Diagnostic accuracy of placental alkaline phosphatase concentrations in gingival crevicular fluid at early pregnancy in predicting the risk of preeclampsia: a prospective cohort study

Alejandra Chaparro[1], Max Monckeberg[2], Ornella Realini[3], Marcela Hernández[4], Fernanda Param[3], Daniela Albers[5], Valeria Ramírez[6], Juan Pedro Kusanovic[7], Roberto Romero[8], Gregory Rice[6], and Sebastian Illanes[2]

[1]Affiliation not available
[2]University of los Andes
[3]Universidad de los Andes ESE Escuela de Negocios
[4]Universidad de Chile
[5]Universidad Mayor
[6]Universidad de los Andes Commercial Engineering
[7]Pontificia Universidad Catolica de Chile
[8]Wayne State University School of Medicine

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Abstract

Objective: To evaluate the accuracy of concentrations of gingival crevicular fluid (GCF) placental alkaline phosphatase (PLAP) during early pregnancy in identifying women at risk of subsequently developing preeclampsia (PE). Design: Prospective cohort study. Setting: Hospital Sotero del Río, Santiago, Chile. Population: Pregnant women recruited at 11-14 weeks of gestation. Methods: Maternal obstetric and periodontal histories were obtained. GCF samples were collected for PLAP determination by ELISA assay. Multiple logistic regression models estimated the association between GCF-PLAP concentrations, maternal variables, and PE development. The accuracy performance of the prediction model was established. Results: 460 women were recruited into the study, and 412 completed their pregnancy follow–up visit. 18 (4.3%) women developed PE. GCF-PLAP concentrations and systolic blood pressure measurements were significantly higher in women who developed PE (p=0.015 and p<0.001, respectively). An association between first-trimester systolic blood pressure, GCF-PLAP, and PE were established. The predictive model had a sensitivity of 83%, specificity of 72%, a positive predictive value (PPV) of 12%, and a negative predictive value (NPV) of 99%. The positive and negative likelihood ratios were 2.9 and 0.3, respectively, thus classifying correctly 72% of women who subsequently developed PE. The area under the receiver operating characteristic curve was 0.77 for PE and 0.85 for preterm PE. Conclusions: An algorithm that includes PLAP concentrations in GCF and blood pressure during early pregnancy may aid in the identification of women at risk of developing PE. Funding: FONDEF IDeA: ID16I10452. NICHD/NIH/DHHS: HHSN275201300006C. Keywords: a cohort study, gestation, hypertension, placental biomarkers, risk prediction model.

1. Introduction

Preeclampsia (PE) is a hypertensive disease that complicates approximately 3-5% of all pregnancies, being one of the leading causes of maternal morbidity and mortality[1–10], as well as adverse perinatal and neonatal outcomes[11–14]. This condition is part of the most important obstetrical syndromes[15,16] and multiple etiologies[17–23] have been proposed to play a role in its pathophysiology, including an imbalance of angiogenic and anti-angiogenic factors[24–40], systemic maternal inflammation [41–43], endothelial dysfunction[44,45], meta-

bolic syndrome[46–51], vascular disorders of the placenta[52–54], abnormal placentation[55–58], and utero-placental ischemia[59–61]. Indeed, PE is characterized by a systemic maternal vascular dysfunction associated with an abnormal placentation that is, in part, attributable to abnormal remodeling of the spiral arteries during early pregnancy[62–69]. An abnormal placentation is associated with the increased release of cellular debris from the trophoblast into the maternal circulation that contributes to systemic inflammation, endothelial dysfunction, and the clinical manifestation of the disease[70–75]. The only effective treatment of PE is the delivery of the fetus, thus removing the deleterious effects of the placenta on maternal physiology[67,76].

Early identification of women at risk for PE would allow for the development and evaluation of timely and appropriate intervention strategies to limit short- and long-term adverse outcomes[77,78]. Given that interventions such as aspirin administration during the first trimester of pregnancy, have demonstrated a reduction of its incidence, the development of accurate methods for identifying women at risk of developing PE is a recognized clinical need[79–82]. Several multiparametric predictive algorithms have been previously reported in literature[83,84], and are based on several combinations of maternal risk factors, uterine artery Doppler pulsatility indices, and different blood-borne biomarkers[85–87]; however, these algorithms have not been universally adopted and accepted for routine obstetric clinical care[78,88–94]. The development of more accurate, inexpensive and effective risk assessment algorithms may increase the adoption of such testing in clinical care and improve patient management and disease outcome.

Recently, we have identified gingival crevicular fluid (GCF) as a source of surrogate biomarkers of placental function[95–97]. GCF is a serum exudate that originates in the gingival sulcus as a result of periodontal inflammation and contains a variety of biological cell types and molecular markers of systemic and local origin[98]. Thus, the determination of the concentration of such biomarkers in GCF may serve as a minimally invasive source of biomarkers for the prediction of placenta-originated diseases[99,100]. Among those biomarkers, placental alkaline phosphatase (PLAP) has been linked to perinatal diseases such as preterm delivery and PE[101,102]. PLAP is a membrane-bound glycoprotein expressed by the maternal microvillous membrane of the syncytiotrophoblast[103–105]. The concentration of PLAP in maternal blood increases throughout gestation in normal pregnancy[103], and has been implicated in regulating fetal/maternal metabolism, the transport of nutrients, and placental differentiation[106,107]. Moreover, in a recent case-control study, we reported a significantly higher concentrations of GCF-PLAP in pregnant women with PE compared to those with a normal pregnancy, even after adjusting for smoking status, body mass index, and periodontal diagnosis[99].

The rationale to utilize GCF for the prediction of PE also relies on the association between periodontal disease and development of hypertensive disorders of pregnancy[108–111]. A recent overview of systematic reviews suggested an association between periodontal disease and PE development (odds ratio [OR] 2.2; 95% confidence interval [CI], 1.4 to 3.4), after the analysis of 15 studies that comprised 5,111 pregnant women[112]. In addition, periodontal bacteria, such as *Porphyromona gingivalis* , *Fusobacterium nucleatum* , and *Treponema denticola* , have been found to be significantly enriched in the placentae of women affected by hypertensive disorders compared to those of healthy controls[113–115], with increased expression of Toll-like receptor 2 in the placentae of patients with PE[115]; this finding suggests that periodontal bacteremia could stimulate the placental tissue, inducing the systemic release of pro-inflammatory cytokines.

Based on this previous considerations, the aims of the present study are (1) to determine whether GCF-PLAP concentrations are increased during early pregnancy in patients who will subsequently develop PE and (2) to assess the diagnostic performance of GCF-PLAP concentrations when combined with other maternal clinical parameters for the identification of patients who will develop PE.

2. Methods

2.1 Study Design and Participants

A prospective, observational cohort study was performed between January 2017 and March 2018 at a public tertiary health center (Hospital Sótero del Río, in Santiago, Chile). Women with a singleton pregnancy less than 14 weeks of gestation and with confirmed fetal viability were invited to participate in the study. Patients under the age of 18 or with an intention of delivery at other medical centers were excluded from the

present research. Patients with incomplete follow-up until delivery or with an unsatisfactory periodontological evaluation or GCF-PLAP measurements were also excluded from participation. The study was approved by the Scientific and Ethical Review Boards of the Hospital Sótero del Río and the Universidad de Los Andes, and all patients read and signed a written informed consent form prior to sampling and evaluation.

A full periodontal evaluation was scheduled between 11 and 13 6/7 weeks of pregnancy. One dentist, specially trained for this study, evaluated all participants and recorded a detailed maternal and obstetric history. Maternal systolic, diastolic, and mean arterial blood pressure, weight, and height were measured with standardized instruments. A full periodontal diagnostic evaluation was performed, and GCF samples were collected to assess concentrations of PLAP, using a standardized sampling procedure. A plasma sample was obtained from 80 randomly selected women to compare to paired plasma and GCF-PLAP concentrations. Patients, researchers, and health-care providers remained blinded to the results of GCF and plasma PLAP determinations.

After this initial periodontal evaluation, patients received standard obstetrical care, and cases with subsequent obstetrical complications were managed according to local hospital protocols. Patients were followed until delivery and immediate postpartum, with special emphasis on patients who developed gestational hypertension and PE. Data regarding pregnancy outcomes were recorded shortly after delivery in a pre-specified database.

2.2 Definitions:

Preeclampsia was defined as a new-onset persistent blood pressure (systolic blood pressure [?] 140 mmHg or diastolic blood pressure [?] 90 mmHg) and proteinuria (based on a 24-hour urine collection with a total protein excretion > 300 mg or a urinary spot measurement of protein-to-creatinine ratio > 0.3) after 20 weeks of gestation, according to the clinical guidelines established by the American College of Obstetricians and Gynecologists[90]. Preterm PE was defined as those cases of diagnosed PE that required delivery before 37 weeks of gestation. The non-PE group was defined as normotensive pregnant women with a normal pregnancy who did not develop serious obstetric disease and who delivered a healthy newborn at term. Periodontitis and its severity were defined according to the classifications established by the 2017 World Workshop[116,117]: (1) interdental clinical attachment level (CAL) detectable in [?] 2 non-adjacent teeth or (2) buccal or oral CAL [?] 3 mm with pocketing > 3 mm detectable in [?] 2 teeth. Gingivitis was defined in subjects who did not exhibit a periodontal probing depth (PPD) [?] 3 mm, who were without CAL, and who had positive bleeding upon probing (BOP) in [?] 10% of probe sites. Gingival health was defined as < 10% BOP sites, with a PPD [?] 3 mm[116–118].

