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The prediction of fetal death with a simple maternal blood test at 24–28 weeks: a role for angiogenic index-1 (PlGF / sVEGFR-1 ratio)

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Abstract

Objective—To determine if maternal plasma concentrations of angiogenic and anti-angiogenic factors measured at 24–28 weeks of gestation can predict subsequent fetal death.

Methods—A case-cohort study was designed to include 1000 randomly selected subjects and all remaining fetal deaths (cases) from a cohort of 4006 women with a singleton pregnancy, enrolled at 6–22 weeks of gestation, in a pregnancy biomarker cohort study. The placentas of all fetal

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deaths were histologically examined by pathologists who used a standardized protocol and were blinded to patient outcomes. Placental growth factor (PlGF), soluble endoglin (sEng), and soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) concentrations were measured by ELISA assays. Quantiles of the analyte concentrations (or concentration ratios) were estimated as a function of gestational age among women who delivered live neonates but did not develop preeclampsia or deliver small-for-gestational–age (SGA) newborns. A positive test was defined as analyte concentrations (or ratios) <2.5th and 10th centiles [PlGF, PlGF/sVEGFR-1 (angiogenic index-1), and PlGF/sEng)] or >90th and 97.5th centiles (sVEGFR-1 and sEng). Inverse probability weighting was used to reflect the parent cohort when estimating the relative risk.

Results—There were 11 fetal deaths and 829 controls with samples available for analysis between 24–28 weeks of gestation. Three fetal deaths occurred prior to 28 weeks and eight at or after 28 weeks of gestation. The rate of placental lesions consistent with maternal vascular underperfusion was 33.3% (1/3) among those who had a fetal death before 28 weeks and 87.5% (7/8) of those who had this complication at or after 28 weeks of gestation.

The maternal plasma angiogenic index-1 value was below the 10th centile in 63.6% (7/11) of the fetal death group and in 11.1% (92/829) of the controls. The angiogenic index-1 value was <2.5th centile in 54.5% (6/11) of the fetal death group and in 3.7% (31/829) of the controls. An angiogenic index-1 value <2.5th centile had the largest positive likelihood ratio for predicting fetal death >24 weeks (14.6; 95% CI, 7.7–27.7) and a relative risk of 29.1 (95% CI, 8.8–97.1), followed by sEng >97.5thcentile and PlGF/sEng <2.5th, both with a positive likelihood ratio of 13.7 (95% CI, 7.3–25.8) and a relative risk of 27.4 (95% CI, 8.2–91.2).

Among women without a fetal death whose plasma angiogenic index-1 concentration ratio was below the 2.5th centile, 61% (19/31) developed preeclampsia or delivered an SGA neonate; when the 10th centile was used as the cut-off, 37% (34/92) of women had these adverse outcomes.

Conclusions—1) A maternal plasma angiogenic index-1 value below the 2.5th centile (0.126) at 24–28 weeks of gestation carries a 29-fold increase in the risk of subsequent fetal death and identifies 55% of subsequent fetal deaths with a false-positive rate of 3.5%; and 2) 61% of women who have a false-positive test result will subsequently experience adverse pregnancy outcomes.

Keywords

Endoglin; maternal vascular underperfusion; preeclampsia; preterm delivery; placenta; placental growth factor (PlGF); small for gestational age (SGA); soluble vascular endothelial growth factor receptor-1 (sVEGFR-1); soluble fms-like tyrosine kinase-1 (sFLT-1)

Introduction

Fetal death is an obstetrical syndrome [1, 2] caused by multiple etiologies rather than the end stage of a single disease process [3–6]. This syndrome affected 23,595 pregnancies in the United States [7] in 2013, and according to the World Health Organization (WHO), an estimated 2.6 million fetal deaths occurred globally during the third trimester [8, 9]. The most common type of fetal death is unexplained stillbirth [10–13], which comprises a progressively larger proportion of all fetal deaths as pregnancy advances. This category of fetal death accounts for approximately 20% of all cases just after 20 weeks of gestation and for about

40% of all cases at term[10–15], while infection and congenital anomalies cause most early fetal deaths (<28 weeks of gestation) [16–19], placental causes leading to fetal death are more frequent after 26 weeks of gestation [14] including placental vascular underperfusion, resulting in an impaired supply of nutrients to the fetus, [20–35] abruptio placentae [36–38], and placental senescence that has been implicated as a mechanism of fetal death at term. [22]

The term "placental malperfusion" (formerly called "maternal vascular lesions of underperfusion") refers to a group of vascular lesions, including villous infarcts, syncytial knots, villous agglutination, increased intervillous fibrin deposition, villous hypoplasia, persistent muscularization of the basal plate arteries, and mural hypertrophy of the decidual arterioles as well as acute atherosis of the basal plate and decidual arteries [39–43]. The frequency and burden of placental vascular lesions of malperfusion is reflected in the maternal plasma by an imbalance between the concentration of angiogenic [placental growth factor (PlGF)] and anti-angiogenic factors [soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and soluble endoglin (sEng)][44].

Several investigators [45–47] have characterized the changes in the plasma concentrations of angiogenic and anti-angiogenic factors in women who subsequently had a fetal death, demonstrating that, from 20 weeks of gestation onward, these patients had a lower maternal plasma PlGF and higher sEng and sVEGFR-1 concentrations than women with a normal pregnancy [45]. Additionally, a maternal plasma PIGF/sVEGFR-1 concentration ratio (angiogenic index-1) of less than 0.12 multiples of the median at 30–34 weeks of gestation in normal pregnancy—which corresponds to the 5^{th}–6^{th} centile of the distribution—identified 4 of 5 fetal deaths that occurred later in pregnancy [48]. Given that an imbalance in angiogenic and anti-angiogenic factors reflects the presence and burden of placental vascular lesions[44] of malperfusion that have been proposed to be the leading cause of fetal death in the late second and third trimesters until term[14], we sought to validate and extend these findings by determining whether plasma angiogenic index-1 at 24–28 weeks of gestation could be used as a biomarker to identify patients at risk for a subsequent fetal death.

