

Angiogenic biomarkers for risk stratification in women with suspected preeclampsia

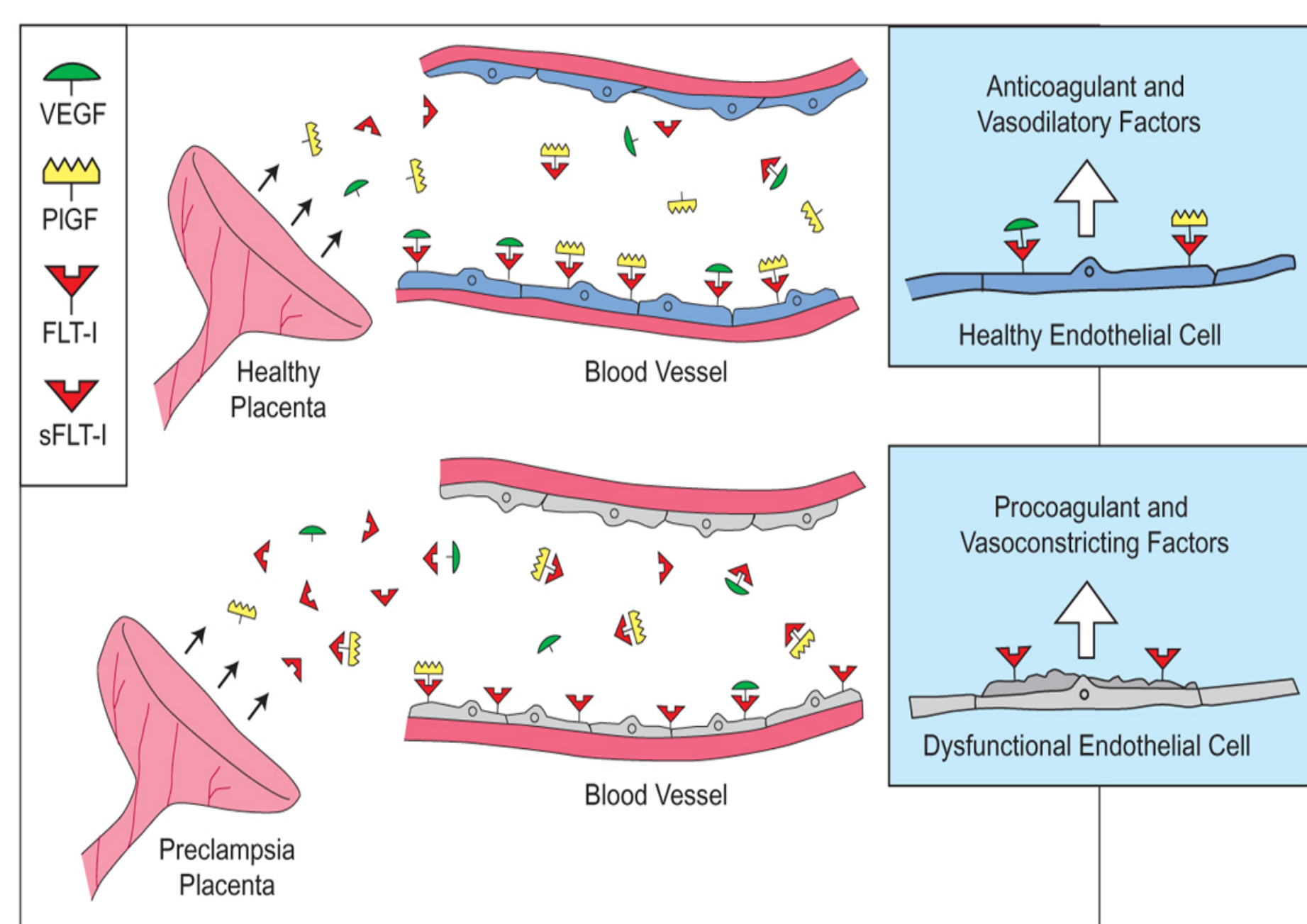
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Introduction

- Preeclampsia is a syndrome defined by the new onset of hypertension (SBP >140 mmHg or DBP >90 mmHg) and proteinuria (24 hour urine protein >300 mg) after 20 weeks of gestation.
- Affects 3-5% of all pregnancies can result in serious maternal and neonatal morbidity and mortality.
- Delivery of the neonate is the only definitive treatment, thus preeclampsia is a major cause of preterm birth.
- Although gestational hypertension is common, only a minority eventually develop severe preeclampsia with serious complications.
- A blood test which could predict maternal or neonatal complications in women with suspected preeclampsia would be useful for clinical risk stratification.

