risks model for PE in twin pregnancies demonstrated that the results from the validation dataset, derived from prospectively collected data from multicenter studies, are consistent with those of the training set used for development of the model.

The competing risks model provided moderate discrimination between affected and unaffected pregnancies in both the training and the validation datasets with values for the AUROC curve of about 0.65. This is not surprising because all twin pregnancies compaired to singletons are at substantially increased risk of PE.

Calibration refers to how well the predicted risk from the model agrees with the observed incidence of PE. The results of the study demonstrate that in both the training and validation datasets the observed incidence of PE was lower than the predicted one and such overestimation of risk was particularly marked for early-PE. It was therefore concluded that the model needs to be adjusted to correct the observed overestimation of risk for early-PE.

In the second study we developed a new model for the prediction of PE in twin pregnancies and demonstrated relatively good calibration in an independent validation dataset. [P4] The basis of the new model is that in twin pregnancies the shift to the left of the distrubution of gestational age at delivery with PE in singleton pregnancies is not uniform, as in our original model, but the effect increases with increasing singleton prior mean.

The implication of this finding is that in a woman who on the basis of her demographic characteristics and medical history has a very high-risk of developing PE, reflected in a mean of ≤34 weeks for the gestational age of delivery with PE, the presence of a DC twin pregnancy does not increase her risk over above that of a singleton pregnancy. In contrast, in a woman at very low-risk of developing PE, reflected in a mean of 65 weeks for the distribution of gestational age at delivery with PE, the presence of a DC twin pregnancy

results in a substantially increased risk of developing PE compared to a singleton pregnancy with a shift of the distribution to the left by about 16 weeks. In a MC twin pregnancy, there is no shift to the left if the prior mean is ≤28 weeks, but if the prior mean is 65 weeks the shift to the left is about 18 weeks.

This finding is analogous to the effect of a history of previously affected pregnancy with Down syndrome on the maternal age-related risk for Down syndrome in the current pregnancy. On the assumption that such history increases the risk by about 1%, in a 50 year old woman with an age-related risk of about 1 in 10 there is a 1.1-fold increase to 1.1 in 10, whereas in a 20 year old woman with an age-related risk of about 1 in 1,000 there is a 10-fold increase to 11 in 1,000; consequently, the increase in risk is inversely proportional to the prior risk.

In the prediction of PE in a mixed population of singleton and twin pregnancies the same risk cut-off should be used in identifying the high-risk group in need of prophylactic pharmacological interventions to prevent the development of PE and closer monitoring for early identification of the clinical signs of the disease in those that will develop PE. In this study we have demonstrated that at a risk cut-off that would classify 10% of a mixed population as being at high-risk for preterm-PE all twins will be classified as screen positive.

# 6. CONCLUSIONS

In the initial development of the competing risks model of PE in twin pregnancies we adopted the simple approach of adjusting the model for singletons; in DC and MC twin pregnancies with the same characteristics as singleton pregnancies the distribution of gestational age of delivery with PE was shifted to the left by 8 and 10 weeks, respectively. [P1] Our first study demonstrated that such approach did not adequately address the effect of twins on risk of PE and this was particularly so for early-PE. It was therefore concluded that a new model needs to be fitted whereby the effect of twins in shifting the distribution of risks in singletons to the left should not be the same for all gestational ages but such shift should be less for lower than higher gestations.

In the second study a new competing risks model in screening for PE by maternal risk factors in twin pregnancies was developed and using this model the predicted risks for early-PE, preterm-PE and all-PE were in good agreement with the observed incidence of the disease.

# 7. ABSTRACT

**Background:** We have previously proposed that the competing risk model for prediction of PE based on maternal characteristics and medical history, developed in singleton pregnancies, can be extended to risk assessment for twins; in DC and MC twin pregnancies with the same characteristics as singleton pregnancies the distribution of gestation of delivery with PE was shifted to the left by 8 and 10 weeks, respectively.

**Objectives:** First, to examine the predictive performance of the model in a training dataset used for development of the model and in an independent validation dataset. Second, to develop a new model in screening for PE by maternal characteristics and medical history in twin pregnancies and to examine the predictive performance of this new model. Third, to demonstrate the application of screening in a mixed population of singleton and twin pregnancies.

**Methods**: The data were obtained from two prospective multicentre studies for PE in twin pregnancies at $11^{+0}$ - $13^{+6}$ weeks. The training and validation datasets consisted of 2,219 and 2,999 women, respectively. We examined the predictive performance of the model both datasets using the AUROC and calibration plots. We used the training dataset to fit the model whereby the effect of twins in shifting the distribution of gestational age of delivery with PE in singletons to the left should not be the same for all gestational ages but the shift should depend on the singleton prior mean; the effect increases with increasing prior mean. Data obtained from the SPREE study were included to examine the performance of screening in a mixed population of singleton and twin pregnancies.

**Results**: We found in the study that using the original model in both the training and validation datasets the observed incidence of PE was lower than the predicted one. Calibration plots and calibration intercept and slope demonstrate superior predictive performance of the new model in the validation dataset. Although the AUROC in twins is lower than in singletons, the performance of screening in a mixed population of singleton and twin pregnancies is superior to that in singletons. For the risk cut offs likely to be used in practice, all twin pregnancies screen positive using maternal characteristics and medical history.

**Conclusions:** A new competing risks model in screening for PE by maternal risk factors in twin pregnancies has been developed and using this model the predicted risk for early-PE, preterm-PE and all-PE are in relatively good agreement with the observed incidence of the disease.

**Háttér:** Korábbi kutatási eredményeink alapján megállapítottuk, hogy az egyes terhességek esetében PE szűrésére kidolgozott versengési modell, alkalmazható ikerterhességekben is. Az anyai jellemzők és az anamnézis alapján felállított modell Gauss görbéje, ikerterhességekre korrigálva egyenletesen, MC ikerterhesség esetén 10-héttel, DC ikerterhesség esetén pedig 8-héttel tolódik balra.

**Célkitűzés:** Jelen kutatásunk elsődleges célja az ikerterhességben kialakuló, PE szűrésére korábban anyai jellemzők és az anamnézis alapján a training adatbázis használatával felállított versengéses modell hatékonyságának vizsgálata, egy független validációs adatbázis segítségével. Második célként a korábbi modell módosítását terveztük. Harmadik cél pedig a szűrőmódszer vizsgálata vegyes populációban, amely iker és egyes terhességeket egyaránt tartalmaz.

**Módszertan:** Két prospektív, multicentrikus szűrővizsgálati kutatás betegcsoportjának adatait vizsgáltuk, melyekben a betegek bevonása a $11^{+0}$ és a $13^{+6}$ terhességi hét között történt. Az training adatbázis 2219 ikerterhes adatát, míg a validációs adatbázis 2999 ikerterhes adatát tartalmazta. A versengéses modell hatékonyságának vizsgálatát mindkét adatbázisban AUROC és kalibrációs módszerekkel végeztük. Ezt követően úgy módosítottuk a modellt, hogy az ikerterhességekben megfigyelt kockázatemelkedés mértéke ne egyenletes, hanem az anyai tényezők alapján meghatározott alapkockázat függvényében változzon. A módosított új modell vegyes populációban történő vizsgálatát a SPREE adatbázis segítségével végeztük el.

**Eredmények:** Mind a training, mind a validációs vizsgálati csoportban megfigyeltük, hogy PE ritkábban fordult elő, mint az az eredeti modell alapján várható lett volna. Az előfordulás várttól való eltérése főként a korai PE esetén volt kiemelkedő. Az új modell alkalmazásával erősebb egyezést tapasztaltunk az előre becsült és a valós incidencia között. Bár az ikerterhességek esetében az AUROC alacsonyabb értékeket vett fel, mint egyes terhességekben, az új modell vegyes populáció szűrésére is alkalmazható. Ebben az esetben azonban minden ikerterhes a magas kockázatú várandósok csoportjába fog tartozni.