Methods

Study Design & Participants

This was a case-cohort study. We randomly selected 1,000 patients from a cohort of 4,006 pregnant women enrolled in a longitudinal study previously reported by our group.[44] The remaining women in the original cohort who had a fetal death, but were not selected in the random sample of 1,000 women, were subsequently added to the case-cohort. Women who had multiple gestations or any of the following conditions at the time of enrollment were excluded from this study: active vaginal bleeding; severe maternal morbidity (i.e., renal insufficiency, congestive heart disease, and chronic respiratory insufficiency); chronic hypertension requiring medication; asthma requiring systemic steroids; requirement of anti-platelet or non-steroidal anti-inflammatory drugs; active hepatitis; or fetal chromosomal abnormalities and congenital anomalies. All study participants provided written informed consent and were followed until delivery. The use of clinical data and biological specimens obtained from these women for research purposes was approved by the Institutional Review Boards of Wayne State University and the *Eunice Kennedy Shriver* National Institute of

Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services (NICHD/NIH/DHHS).

Clinical Definitions

The following definitions were used in this study:

1. Fetal death: diagnosed as the death of the fetus after 20 weeks of gestation and confirmed by ultrasound examination [49];
2. Preeclampsia: diagnosed by the presence of systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on at least two occasions, 4 hours to 1 week apart, and of proteinuria ≥300 mg in a 24-hour urine collection or by one dipstick with ≥1+ [50, 51]. We have used this definition after analysis from the Collaborative Perinatal Project, which was reported in detail by Friedman and Neff[52], and the reasons described by one of the authors[53]. Moreover, the definition outlined above was the one used in our Center to collect outcome information when the patients were recruited and delivered;
3. Small-for-gestational-age (SGA) neonate: a birth weight <10th centile for gestational age at delivery according to a U.S. reference population[54]; and
4. Preterm delivery: a delivery occurring prior to the 37th week of gestation.

Sample collection and immunoassays

Patients were scheduled to donate maternal plasma in EDTA tubes at enrollment, then every four weeks until the 24th week of gestation, and bi-weekly thereafter until delivery. Samples were centrifuged and stored at −70^{0} C. Maternal plasma concentrations of sVEGFR-1, PlGF, and sEng were measured by immunoassays (R&D Systems, Minneapolis, MN, USA) as previously described [55]. The inter- and intra-assay coefficients of variation of the assays were 1.4% and 3.9% for sVEGFR-1, 2.3% and 4.6% for sEng, and 6.02% and 4.8% for PlGF, respectively. The sensitivity of each assay was 16.97 pg/ml for sVEGFR-1, 0.08 ng/ml for sEng, and 9.52 pg/ml for PlGF. Laboratory personnel performing the assays were blinded to the clinical information.

Histologic Placental Examination

Placentas were examined according to standardized protocols by perinatal pathologists blinded to clinical diagnoses and obstetrical outcomes. Placental lesions consistent with maternal vascular lesions of underperfusion (now known as placental malperfusion) were diagnosed using criteria established by the Perinatal Section of the Society for Pediatric Pathology[56] and were classified as the following: 1) villous changes, which are further subdivided into abrupt onset (remote villous infarcts, recent villous infarcts), gradual onset with intermediate duration (increased syncytial knots, villous agglutination, increased intervillous fibrin), or gradual onset with prolonged duration (decreased placental weight/ increased feto-placental weight ratio, distal villous hypoplasia); and 2) vascular lesions (persistent muscularization of the basal plate arteries, mural hypertrophy of the decidual arterioles, acute atherosis of the basal plate arteries and/or the decidual arterioles).[40]

Statistical Analysis

Using quantile regression, which does not assume data normality, we estimated the percentiles of maternal plasma concentration of analytes and their ratios in a subset of controls (i.e. women who delivered live babies at term and did not have preeclampsia or an SGA neonate). Data was first log-transformed, a standard approach to improve normality of distribution[57] and to reduce the non-linearity of the relation between the response and gestational age at sample collection. Linear quantile regression was then used iteratively to estimate the quantiles at discrete values of gestation, in narrow windows of gestational age, over which the linear assumption holds. The resulting estimated percentiles were not smooth because they were obtained from different models (fitted to the different narrow gestational-age windows); therefore, we fitted a 5th degree polynomial function to smooth the estimated percentiles[48, 58]. The quantile regression was performed using the *quantreg* package of the R open source statistical software. [59, 60]

Positive tests were defined as analyte concentrations (or their ratios) below the 2.5th and 10th centiles (PlGF, PlGF/sVEGFR-1, and PlGF/sEng) or above the 90th and 97.5th centiles (sVEGFR-1 and sEng). Data from the 11 fetal deaths and 829 controls with available measurements were used to construct Receiver Operating Characteristic (ROC) curves and to determine the sensitivity, specificity, and likelihood ratios (positive and negative). Likelihood ratios for a positive test result above 10 and likelihood ratios for a negative test result below 0.1 were taken as strong predictive evidence under most circumstances. Moderate prediction could be achieved with likelihood ratios of 5–10 and 0.1–0.2, whereas ratios <5 and >0.2 were only minimally predictive.[61] Modified Poisson regression was used to estimate relative risk (RR) with 95% confidence intervals (CI)[62]. To estimate positive predictive values (PPV), negative predictive values (NPV), and RR, we weighted the data by the inverse probability of selection to reflect the parent cohort [63].

For demographic data analysis, we used a Chi-square or Fisher's exact test and reported proportions for categorical variables, and medians and interquartile ranges (IQR) for continuous variables.

Results

There were 24 fetal deaths (0.6%, 24/4,006): six were initially selected with 994 controls in the random sample of 1,000 women. Following this selection process, we added the remaining 18 patients with a fetal death from the parent cohort to the cases group, reaching a total of 24 patients. Of the 24 fetal deaths, 12 were diagnosed < 28 weeks, and 12 ≥ 28 weeks of gestation.

Table 1 shows the characteristics of the cases and controls.. Cases delivered at a lower median gestational age ($p < 0.0001$) had lower median birth weights ($p < 0.0001$). There were no significant differences between cases and controls in median maternal age and the proportion of smokers, nulliparity, and African-American ethnicity.