Pathophysiology of preeclampsia:



Materials and Methods

• A single-center prospective cohort study of 315 pregnant women who presented to GWUH Labor and Delivery service with a clinical suspicion of preeclampsia between February 2007 and November 2011. Informed consent was obtained.

• Baseline demographic information and medical history was collected on each patient including maternal age, ethnicity, body mass index, tobacco and other substance use, gestational age, medical problems and obstetric history.

• Serum samples were obtained from each enrolled subject within 24 hours of admission, and sFlt1, PIGF and sEng ELISA assays were performed in duplicate by a blinded laboratory technician at the University of Massachusetts.

• **Primary outcome variable:** Preeclampsia defined as the new onset of hypertension and proteinuria after 20 weeks gestation, according to guidelines published by NHLBI and ACOG. Hypertension was defined as SBP≥140 or DBP≥90 on at least 2 occasions at least 4 hours apart. Proteinuria was defined as >300mg of protein in a 24hr urine specimen, spot urine protein:creatinine ratio >0.3, or urine dipstick of 1+ or higher. Additionally:

- Women with chronic hypertension and without proteinuria prior to 20 wks gestation: diagnosis of preeclampsia required new onset proteinuria after 20 wks gestation
- Women with baseline proteinuria, but without chronic hypertension: diagnosis of preeclampsia required new onset hypertension and worsening proteinuria
- Women with chronic hypertension and baseline proteinuria prior to 20 wks: preeclampsia required worsening hypertension and one of the following: (1) elevated AST or ALT; (2) worsening proteinuria; (3) thrombocytopenia; or (4) eclampsia

• **Secondary outcome** was a composite of adverse maternal and neonatal complications, including any one of the following. Maternal complications: elevated liver enzymes, thrombocytopenia, oliguria, seizures, pulmonary edema or cerebral hemorrhage. Neonatal complications: early preterm birth (<34 weeks), placental abruption, small for gestational age, NICU admission, fetal or neonatal demise.

• **Statistical analysis:** Wilcoxon rank sum tests were used to compare serum levels of biomarkers among women with and without preeclampsia. A Kruskal-Wallis test with post-hoc comparisons was used to do a three-way comparison of the serum levels of the angiogenic factors between women without preeclampsia, women with preeclampsia and no complications and women with preeclampsia and complications. A logistic regression analysis, adjusted for race, obesity, renal disease, chronic hypertension, gestational age at time of sample collection was used to identify correlating variables.

Results

Table 1. Characteristics of women who were enrolled in the study and later identified to have or not have preeclampsia.

| Variable | Pre-eclampsia (n=85) | No pre-eclampsia (n=217) |
|---|----------------------|--------------------------|
| Mean maternal age in years (SE) | 29.5 (0.7) | 30.8 (0.4) |
| Race | % (N) | % (N) |
| White | 18 (15) | 27 (59) |
| Black | 71 (60) | 69 (133) |
| Other | 12 (10) | 12 (26) |
| Smoking | | |
| Never | 82 (70) | 92 (201) |
| Former | 13 (11) | 5 (11) |
| Current | 5 (4) | 3 (6) |
| Chronic HTN | 35 (30) | 20 (43) |
| Diabetes | 6 (5) | 6 (13) |
| Renal disease | 2 (2) | 2 (4) |
| Gravidity (excluding current pregnancy) | | |
| 0 | 33 (28) | 32 (69) |
| 1-3 | 44 (37) | 51 (111) |
| 4+ | 24 (20) | 17 (37) |
| Prior history of preeclampsia | 15 (13) | 10 (21) |
| Delivery method | | |
| C-section | 58 (49) | 49 (111) |
| Vaginal induced | 24 (20) | 25 (54) |
| Vaginal spontaneous | 19 (16) | 26 (57) |
| Gestational age at delivery (weeks) | | |
| 37+ | 62 (53) | 76 (163) |
| <37 | 38 (32) | 24 (51) |

*17 women were excluded due to failure to contribute a blood sample

Table 4 Legend. Logistic regression analysis was performed and adjusted for race, renal disease, hypertension status, gravity and prior history of pre-eclampsia. Significant correlations are indicated in bold.

Results, cont.