**Következtetés:** A korrigált modell létrehozásával mindkét vizsgálati csoportban erős korrelációt figyeltünk meg a különböző terhességi korokban kialakuló PE előfordulásának kockázatbecslése és tényleges előfordulása között.

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# 9. PUBLICATIONS

**PUBLISHED ARTICLES**

PAPER 1.

Francisco C, Wright D, Benkő Z, Syngelaki A, Nicolaides KH. (2017) Hidden high rate of preeclampsia in twin compared to singleton pregnancies. Ultrasound Obstet Gynecol 50:88-92.

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PAPER 2.

Francisco C, Wright D, Benkő Z, Syngelaki A, Nicolaides KH. (2017) Competing-risks model in screening for pre-eclampsia in twin pregnancy by maternal characteristics and medical history. Ultrasound Obstet Gynecol 50:501-506.

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**PAPER 3.**

**Benkő Z, Chaveeva P, de Paco Matallana C, Zingler E, Wright A, Wright D, Nicolaides KH. (2019) Validation of competing risks model in screening for pre-eclampsia in twin pregnancy by maternal factors. Ultrasound Obstet Gynecol 53:649-654.**

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**Benkő Z, Chaveeva P, de Paco Matallana C, Zingler E, Wright D, Nicolaides KH. (2019) Revised competing-risks model in screening for pre-eclampsia in twin pregnancy by maternal characteristics and medical history. Ultrasound Obstet Gynecol 54:617-624.**

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# Validation of competing-risks model in screening for pre-eclampsia in twin pregnancy by maternal factors

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**KEYWORDS:** calibration; competing-risks model; discrimination; first-trimester screening; performance of screening; pre-eclampsia; survival model; twin pregnancy

## ABSTRACT

***Objective*** *To examine the predictive performance of the competing-risks model in screening for pre-eclampsia (PE) by maternal demographic characteristics and medical history in twin pregnancy, in a training dataset used for development of the model and a validation dataset.*

***Methods*** *The data for this study were derived from two prospective non-intervention multicenter screening studies for PE in twin pregnancies at 11 + 0 to 13 + 6 weeks' gestation. The first study of 2219 women, which was reported previously, was used to develop the competing-risks model for prediction of PE and is therefore considered to be the training set. The validation study comprised 2877 women. Patient-specific risks of delivery with PE at < 34 (early), < 37 (preterm) and < 41 + 3 (all) weeks' gestation were calculated using the competing-risks model and the performance of screening for PE in the training and validation datasets was assessed. We examined the predictive performance of the model by, first, its ability to discriminate between the PE and no-PE groups using the area under the receiver–operating characteristics curve (AUC) and, second, calibration, which assesses agreement between the predicted risk and observed incidence of PE.*

***Results*** *The incidence of early PE, preterm PE and all PE in the training and validation datasets was similar (1.8% vs 1.4%, 5.6% vs 5.6% and 7.7% vs 7.2%, respectively) and this was substantially higher than in our previous studies in singleton pregnancies. The training and validation datasets had similar AUCs for early PE (0.670 (95% CI, 0.593–0.747) vs 0.677 (95% CI, 0.594–0.760)), preterm PE (0.666 (95% CI, (0.617–0.715) vs 0.652 (95% CI, 0.609–0.694)) and all PE (0.656 (95% CI, 0.615–0.697) vs 0.644 (95% CI, 0.606–0.682)). Calibration plots of the predictive performance of the competing-risks model demonstrated that, in both the training and validation datasets, the observed incidence of PE was lower than the predicted one and such overestimation of risk was particularly marked for early PE.*

***Conclusions*** *Discrimination and calibration of the competing-risks model for PE in a validation dataset are consistent with those in the training dataset. However, the model needs to be adjusted to correct the observed overestimation of risk for early PE. Copyright © 2019 ISUOG. Published by John Wiley & Sons Ltd.*

## INTRODUCTION

In twin pregnancy, the incidence of pre-eclampsia (PE) is about 9%[1–11], which is three-times higher than in singleton pregnancies. However, twins are delivered at an earlier gestational age than singletons and, consequently, comparison of the overall rates of PE between twin and singleton pregnancies underestimates the relative risk of preterm PE in twins, which is nine-times higher[11]. In screening for PE in singleton pregnancies, we proposed the competing-risks approach, which is based on a survival-time model for the gestational age at delivery with PE[12–14]. Each woman has a personalized distribution of gestational age at delivery with PE, and the risk of delivery with PE before a specified gestational age, assuming no other cause of delivery, is given by the area under the probability density curve. In this approach, it is assumed that, if the pregnancy was to continue indefinitely, all women would develop PE, and whether they do so or not before a specified gestational age depends on competition between delivery before and after development of PE. The effect of variables from maternal factors and biomarkers

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is to modify the distribution of gestational age at delivery with PE so that, in pregnancies at low risk for PE, the gestational age distribution is shifted to the right with the implication that, in most pregnancies, delivery will actually occur before development of PE. In high-risk pregnancies, the distribution is shifted to the left and the smaller the mean gestational age the higher the risk for PE.

We have examined previously 2219 twin pregnancies and proposed that the same competing-risks model developed in singleton pregnancies can be adapted for use in twins[15]. In this model, the mean gestational age at delivery with PE was 55 weeks in a reference population (white race, weight 69 kg, height 164 cm, nulliparous, spontaneous conception, no family history of PE and no history of diabetes mellitus, systemic lupus erythematosus or antiphospholipid syndrome). In dichorionic (DC) and monochorionic (MC) twin pregnancies with the same characteristics as singleton pregnancies, the distribution of gestational age at delivery with PE was shifted to the left by 8 and 10 weeks, respectively. The estimated risk of PE at < 37 weeks' gestation was 0.6% for singletons, 9.0% for DC twins and 14.2% for MC twins; the respective values for PE at < 42 weeks were 3.6%, 27.0% and 36.5%. A limitation of the study was that the performance of screening by a model derived and tested using the same dataset is overestimated and we suggested the necessity for external validation using independent data from different sources.

The objective of this study was to examine the predictive performance of the competing-risks model in screening for PE with delivery < 34 weeks (early PE), < 37 weeks (preterm PE) and at any gestational age (all PE) in twins in the training dataset[15] used for development of the model and in a validation dataset.

## METHODS

### Study population

The data for this study were derived from prospective screening for adverse obstetric outcome in women attending for their routine hospital visit at 11 + 0 to 13 + 6 weeks' gestation. At this visit, we recorded maternal demographic characteristics and medical history, measured maternal weight and height and performed an ultrasound scan to determine if both fetuses were alive and had any major abnormalities, estimate gestational age from the measurement of fetal crown–rump length[16] of the larger twin, and determine chorionicity by examining the intertwin membrane at its junction with the placenta[17].

The training dataset was derived from pregnancies examined at King's College Hospital, London and Medway Maritime Hospital, Gillingham, UK, between January 2006 and December 2015[15].

The validation dataset was derived from pregnancies examined at five hospitals in the UK (King's College Hospital and Medway Maritime Hospital, between December 2015 and April 2018; Homerton University Hospital, London, between January 2014 and April 2018; North Middlesex University Hospital, London, between May 2015 and April 2018; and Southend University Hospital, Essex, between June 2015 and April 2018), one hospital in Bulgaria (Dr. Shterev Hospital, Sofia, between January 2013 and April 2018) and one hospital in Spain (Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, between March 2009 and April 2018). The study was approved by the NHS Research Ethics Committee in England and the Hospital Ethics Committees of the participating hospitals in Bulgaria and Spain.