Patients with a fetal death had a higher rate of placental lesions consistent with maternal vascular underperfusion than the remainder of the study cohort [fetal death, 58.3% (14/24)

versus the remainder of the cohort, 21.3% (206/968), and an odds ratio of 5.2 (2.1–13.2), p=0.0001]. Among women with a fetal death, the rate of placental lesions consistent with maternal vascular underperfusion was 42% (5/12) in those diagnosed prior to 28 weeks of gestation and 75% (9/12) in those diagnosed ≥28 weeks of gestation (Fisher's exact test, p=0.2).

The Changes in Angiogenic and Anti-Angiogenic Profiles between 24 and 28 Weeks of Gestation in Women with a Subsequent Fetal Death

This analysis included only patients whose blood samples were collected between 24 and 28 weeks of gestation. Seven women had fetal deaths before 24 weeks of gestation and were excluded from further analysis. Additionally, six patients were excluded, as samples were not available at the desired gestational-age interval.

Eleven women with a fetal death were available for further analysis; eight had a fetal death ≥28 weeks and three of these women had a fetal death <28 weeks of gestation. From the remaining case-cohort, blood samples collected at 24–28 weeks of gestation were available from 829 controls.

Figure 1(a–d) shows the changes in the maternal plasma soluble VEGFR-1, Eng, PlGF concentrations and the PlGF/ sEng ratio for fetal death cases superimposed on the estimated quantiles for the controls (after excluding patients with preeclampsia and those with an SGA neonate; see Table 2 for quantile values of all analytes and ratios). Similar data for maternal plasma angiogenic index-1 are shown in Figure 2. The value of the 2.5^{th} centile of angiogenic index-1 was constant (0.126) between 24 and 28 weeks of gestation and corresponds to 0.2 multiples of the median (MOM).

Table 3 shows the prediction performance for fetal death >24 weeks when defining a positive test based on abnormal angiogenic and anti-angiogenic factor concentrations and ratios measured at 24–28 weeks of gestation. An angiogenic index-1 value $<2.5^{th}$ centile had the largest positive likelihood ratio (14.6; 95% CI, 7.7–27.7) and relative risk (29.1; 95% CI, 8.8–97.1), followed by sEng concentrations $>97.5^{th}$centile and PlGF/sEng ratios $<2.5^{th}$, both of which had a positive likelihood ratio of 13.7 (95% CI, 7.3–25.8) and a relative risk of 27.4 (95% CI, 8.2–91.2).

Table 4 presents the number of cases and controls with maternal plasma angiogenic index-1 values below the 10^{th} and 2.5^{th} centiles. Using the 10^{th} percentile cut-off, maternal plasma angiogenic index-1 had a sensitivity of 63.6% (7/11), a false-positive rate of 11.1% (92/829), and a positive likelihood ratio (LR+) of 5.7. Using the 2.5^{th} centile cut-off, maternal plasma angiogenic index-1 had a sensitivity of 54.5% (6/11), a false-positive rate of 3.7% (31/829), and a LR+ of 14.6 (Tables 2 and 3). The whole range of sensitivities and corresponding false-positive rates of angiogenic index-1 is shown by the Receiver Operating Characteristic (ROC) curve in Figure 3 (AUC= 0.79, 95% confidence interval (CI) 0.61–0.97, p=0.0005.).

Eight of the 11 cases had placental findings consistent with maternal vascular lesions of underperfusion. The positive likelihood ratio of angiogenic index-1 in these 8 cases increased from 5.7 to 7.9 at the 10^{th} centile cut-off, and from 14.6 to 20.0 at the 2.5^{th} centile

cut-off, as three of the four cases missed by a positive angiogenic index-1 at the 10th centile did not have placental lesions consistent with maternal vascular underperfusion. In other words, angiogenic index-1 identified 7 of 8 (87.58%) subsequent fetal deaths associated with placental lesions consistent with maternal underperfusion (Figure 2). The performance of the test in the detection of fetal death in patients with maternal vascular lesions of underperfusion is shown by the ROC curve in Figure 3 (AUC=0.89, 95% CI 0.71–1.00, p=0.00007).

The risk of fetal death in patients with abnormal angiogenic index-1

In the tested cohort in which the prevalence of fetal death after 24 weeks was 1.3% (11/840), 7% of women who had a 'positive' angiogenic index-1 value at the 10th centile had a subsequent fetal death, and 16% [6/(6+31)] of those who had a 'positive' angiogenic index-1 value at the 2.5th centile had a subsequent fetal death (Table 4). When weighting of cases and controls in the tested cohort was performed so that the weight of cases was the same as the prevalence of fetal death in the full cohort (0.4%, 17/4006), the risk of fetal death >24 weeks was determined to be 2% (PPV) for a 'positive' angiogenic index-1 value at the 10th centile, and 6% [6*1.55/(6*1.55+31*4.81)] (PPV) for a 'positive' angiogenic index-1 value at the 2.5th centile.

Pregnancy outcomes of women with abnormal angiogenic index-1 values who did not have a fetal death?

Of the 31 women who had "false-positive" test results at the 2.5th centile cut-off, 48% (15/31) developed preeclampsia, 39% (12/31) delivered an SGA neonate, and 61% (19/31) had at least one of these outcomes. Of the 92 women who had "false-positive" test results at the 10th centile cut-off, 23% (21/92) developed preeclampsia, 24% (22/92) delivered an SGA neonate, and 37% (34/92) had at least one of these outcomes.

Discussion

Principal findings of the study

1) The maternal plasma angiogenic index-1 value (PlGF/sVEGFR-1 maternal plasma concentration ratio) at 24–28 weeks can identify women who subsequently have a fetal death; 2) an angiogenic index-1 < 2.5th centile had a positive likelihood ratio of 14.6 for identifying a subsequent fetal death and can detect one-half of all fetal deaths (sensitivity, 54.5%) in the late second and third trimesters of pregnancy; 3) in the subset of fetal deaths associated with placental lesions consistent with maternal vascular underperfusion, the positive likelihood ratio of angiogenic index-1 < 2.5th centile increased to 20.0; and 4) 61% of women whose angiogenic index-1 at 24–28 weeks was < 2.5th centile, who did not have a subsequent fetal death, developed preeclampsia or delivered an SGA neonate.