2.3 Gingival crevicular fluid sample collection and elution protocol:

Oral examination and the collection of GCF samples were performed between 11 0/7 and 13 6/7 weeks of gestation. After a tooth was isolated with a cotton roll, the supragingival plaque was removed with curettes but without contacting the gingival margin. The gingival sulcus was then gently dried with an air syringe. GCF was collected using paper strips. The strips were placed into the sulci/pocket until mild resistance was sensed and left in place for 30 seconds. Strips contaminated by saliva or blood were excluded from the study. After GCF collection, the strips were placed into an Eppendorf tube containing 100 µl of phosphate-buffered saline with 0.05% (v/v) Tween-20. GCF was eluted from the strips by centrifugation (10,000 g for 5 min). This procedure was repeated twice. GCF samples were obtained from four periodontal sulcus/pockets (1 x quadrant) at the most affected periodontal site, representative of the periodontal diagnosis of the patient, and then the strips were pooled to make one sample[119].

2.3 Blood samples:

Blood samples were collected by venipuncture into EDTA-containing tubes between 8:00 a.m. and 10:00 a.m. and then were separated by centrifugation at 1000 g for 15 minutes at 4°C. All the samples were frozen and stored at -80degC until analysis.

2.4 ELISA assays:

PLAP concentrations in GCF and plasma were quantified using the commercially available Placental Alkaline Phosphatase ELISA kit (catalog no. MBS701995; MyBiosource, San Diego, CA, USA). The sensitivity was 0.39 ng/ml, the intra-assay coefficient of variation (CV) was < 8%, and the inter-assay precision CV was < 10%. The GCF and plasma samples were incubated in a pre-coated microplate according to the manufacturer's instructions. The samples were read at a wavelength of 450 nm in an automatic ELISA plate reader (CM Sunrise 350-700 nm, Tecan US, Inc., AG, Switzerland).

2.5 Sample size calculation

To test the hypothesis that GCF-PLAP concentrations are significantly higher during the first trimester in pregnancies that will subsequently develop PE, the estimated sample size was calculated based on previously published data[41] and in our previous observations of differences between GCF-PLAP concentrations in women with PE and normotensive pregnant women (2044 +- 217 and 1880 +- 82 pg/ml; mean +- standard deviation, respectively)[99]. To test the hypothesis of mean differences in GCF-PLAP between patients affected by PE and heathy controls, a minimum cohort size of 406 pregnant women was calculated based on the following assumptions: a 6.4% prevalence of PE in the entire cohort; a significance level of 5%; a power of 80%; a two-sided test; and a loss-to-follow-up of 5%.

2.6 Statistical analyses:

Shapiro-Wilk tests were used to assess data normality. Maternal GCF-PLAP concentrations were not normally distributed; therefore, non-parametric tests were used to assess statistical differences. Comparisons between proportions were performed with a chi-squared or Fisher's exact test, and the Mann-Whitney U test was used to compare continuous variables. The association strength was assessed by using a multiple logistic regression model, which was adjusted by systolic blood pressure and PLAP-GCF concentrations. Receiver Operating Characteristic (ROC) curves summarized the classification performance of biomarkers. Goodness of fit and internal validation of the model were assessed using the Bayesian Information Criterion (BIC) and bootstrapping. The statistical analysis was performed using a commercially available software package (STATA software, StataCorp version 14.1, Lakeway Drive College Station, TX, USA). A $p < 0.05$ was considered statistically significant.

3. Results

A study design flowchart is presented in Figure 1. Of the 460-singleton pregnant woman recruited into this study, 423 (92%) completed the follow-up until delivery. In 11 cases (2.6%), GCF-PLAP samples were unsatisfactory for analysis and were excluded from the study; therefore, 412 cases (89.6%) were available for analysis.

The baseline characteristics of the study population are summarized in Table 1. Of the 423 pregnant women recruited and followed throughout pregnancy, 18 of them subsequently developed PE (4.3%), and five (1.2%) required delivery before 37 weeks of gestation. Maternal age, maternal weight and height, body mass index, and smoking status did not differ significantly between patients with PE and controls. Systolic blood pressure (p<0.001), diastolic blood pressure (p=0.007), and median arterial blood pressure (p=0.006), measured during early pregnancy, were significantly higher in women who developed PE when compared to controls. No statistically significant differences in periodontal parameters were identified between patients who developed PE and controls, and no statistically significant association was identified between periodontal clinical diagnosis and the subsequent development of PE (p=0.617) (Table 2). The median maternal GCF-PLAP concentration was 63.7 (interquartile range [IQR]: 88.9) pg/ml in healthy patients, 46.6 (IQR: 47.2) pg/ml in patients with gingivitis, and 42.4 (IQR: 46.7), 41.1 (IQR: 51.8), 34.7 (IQR: 46.88) pg/ml at periodontitis stage I, stage II – III and stage IV, respectively, without statistically significant differences among them (p=0.407).

GCF-PLAP concentrations at 11-14 weeks were compared between patients who subsequently developed PE and controls. The median maternal GCF-PLAP concentration was significantly higher in the PE group than that of the controls (77.5 pg/ml (IQR: 41.5) vs. 41.3 pg/ml (IQR: 50.1), p=0.015) (Figure 2A). In addition,

PLAP concentrations were also measured in paired plasma and GCF samples from 80 women from the same cohort. The median plasma PLAP concentrations were 24.2 pg/ml (IQR: 2.5) and 24.6 pg/ml (IQR: 7.6) in the control and PE groups, respectively. In the paired GCF samples, median PLAP concentrations were 66.1 pg/ml (IQR: 4.3) and 99 pg/ml (IQR: 17.8) in the control and PE groups, respectively (p=0.011) (Figure 2B). There was no correlation between plasma and GCF-PLAP concentrations. The observed amount of PLAP in GCF was 3- to 6-fold higher than in plasma samples.

Multiple logistic regression analysis identified an association between first-trimester systolic blood pressure (OR: 1.07; 95% CI 1.00-1.015; p=0.004) and GCF-PLAP concentrations (OR: 1.008, 95% CI 1.000-1.015; p=0.034) (Table 3) in women who subsequently developed PE. The results of the bootstrap analysis were similar to those observed in the logistic regression model (Table 3). The GCF-PLAP concentration combined with systolic blood pressure at 11-14 weeks of gestation was found to be a good predictor of PE, with a specificity of 72%, a sensitivity of 83%, a PPV of 12%, and an NPV of 99%. The positive likelihood ratio was 2.9, and the negative likelihood ratio was 0.3. The model correctly classified 72% of the women who developed PE. The AUC for GCF-PLAP concentrations alone at 11-14 weeks of gestation was 0.67; for systolic blood pressure, 0.74, and for GCF-PLAP concentrations and systolic blood pressure, 0.77 (95% CI: 0.70 – 0.85) (Figure 2C). In the sub-analysis, dividing the PE pregnancies into preterm PE ([?] 37 weeks) and term PE (> 37 weeks), the observed AUC was 0.85 (95% CI: 0.81 – 0.93) for preterm PE and 0.72 (95% CI: 0.58 - 0.82) for term PE (Figure 2D and 2D, respectively). All five cases (100%) of preterm PE observed in the current study were correctly classified by the model.

4. Discussion

Main Findings

The data obtained in the present study support the hypothesis that GCF-PLAP concentrations are significantly increased in asymptomatic women who develop PE later in pregnancy. Specifically, GCF-PLAP concentrations, measured at 11-14 weeks of gestation, were predictive of PE when combined with maternal systolic blood pressure in a multivariate predictive model. The performance of the predictive model was satisfactory, with an observed AUC of 0.77 (95% CI: 0.70 – 0.85) for all PE cases, 0.85 (95% CI: 0.81 – 0.93) for preterm PE, and 0.72 (95% CI: 0.58 - 0.82) for term PE. Bootstrap analysis confirmed the stability of our results.

Strengths and limitations

To our knowledge, this is the first cohort study evaluating the predictive potential of GCF-PLAP concentrations for early-pregnancy risk assessment of PE. These data are consistent with and extend our previous observations[99]. PLAP concentrations in GCF were significantly greater than those measured in paired plasma samples. In women who subsequently developed PE, GCF-PLAP concentrations were 3- to 6-fold greater than those measured in matched plasma samples. These findings suggest that potential biomarkers of obstetrical diseases can be concentrated in GCF, highlighting the opportunity to use placental biomarkers measured in GCF to improve the performance of prediction models.