Patient characteristics included maternal age and racial origin (white, black, South Asian, East Asian and mixed), method of conception (spontaneous or assisted requiring *in-vitro* fertilization or the use of ovulation drugs), cigarette smoking during pregnancy, history of chronic hypertension, diabetes mellitus, systemic lupus erythematosus or antiphospholipid syndrome, family history of PE in the mother of the patient and obstetric history including parity (parous or nulliparous if no previous pregnancy ≥ 24 weeks), previous pregnancy with PE, gestational age at delivery and birth weight of the neonate in the last pregnancy, and interval in years between delivery of the previous pregnancy and estimated date of conception of the current pregnancy.

The inclusion criteria for this study on screening for PE were twin pregnancy with delivery of a phenotypically normal liveborn or stillborn neonate at ≥ 24 weeks' gestation. We excluded pregnancies with aneuploidy or major fetal abnormality, those ending in termination, miscarriage or fetal death before 24 weeks and those with an interval of more than 3 days between death of one fetus and live birth of the second twin.

### Outcome measures

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was PE, as defined by the International Society for the Study of Hypertension in Pregnancy[18].

### Statistical analysis

Patient-specific risks of delivery with PE at < 34, < 37 and < 41 + 3 weeks' gestation were calculated using the competing-risks model based on maternal characteristics and medical history[13]. The performance of screening for early PE, preterm PE and all PE in the training and validation datasets was assessed. The number of affected cases was too small to provide separate results for DC and MC twins.

We examined the predictive performance of the model by, first, its ability to discriminate between the PE and

no-PE groups using the area under the receiver–operating characteristics (ROC) curve (AUC) (a value of 1 indicates perfect discrimination and 0.5 indicates no discrimination beyond chance) and, second, calibration, which assesses agreement between predicted risk and outcome. Calibration was assessed visually through a series of figures showing the observed incidence against that predicted from risk for PE < 34, < 37 and < 41 + 3 weeks' gestation. The plots were produced by grouping the data into bins according to risk. The observed incidence in each group was then plotted against the incidence predicted by the model (i.e. the mean risk within each group).

The risks produced from our competing-risks model are for delivery with PE before a specific gestational age assuming no other cause for delivery. Because other causes of delivery are effectively censored observations, the actual incidence of PE would be expected to be lower than predicted. Consequently, we applied survival analysis (Kaplan–Meier) to estimate the incidence of delivery with PE, treating deliveries due to other causes as censored observations.

The statistical software package R was used for data analyses[19]. The package pROC was used for the ROC curve analysis and the package 'survival' was used for survival analysis[20–22].

## RESULTS

Maternal and pregnancy characteristics in the training and validation datasets are provided and compared in Table 1. The incidence of early PE, preterm PE and all PE in the two datasets was similar.

The ROC curves for the performance of screening for early PE, preterm PE and all PE in the two datasets and their combination are shown in Figure 1. The two datasets had similar AUCs for early PE (training dataset 0.670 (95% CI, 0.593–0.747); validation dataset 0.677 (95% CI, 0.594–0.760)), preterm PE (training dataset 0.666 (95% CI, 0.617–0.715); validation dataset 0.652 (95% CI, 0.609–0.694)) and all PE (training dataset 0.656 (95% CI, 0.615–0.697); validation dataset 0.644 (95% CI, 0.606–0.682)). Calibration plots of the predictive

**Table 1** Maternal and pregnancy characteristics in women with twin pregnancy included in training and validation datasets for pre-eclampsia (PE) screening model

| *Characteristic* | *Training set* (n = *2219*) | *Validation set* (n = *2999*) | P |
|---|---|---|---|
| Maternal age (years) | 32.9 (28.7–36.3) | 33.7 (30.1–36.9) | < 0.00001 |
| Maternal weight (kg) | 68.0 (60.0–79.0) | 66.0 (58.8–76.0) | < 0.00001 |
| Maternal height (cm) | 165 (160–170) | 165 (161–170) | 0.739 |
| Maternal body mass index (kg/m$^2$) | 24.9 (22.3–28.6) | 23.9 (21.6–27.7) | < 0.00001 |
| Gestational age (weeks) | 12.9 (12.5–13.3) | 12.6 (12.1–13.1) | < 0.00001 |
| Racial origin | | | < 0.00001 |
| White | 1710 (77.1) | 2627 (87.6) | |
| Black | 353 (15.9) | 240 (8.0) | |
| South Asian | 80 (3.6) | 78 (2.6) | |
| East Asian | 33 (1.5) | 20 (0.7) | |
| Mixed | 43 (1.9) | 34 (1.1) | |
| Conception | | | < 0.00001 |
| Natural | 1547 (69.7) | 1619 (54.0) | |
| Assisted by use of ovulation drugs | 55 (2.5) | 63 (2.1) | |
| *In-vitro* fertilization | 617 (27.8) | 1317 (43.9) | |
| Medical history | | | |
| Chronic hypertension | 30 (1.4) | 57 (1.9) | < 0.00001 |
| Diabetes mellitus | 23 (1.0) | 17 (0.6) | < 0.00001 |
| SLE/APS | 4 (0.2) | 12 (0.4) | 0.243 |
| Cigarette smoker | 203 (9.1) | 190 (6.3) | < 0.001 |
| Family history of PE | 97 (4.4) | 35 (1.2) | < 0.00001 |
| Parity | | | < 0.00001 |
| Nulliparous | 1184 (53.4) | 1877 (62.6) | |
| Parous with no previous PE | 967 (43.6) | 1095 (36.5) | |
| Parous with previous PE | 68 (3.1) | 27 (0.9) | |
| Chorionicity | | | 0.103 |
| Dichorionic | 1789 (80.6) | 2472 (82.4) | |
| Monochorionic | 430 (19.4) | 527 (17.6) | |
| PE | | | |
| Total | 171 (7.7) | 215 (7.2) | 0.497 |
| Delivery < 37 weeks | 124 (5.6) | 167 (5.6) | 1 |
| Delivery < 34 weeks | 41 (1.8) | 43 (1.4) | 0.288 |

Data are given as median (interquartile range) or *n* (%). Comparisons between outcome groups were by chi-square or Fisher's exact test for categorical variables and Mann–Whitney *U*-test for continuous variables. APS, antiphospholipid syndrome; SLE, systemic lupus erythematosus.

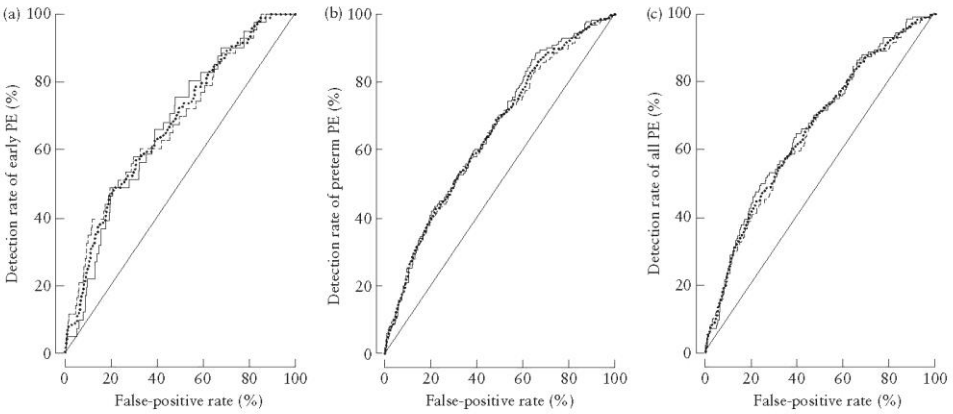


**Figure 1** Receiver–operating characteristics curves in screening for early (< 34 weeks) pre-eclampsia (PE) (a), preterm (< 37 weeks) PE (b) and all (< 41 + 3 weeks) PE (c) in training dataset (——), validation dataset (– – –) and combination of the two datasets (·····).

performance of the competing-risks model for early PE, preterm PE and all PE in the two datasets are shown in Figure 2. In both the training and validation datasets, there was a general tendency for overestimation of risk, which was most marked for early PE.