Placental lesions and fetal death—The placenta is considered to be a record of fetal life[64], and by examining it histologically, one can often glean the intrauterine pathological processes that may have led to obstetrical complications such as fetal death[32]. The pathologic processes implicated in fetal death include: infection[65–84], placental abruption,[5, 37, 70, 85–92] vascular lesions of the placenta,[28, 35, 93–100] preeclampsia,[41, 101–105] fetal

growth restriction[9, 106–114], maternal anti-fetal rejection, [35, 115–120] metabolic disorders, [121–133] genetic disorders,[80, 131, 134–137] umbilical cord accident,[97, 138–145] trauma[146–148], and placental senescence[22]; however, most of the 47,000 stillbirths reported from developed countries in 2015[149] were classified as unknown etiology. It is currently believed that the causes of fetal loss change with gestational age: chromosomal abnormalities[150–153] and infection[75] are the most common causes during the first half of pregnancy, placental causes (abruption or vascular abnormalities)[154–156], and maternal anti-fetal rejection[118] become the most common causes after 26 weeks until term[14], after which the etiology of in most cases, especially after 40 weeks, is unknown[14]. In this study, the frequency of placental lesions consistent with maternal vascular underperfusion was significantly higher in cases than controls, supporting the view that the most common cause of fetal death in the late second and third trimesters of pregnancy is placental in origin.

What is the association between fetal death and maternal plasma angiogenic index-1?

The placenta is a network of blood vessels surrounded by trophoblast [64], and the processes of vasculogenesis and angiogenesis are crucial for the success of pregnancy. Indeed, deletion of a single VEGF allele in an embryo (haploinsufficiency) is lethal [157]. An imbalance in the maternal plasma angiogenic and anti-angiogenic factors has been reported in women with preeclampsia[48, 55, 58, 158–172], SGA neonates [166, 169, 170, 173], a subset of those who had a spontaneous preterm parturition[174], mirror syndrome [175–177], twin-to-twin transfusion syndrome [178], and fetal death [45, 46]. These alterations in the maternal plasma concentrations of PlGF and sVEGFR-1 can be detected at the time of disease and at several weeks prior to the onset of the different obstetrical syndromes.

What are the clinical implications of a low maternal plasma angiogenic index-1?

In previous studies[44], we reported that maternal plasma angiogenic index-1 measured at 30–34 weeks of gestation identified 80% of subsequent late fetal deaths. Among those who had a fetal death, identified by the maternal plasma angiogenic index-1 value, 80% had maternal vascular underperfusion.[44] This result, coupled with our findings that women who subsequently had a fetal death have higher maternal plasma sVEGFR-1 and lower PIGF concentrations, as early as 20 weeks of gestation, compared to those with a normal pregnancy, led us to explore whether the ratio between these two analytes was predictive of fetal death as early as 24 weeks of gestation. This study found that a maternal plasma angiogenic index-1 below the 2.5th centile at 24–28 weeks of gestation (i.e., below 0.126) could identify 54.5% of subsequent fetal deaths A patient whose plasma angiogenic index-1 at 24–28 weeks of gestation is < 0.126 has a 6% chance of a fetal death, while a patient whose plasma angiogenic index-1 is > 0.126 only a 0.2% chance of a fetal death.

Two factors support choosing the 2.5th centile over the 10th centile as the cut-off to define a ‘positive’ angiogenic index-1. First, angiogenic index-1 is constant below the 2.5th centile but not below the 10th centile, so a single value can be used to determine whether a test is ‘positive’ or ‘negative’ using the 2.5th centile as the cut-off. Second, the proportion of false-positives associated with preeclampsia or SGA neonates was higher for false positives below the 2.5th centile (61%) than for the 10th centile (37%), making a positive result below the

2.5th centile more clinically significant than a positive result below the 10th centile, even if the latter cut-off is slightly more predictive of a subsequent fetal death.

A preliminary report indicating that statins may improve pregnancy outcome by reducing the anti-angiogenic state in pregnancy[179] has increased the potential value of screening pregnancies for the PIGF/sVEGFR-1 concentration. We previously reported a case in which a patient with recurrent fetal losses due to maternal perivillous fibrin deposition was successfully treated with pravastatin, and the treatment improved her PIGF/sVEGFR-1 concentration ratio, leading to a successful pregnancy. Maternal perivillous fibrin deposition has a stereotypic pattern for angiogenic index-1[180], and we have provided evidence that this may reflect (at least, in some cases) maternal anti-fetal rejection[120]. Since treatment with pravastatin can reverse low plasma PIGF/sVEGFR-1 concentration ratios, therapeutic trials of pravastatin in women who have low plasma PIGF/sVEGFR-1 concentration ratios are warranted, especially given the negligible risks resulting from a false-positive screening test result. Other interventions that could be effective include metformin[181] and proton pump inhibitors.[182, 183]

Thus, the maternal plasma angiogenic index-1 measured at 24–28 weeks of gestation can serve as a biomarker to identify women at risk of having a subsequent fetal death or developing other obstetrical complications. The availability of potentially effective treatments makes the formulation of practical screening programs using plasma angiogenic index-1 a clinical priority.

Strengths and limitations of this study

The strengths of this study are as follows: 1) the use of a case-cohort study design included a random sample of about one-quarter of the full cohort of 4,006 women and all remaining women with a fetal death in the parent cohort. Unlike a nested case-control study, this case-cohort design allowed us to estimate relative risk and to account for other complications that may involve a common pathway with fetal death; 2) the demonstration that angiogenic index-1 below the 2.5th centile has a higher positive likelihood ratio for patients whose placenta had histologic lesions of maternal vascular underperfusion, emphasizing that this risk-assessment method is also related to the underlying mechanism of disease leading to fetal death; and 3) the proportion of placental-associated fetal deaths in this study is comparable to that reported in another study[34].