Currently, there is considerable debate about the use multivariate algorithms for the prediction of PE, and most obstetric societies do not recommend their use as an screening strategy in routine obstetrical care[78,88–94], arguing the lack of solid evidence of external validation and/or randomization of many of such existing models[84,128,129]. In fact, many societies only recommend the use of maternal risk factors to identify those women who may be at increased risk of PE. Nevertheless, this screening approach based on identification of clinical risk factors has several limitations, especially considering their limited detection capabilities. When only clinical assessment of risk factors is used to identify patients at risk of PE, a minor proportion of PE cases are detected (approximately 30 - 40% of preterm PE and 20 to 35% of late PE cases)[130–133], with very high rates of false-positive screening results. In contrast, uterine artery pulsatility index with or without the combination of several maternal risk factors, measured at 11 to 14 weeks of, increases the detection rates of early PE (<34 weeks of gestation) to about 40% to 65% at a fixed false

positive rate of 5%, and with an overall sensitivity for early PE of nearly 50%[134–152]. The most recently developed predictive algorithms, using different combinations of maternal risk factors, biophysical variables like mean maternal blood pressure, uterine artery pulsatility index, and maternal plasmatic biomarkers like placental growth factor and/or pregnancy-associated plasma protein A at 11-14 weeks of gestation, have consistently demonstrated detection rates of preterm PE over nearly 70% at false-positive rates of 10%[153–167]. The results obtained in this study further support the use of multiparametric algorithms for improving the prediction of PE. Future studies are needed to confirm our results and to address the predictive capabilities of CGF-PLAP concentration alone and/or in combination with more variables and risk factors, such as the uterine artery Doppler value, to further increase the performance of the algorithm.

Regarding the link between periodontal disease and the risk of PE, our study did not confirm the association between periodontal diagnoses and PE that had been previously described in the literature[108–112]. These results, however, should be interpreted with caution given that the present study was powered to determine the association between GCF-PLAP concentrations and PE but not the association between PE and periodontal diagnosis. Moreover, this link has not been confirmed in all populations[120,121]. In fact, in a cohort study conducted in 1,562 pregnant women from Argentina[122], no significant association between periodontal disease and PE was identified. In addition, in our study, periodontal disease was assessed during early pregnancy. It is known that periodontal disease usually worsens during pregnancy[123–125] and that its evaluation at a later stage of pregnancy may be more related to the development of PE.

Interpretation

PLAP, a membrane-bound glycosylated enzyme, is highly expressed in the syncytiotrophoblastic membranes during gestation[104,107]. PLAP concentrations in maternal plasma are significantly increased in pregnant women with hypertensive disorders than in those with a normotensive pregnancy[101,126,127]. Increased PLAP concentrations associated with PE may be a result of placental dysfunction, and they may also represent an informative biomarker of the syncytiotrophoblast function[66,126]. During pregnancy, syncytiotrophoblastic debris is normally shed into the maternal circulation; however, shedding is significantly increased in pregnancies complicated by PE[75,105]. Furthermore, replenishment of the syncytiotrophoblast is intense, complicated by necrosis and aponecrosis with increased liberation into the circulation of syncytiotrophoblast-derived particles in PE[66]. In light with our result, syncytiotrophoblast-derived particles, such as PLAP, can reach the gingival sulcus during early pregnancy, and can be detected in the GCF. Its concentrations are informative of the risk of developing PE and may be potentially used in future multiparametric algorithm for the prediction of the disease.

In recent years, systematic reviews and meta-analyses of randomized clinical trials have suggested that low-dose aspirin administration, particularly in the early stage of pregnancy (<16 weeks of gestation), to women at high risk for PE is associated with a significant risk reduction in the incidence of this disease[79,168–173]. Furthermore, the ASPRE randomized clinical trial[80], inclusive of more than 1,600 participants, reported that administration of aspirin (150 mg /day) between 11 and 14 weeks of gestation in patients screened for PE risk significantly reduced the risk of preterm PE by 62% compared with placebo [OR: 0.38 (95% CI: 0.20–0.74)]. This finding has been recently confirmed by a systematic review and meta-analysis[81] of six randomized clinical trials[80,174–177]. This evidence highlights the importance of implementing effective predictive models to identify patients at risk of developing PE for whom aspirin administration would be of clinical benefit. The predictive model reported herein is based on the measurement of a single biomarker in GCF and systolic blood pressure; therefore, it is non-invasive and inexpensive. Moreover, it displays a potential clinical utility in identifying women during early pregnancy who are at increased risk for developing PE, especially preterm PE, and, as such, warrants further clinical evaluation.

5. Conclusions

In summary, the development of more innovative diagnostic tests that allow the early identification of women at risk for developing PE is a recognized clinical need. The data obtained in this study are consistent with the hypothesis that the accumulation of placental molecules within GCF during early pregnancy are informative

of the risk of developing PE and could be surrogate markers of placental function. The practical translation of these data to the clinical setting requires further validation to determine its clinical implications.

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References

1. Romero R, Lockwood C, Oyarzun E, Hobbins JC. Toxemia: new concepts in an old disease. *Semin Perinatol* . 1988;12(4):302-323.

2. MacKay AP, Berg CJ, Atrash HK. Pregnancy-related mortality from preeclampsia and eclampsia. *Obstet Gynecol* . 2001;97(4):533-538. doi:10.1016/s0029-7844(00)01223-0

3. von Dadelszen P, Menzies J, Magee LA. The complications of hypertension in pregnancy. *Minerva Med* . 2005;96(4):287-302.

4. Sibai BM. Hypertensive disorders of pregnancy: the United States perspective. *Curr Opin Obstet Gynecol* . 2008;20(2):102-106. doi:10.1097/GCO.0b013e3282f73380

5. Berg CJ, Mackay AP, Qin C, Callaghan WM. Overview of maternal morbidity during hospitalization for labor and delivery in the United States: 1993-1997 and 2001-2005. *Obstet Gynecol* . 2009;113(5):1075-1081. doi:10.1097/AOG.0b013e3181a09fc0

6. Hutcheon JA, Lisonkova S, Joseph KS. Epidemiology of pre-eclampsia and the other hypertensive disorders of pregnancy. *Best Pract Res Clin Obstet Gynaecol* . 2011;25(4):391-403. doi:10.1016/j.bpobgyn.2011.01.006

7. Ghulmiyyah L, Sibai B. Maternal mortality from preeclampsia/eclampsia. *Semin Perinatol* . 2012;36(1):56-59. doi:10.1053/j.semperi.2011.09.011

8. Lisonkova S, Sabr Y, Mayer C, Young C, Skoll A, Joseph KS. Maternal morbidity associated with early-onset and late-onset preeclampsia. *Obstet Gynecol* . 2014;124(4):771-781. doi:10.1097/AOG.0000000000000472

9. Say L, Chou D, Gemmill A, et al. Global causes of maternal death: a WHO systematic analysis. *Lancet Glob Health* . 2014;2(6):e323-333. doi:10.1016/S2214-109X(14)70227-X

10. Tooher J, Thornton C, Makris A, et al. Hypertension in pregnancy and long-term cardiovascular mortality: a retrospective cohort study. *Am J Obstet Gynecol* . 2016;214(6):722.e1-6. doi:10.1016/j.ajog.2015.12.047

11. Madazli R, Yuksel MA, Imamoglu M, et al. Comparison of clinical and perinatal outcomes in early- and late-onset preeclampsia. *Arch Gynecol Obstet* . 2014;290(1):53-57. doi:10.1007/s00404-014-3176-x

12. Sharma KJ, Esakoff TF, Guillet A, Burwick RM, Caughey AB. Pregnancies complicated by both preeclampsia and growth restriction between 34 and 37 weeks' gestation are associated with adverse perinatal outcomes. *J Matern Fetal Neonatal Med* . 2017;30(19):2342-2345. doi:10.1080/14767058.2016.1248394

13. van Esch JJA, van Heijst AF, de Haan AFJ, van der Heijden OWH. Early-onset preeclampsia is associated with perinatal mortality and severe neonatal morbidity. *J Matern Fetal Neonatal Med* . 2017;30(23):2789-2794. doi:10.1080/14767058.2016.1263295

14. Marins LR, Anizelli LB, Romanowski MD, Sarquis AL. How does preeclampsia affect neonates? Highlights in the disease's immunity.*J Matern Fetal Neonatal Med* . 2019;32(7):1205-1212. doi:10.1080/14767058.2017.1401996

15. Di Renzo GC. The great obstetrical syndromes. *J Matern Fetal Neonatal Med* . 2009;22(8):633-635. doi:10.1080/14767050902866804

16. Erez O, Gotsch F, Mazaki-Tovi S, et al. Evidence of maternal platelet activation, excessive thrombin generation, and high amniotic fluid tissue factor immunoreactivity and functional activity in patients with fetal death. *J Matern Fetal Neonatal Med* . 2009;22(8):672-687. doi:10.1080/14767050902853117

17. Dekker GA, Sibai BM. Etiology and pathogenesis of preeclampsia: current concepts. *Am J Obstet Gynecol* . 1998;179(5):1359-1375. doi:10.1016/s0002-9378(98)70160-7

18. von Dadelszen P, Magee LA, Roberts JM. Subclassification of preeclampsia. *Hypertens Pregnancy* . 2003;22(2):143-148. doi:10.1081/PRG-120021060

19. Vatten LJ, Skjaerven R. Is pre-eclampsia more than one disease?*BJOG* . 2004;111(4):298-302. doi:10.1111/j.1471-0528.2004.00071.x

20. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science* . 2005;308(5728):1592-1594. doi:10.1126/science.1111726

21. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet* . 2005;365(9461):785-799. doi:10.1016/S0140-6736(05)17987-2

22. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia part 1: current understanding of its pathophysiology. *Nat Rev Nephrol* . 2014;10(8):466-480. doi:10.1038/nrneph.2014.102

23. Burton GJ, Redman CW, Roberts JM, Moffett A. Pre-eclampsia: pathophysiology and clinical implications. *BMJ* . 2019;366:l2381-l2381. doi:10.1136/bmj.l2381

24. Torry DS, Wang HS, Wang TH, Caudle MR, Torry RJ. Preeclampsia is associated with reduced serum levels of placenta growth factor. *Am J Obstet Gynecol* . 1998;179(6 Pt 1):1539-1544. doi:10.1016/s0002-9378(98)70021-3

25. Reuvekamp A, Velsing-Aarts FV, Poulina IE, Capello JJ, Duits AJ. Selective deficit of angiogenic growth factors characterises pregnancies complicated by pre-eclampsia. *Br J Obstet Gynaecol* . 1999;106(10):1019-1022. doi:10.1111/j.1471-0528.1999.tb08107.x

26. Tidwell SC, Ho HN, Chiu WH, Torry RJ, Torry DS. Low maternal serum levels of placenta growth factor as an antecedent of clinical preeclampsia. *Am J Obstet Gynecol* . 2001;184(6):1267-1272. doi:10.1067/mob.2001.113129

27. Chaiworapongsa T, Romero R, Espinoza J, et al. Evidence supporting a role for blockade of the vascular endothelial growth factor system in the pathophysiology of preeclampsia. Young Investigator Award. *Am J Obstet Gynecol* . 2004;190(6):1541-1547; discussion 1547-1550. doi:10.1016/j.ajog.2004.03.043

28. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* . 2004;350(7):672-683. doi:10.1056/NEJMoa031884

29. Bujold E, Romero R, Chaiworapongsa T, et al. Evidence supporting that the excess of the sVEGFR-1 concentration in maternal plasma in preeclampsia has a uterine origin. *J Matern Fetal Neonatal Med* . 2005;18(1):9-16. doi:10.1080/14767050500202493

30. Levine RJ, Thadhani R, Qian C, et al. Urinary placental growth factor and risk of preeclampsia. *JAMA* . 2005;293(1):77-85. doi:10.1001/jama.293.1.77

31. Maynard SE, Venkatesha S, Thadhani R, Karumanchi SA. Soluble Fms-like tyrosine kinase 1 and endothelial dysfunction in the pathogenesis of preeclampsia. *Pediatr Res* . 2005;57(5 Pt 2):1R-7R. doi:10.1203/01.PDR.00001595

32. Crispi F, Dominguez C, Llurba E, Martin-Gallan P, Cabero L, Gratacos E. Placental angiogenic growth factors and uterine artery Doppler findings for characterization of different subsets in preeclampsia and in isolated intrauterine growth restriction. *Am J Obstet Gynecol* . 2006;195(1):201-207. doi:10.1016/j.ajog.2006.01.014

33. Levine RJ, Lam C, Qian C, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med* . 2006;355(10):992-1005. doi:10.1056/NEJMoa055352

34. Lindheimer MD, Romero R. Emerging roles of antiangiogenic and angiogenic proteins in pathogenesis and prediction of preeclampsia.*Hypertension* . 2007;50(1):35-36. doi:10.1161/HYPERTENSIONAHA.107.089045

35. Widmer M, Villar J, Benigni A, Conde-Agudelo A, Karumanchi SA, Lindheimer M. Mapping the theories of preeclampsia and the role of angiogenic factors: a systematic review. *Obstet Gynecol* . 2007;109(1):168-180. doi:10.1097/01.AOG.0000249609.04831.7c

36. Masuyama H, Segawa T, Sumida Y, et al. Different profiles of circulating angiogenic factors and adipocytokines between early- and late-onset pre-eclampsia. *BJOG* . 2010;117(3):314-320. doi:10.1111/j.1471-0528.2009.02453.x

37. Baltajian K, Bajracharya S, Salahuddin S, et al. Sequential plasma angiogenic factors levels in women with suspected preeclampsia. *Am J Obstet Gynecol* . 2016;215(1):89.e1-89.e10. doi:10.1016/j.ajog.2016.01.168

38. Holme AM, Roland MCP, Henriksen T, Michelsen TM. In vivo uteroplacental release of placental growth factor and soluble Fms-like tyrosine kinase-1 in normal and preeclamptic pregnancies. *Am J Obstet Gynecol* . 2016;215(6):782.e1-782.e9. doi:10.1016/j.ajog.2016.07.056

39. Cerdeira AS, Agrawal S, Staff AC, Redman CW, Vatish M. Angiogenic factors: potential to change clinical practice in pre-eclampsia?*BJOG* . 2018;125(11):1389-1395. doi:10.1111/1471-0528.15042

40. Karumanchi SA. Angiogenic factors in pre-eclampsia: implications for clinical practice. *BJOG* . 2018;125(11):1396. doi:10.1111/1471-0528.15180

41. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. *Am J Obstet Gynecol* . 1999;180(2 Pt 1):499-506. doi:10.1016/s0002-9378(99)70239-5

42. Djurovic S, Clausen T, Wergeland R, Brosstad F, Berg K, Henriksen T. Absence of enhanced systemic inflammatory response at 18 weeks of gestation in women with subsequent pre-eclampsia. *BJOG* . 2002;109(7):759-764. doi:10.1111/j.1471-0528.2002.01330.x

43. Redman CWG, Sargent IL. Immunology of pre-eclampsia. *Am J Reprod Immunol* . 2010;63(6):534-543. doi:10.1111/j.1600-0897.2010.00831.x

44. Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, McLaughlin MK. Preeclampsia: an endothelial cell disorder. *Am J Obstet Gynecol* . 1989;161(5):1200-1204. doi:10.1016/0002-9378(89)90665-0

45. Dekker GA, van Geijn HP. Endothelial dysfunction in preeclampsia. Part I: Primary prevention. Therapeutic perspectives. *J Perinat Med* . 1996;24(2):99-117. doi:10.1515/jpme.1996.24.2.99

46. Wolf M, Sandler L, Munoz K, Hsu K, Ecker JL, Thadhani R. First trimester insulin resistance and subsequent preeclampsia: a prospective study. *J Clin Endocrinol Metab* . 2002;87(4):1563-1568. doi:10.1210/jcem.87.4.8405

47. Berkowitz KM. Insulin resistance and preeclampsia. *Clin Perinatol* . 1998;25(4):873-885.

48. Parretti E, Lapolla A, Dalfra M, et al. Preeclampsia in lean normotensive normotolerant pregnant women can be predicted by simple insulin sensitivity indexes. *Hypertension* . 2006;47(3):449-453. doi:10.1161/01.HYP.0000205122.47333.7f

49. Mastrogiannis DS, Spiliopoulos M, Mulla W, Homko CJ. Insulin resistance: the possible link between gestational diabetes mellitus and hypertensive disorders of pregnancy. *Curr Diab Rep* . 2009;9(4):296-302. doi:10.1007/s11892-009-0046-1

50. Scioscia M, Gumaa K, Rademacher TW. The link between insulin resistance and preeclampsia: new perspectives. *J Reprod Immunol* . 2009;82(2):100-105. doi:10.1016/j.jri.2009.04.009

51. Anim-Nyame N, Gamble J, Sooranna SR, Johnson MR, Steer PJ. Relationship between insulin resistance and tissue blood flow in preeclampsia. *J Hypertens* . 2015;33(5):1057-1063. doi:10.1097/HJH.0000000000000494

52. Robertson WB, Brosens I, Dixon G. Maternal uterine vascular lesions in the hypertensive complications of pregnancy. *Perspect Nephrol Hypertens* . 1976;5:115-127.