## DISCUSSION

### Main findings

In both the training and validation datasets, the incidence of early PE and preterm PE in twin pregnancies was substantially higher than in our previous studies in singleton pregnancies[12–14]. The findings on the predictive performance of the competing-risks model for PE in twin pregnancy demonstrate that the results in the validation dataset, derived from prospectively collected data from multicenter studies, are consistent with those in the training set used for development of the model.

The competing-risks model provided moderate discrimination between affected and unaffected pregnancies in both the training and validation datasets, with AUC values of about 0.65. This is not surprising because all twin pregnancies, compared to singletons, are at substantially increased risk of PE.

Calibration refers to how well the predicted risk from the model agrees with the observed incidence of PE. The results of the study demonstrate that, in both the training and validation datasets, the observed incidence of PE was lower than the predicted one and such overestimation of risk was particularly marked for early PE.

### Strengths and limitations

The strengths of this study include, first, prospective evaluation of discrimination and calibration of the prespecified model in an independent multicenter study and, second, assessment of calibration allowing for the effect of censoring due to births from causes other than PE. A limitation of the study is that the number of twin pregnancies was too small to be divided according to chorionicity.

### Comparison with previous studies

In previous studies, we established a competing-risks approach for the prediction of PE in singleton pregnancies based on maternal factors and extended this model to include twin pregnancies[13,15]. Other studies in twin pregnancies merely reported that the rate of PE is about three-times higher than in singleton pregnancies[1–11]. In a previous study, we evaluated the predictive performance of the competing-risks model in singleton pregnancies using two validation datasets and demonstrated very high discrimination between affected and unaffected pregnancies and very good agreement between the predicted risk and observed incidence of PE[15,23–25]. In this study, we compared the predictive performance of the model developed for twin pregnancies[15].

### Implications for further research

In the initial development of the competing-risks model of PE in twin pregnancies, we adopted the simple approach of adjusting the model for singletons; in DC and MC twin pregnancies with the same characteristics as singleton pregnancies, the distribution of gestational age at delivery with PE was shifted to the left by 8 and 10 weeks, respectively[15]. This study has demonstrated that such an approach did not adequately address the effect of twin pregnancy on risk of PE and this was particularly so for early PE. Therefore, a new model needs to be fitted in which the effect of twins in shifting the distribution of risk in singletons to the left should not be the same for

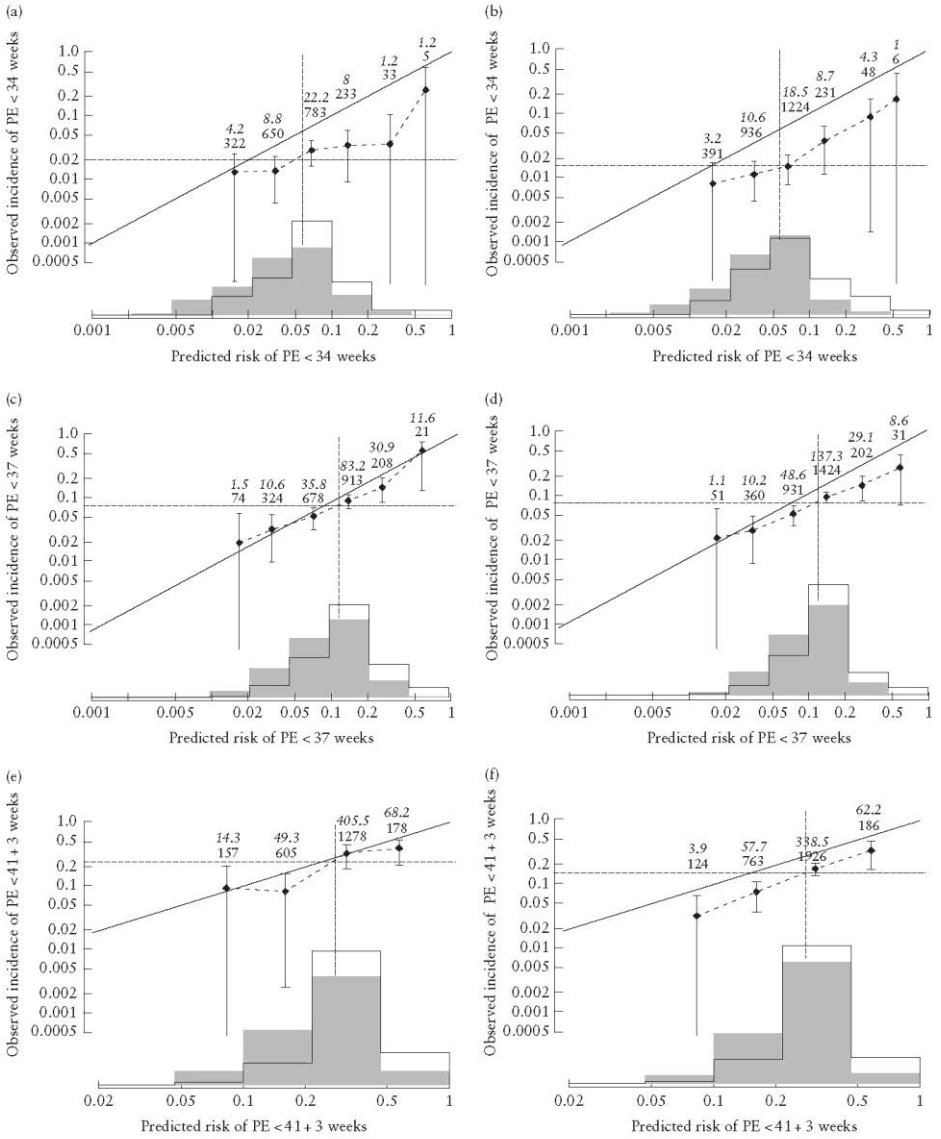


**Figure 2** Calibration plots for screening using competing-risks model for prediction of early (< 34 weeks) pre-eclampsia (PE) (a,b), preterm (< 37 weeks) PE (c,d) and all (< 41 + 3 weeks) PE (e,f) in training (a,c,e) and validation (b,d,f) datasets after adjustment for effect of censoring due to births from causes other than PE. Diagonal line is line of perfect agreement. Overall mean risk is shown by vertical dashed line and overall incidence by horizontal dashed line. Vertical solid lines are confidence intervals. Numbers of women with PE are shown in italics above the total number in that predicted-risk group. Histograms show distribution of risk in affected (□) and unaffected (▨) pregnancies.

all gestational ages but such shift should be less for lower than for higher gestational ages.

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# Revised competing-risks model in screening for pre-eclampsia in twin pregnancy by maternal characteristics and medical history

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**KEYWORDS:** calibration; competing-risks model; discrimination; first-trimester screening; performance of screening; pre-eclampsia; survival model; twin pregnancy

## CONTRIBUTION

*What are the novel findings of this work?*
In a new extension of the competing-risks model in screening for pre-eclampsia (PE) by maternal factors in twin pregnancy, the effect of twins on shifting the distribution of gestational age at delivery with PE in singletons to the left is not constant but increases with increasing prior mean.