Limitations of this study include: 1) In the study design, the sub-cohort of 1000 random individuals was not selected completely at random from the full cohort but rather from all patients who had at least three blood samples available for analysis. Such longitudinal data are best-suited for establishing reference intervals in the control group; 2) the fact that not all of the patients selected for inclusion in the case-cohort had a sample during the 24–28 week interval for analysis; 3) the small number of late fetal deaths included in the analysis; however, this reflects the prevalence of the disease in the population, and, in spite of this limitation, we demonstrated a highly positive result; and 4) although an abnormal angiogenic index-1 had the highest relative risk and positive likelihood ratio for fetal death, a cost-effectiveness analysis is still warranted to determine the optimal screening method, since sEng (Figure 1b) alone had a similar prediction performance for this outcome.

Conclusions

1) A maternal plasma angiogenic index-1 value below the 2.5th centile at 24–28 weeks of gestation carries a 29-fold increase for the risk of subsequent fetal death and identifies 55% of these patients at a very low (3.7%) false-positive rate; and 2) 61% of the women who had a false-positive result subsequently had adverse pregnancy outcomes (preeclampsia or an SGA neonate).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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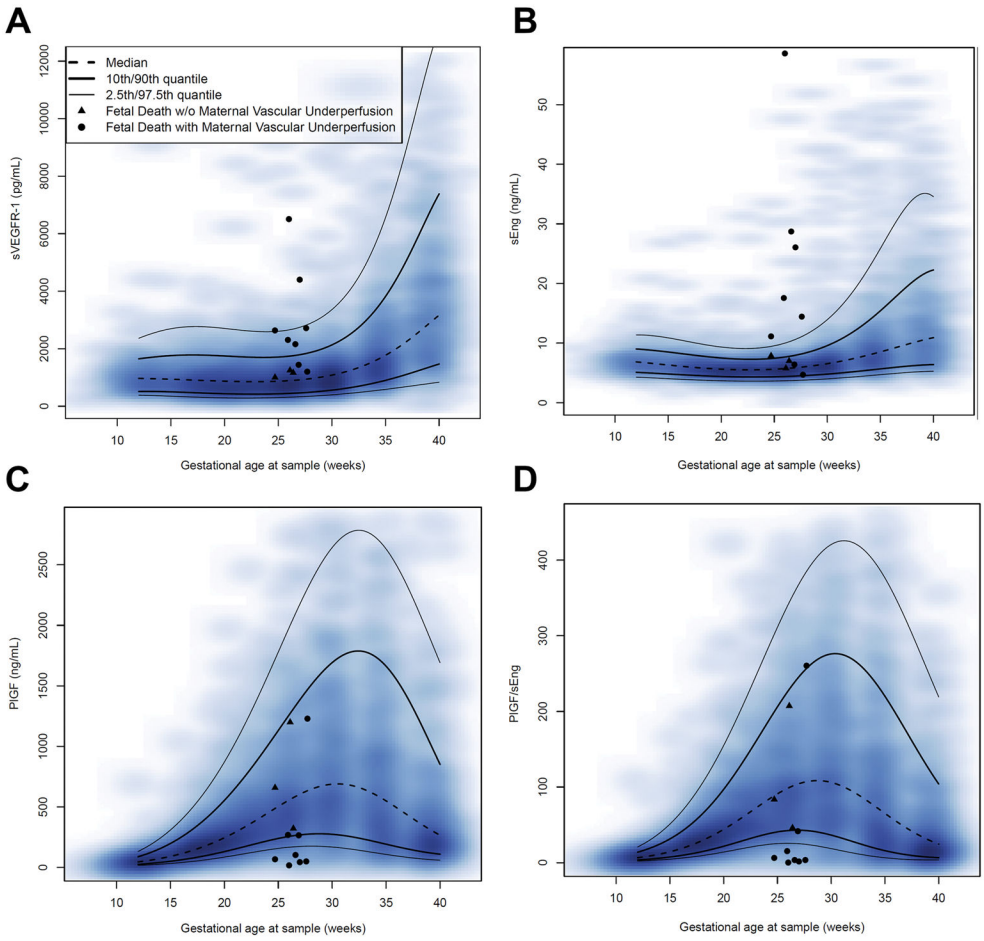

Figure 1. Concentrations of maternal plasma angiogenic and anti-angiogenic factors in patients with fetal death

For each fetal death case, data for one sample collected at 24–28 weeks are shown; triangles identify cases without placental lesions of maternal vascular underperfusion (MVU) and filled circles represent cases with MVU. The lines correspond with the percentiles of the analytes (or ratios) (2.5^{th}, 10^{th}, 50^{th}, 90^{th}, and 97.5^{th} centiles). The distribution of the raw longitudinal data used to derive the percentiles is shown using a heat map; the color intensity denotes the frequency of the data points for the corresponding gestational age: A) soluble vascular endothelial growth factor receptor-1, B) soluble endoglin, C) placental growth factor and D) placental growth factor/soluble endoglin ratio.