53. Salafia CM, Pezzullo JC, Lopez-Zeno JA, Simmens S, Minior VK, Vintzileos AM. Placental pathologic features of preterm preeclampsia.*Am J Obstet Gynecol* . 1995;173(4):1097-1105. doi:10.1016/0002-9378(95)91333-5

54. Egbor M, Ansari T, Morris N, Green CJ, Sibbons PD. Morphometric placental villous and vascular abnormalities in early- and late-onset pre-eclampsia with and without fetal growth restriction. *BJOG* . 2006;113(5):580-589. doi:10.1111/j.1471-0528.2006.00882.x

55. Silasi M, Cohen B, Karumanchi SA, Rana S. Abnormal placentation, angiogenic factors, and the pathogenesis of preeclampsia. *Obstet Gynecol Clin North Am* . 2010;37(2):239-253. doi:10.1016/j.ogc.2010.02.013

56. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. *Am J Obstet Gynecol* . 2011;204(3):193-201. doi:10.1016/j.ajog.2010.08.009

57. Khong Y, Brosens I. Defective deep placentation. *Best Pract Res Clin Obstet Gynaecol* . 2011;25(3):301-311. doi:10.1016/j.bpobgyn.2010.10.012

58. Brosens I, Puttemans P, Benagiano G. Placental bed research: I. The placental bed: from spiral arteries remodeling to the great obstetrical syndromes. *Am J Obstet Gynecol* . 2019;221(5):437-456. doi:10.1016/j.ajog.2019.05.044

59. Granger JP, Alexander BT, Llinas MT, Bennett WA, Khalil RA. Pathophysiology of preeclampsia: linking placental ischemia/hypoxia with microvascular dysfunction. *Microcirculation* . 2002;9(3):147-160. doi:10.1038/sj.mn.7800137

60. Gilbert JS, Gilbert SAB, Arany M, Granger JP. Hypertension produced by placental ischemia in pregnant rats is associated with increased soluble endoglin expression. *Hypertension* . 2009;53(2):399-403. doi:10.1161/HYPERTENSIONAHA.108.123513

61. Makris A, Yeung KR, Lim SM, et al. Placental Growth Factor Reduces Blood Pressure in a Uteroplacental Ischemia Model of Preeclampsia in Nonhuman Primates. *Hypertension* . 2016;67(6):1263-1272. doi:10.1161/HYPERTENSIONAHA.116.07286

62. Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of pre-eclampsia. *J Pathol* . 1970;101(4). https://pubmed.ncbi.nlm.nih.gov/5504740.

63. Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of preeclampsia. *Obstet Gynecol Annu* . 1972;1:177-191.

64. Pijnenborg R, Anthony J, Davey DA, et al. Placental bed spiral arteries in the hypertensive disorders of pregnancy. *Br J Obstet Gynaecol* . 1991;98(7):648-655. doi:10.1111/j.1471-0528.1991.tb13450.x

65. Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR, van Asshe A. A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. *Br J Obstet Gynaecol* . 1994;101(8):669-674. doi:10.1111/j.1471-0528.1994.tb13182.x

66. Hutchinson ES, Brownbill P, Jones NW, et al. Utero-placental haemodynamics in the pathogenesis of pre-eclampsia. *Placenta* . 2009;30(7):634-641. doi:10.1016/j.placenta.2009.04.011

67. Steegers EAP, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet* . 2010;376(9741):631-644. doi:10.1016/S0140-6736(10)60279-6

68. Powe CE, Levine RJ, Karumanchi SA. Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease. *Circulation* . 2011;123(24):2856-2869. doi:10.1161/CIRCULATIONAHA.109.853127

69. Pijnenborg R, Vercruysse L, Hanssens M, Brosens I. Endovascular trophoblast and preeclampsia: A reassessment. *Pregnancy Hypertens* . 2011;1(1):66-71. doi:10.1016/j.preghy.2010.10.010

70. Germain SJ, Sacks GP, Sooranna SR, Sargent IL, Redman CW. Systemic inflammatory priming in normal pregnancy and preeclampsia: the role of circulating syncytiotrophoblast microparticles. *J Immunol* . 2007;178(9):5949-5956. doi:10.4049/jimmunol.178.9.5949

71. Redman CWG, Sargent IL. Circulating microparticles in normal pregnancy and pre-eclampsia. *Placenta* . 2008;29 Suppl A:S73-S77. doi:10.1016/j.placenta.2007.11.016

72. van der Post JAM, Lok CAR, Boer K, Sturk A, Sargent IL, Nieuwland R. The functions of microparticles in pre-eclampsia. *Semin Thromb Hemost* . 2011;37(2):146-152. doi:10.1055/s-0030-1270342

73. Lok CAR, Van der Post JAM, Sturk A, Sargent IL, Nieuwland R. The functions of microparticles in preeclampsia. *Pregnancy Hypertens* . 2011;1(1):59-65. doi:10.1016/j.preghy.2010.10.006

74. Fisher SJ. Why is placentation abnormal in preeclampsia? *Am J Obstet Gynecol* . 2015;213(4 Suppl):S115-122. doi:10.1016/j.ajog.2015.08.042

75. Salomon C, Rice GE. Role of Exosomes in Placental Homeostasis and Pregnancy Disorders. *Prog Mol Biol Transl Sci* . 2017;145:163-179. doi:10.1016/bs.pmbts.2016.12.006

76. Mol BWJ, Roberts CT, Thangaratinam S, Magee LA, de Groot CJM, Hofmeyr GJ. Pre-eclampsia. *Lancet* . 2016;387(10022):999-1011. doi:10.1016/S0140-6736(15)00070-7

77. Poon LC, Nicolaides KH. First-trimester maternal factors and biomarker screening for preeclampsia. *Prenat Diagn* . 2014;34(7):618-627. doi:10.1002/pd.4397

78. Committee Opinion No. 638: First-Trimester Risk Assessment for Early-Onset Preeclampsia. *Obstet Gynecol* . 2015;126(3):e25-27. doi:10.1097/AOG.0000000000001049

79. Roberge S, Nicolaides KH, Demers S, Villa P, Bujold E. Prevention of perinatal death and adverse perinatal outcome using low-dose aspirin: a meta-analysis. *Ultrasound Obstet Gynecol* . 2013;41(5):491-499. doi:10.1002/uog.12421

80. Rolnik DL, Wright D, Poon LC, et al. Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. *N Engl J Med* . 2017;377(7):613-622. doi:10.1056/NEJMoa1704559

81. Roberge S, Bujold E, Nicolaides KH. Aspirin for the prevention of preterm and term preeclampsia: systematic review and metaanalysis.*Am J Obstet Gynecol* . 2018;218(3):287-293.e1. doi:10.1016/j.ajog.2017.11.561

82. Duley L, Meher S, Hunter KE, Seidler AL, Askie LM. Antiplatelet agents for preventing pre-eclampsia and its complications.*Cochrane Database Syst Rev* . 2019;2019(10). doi:10.1002/14651858.CD004659.pub3

83. Pedrosa AC, Matias A. Screening for pre-eclampsia: a systematic review of tests combining uterine artery Doppler with other markers.*J Perinat Med* . 2011;39(6):619-635. doi:10.1515/JPM.2011.077

84. Brunelli VB, Prefumo F. Quality of first trimester risk prediction models for pre-eclampsia: a systematic review. *BJOG* . 2015;122(7):904-914. doi:10.1111/1471-0528.13334

85. Rana S, Karumanchi SA, Lindheimer MD. Angiogenic factors in diagnosis, management, and research in preeclampsia.*Hypertension* . 2014;63(2):198-202. doi:10.1161/HYPERTENSIONAHA.113.02293

86. Widmer M, Cuesta C, Khan KS, et al. Accuracy of angiogenic biomarkers at ?20weeks' gestation in predicting the risk of pre-eclampsia: A WHO multicentre study. *Pregnancy Hypertens* . 2015;5(4):330-338. doi:10.1016/j.preghy.2015.09.004

87. Duckworth S, Griffin M, Seed PT, et al. Diagnostic Biomarkers in Women With Suspected Preeclampsia in a Prospective Multicenter Study.*Obstet Gynecol* . 2016;128(2):245-252. doi:10.1097/AOG.0000000000001508

88. Magee LA, Helewa M, Rey E, HYPERTENSION GUIDELINE COMMITTEE, STRATEGIC TRAINING INITIATIVE IN RESEARCH IN THE REPRODUCTIVE HEALTH SCIENCES (STIRRHS) SCHOLARS. Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy. *J Obstet Gynaecol Can* . 2008;30(3 Suppl):S1-S2. doi:10.1016/S1701-2163(16)32776-1

89. National Collaborating Centre for Women's and Children's Health (UK). *Hypertension in Pregnancy: The Management of Hypertensive Disorders During Pregnancy* . London: RCOG Press; 2010. http://www.ncbi.nlm.nih.gov/b

90. American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol* . 2013;122(5):1122-1131. doi:10.1097/01.AOG.0000437382.03963.88

91. Magee LA, Pels A, Helewa M, Rey E, von Dadelszen P, Canadian Hypertensive Disorders of Pregnancy (HDP) Working Group. Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy.*Pregnancy Hypertens* . 2014;4(2):105-145. doi:10.1016/j.preghy.2014.01.003

92. Lowe SA, Bowyer L, Lust K, et al. The SOMANZ Guidelines for the Management of Hypertensive Disorders of Pregnancy 2014. *Aust N Z J Obstet Gynaecol* . 2015;55(1):11-16. doi:10.1111/ajo.12253

93. US Preventive Services Task Force, Bibbins-Domingo K, Grossman DC, et al. Screening for Preeclampsia: US Preventive Services Task Force Recommendation Statement. *JAMA* . 2017;317(16):1661-1667. doi:10.1001/jama.2017.3439

94. Wertaschnigg D, Reddy M, Mol BWJ, Rolnik DL, da Silva Costa F. Prenatal screening for pre-eclampsia: Frequently asked questions.*Aust N Z J Obstet Gynaecol* . 2019;59(4):477-483. doi:10.1111/ajo.12982

95. Ebersole JL, Nagarajan R, Akers D, Miller CS. Targeted salivary biomarkers for discrimination of periodontal health and disease(s).*Front Cell Infect Microbiol* . 2015;5:62. doi:10.3389/fcimb.2015.00062