*What are the clinical implications of this work?*
Calibration plots and calibration intercept and slope demonstrate that the new model has a superior predictive performance and provides more accurate patient-specific risk of PE than does the previous model.

## ABSTRACT

***Background*** *We have proposed previously that the competing-risks model for prediction of pre-eclampsia (PE) based on maternal characteristics and medical history (prior model), developed in singleton pregnancies, can be extended to risk assessment for twins; in dichorionic (DC) and monochorionic (MC) twin pregnancies with the same characteristics as in singleton pregnancies, the distribution of gestational age at delivery with PE was shifted to the left by 8 and 10 weeks, respectively. However, in a subsequent validation study, we found that, in both the training and validation datasets, the observed incidence of PE was lower than the predicted one and such overestimation of risk was particularly marked for early PE.*

***Objectives*** *First, to develop a new extension of the competing-risks prior model in screening for PE by maternal demographic characteristics and medical history in twin pregnancies in a training dataset. Second, to examine the predictive performance of this model in screening for PE with delivery < 34 weeks (early PE), < 37 weeks (preterm PE) and at any gestational age (all PE) in twins in a validation dataset. Third, to demonstrate the application of screening in a mixed population of singleton and twin pregnancies.*

***Methods*** *The data for this study were obtained from two prospective non-intervention multicenter screening studies for PE in twin pregnancies at 11 + 0 to 13 + 6 weeks' gestation. The training and validation datasets consisted of 2219 and 2999 women, respectively. We used the training dataset to fit a model in which the effect of twins on shifting the distribution of gestational age at delivery with PE in singletons to the left should not be the same for all gestational ages but the shift should depend on the singleton prior mean; the effect increases with increasing prior mean. We examined the predictive performance of the model in the training and validation datasets using the area under the receiver–operating characteristics curve (AUC) and calibration plots. Data on 16 747 singleton pregnancies obtained from the Screening ProgRamme for prE-Eclampsia (SPREE) study were included to examine the performance of screening in a mixed population of singleton and twin pregnancies.*

***Results*** *Calibration plots and calibration intercept and slope demonstrate superior predictive performance of*

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*the new model in the validation dataset. Although the AUC for twin pregnancies is lower than in singleton pregnancies, performance of screening in a mixed population of singleton and twin pregnancies is superior to that in singletons (AUC of 0.790 in a mixed population comprising 2% twins and 98% singletons compared to 0.775 in singletons). For the risk cut-offs likely to be used in practice, all twin pregnancies screen positive using maternal characteristics and medical history.*

***Conclusions*** *A new competing-risks model in screening for PE by maternal risk factors in twin pregnancy has been developed and, using this model, the predicted risks for early PE, preterm PE and all PE are in relatively good agreement with the observed incidence of the disease.* 

## INTRODUCTION

In screening for pre-eclampsia (PE) in singleton pregnancy, we proposed the competing-risks approach, which is based on a survival-time model for gestational age at delivery with PE[1–3]. Each woman has a personalized distribution of gestational age at delivery with PE and the risk of delivery with PE before a specified gestational age, assuming no other cause of delivery, is given by the area under the probability-density curve. In this approach, it is assumed that, if the pregnancy was to continue indefinitely, all women would develop PE, and whether they do so or not before a specified gestational age depends on competition between delivery due to PE or other causes. The personalized distribution is obtained by applying Bayes' theorem to combine a prior distribution determined from maternal characteristics and medical history with a likelihood function determined from biomarkers. The effects of variables from maternal factors and biomarkers is to modify the distribution of gestational age at delivery with PE so that, in pregnancies at low risk for PE, gestational age at delivery with PE is increased, with the implication that, in more pregnancies, delivery from other causes occurs before development of PE. In high-risk pregnancies, gestational age at delivery with PE is decreased so delivery with PE occurs more often.

In twin pregnancies, the rate of PE is about 9%, which is 3-times higher than in singleton pregnancies, but twins are delivered at an earlier gestational age than are singletons and, consequently, comparison of the overall rates of PE between twin and singleton pregnancies underestimates the relative risk of preterm PE in twins, which is 9-times higher[4]. In a study of 2219 twin pregnancies, we proposed that the same competing-risks model developed in singleton pregnancies can be adapted for use in twins; in dichorionic (DC) and monochorionic (MC) twin pregnancies with the same characteristics as in singleton pregnancies, the distribution of gestational age at delivery with PE was shifted to the left by 8 and 10 weeks, respectively[5]. In a subsequent validation study involving 2999 twin pregnancies, we found that the predictive performance for PE was consistent with that in the training set used for development of the model; however, calibration plots of the predictive performance of the competing-risks model demonstrated that, in both the training and validation datasets, the observed incidence of PE was lower than the predicted one and such overestimation of risk was particularly marked for early PE[6]. This suggested the need for a model in which the effect of twins relative to singletons in decreasing the gestational age at delivery with PE should increase with gestational age.

The objectives of this study were, first, to develop a new extension of the competing-risks prior model in screening for PE in twin pregnancies in the original training dataset[5], second, to examine the predictive performance of this model in screening for PE with delivery < 34 weeks (early PE), < 37 weeks (preterm PE) and at any gestational age (all PE) in twins in a validation dataset[6], and, third, to demonstrate the application of screening in a mixed population of singleton and twin pregnancies.

## METHODS

### Study population

Three datasets were used for this study. First, 2219 twin pregnancies (training dataset) that were examined at King's College Hospital and Medway Maritime Hospital, UK, between January 2006 and December 2015[5]. Second, 2999 twin pregnancies (validation dataset) that were examined at five hospitals in England (King's College Hospital and Medway Maritime Hospital, between December 2015 and April 2018; Homerton University Hospital, between January 2014 and April 2018; North Middlesex University Hospital, between May 2015 and April 2018; and Southend University Hospital, between June 2015 and April 2018), one hospital in Bulgaria (Dr. Shterev Hospital in Sofia, between January 2013 and April 2018) and one hospital in Spain (Hospital Clínico Universitario Virgen de la Arrixaca in Murcia, between March 2009 and April 2018)[6]. Third, the validation dataset of 16 747 singleton pregnancies from the Screening ProgRamme for prE-Eclampsia (SPREE) study; this was a prospective multicenter study in seven National Health Service (NHS) maternity hospitals in England[7]. This study was approved by the NHS Research Ethics Committee in England and the Hospital Ethics Committees of the participating hospitals in Bulgaria and Spain.

In all three datasets, women had a routine hospital visit at 11 + 0 to 13 + 6 weeks' gestation, which included recording of maternal demographic characteristics and medical history, measurement of maternal weight and height and ultrasound examination to, first, determine if the fetuses were alive and had any major abnormalities, second, estimate gestational age from the measurement of fetal crown–rump length[8] (in twin pregnancies, the measurement from the larger twin was used), and, third,

determine chorionicity in twin pregnancies by examining the intertwin membrane at its junction with the placenta[9].

Patient characteristics recorded included maternal age, racial origin (white, black, South Asian, East Asian or mixed), method of conception (spontaneous or assisted requiring *in-vitro* fertilization or the use of ovulation drugs), cigarette smoking during pregnancy, history of chronic hypertension, diabetes mellitus, systemic lupus erythematosus or antiphospholipid syndrome, family history of PE in the mother of the patient and obstetric history including parity (parous or nulliparous if no previous pregnancy at $\geq 24$ weeks), previous pregnancy with PE, gestational age at delivery and birth weight of the neonate in the last pregnancy and interval in years between delivery of the last child and estimated date of conception of the current pregnancy.