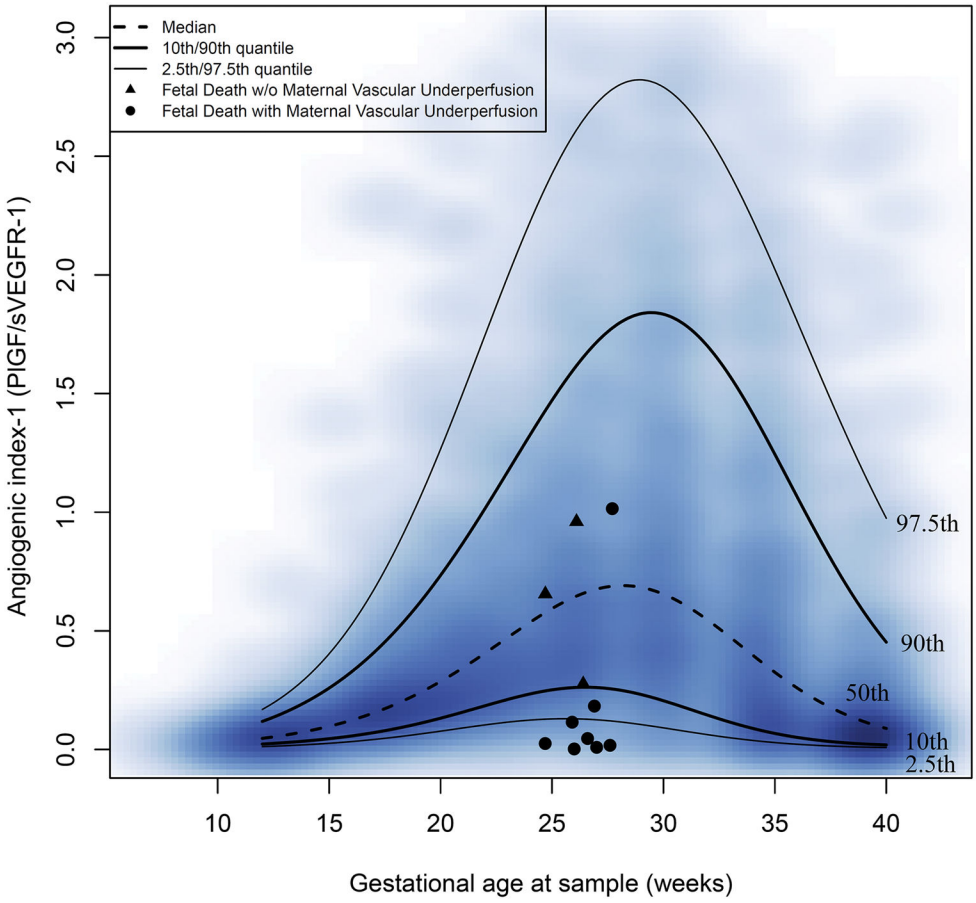

Figure 2. Angiogenic index-1 in patients with a fetal death

For each fetal death case, data for one sample collected at 24–28 weeks are shown; triangles identify cases without placental lesions of maternal vascular underperfusion (MVU) and filled circles represent cases with MVU. The lines correspond with the percentiles of angiogenic index-1 as described by the labels in the graph (2.5^{th}, 10^{th}, 50^{th}, 90^{th}, and 97.5^{th} centiles). The distribution of the raw longitudinal data used to derive the percentiles is shown using a heat map; the color intensity denotes the frequency of the data points for the corresponding gestational age and angiogenic index-1 value.

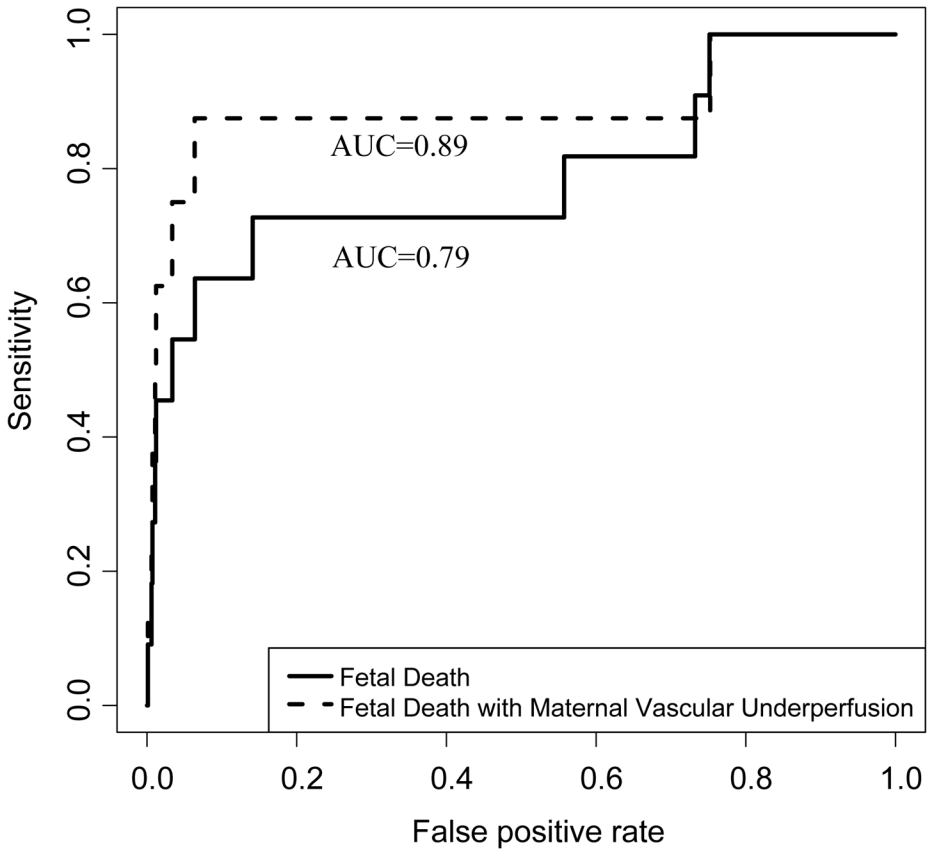

Figure 3. The Receiver Operating Characteristic (ROC) curves of the performance of angiogenic index-1 in the detection of patients who will subsequently have a fetal death

The area under the curve (AUC) of the ROC curve for the detection of all patients with a subsequent fetal death (Bold line) is 0.79, 95% confidence interval (CI) 0.61–0.97, p=0.0005. The area under the curve (AUC) of the ROC curve for the detection of subsequent fetal death of patients who also had placental lesions consistent with maternal vascular under perfusion (broken line) is 0.89, 95% CI 0.71–1.00, p=0.00007.

Table 1

Descriptive characteristics of THE study participants

Characteristic	No Fetal Death (n=994)	Fetal Death (n=24)	P
Maternal age, years	23 (20–27)	22.5 (20–29.5)	0.59
Smoker	206 (20.8)	5 (20.8)	1
Nulliparity	381 (38.7)	8 (33.3)	0.64
African Americans	921 (92.7)	23 (95.8)	1
Pre-Pregnancy BMI	26.6 (22.5–32.5)	26.8 (23.2–33.3)	0.54
GA at delivery	39.1 (37.9–40.1)	28.3 (23–31.5)	<0.0001
Birthweight	3172.5 (2800–3485)	924.5 (493.5–1400)	<0.0001

Data presented as median (interquartile range) for continuous variables and number (%) for categorical variables.

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TABLE 2

the 2.5th, 10th, 50th, 90th, and 97.5th centiles of the concentrations of PlGF, sVEGFR-1, and sEng, and the ratios of PlGF/sVEGFR-1 and PlGF/sEng, according to gestational age at sample collection.