96. Barros SP, Williams R, Offenbacher S, Morelli T. Gingival crevicular fluid as a source of biomarkers for periodontitis. *Periodontol 2000* . 2016;70(1):53-64. doi:10.1111/prd.12107

97. Wassall RR, Preshaw PM. Clinical and technical considerations in the analysis of gingival crevicular fluid. *Periodontol 2000* . 2016;70(1):65-79. doi:10.1111/prd.12109

98. Taylor JJ, Preshaw PM. Gingival crevicular fluid and saliva.*Periodontol 2000* . 2016;70(1):7-10. doi:10.1111/prd.12118

99. Chaparro A, Gaedechens D, Ramirez V, et al. Placental biomarkers and angiogenic factors in oral fluids of patients with preeclampsia.*Prenat Diagn* . 2016;36(5):476-482. doi:10.1002/pd.4811

100. Chaparro A, Zuniga E, Varas-Godoy M, et al. Periodontitis and placental growth factor in oral fluids are early pregnancy predictors of gestational diabetes mellitus. *J Periodontol* . 2018;89(9):1052-1060. doi:10.1002/JPER.17-0497

101. Rajagambeeram R, Abu Raghavan S, Ghosh S, Basu S, Ramasamy R, Murugaiyan SB. Diagnostic utility of heat stable alkaline phosphatase in hypertensive disorders of pregnancy. *J Clin Diagn Res* . 2014;8(11):CC10-13. doi:10.7860/JCDR/2014/10895.5084

102. Pillay P, Maharaj N, Moodley J, Mackraj I. Placental exosomes and pre-eclampsia: Maternal circulating levels in normal pregnancies and, early and late onset pre-eclamptic pregnancies. *Placenta* . 2016;46:18-25. doi:10.1016/j.placenta.2016.08.078

103. Moss DW. Alkaline phosphatase isoenzymes. *Clin Chem* . 1982;28(10):2007-2016.

104. Griffiths J, Black J. Separation and identification of alkaline phosphatase isoenzymes and isoforms in serum of healthy persons by isoelectric focusing. *Clin Chem* . 1987;33(12):2171-2177.

105. Adam S, Elfeky O, Kinhal V, et al. Review: Fetal-maternal communication via extracellular vesicles - Implications for complications of pregnancies. *Placenta* . 2017;54:83-88. doi:10.1016/j.placenta.2016.12.001

106. Bashiri A, Katz O, Maor E, Sheiner E, Pack I, Mazor M. Positive placental staining for alkaline phosphatase corresponding with extreme elevation of serum alkaline phosphatase during pregnancy. *Arch Gynecol Obstet* . 2007;275(3):211-214. doi:10.1007/s00404-006-0212-5

107. She QB, Mukherjee JJ, Huang JS, Crilly KS, Kiss Z. Growth factor-like effects of placental alkaline phosphatase in human fetus and mouse embryo fibroblasts. *FEBS Lett* . 2000;469(2-3):163-167. doi:10.1016/s0014-5793(00)01273-4

108. Kunnen A, Blaauw J, van Doormaal JJ, et al. Women with a recent history of early-onset pre-eclampsia have a worse periodontal condition.*J Clin Periodontol* . 2007;34(3):202-207. doi:10.1111/j.1600-051X.2006.01036.x

109. Conde-Agudelo A, Villar J, Lindheimer M. Maternal infection and risk of preeclampsia: systematic review and metaanalysis. *Am J Obstet Gynecol* . 2008;198(1):7-22. doi:10.1016/j.ajog.2007.07.040

110. Siqueira FM, Cota LOM, Costa JE, Haddad JPA, Lana AMQ, Costa FO. Maternal periodontitis as a potential risk variable for preeclampsia: a case-control study. *J Periodontol* . 2008;79(2):207-215. doi:10.1902/jop.2008.070174

111. Huang X, Wang J, Liu J, et al. Maternal periodontal disease and risk of preeclampsia: a meta-analysis. *J Huazhong Univ Sci Technol Med Sci* . 2014;34(5):729-735. doi:10.1007/s11596-014-1343-8

112. Daalderop LA, Wieland BV, Tomsin K, et al. Periodontal Disease and Pregnancy Outcomes: Overview of Systematic Reviews. *JDR Clin Trans Res* . 2018;3(1):10-27. doi:10.1177/2380084417731097

113. Barak S, Oettinger-Barak O, Machtei EE, Sprecher H, Ohel G. Evidence of periopathogenic microorganisms in placentas of women with preeclampsia. *J Periodontol* . 2007;78(4):670-676. doi:10.1902/jop.2007.060362

114. Swati P, Ambika Devi K, Thomas B, Vahab SA, Kapaettu S, Kushtagi P. Simultaneous detection of periodontal pathogens in subgingival plaque and placenta of women with hypertension in pregnancy. *Arch Gynecol Obstet* . 2012;285(3):613-619. doi:10.1007/s00404-011-2012-9

115. Chaparro A, Blanlot C, Ramirez V, et al. Porphyromonas gingivalis, Treponema denticola and toll-like receptor 2 are associated with hypertensive disorders in placental tissue: a case-control study.*J Periodont Res* . 2013;48(6):802-809. doi:10.1111/jre.12074

116. Chapple ILC, Mealey BL, Van Dyke TE, et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol* . 2018;89 Suppl 1:S74-S84. doi:10.1002/JPER.17-0719

117. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J*

Periodontol . 2018;89 Suppl 1:S173-S182. doi:10.1002/JPER.17-0721

118. Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. *J Clin Periodontol* . 2018;45 Suppl 20:S1-S8. doi:10.1111/jcpe.12935

119. Hernandez M, Martinez B, Tejerina JM, Valenzuela MA, Gamonal J. MMP-13 and TIMP-1 determinations in progressive chronic periodontitis.*J Clin Periodontol* . 2007;34(9):729-735. doi:10.1111/j.1600-051X.2007.01107.x

120. Khader YS, Jibreal M, Al-Omiri M, Amarin Z. Lack of association between periodontal parameters and preeclampsia. *J Periodontol* . 2006;77(10):1681-1687. doi:10.1902/jop.2006.050463

121. Armitage GC. Bi-directional relationship between pregnancy and periodontal disease. *Periodontol 2000* . 2013;61(1):160-176. doi:10.1111/j.1600-0757.2011.00396.x

122. Castaldi JL., Berin MS., Gimenez F., Lede R. Periodontal disease: is it a risk factor for premature labor, low birth weight or preeclampsia? *Rev Panam Salud Publica* . 2006;19:253–258.

123. Wu M, Chen S-W, Jiang S-Y. Relationship between gingival inflammation and pregnancy. *Mediators Inflamm* . 2015;2015:623427. doi:10.1155/2015/623427

124. Silva de Araujo Figueiredo C, Goncalves Carvalho Rosalem C, Costa Cantanhede AL, Abreu Fonseca Thomaz EB, Fontoura Nogueira da Cruz MC. Systemic alterations and their oral manifestations in pregnant women.*J Obstet Gynaecol Res* . 2017;43(1):16-22. doi:10.1111/jog.13150

125. Martelli ML, Brandi ML, Martelli M, Nobili P, Medico E, Martelli F. Periodontal disease and women's health. *Curr Med Res Opin* . 2017;33(6):1005-1015. doi:10.1080/03007995.2017.1297928

126. Mangal A., Shrivastava P., Gaur U., Jain A., Goyal U., Rath G. Histochemical Analysis of Placental Alkaline Phosphatase in Hypertensive Disorders complicating Pregnancy. *Journal of the Anatomical Society of India* . 2005;54 (2):2005-2012.

127. Mangal A., Gaur U., Jain A., Goyal U., Tripathi R., Rath R. Alkaline phosphatase and placental alkaline phosphatase activity in serum of normal and pregnancy induced hypertensive mothers.*JIMSA* . 2007;20 (2):117-120.