The inclusion criteria for this study on screening for PE were delivery of a phenotypically normal liveborn or stillborn neonate at $\geq 24$ weeks' gestation. We excluded pregnancies with aneuploidy or major fetal abnormality, those ending in termination, miscarriage or fetal death before 24 weeks and, in twin pregnancies, those with an interval of $> 3$ days between the death of one fetus and live birth of the second twin.

## Outcome measures

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was PE as defined by the International Society for the Study of Hypertension in Pregnancy[10].

## Statistical analysis

### *Model development*

Using data on 120 492 singleton pregnancies, we developed a parametric survival model in which the distribution of gestational age at delivery with PE has a Gaussian distribution with a mean determined from maternal characteristics and a constant standard deviation[2]. We extended this model using data on the 2219 pregnancies in the training dataset by including effects for DC and MC twins[5]. Using this model, the prior distribution of gestational age at delivery with PE is the same as that in singleton pregnancy with the same maternal characteristics but with the mean reduced by 8 weeks in DC twins and 10 weeks in MC twins.

Here, we develop an alternative extension of the singleton model for twins by including the singleton prior mean as a covariate in a parametric survival model. The relationship between the singleton prior mean and gestational age at delivery with PE was examined by, first, treating the prior mean as a factor with levels determined by deciles (10 groups of equal size). Effects plots showed a linear relationship for both DC and MC twins. We therefore fitted a model with a constant slope but different intercepts for DC and MC twins in the training dataset[5] and tested the model on the validation dataset[6].

### *Choice of gestational ages for risk assessment*

The model we have adopted gives risk of delivery with PE before a specified gestational age, assuming no other cause of delivery. For singleton pregnancies, we focused on risks of delivery with PE at $< 34$, $< 37$ and $< 41 + 3$ weeks' gestation[11]. Of singleton pregnancies, 12% reach $41 + 3$ weeks' gestation, but, in the case of twins, $< 0.1\%$ reach $41 + 3$ weeks; consequently, in the case of twins, the risk of delivery with PE $< 41 + 3$ weeks is hypothetical and unrealistically high. Therefore, in twin pregnancies, it is more appropriate to use a risk of delivery with PE at $< 39$ weeks, with 2.7% (95% CI, 2.1–3.5%) of those in the training dataset and 1.4% (95% CI, 1.0–1.9%) of those in the validation dataset reaching 39 weeks' gestation.

### *Risk calibration*

Calibration was assessed visually by plotting the observed incidence against the predicted risk for PE $< 34$, $< 37$ and $< 39$ weeks' gestation. The plots were produced by grouping the data into bins according to risk. The observed incidence in each group was then plotted against the incidence predicted by the model (i.e. the mean risk within each group). The risks produced from our competing-risks model are for delivery with PE before a specific gestational age, assuming no other cause for delivery. Because other causes of delivery are effectively censored observations, the actual incidence of PE would be expected to be lower than predicted. Consequently, we applied survival analysis (Kaplan–Meier) to estimate the incidence of delivery with PE, treating deliveries from other causes as censored observations. Statistical assessment of calibration of the fitted survival model was undertaken with calibration-in-the-large and calibration slope with correction for censoring. The calibration of the previous model and the new model, both fitted to the training dataset, is compared in the validation dataset.

### *Screening performance in mixed population of twin and singleton pregnancies*

Performance of screening in a mixed population of twin and singleton pregnancies was examined using stratified analysis of the population of twins described above and the singleton population of 16 747 pregnancies from the SPREE study[7]. The strata weights for the detection rates are proportional to the incidence rates in twins and singletons in the mixed population. Those for the false-positive rate are proportional to 1 – incidence, and those for the screen-positive rate are proportional to the proportions of twins and singletons.

The statistical software package R was used for data analyses[12]. The package pROC was used for

receiver–operating characteristics (ROC) curve analysis and the package survival was used for survival analysis[13–15].

## RESULTS

Maternal and pregnancy characteristics in the training and validation datasets are provided and compared in Table 1. In the validation dataset, compared with the training dataset, median maternal age was higher, but median weight and body mass index were lower, the incidences of conception by *in-vitro* fertilization, chronic hypertension and nulliparity were higher and the incidences of diabetes mellitus, cigarette smoking and family history of PE were lower. The incidences of early PE, preterm PE and all PE in the two datasets were similar.

### Model development

Estimates for the effect of twins (DC and MC grouped together) on gestational age at delivery with PE, grouped according to decile of the mean of the Gaussian distribution for gestational age at delivery with PE in singletons, are shown in Figure 1. The effect of twins in reducing gestational age at delivery with PE is not uniform but increases with increasing singleton prior mean. On the basis of this, a model in which the effect of twins depends linearly on the singleton prior mean with a common slope but different intercepts for DC and MC twins was fitted to the training dataset. Table 2 shows the coefficients of the regression model fitted to the training dataset alone and the training and validation datasets combined. The fitted regression lines for DC and MC twins with 95% CI are shown in Figure 2. The regression lines have the same slope but different intercepts; in MC twin pregnancies, delivery with PE was an estimated 1.48 (95% CI, 0.51–2.46) weeks earlier than in DC twins ($P = 0.0028$).

### Risk calibration

Calibration intercept and slope statistics for the predictive performance for early PE, preterm PE and all PE of the previous model and the new model are given in Table 3. The corresponding calibration plots showing the predictive performance for early PE and preterm PE are shown in Figure 3. Using the new model, the

**Table 1** Maternal and pregnancy characteristics in training and validation datasets of twin pregnancies

| *Variable* | *Training set* (n = *2219*) | *Validation set* (n = *2999*) | P |
|---|---|---|---|
| Maternal age (years) | 32.9 (28.7–36.3) | 33.7 (30.1–36.9) | < 0.00001 |
| Maternal weight (kg) | 68.0 (60.0–79.0) | 66.0 (58.8–76.0) | < 0.00001 |
| Maternal height (cm) | 165 (160–170) | 165 (161–170) | 0.739 |
| Body mass index (kg/m$^2$) | 24.9 (22.3–28.6) | 23.9 (21.6–27.7) | < 0.00001 |
| Gestational age (weeks) | 12.9 (12.5–13.3) | 12.6 (12.1–13.1) | < 0.00001 |
| Racial origin | | | < 0.00001 |
| White | 1710 (77.1) | 2627 (87.6) | |
| Black | 353 (15.9) | 240 (8.0) | |
| South Asian | 80 (3.6) | 78 (2.6) | |
| East Asian | 33 (1.5) | 20 (0.7) | |
| Mixed | 43 (1.9) | 34 (1.1) | |
| Conception | | | < 0.00001 |
| Natural | 1547 (69.7) | 1619 (54.0) | |
| Assisted by use of ovulation drugs | 55 (2.5) | 63 (2.1) | |
| *In-vitro* fertilization | 617 (27.8) | 1317 (43.9) | |
| Medical history | | | |
| Chronic hypertension | 30 (1.4) | 57 (1.9) | < 0.00001 |
| Diabetes mellitus | 23 (1.0) | 17 (0.6) | < 0.00001 |
| SLE/APS | 4 (0.2) | 12 (0.4) | 0.243 |
| Cigarette smoker | 203 (9.1) | 190 (6.3) | < 0.001 |
| Family history of PE | 97 (4.4) | 35 (1.2) | < 0.00001 |
| Parity | | | < 0.00001 |
| Nulliparous | 1184 (53.4) | 1877 (62.6) | |
| Parous with no previous PE | 967 (43.6) | 1095 (36.5) | |
| Parous with previous PE | 68 (3.1) | 27 (0.9) | |
| Chorionicity | | | 0.103 |
| Dichorionic | 1789 (80.6) | 2472 (82.4) | |
| Monochorionic | 430 (19.4) | 527 (17.6) | |
| PE | | | |
| Total | 171 (7.7) | 215 (7.2) | 0.497 |
| Delivery < 37 weeks | 124 (5.6) | 167 (5.6) | 1 |
| Delivery < 34 weeks | 41 (1.8) | 43 (1.4) | 0.288 |

Data are given as median (interquartile range) or *n* (%). Comparisons between outcome groups were by chi-square or Fisher's exact test for categorical variables and Mann–Whitney *U*-test for continuous variables. APS, antiphospholipid syndrome; PE, pre-eclampsia; SLE, systemic lupus erythematosus.