PlGF (pg/mL)						sVEGFR1 (pg/mL)						sEng (ng/mL)						PlGF/sVEGFR1						(PlGF/sEng) x 1000					
GA(weeks)	2.5th	10th	50th	90th	97.5th	GA(weeks)	2.5th	10th	50th	90th	97.5th	GA(weeks)	2.5th	10th	50th	90th	97.5th	GA(weeks)	2.5th	10th	50th	90th	97.5th	GA(weeks)	2.5th	10th	50th	90th	97.5th
12	17.3	25.3	45.4	88.4	132.4	12	384.8	513.9	970.8	1650.2	2364.6	12	4.28	5.09	6.84	9.01	11.37	12	0.012	0.023	0.047	0.118	0.169	12	2.2	3.6	6.6	13.9	20.9
13	21.5	32.3	58.0	117.8	180.2	13	376.2	516.6	962.1	1695.5	2501.5	13	4.21	5.01	6.68	8.88	11.32	13	0.016	0.029	0.060	0.156	0.228	13	2.8	4.6	8.6	18.4	28.7
14	26.6	40.8	73.3	154.1	238.9	14	363.8	511.9	951.9	1733.5	2613.8	14	4.12	4.91	6.50	8.70	11.14	14	0.020	0.036	0.077	0.202	0.306	14	3.6	5.9	11.2	24.2	38.7
15	32.8	50.9	91.6	198.0	309.5	15	349.6	502.0	940.1	1761.7	2696.7	15	4.03	4.82	6.32	8.50	10.88	15	0.026	0.046	0.098	0.259	0.404	15	4.6	7.5	14.4	31.4	51.1
16	40.3	62.8	113.5	250.3	392.9	16	335.2	488.9	926.8	1778.5	2748.6	16	3.95	4.72	6.15	8.29	10.56	16	0.033	0.057	0.123	0.327	0.525	16	5.9	9.4	18.4	40.3	66.1
17	49.3	76.7	139.4	311.6	490.0	17	321.7	474.4	912.6	1783.9	2770.6	17	3.87	4.62	5.99	8.07	10.22	17	0.042	0.071	0.154	0.408	0.673	17	7.5	11.8	23.3	51.2	84.0
18	59.8	92.8	169.8	382.4	601.8	18	309.9	460.1	898.2	1779.2	2766.8	18	3.79	4.54	5.85	7.86	9.88	18	0.053	0.088	0.192	0.502	0.846	18	9.4	14.6	29.2	64.3	104.8
19	71.9	110.8	205.0	463.1	728.6	19	300.5	446.9	884.3	1766.5	2743.0	19	3.73	4.46	5.73	7.66	9.58	19	0.064	0.109	0.236	0.611	1.045	19	11.6	18.0	36.1	79.5	128.5
20	85.4	130.8	245.2	553.7	871.0	20	293.6	436.0	872.1	1748.8	2706.7	20	3.68	4.40	5.63	7.50	9.34	20	0.077	0.132	0.287	0.734	1.266	20	14.0	21.7	44.1	97.0	155.0
21	100.0	152.4	290.2	654.0	1028.5	21	289.5	427.8	862.3	1729.5	2665.5	21	3.64	4.36	5.56	7.38	9.17	21	0.091	0.158	0.344	0.870	1.503	21	16.7	25.8	52.9	116.6	184.0
22	115.2	174.9	339.8	763.3	1200.5	22	288.3	423.0	856.2	1712.4	2627.5	22	3.62	4.33	5.53	7.30	9.09	22	0.104	0.185	0.406	1.016	1.748	22	19.4	30.0	62.5	137.9	214.9
23	130.4	197.6	392.9	880.5	1385.0	23	290.2	422.0	854.7	1701.2	2600.3	23	3.61	4.32	5.52	7.29	9.12	23	0.115	0.211	0.471	1.169	1.991	23	21.9	34.1	72.3	160.4	247.0
24	144.7	219.6	448.2	1004.0	1579.4	24	295.2	425.2	859.0	1699.7	2591.1	24	3.61	4.33	5.55	7.33	9.26	24	0.124	0.234	0.535	1.324	2.220	24	23.9	37.8	82.0	183.5	279.5
25	157.2	239.6	503.7	1131.4	1779.6	25	303.5	433.1	870.2	1711.7	2607.2	25	3.64	4.35	5.62	7.46	9.55	25	0.129	0.252	0.594	1.472	2.426	25	25.2	40.7	91.0	206.1	311.4
26	167.0	256.4	556.8	1259.6	1980.3	26	315.1	446.0	889.7	1741.3	2655.8	26	3.68	4.40	5.73	7.66	9.99	26	0.129	0.261	0.642	1.605	2.596	26	25.6	42.5	98.7	227.3	341.4
27	173.4	268.9	604.7	1384.7	2174.8	27	330.2	464.6	918.8	1792.7	2744.9	27	3.73	4.47	5.87	7.96	10.62	27	0.125	0.261	0.676	1.716	2.723	27	25.1	43.0	104.5	245.9	368.5
28	175.9	276.3	644.4	1502.1	2355.7	28	349.0	489.3	959.4	1871.0	2883.9	28	3.81	4.56	6.05	8.37	11.46	28	0.117	0.251	0.690	1.796	2.799	28	23.6	42.0	107.8	260.8	391.4
29	174.3	278.0	672.9	1606.6	2514.5	29	371.6	520.9	1013.3	1981.9	3083.9	29	3.90	4.67	6.28	8.91	12.56	29	0.105	0.232	0.683	1.837	2.822	29	21.3	39.8	108.4	271.1	409.1
30	168.7	273.9	688.0	1692.5	2643.0	30	398.0	560.3	1082.9	2132.4	3358.8	30	4.00	4.80	6.55	9.58	13.93	30	0.091	0.206	0.655	1.834	2.792	30	18.6	36.4	106.1	276.0	420.7
31	159.7	264.3	688.1	1753.8	2733.6	31	428.4	608.2	1171.0	2330.8	3725.7	31	4.12	4.95	6.86	10.40	15.63	31	0.077	0.175	0.607	1.787	2.711	31	15.7	32.3	101.2	275.2	425.5
32	148.2	250.2	672.9	1785.2	2780.1	32	462.8	665.5	1280.5	2587.1	4205.1	32	4.25	5.11	7.22	11.40	17.69	32	0.062	0.144	0.545	1.698	2.585	32	12.9	27.9	94.0	268.7	423.3
33	135.0	232.5	643.3	1782.1	2778.6	33	501.0	733.1	1415.0	2912.5	4820.9	33	4.40	5.29	7.62	12.57	20.11	33	0.049	0.115	0.473	1.571	2.423	33	10.3	23.5	85.2	256.8	414.0
34	121.1	212.6	601.2	1741.4	2728.1	34	542.7	811.6	1577.7	3318.7	5597.7	34	4.55	5.48	8.06	13.91	22.87	34	0.038	0.089	0.398	1.416	2.233	34	8.2	19.4	75.3	240.2	398.1
35	107.4	191.9	549.7	1662.3	2630.7	35	587.7	901.2	1771.9	3816.6	6557.3	35	4.70	5.67	8.53	15.42	25.87	35	0.029	0.068	0.325	1.244	2.023	35	6.5	15.8	65.1	219.9	376.3
36	94.5	171.5	492.2	1546.7	2491.3	36	635.3	1001.8	1999.4	4412.4	7709.5	36	4.86	5.86	9.02	17.03	28.95	36	0.022	0.051	0.259	1.065	1.804	36	5.2	12.9	55.1	197.2	349.6
37	83.1	152.4	432.1	1399.3	2317.9	37	684.7	1112.1	2260.1	5102.6	9038.7	37	5.00	6.04	9.53	18.68	31.80	37	0.017	0.039	0.202	0.889	1.584	37	4.3	10.5	45.8	173.1	319.2
38	73.3	135.4	372.8	1227.6	2119.9	38	735.3	1229.7	2549.7	5866.0	10483.9	38	5.13	6.20	10.03	20.23	34.01	38	0.013	0.030	0.155	0.725	1.368	38	3.8	8.7	37.6	148.9	286.4
39	65.5	120.9	317.0	1041.4	1907.6	39	786.1	1350.7	2858.3	6654.4	11917.8	39	5.23	6.32	10.50	21.49	35.09	39	0.010	0.023	0.117	0.578	1.164	39	3.5	7.4	30.4	125.6	252.6
40	59.6	109.4	266.4	851.6	1691.3	40	836.3	1469.3	3167.7	7383.6	13132.3	40	5.29	6.40	10.91	22.26	34.55	40	0.008	0.019	0.089	0.452	0.975	40	3.7	6.6	24.5	103.9	219.0