128. Conde-Agudelo A, Villar J, Lindheimer M. World Health Organization systematic review of screening tests for preeclampsia. *Obstet Gynecol* . 2004;104(6):1367-1391. doi:10.1097/01.AOG.0000147599.47713.5d

129. Townsend R, Khalil A, Premakumar Y, et al. Prediction of pre-eclampsia: review of reviews. *Ultrasound Obstet Gynecol* . 2019;54(1):16-27. doi:10.1002/uog.20117

130. Poon LCY, Kametas NA, Chelemen T, Leal A, Nicolaides KH. Maternal risk factors for hypertensive disorders in pregnancy: a multivariate approach. *J Hum Hypertens* . 2010;24(2):104-110. doi:10.1038/jhh.2009.45

131. O'Gorman N, Wright D, Poon LC, et al. Multicenter screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation: comparison with NICE guidelines and ACOG recommendations.*Ultrasound Obstet Gynecol* . 2017;49(6):756-760. doi:10.1002/uog.17455

132. Tan MY, Wright D, Syngelaki A, et al. Comparison of diagnostic accuracy of early screening for pre-eclampsia by NICE guidelines and a method combining maternal factors and biomarkers: results of SPREE.*Ultrasound Obstet Gynecol* . 2018;51(6):743-750. doi:10.1002/uog.19039

133. Poon LC, Rolnik DL, Tan MY, et al. ASPRE trial: incidence of preterm pre-eclampsia in patients fulfilling ACOG and NICE criteria according to risk by FMF algorithm. *Ultrasound Obstet Gynecol* . 2018;51(6):738-742. doi:10.1002/uog.19019

134. Martin AM, Bindra R, Curcio P, Cicero S, Nicolaides KH. Screening for pre-eclampsia and fetal growth restriction by uterine artery Doppler at 11-14 weeks of gestation. *Ultrasound Obstet Gynecol* . 2001;18(6):583-586. doi:10.1046/j.0960-7692.2001.00594.x

135. Schuchter K, Metzenbauer M, Hafner E, Philipp K. Uterine artery Doppler and placental volume in the first trimester in the prediction of pregnancy complications. *Ultrasound Obstet Gynecol* . 2001;18(6):590-592. doi:10.1046/j.0960-7692.2001.00596.x

136. Carbillon L, Uzan M, Largilliere C, et al. Prospective evaluation of uterine artery flow velocity waveforms at 12-14 and 22-24 weeks of gestation in relation to pregnancy outcome and birth weight. *Fetal Diagn Ther* . 2004;19(4):381-384. doi:10.1159/000077971

137. Prefumo F, Sebire NJ, Thilaganathan B. Decreased endovascular trophoblast invasion in first trimester pregnancies with high-resistance uterine artery Doppler indices. *Hum Reprod* . 2004;19(1):206-209. doi:10.1093/humrep/deh03

138. Gomez O, Martinez JM, Figueras F, et al. Uterine artery Doppler at 11-14 weeks of gestation to screen for hypertensive disorders and associated complications in an unselected population. *Ultrasound Obstet Gynecol* . 2005;26(5):490-494. doi:10.1002/uog.1976

139. Parra M, Rodrigo R, Barja P, et al. Screening test for preeclampsia through assessment of uteroplacental blood flow and biochemical markers of oxidative stress and endothelial dysfunction. *Am J Obstet Gynecol* . 2005;193(4):1486-1491. doi:10.1016/j.ajog.2005.02.109

140. Vainio M, Kujansuu E, Koivisto A-M, Maenpaa J. Bilateral notching of uterine arteries at 12–14 weeks of gestation for prediction of hypertensive disorders of pregnancy. *Acta Obstet Gynecol Scand* . 2005;84(11):1062-1067. doi:10.1111/j.0001-6349.2005.00889.x

141. Gomez O, Figueras F, Martinez JM, et al. Sequential changes in uterine artery blood flow pattern between the first and second trimesters of gestation in relation to pregnancy outcome. *Ultrasound Obstet Gynecol* . 2006;28(6):802-808. doi:10.1002/uog.2814

142. Pilalis A, Souka AP, Antsaklis P, et al. Screening for pre-eclampsia and small for gestational age fetuses at the 11-14 weeks scan by uterine artery Dopplers. *Acta Obstet Gynecol Scand* . 2007;86(5):530-534. doi:10.1080/00016340601155056

143. Plasencia W, Maiz N, Bonino S, Kaihura C, Nicolaides KH. Uterine artery Doppler at 11 + 0 to 13 + 6 weeks in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* . 2007;30(5):742-749. doi:10.1002/uog.5157

144. Fratelli N, Rampello S, Guala M, Platto C, Frusca T. Transabdominal uterine artery Doppler between 11 and 14 weeks of gestation for the prediction of outcome in high-risk pregnancies. *J Matern Fetal Neonatal Med* . 2008;21(6):403-406. doi:10.1080/14767050802053073

145. Onwudiwe N, Yu CKH, Poon LCY, Spiliopoulos I, Nicolaides KH. Prediction of pre-eclampsia by a combination of maternal history, uterine artery Doppler and mean arterial pressure. *Ultrasound Obstet Gynecol* . 2008;32(7):877-883. doi:10.1002/uog.6124

146. Melchiorre K, Wormald B, Leslie K, Bhide A, Thilaganathan B. First-trimester uterine artery Doppler indices in term and preterm pre-eclampsia. *Ultrasound Obstet Gynecol* . 2008;32(2):133-137. doi:10.1002/uog.5400

147. Plasencia W, Maiz N, Poon L, Yu C, Nicolaides KH. Uterine artery Doppler at 11 + 0 to 13 + 6 weeks and 21 + 0 to 24 + 6 weeks in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* . 2008;32(2):138-146. doi:10.1002/uog.5402

148. Rizzo G, Capponi A, Cavicchioni O, Vendola M, Arduini D. First trimester uterine Doppler and three-dimensional ultrasound placental volume calculation in predicting pre-eclampsia. *Eur J Obstet Gynecol Reprod Biol* . 2008;138(2):147-151. doi:10.1016/j.ejogrb.2007.08.015

149. Poon LCY, Staboulidou I, Maiz N, Plasencia W, Nicolaides KH. Hypertensive disorders in pregnancy: screening by uterine artery Doppler at 11-13 weeks. *Ultrasound Obstet Gynecol* . 2009;34(2):142-148. doi:10.1002/uog.6452

150. Akolekar R, Syngelaki A, Sarquis R, Zvanca M, Nicolaides KH. Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks.*Prenat Diagn* . 2011;31(1):66-74. doi:10.1002/pd.2660

151. Caradeux J, Serra R, Nien J-K, et al. First trimester prediction of early onset preeclampsia using demographic, clinical, and sonographic data: a cohort study. *Prenat Diagn* . 2013;33(8):732-736. doi:10.1002/pd.4113

152. Velauthar L, Plana MN, Kalidindi M, et al. First-trimester uterine artery Doppler and adverse pregnancy outcome: a meta-analysis involving 55,974 women. *Ultrasound Obstet Gynecol* . 2014;43(5):500-507. doi:10.1002/uog.13275

153. Poon LCY, Kametas NA, Maiz N, Akolekar R, Nicolaides KH. First-trimester prediction of hypertensive disorders in pregnancy.*Hypertension* . 2009;53(5):812-818. doi:10.1161/HYPERTENSIONAHA.108.127977

154. Poon LCY, Stratieva V, Piras S, Piri S, Nicolaides KH. Hypertensive disorders in pregnancy: combined screening by uterine artery Doppler, blood pressure and serum PAPP-A at 11-13 weeks. *Prenat Diagn* . 2010;30(3):216-223. doi:10.1002/pd.2440

155. Seed PT, Chappell LC, Black MA, et al. Prediction of preeclampsia and delivery of small for gestational age babies based on a combination of clinical risk factors in high-risk women. *Hypertens Pregnancy* . 2011;30(1):58-73. doi:10.3109/10641955.2010.486460

156. Wright D, Akolekar R, Syngelaki A, Poon LCY, Nicolaides KH. A competing risks model in early screening for preeclampsia. *Fetal Diagn Ther* . 2012;32(3):171-178. doi:10.1159/000338470

157. Akolekar R, Syngelaki A, Poon L, Wright D, Nicolaides KH. Competing risks model in early screening for preeclampsia by biophysical and biochemical markers. *Fetal Diagn Ther* . 2013;33(1):8-15. doi:10.1159/000341264

158. Parra-Cordero M, Rodrigo R, Barja P, et al. Prediction of early and late pre-eclampsia from maternal characteristics, uterine artery Doppler and markers of vasculogenesis during first trimester of pregnancy.*Ultrasound Obstet Gynecol* . 2013;41(5):538-544. doi:10.1002/uog.12264

159. Scazzocchio E, Figueras F, Crispi F, et al. Performance of a first-trimester screening of preeclampsia in a routine care low-risk setting. *Am J Obstet Gynecol* . 2013;208(3):203.e1-203.e10. doi:10.1016/j.ajog.2012.12.016

160. Crovetto F, Figueras F, Triunfo S, et al. Added value of angiogenic factors for the prediction of early and late preeclampsia in the first trimester of pregnancy. *Fetal Diagn Ther* . 2014;35(4):258-266. doi:10.1159/000358302

161. Kenny LC, Black MA, Poston L, et al. Early pregnancy prediction of preeclampsia in nulliparous women, combining clinical risk and biomarkers: the Screening for Pregnancy Endpoints (SCOPE) international cohort study. *Hypertension* . 2014;64(3):644-652. doi:10.1161/HYPERTENSIONAHA.114.03578

162. Wright D, Syngelaki A, Akolekar R, Poon LC, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal characteristics and medical history. *Am J Obstet Gynecol* . 2015;213(1):62.e1-62.e10. doi:10.1016/j.ajog.2015.02.018

163. O'Gorman N, Wright D, Syngelaki A, et al. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks gestation. *Am J Obstet Gynecol* . 2016;214(1):103.e1-103.e12. doi:10.1016/j.ajog.2015.08.034

164. O'Gorman N, Wright D, Poon LC, et al. Accuracy of competing-risks model in screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. *Ultrasound Obstet Gynecol* . 2017;49(6):751-755. doi:10.1002/uog.17399

165. Rolnik DL, Wright D, Poon LCY, et al. ASPRE trial: performance of screening for preterm pre-eclampsia. *Ultrasound Obstet Gynecol* . 2017;50(4):492-495. doi:10.1002/uog.18816