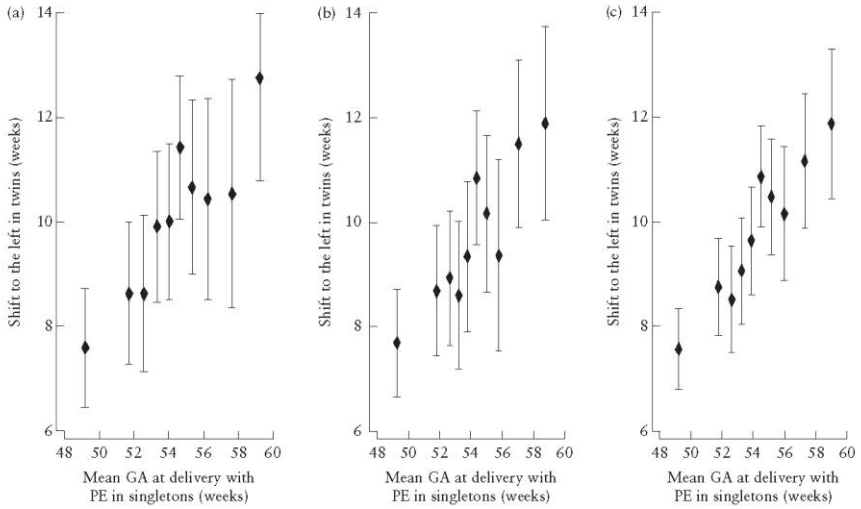


**Figure 1** Estimates with 95% CI for effect of twins on gestational age (GA) at delivery with pre-eclampsia (PE) in training (a), validation (b) and combined (c) datasets, according to decile of mean of Gaussian distribution for GA at delivery with PE in singletons.

**Table 2** Fitted regression model for prediction of pre-eclampsia in dichorionic and monochorionic twin pregnancies in training dataset alone and in training and validation datasets combined

| | *Value (95% CI)* | P |
|---|---|---|
| Training data | | |
| Singleton mean* | 0.487 (0.3588–0.6158) | < 0.00001 |
| Dichorionic | 17.268 (10.634–23.902) | < 0.00001 |
| Monochorionic | 15.783 (8.989–22.578) | < 0.00001 |
| SD | 4.5058 (4.0073–5.0663) | |
| Combined data | | |
| Singleton mean* | 0.492 (0.4036–0.5811) | < 0.00001 |
| Dichorionic | 17.115 (12.532–21.698) | < 0.00001 |
| Monochorionic | 15.768 (11.059–20.477) | < 0.00001 |
| SD | 4.6019 (4.2557–4.9761) | |

*Singleton mean obtained from Wright *et al.*[2].

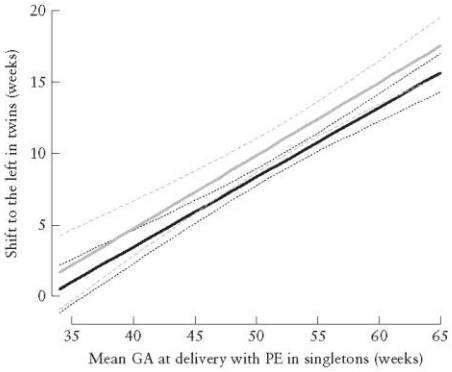


**Figure 2** Relationship between effect of dichorionic (black) and monochorionic (gray) twin pregnancy in reducing gestational age (GA) at delivery with pre-eclampsia (PE) and prior mean of GA at delivery with PE in singleton pregnancies. Dashed lines are 95% CI.

observed incidence of early PE and preterm PE is close to that predicted, and it is substantially better than the previous model. Calibration of the 39-week risk, when used for prediction of PE at any gestational age, is also satisfactory.

## Performance of screening

ROC curves for twins, singletons and for a mixed population comprising 98% singletons and 2% twins are shown in Figure 4. The area under the ROC curve for the mixed population is 0.790 (95% CI, 0.755–0.826) compared to 0.775 (95% CI, 0.735–0.815) for singletons and 0.647 (95% CI, 0.604–0.690) for twins, and the performance of screening in the mixed population is superior to that in the subpopulations comprising the mixture. This is because twins are at higher risk than singletons, and whether a pregnancy is a singleton or twin is informative, improving screening performance over that achieved in singletons. To illustrate this, we considered screening for PE < 37 weeks with a screen-positive rate of 10%. In the mixed population, a cut-off of 1 in 60 gives an overall screen-positive rate of 10% (8.2% for singletons and 100% for twins) with an overall detection rate of 45%, including 38% for singletons and 100% for twins. In contrast, for singletons, a risk cut-off of 1 in 70 gives a screen-positive rate of 10% with a detection

**Table 3** Risk calibration in validation dataset for prediction of pre-eclampsia (PE) in twin pregnancy

| *Model* | *Calibration intercept* | *Calibration slope* |
|---|---|---|
| Early PE (< 34 weeks) | | |
| Previous | −1.244 (−1.544 to −0.944) | 0.746 (0.308 to 1.184) |
| New | −0.353 (−0.641 to −0.066) | 0.891 (0.433 to 1.349) |
| Preterm PE (< 37 weeks) | | |
| Previous | −0.464 (−0.629 to −0.300) | 0.771 (0.553 to 0.988) |
| New | −0.100 (−0.274 to 0.074) | 0.941 (0.655 to 1.228) |
| All PE (< 39 weeks) | | |
| Previous | −0.293 (−0.538 to −0.047) | 0.802 (0.578 to 1.026) |
| New | −0.263 (−0.486 to −0.039) | 1.096 (0.693 to 1.500) |

Results are given for our previous model[5] and for new model (Table 2). Perfectly calibrated model should have intercept of 0 and calibration slope of 1.0.