Concentrations are given in pg/mL for PlGF and sVEGFR-1 and ng/mL for sENG. Values for the PlGF/sENG ratio were multiplied by 1,000. Of note, by dividing the percentile values to the corresponding median value (50^{th} percentile), one can obtain multiple of median (MOM) cut-offs.

Table 3

Prediction performance of maternal plasma angiogenic and anti-angiogenic factors concentrations and ratios (at 24–28 weeks of gestation) for subsequent fetal death (from 24–37.6 weeks of gestation).

Predictor	Cutoff*	Relative Risk	AUC	Sensitivity	Specificity	Likelihood ratio (+)	Likelihood ratio (−)
sVEGFR1	>90	9.4 (2.9–31.1)	0.85	0.55 (0.23–0.83)	0.89 (0.87–0.91)	4.9 (2.8–8.7)	0.51 (0.27–0.98)
sVEGFR1	>97.5	10.8 (2.8–41.4)	0.85	0.27 (0.06–0.61)	0.97 (0.95–0.98)	8.4 (3–23.5)	0.75 (0.52–1.08)
sEng	>90	14 (4.1–48.3)	0.82	0.64 (0.31–0.89)	0.89 (0.87–0.91)	5.9 (3.6–9.5)	0.41 (0.19–0.89)
sEng	>97.5	27.4 (8.2–91.2)	0.82	0.55 (0.23–0.83)	0.96 (0.94–0.97)	13.7 (7.3–25.8)	0.47 (0.25–0.9)
PlGF	<10	7.7 (2.3–25.5)	0.73	0.55 (0.23–0.83)	0.87 (0.84–0.89)	4.1 (2.3–7.2)	0.52 (0.27–1)
PlGF	<2.5	16.7 (5–55.4)	0.73	0.45 (0.17–0.77)	0.95 (0.94–0.97)	9.9 (4.8–20.3)	0.57 (0.33–0.98)
PlGF/sVEGFR1	<10	13.7 (4–47.1)	0.79	0.64 (0.31–0.89)	0.89 (0.87–0.91)	5.7 (3.5–9.3)	0.41 (0.19–0.89)
PlGF/sVEGFR1	<2.5	29.1 (8.8–97.1)	0.79	0.55 (0.23–0.83)	0.96 (0.95–0.97)	14.6 (7.7–27.7)	0.47 (0.25–0.9)
PlGF/sEng	<10	12 (3.5–41.1)	0.77	0.64 (0.31–0.89)	0.87 (0.85–0.9)	5.1 (3.1–8.2)	0.42 (0.19–0.91)
PlGF/sEng	<2.5	27.4 (8.2–91.2)	0.77	0.55 (0.23–0.83)	0.96 (0.94–0.97)	13.7 (7.3–25.8)	0.47 (0.25–0.9)

Data is presented as pecentile for cutoff values; as number(95% confidence interval) for sensitivity, specificity and likelihood ratios. sVEGFR-1- soluble vascular endothelial growth factor receptor -1;sEng- soluble endoglin; PlGF- placental growth factor; AUC: the Area Under the Receiver Operating Characteristic curve.

Table 4

Contingency table for the association between an abnormal maternal plasma angiogenic index-1 and fetal death at>24 weeks.

Angiogenic index-1	Fetal death>24 weeks	Controls
< 10th centile	7	92
≥ 10th centile	4	737
Total	**11**	**829**
< 2.5th centile	6	31
≥ 2.5th centile	5	798
Total	**11**	**829**