166. Chaemsaithong P, Pooh RK, Zheng M, et al. Prospective evaluation of screening performance of first-trimester prediction models for preterm preeclampsia in an Asian population. *Am J Obstet Gynecol* . 2019;221(6):650.e1-650.e16. doi:10.1016/j.ajog.2019.09.041

167. Wright D, Wright A, Nicolaides KH. THE COMPETING RISK APPROACH FOR PREDICTION OF PREECLAMPSIA. *Am J Obstet Gynecol* . November 2019. doi:10.1016/j.ajog.2019.11.1247

168. Askie LM, Duley L, Henderson-Smart DJ, Stewart LA, PARIS Collaborative Group. Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data.*Lancet* . 2007;369(9575):1791-1798. doi:10.1016/S0140-6736(07)60712-0

169. Bujold E, Roberge S, Lacasse Y, et al. Prevention of preeclampsia and intrauterine growth restriction with aspirin started in early pregnancy: a meta-analysis. *Obstet Gynecol* . 2010;116(2 Pt 1):402-414. doi:10.1097/AOG.0b013e3181e9322a

170. Roberge S, Villa P, Nicolaides K, et al. Early administration of low-dose aspirin for the prevention of preterm and term preeclampsia: a systematic review and meta-analysis. *Fetal Diagn Ther* . 2012;31(3):141-146. doi:10.1159/000336662

171. Xu T, Zhou F, Deng C, Huang G, Li J, Wang X. Low-Dose Aspirin for Preventing Preeclampsia and Its Complications: A Meta-Analysis. *J Clin Hypertens (Greenwich)* . 2015;17(7):567-573. doi:10.1111/jch.12541

172. Meher S, Duley L, Hunter K, Askie L. Antiplatelet therapy before or after 16 weeks' gestation for preventing preeclampsia: an individual participant data meta-analysis. *Am J Obstet Gynecol* . 2017;216(2):121-128.e2. doi:10.1016/j.ajog.2016.10.016

173. Roberge S, Nicolaides K, Demers S, Hyett J, Chaillet N, Bujold E. The role of aspirin dose on the prevention of preeclampsia and fetal growth restriction: systematic review and meta-analysis. *Am J Obstet Gynecol* . 2017;216(2):110-120.e6. doi:10.1016/j.ajog.2016.09.076

174. Bakhti A, Vaiman D. Prevention of gravidic endothelial hypertension by aspirin treatment administered from the 8th week of gestation.*Hypertens Res* . 2011;34(10):1116-1120. doi:10.1038/hr.2011.111

175. Villa PM, Kajantie E, Raikkonen K, et al. Aspirin in the prevention of pre-eclampsia in high-risk women: a randomised placebo-controlled PREDO Trial and a meta-analysis of randomised trials. *BJOG* . 2013;120(1):64-74. doi:10.1111/j.1471-0528.2012.03493.x

176. Scazzocchio E, Oros D, Diaz D, et al. Impact of aspirin on trophoblastic invasion in women with abnormal uterine artery Doppler at 11-14 weeks: a randomized controlled study. *Ultrasound Obstet Gynecol* . 2017;49(4):435-441. doi:10.1002/uog.17351

177. Stanescu A-D, Banica R, Sima R-M, Ples L. Low dose aspirin for preventing fetal growth restriction: a randomised trial. *J Perinat Med* . 2018;46(7):776-779. doi:10.1515/jpm-2017-0184

Tables and Figures legends:

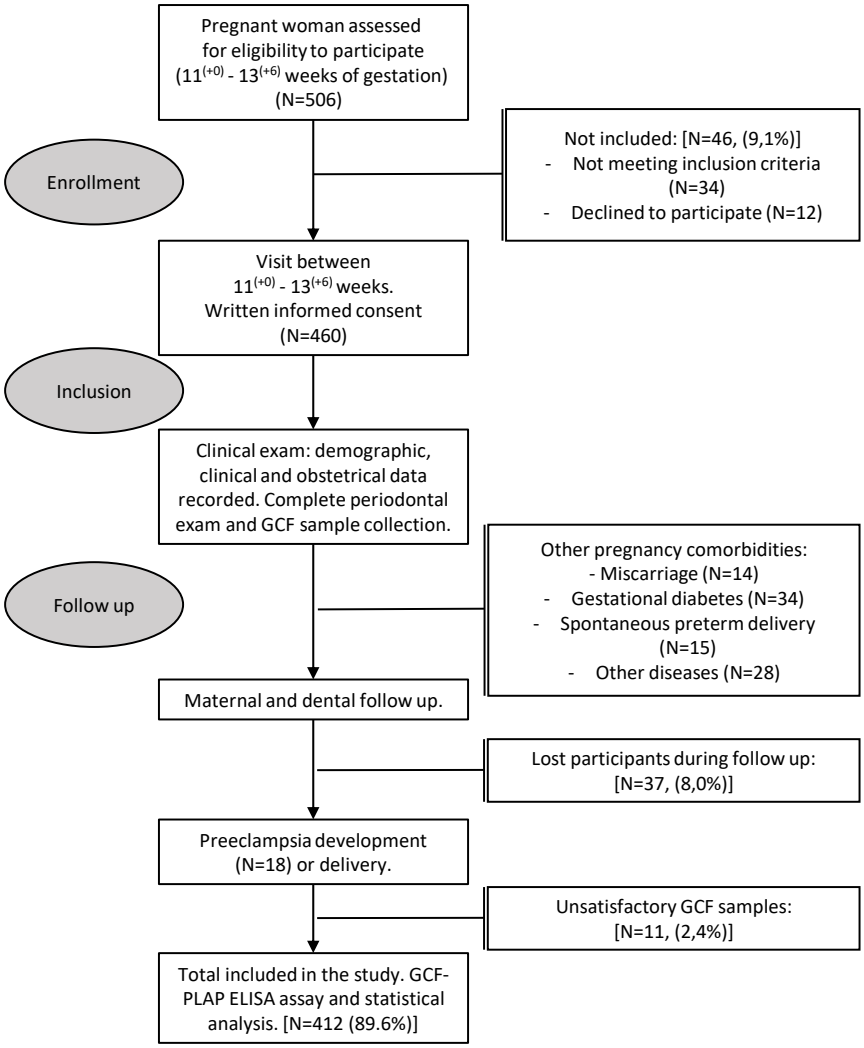
Table. 1: Clinical, demographic, and periodontal descriptions of pregnant women at 11-14 weeks of gestation

Table. 2: Frequency and percentage of the different periodontal diagnoses in the total cohort (11-14 weeks of gestation).

Table 3: Association between GCF-PLAP concentration and systolic blood pressure at 11-14 weeks of gestation, according to the presence or absence of preeclampsia: multiple regression logistic models (A) and bootstrap estimation of the multiple logistic regression model (B).

Figure 1: Flow chart of the study population.

Figure 2: A: Placental alkaline phosphatase (PLAP) concentrations (pg/ml) in gingival crevicular fluid (GCF) in pregnancy according to the presence or absence of preeclampsia. B: Plasma and GCF-PLAP concentrations at 11-14 weeks of gestation in women with and without preeclampsia. GCF, gingival crevicular fluid; PLAP, placental alkaline phosphatase. C: Area under the receiver operating characteristic (ROC) curve (AUC) of PLAP-GCF concentration and systolic blood pressure at 11-14 weeks of gestation versus the development of preeclampsia. D: Area under the receiver operating characteristic (ROC) curve (AUC) of the concentration of PLAP-GCF and systolic blood pressure versus the development of preterm preeclampsia. E: Area under the Receiver Operating Characteristic (ROC) curve (ROC-AUC) of the concentration of PLAP-GCF and systolic blood pressure at 11-14 weeks gestation versus preeclampsia >37 weeks of gestation. GCF, gingival crevicular fluid; PLAP, placental alkaline phosphatase



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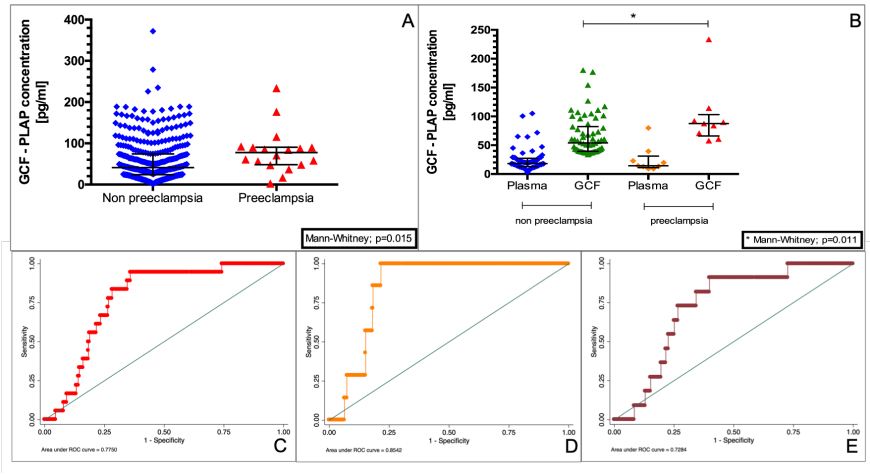

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