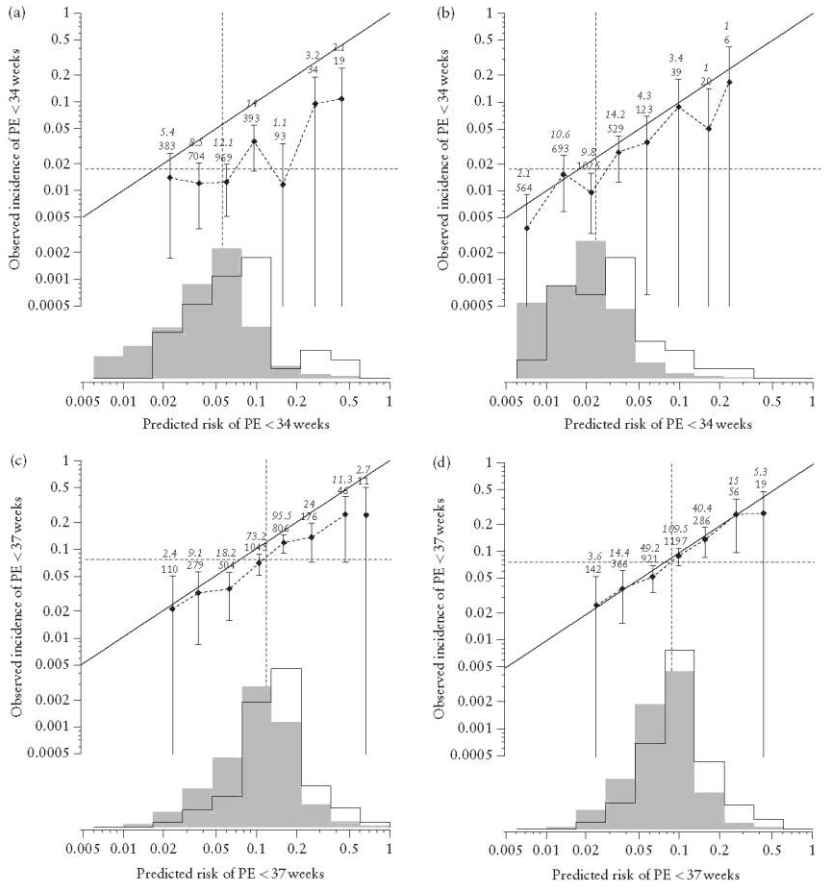


**Figure 3** Calibration plots for screening using competing-risks model for prediction of early (a,b) and preterm (c,d) pre-eclampsia (PE) in validation dataset, according to previous[5] (a,c) and new (b,d) models, after adjustment for effect of censoring due to births from causes other than PE. Diagonal line is line of perfect agreement. Overall mean risk is shown by vertical dashed line and overall incidence by horizontal dashed line. Vertical solid lines are confidence intervals. Numbers of women with PE are shown in italics above total number in that predicted-risk group. Histograms show distribution of risk in affected (□) and unaffected (▩) pregnancies.

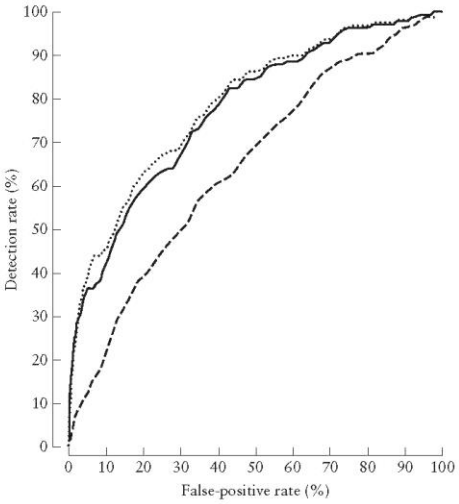


**Figure 4** Receiver–operating characteristics curves for preterm pre-eclampsia in singletons (——), twins (– – –) and mixed population comprising 98% singleton and 2% twin pregnancies (·····).

rate of 41% and, for twins, a risk cut-off of 1 in 7 gives a screen-positive rate of 10% and a detection rate of only 19%.

## DISCUSSION

### Main findings

In this study, we developed a new model for the prediction of PE in twin pregnancies and demonstrated relatively good calibration in an independent validation dataset. The basis of the new model is that, in twin pregnancies, the shift to the left of the distrubution of gestational age at delivery with PE in singleton pregnancies is not uniform, as in our original model[5], but the effect increases with increasing singleton prior mean.

The implication of this finding is that, in a woman who, on the basis of her demographic characteristics and medical history, has a very high risk of developing PE, reflected in a mean of $\leq 34$ weeks for the gestational age at delivery with PE, the presence of a DC twin pregnancy does not increase her risk above that of a singleton pregnancy. In contrast, in a woman at very low risk of developing PE, reflected in a mean of 65 weeks for the distribution of gestational age at delivery with PE, the presence of a DC twin pregnancy results in a substantially increased risk of developing PE compared to that of a singleton pregnancy, with a shift of the distribution to the left by about 16 weeks. In a MC twin pregnancy, there is no shift to the left if the prior mean is $\leq 28$ weeks, but if the prior mean is 65 weeks, the shift to the left is about 18 weeks.

This finding is analogous to the effect of history of pregnancy affected by fetal Down syndrome on the maternal age-related risk for Down syndrome in the current pregnancy. On the assumption that such history increases the risk by about 1%, in a 50-year-old woman with an age-related risk of about 1 in 10, there is a 1.1-fold increase to 1.1 in 10, whereas, in a 20-year-old woman with an age-related risk of about 1 in 1000, there is a 10-fold increase to 11 in 1000; consequently, the increase in risk is inversely proportional to the prior risk.

In the prediction of PE, in a mixed population of singleton and twin pregnancies, the same risk cut-off should be used in identifying the high-risk group in need of prophylactic pharmacological interventions to prevent the development of PE and closer monitoring for early identification of the clinical signs of the disease in those that will develop PE. In this study, we have demonstrated that, at a risk cut-off that would classify 10% of a mixed population as being at high risk for preterm PE, all twins will be classified as screen positive.

### Comparison with previous studies

In a previous study, we evaluated the predictive performance for PE of the competing-risks model in singleton pregnancies using two validation datasets and demonstrated very good discrimination between affected and unaffected pregnancies and good agreement between predicted risk and observed incidence of PE[3,7,16]. In contrast, a validation study of our competing-risks model for twin pregnancies[5] found that, in both the training and validation datasets, the observed incidence of PE was lower than the predicted one, especially for early PE[6]. In this study, we developed a new model and demonstrated good agreement between predicted risk and observed incidence of PE $< 34$, $< 37$ and $< 39$ weeks' gestation. Previously, we used risks of PE $< 41 + 3$ weeks for assessment of risk for all PE in twins[5,6], the same as we have adopted in singletons. Because the vast majority of twins are delivered earlier than $41 + 3$ weeks, these risks are unrealistic and inappropriate for twin pregnancies and, therefore, in this study, we have used risks before 39 weeks for all PE in twin pregnancies.

### Clinical implications

Estimation of accurate patient-specific risk of PE can help stratify the monitoring of twin pregnancies for early identification of those that will develop the disease. In singleton pregnancies at high risk of PE, prophylactic use of aspirin (150 mg/day from 11–14 until 36 weeks' gestation) reduces the incidence of early PE by about 90% and preterm PE by 60%, with no significant effect on the incidence of term PE[17,18]. A systematic review on the prophylactic use of aspirin in twin pregnancies identified five trials[19]. Use of aspirin was not associated

with a reduction in the incidence of PE in any of the trials but a meta-analysis of the trials reported that, first, aspirin reduced the incidence of mild PE but not severe PE and, second, there was significant reduction in PE if aspirin was initiated > 16 weeks' gestation but not < 16 weeks[19]. These results are inconsistent with findings in singleton pregnancies and it was therefore recommended that additional studies are required before recommending that low-dose aspirin should be initiated early in pregnancy for all twin pregnancies. Our results suggest that, when such trials are carried out, all twin pregnancies should be included because they are, by comparison with singleton pregnancies, all at increased risk of developing PE.

### Strengths and limitations

The strengths of this study include, first, development of a new model for the prediction of PE in twin pregnancies in a training dataset and evaluation of discrimination and calibration in a validation dataset derived from an independent multicenter study, and, second, assessment of calibration, allowing for the effect of censoring due to births from causes other than PE. A limitation of this study is that the number of twin pregnancies, by comparison with the number of singleton pregnancies, was relatively small and the model may require further adjustments based on results of future large multicenter studies.

### Conclusions

A new competing-risks model in screening for PE by maternal risk factors in twin pregnancies has been developed and, using this model, the predicted risks for early PE, preterm PE and all PE are in good agreement with the observed incidence of the disease.

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