Table 3 Serum levels of PIGF are significantly lower in women with pre-eclampsia and even lower in women with pre-eclampsia and complications compared with women with gestational hypertension alone.

| Variable | No Preeclampsia (n=215) | Preeclampsia only (n=60) | Preeclampsia + complications (n=24) | Kruskal-Wallis p-value |
|-------------|-------------------------|--------------------------|-------------------------------------|------------------------|
| PIGF | 155 (3-3575) | 129 (24-798) | 78 (8.5-653) | 0.0285 |
| sFlt1/PIGF | 69.4 (2.2-1619) | 79.5 (3.3-470) | 145 (2-1985) | 0.11 |
| sFlt1 | 9609 (140-119,564) | 10,674 (1904-26,774) | 10,123 (959-76,154) | 0.87 |
| sEng | 15.5 (2.5, 297) | 18.6 (3.6-65) | 32.4 (3.2-161) | 0.28 |

Serum levels of PIGF are significantly lower in women with preeclampsia compared to women without. Most importantly, PIGF is further decreased in women with both preeclampsia and documented maternal and neonatal complications. Serum levels of sFlt1 and Eng were not significantly different among the two groups and there was no difference in serum levels among the women who developed complications. The ratio of sFlt1 to PIGF failed to show a statistically significant difference between any groups.

Table 4. Serum levels of PIGF, gestational age, current smoking status and maternal age are correlated with an increased incidence of preeclampsia.

| Variable | Adjusted OR | 95% CI |
|--|-------------|--------------------|
| PIGF per 100 unit change | 0.83 | 0.69, 0.99 |
| Race | | |
| Black vs. White | 1.04 | 0.48, 2.26 |
| Other vs. White | 1.22 | 0.44, 3.37 |
| Gestational age at sample (wks) | 0.91 | 0.85, 0.98 |
| Gravidity | 1.07 | 0.93, 1.25 |
| Preeclampsia history | 1.12 | 0.46, 2.69 |
| History of renal disease | 0.35 | 0.05, 2.56 |
| Chronic hypertension | 1.62 | 0.84, 3.11 |
| Smoking status | | |
| Former vs. Never | 1.85 | 0.46, 7.37 |
| Current vs. Never | 4.43 | 1.64, 11.95 |
| Maternal age (yrs) | 0.95 | 0.90, 0.99 |

Discussion

• Among a cohort of women presenting acutely with signs and symptoms suggestive of preeclampsia, women who eventually met diagnostic criteria for preeclampsia had lower serum PIGF levels at presentation as compared to women who did not meet preeclampsia criteria.

• sFlt1, sEng, and the sFlt1/PIGF ratio were not significantly different in women who did vs. those who did not meet criteria for preeclampsia

• Women who developed preeclampsia with maternal or neonatal complications had lower serum PIGF levels at presentation as compared to women who developed preeclampsia without complications and women who did not meet criteria for preeclampsia.

• Serum PIGF may be a useful tool for risk stratification in women who present with signs/symptoms suggestive of preeclampsia.

• This tool, rooted in the fundamental pathogenesis of preeclampsia, could arm clinicians with a significant advance in the identification of high-risk patients, ensuring that women with a high likelihood of developing severe preeclampsia complications receive aggressive monitoring and intervention, while allowing women at low risk of complications to be managed conservatively.

• This is the first study to evaluate the utility of biomarkers for risk stratification in the triage setting. Larger studies are needed to confirm our result, and to establish the utility of PIGF for risk stratification in the clinical setting.

Table 2. Serum levels of PIGF are significantly lower in women with preeclampsia compared to women without pre-eclampsia.

| Variable | No Pre-eclampsia (n=215) | Pre-eclampsia (n=83) | Wilcoxon p-value |
|-------------|--------------------------|----------------------|------------------|
| PIGF | 155 (3-3575) | 117 (8.5-797) | 0.0295 |
| sFlt1/PIGF | 69.4 (2.2-1619) | 95.7 (2-1982) | 0.0843 |
| sFlt1 | 9609 (140-119,564) | 10689 (939-76,154) | 0.56 |
| Eng | 15.5 (2.5, 297) | 20.3 (3.2-161) | 0.17 |

Serum samples were processed as per methods and analyzed by ELISA. Statistical analysis showed that PIGF levels are significantly lower in the pre-eclamptic women. Serum levels of sFlt1 and sEng are not significantly different between the two groups. The ratio of sFlt1 to PIGF did not reach significance when compared between groups, though a trend towards an increase was noted (p=0.08).

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