POSTER B-319

Novel Biochemical Markers Help Aid in Stratifying Patients at Risk of Preeclampsia and Adverse Events*

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ABSTRACT

Objective: To develop sensitive and specific objective biochemical markers to help aid in diagnosing preeclampsia.

Relevance: Preeclampsia is a pregnancy complication characterized by high blood pressure, presence of protein in the urine, edema, sudden weight gain, headaches, and changes in vision. Preeclampsia occurs in five to eight percent of all pregnancies. In the United States alone, Preeclampsia is responsible for about eighteen percent of all maternal deaths and fifteen percent of premature births. It is also the leading cause of premature delivery. To date, no objective biochemical marker has been found with high sensitivity and specificity to diagnose preeclampsia accurately. The current strategy to diagnose preeclampsia is through the detection of protein in the urine and onset of high blood pressure during the late second and third trimester pregnancy. However, these symptoms are also present in some normal and many other pregnancy complications such as gestation hypertension, thus increasing the number of false positives. Recent studies on maternal serum protein analysis by proteomics have shown upregulation of placental and hepatic proteins. Two of the upregulated proteins, Pappalysin (PAPP-A, a IGFBP-4 protease and PAPP-A2, a IGFBP-5 protease, produced by placenta) and glycosylated form of fibronectin (preferential binding to SNA and other lectins reflecting sialic acid and fucose carbohydrates) mostly produced by the liver were studied.

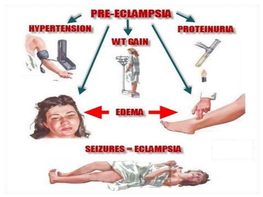
Methodology: Specific monoclonal antibody based ELISAs for GlyFn (AL-160), Pregnancy-Associated Plasma Protein A2 (PAPP-A2, AL-109 C_{cap}-C_{det}, AL-167 C_{cap}-N_{det}), Eosinophil Major Basic Protein (proMBP) (AL-159, $proMBP_{cap}$-$proMBP_{det}$) PAPP-A-proMBP Complex (AL-112, PAPP-A_{cap}-$proMBP_{det}$,) and proMBP-Angiotensinogen (proMBPAGT, AL-111, $proMBP_{cap}$-AGT_{det}) were developed and validated. Preeclampsia status was evaluated using these biomarkers in serum samples from 545 pregnant women (PE, Control, PIH, Undiagnosed) with gestation age 20 to 35 weeks in two subsets of samples. A mathematical algorithm based on 2 decision point using PAPP-A2, GlyFn, protein urea, blood pressure have been evaluated for stratifying the patients the risk of PE and adverse events.

Validation: ELISAs were very specific to the measured analyte and did not cross-react with other related analytes in the family. ROC analysis for each ELISA was used to calculate the area under the curve (sensitivity and specificity) of diagnosing PE vs Controls. GlyFn and PAPP-A2 ELISAs resulted in AUROC of 1.0 and 0.99 for study 1 and ROC of 0.98 and 0.99 for study 2. PAPP-A-proMBP, proMBP-proMBP and proMBP-AGT had low AUORC of 0.72, 0.64, and 0.52, respectively. Clinal cut-off were established for GlyFn and PAPP-A2 and their serum measurements showed a good concordance with the delivery status (concentrations near the cutoff delivered close to term and elevated concentrations delivered very pre-term).

Conclusions: GlyFn and PAPP-A2 serum measurements suggest that these proteins play a critical role in preeclampsia and PAPP-A-proMBP, proMBP-proMBP and proMBP-AGT serum levels may not play a significant role in preeclampsia diagnosis. The unique combination of placental (PAPP-A2) and hepatic (GlyFn) protein biomarkers increases the sensitivity and specificity of PE diagnosis over 95%.

INTRODUCTION

- ❑ Preeclampsia (PE) is a multi-system disorder characterized by the new onset of hypertension and elevated proteinuria in the last half of pregnancy. However, PE can be asymptomatic.
- ❑ PE affects **5-8% of all pregnancies** worldwide and in the USA, PE is responsible for approximately **18% of all maternal deaths.**

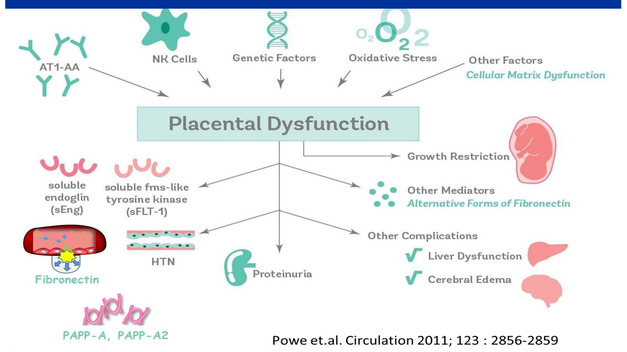


http://mybabysheartbeatbear.com/blog/preeclampsia-is-scary-know-the-symptoms/

- ❑ PE causes **15% of premature births** in industrialized countries and is the **number one reason** doctors decide to **deliver a baby prematurely**.
- ❑ If undetected, PE can lead to Eclampsia, which is one of the top five causes of maternal and infant illness and death. **PE corresponds to one maternal death every 12 minutes.**

For every maternal death, there are 50-100 women who experience "near miss" significant morbidity.

THE PATHOGENESIS OF PE REMAINS UNCLEAR



Powe et.al. Circulation 2011; 123 : 2856-2859

RESEARCH QUESTIONS

1. Can blood test(s) accurately measure PAPP-A, PAPP-A2, proMBP, proMBP-AGT, and GlyFn in pregnancy serum?
2. Can these **blood tests differentiate** Preeclampsia subjects from Control subjects and help predict the pregnancy outcome?

HYPOTHESES

- ❑ If the serum levels of PAPP-A, PAPP-A2, GlyFn, proMBP, and proMBP-AGT is elevated in preeclampsia (as per proteomics analysis), than these circulating proteins should play a critical role in preeclampsia diagnosis.
- ❑ If the sensitivity and specificity of these blood test(s) is/are ≥ 80%, then the test(s) can help manage preeclampsia and time to delivery.

STUDY DESIGN AND DEFINITIONS

Pregnant woman with blood pressure higher than 140/90 mm Hg

- **Before 20 weeks of gestation**
  - **PIH** (Chronic hypertension) No or stable proteinuria
  - **Preeclampsia, PE** (Superimposed on chronic hypertension) New or increased proteinuria, increasing blood pressure, or HELLP syndrome
- **After 20 weeks of gestation**
  - **Preeclampsia** Proteinuria (24 hrs Protein:Creatinine ratio of ≥ 0.3 mg/dL in urine)
  - **PIH** (Gestational hypertension) No proteinuria

HELLP = Hemolysis, Elevated Liver enzymes, Low Platelet count

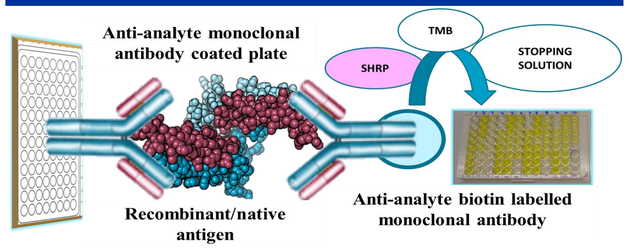
Pregnant woman with normal blood pressure 120/80 mm Hg

- **Controls** (Pregnant women with normal blood pressure and no proteinuria)
- **Renal** (Pregnant women with normal blood pressure and high proteinuria)

Undiagnosed (Pregnant women with high risk pregnancies)

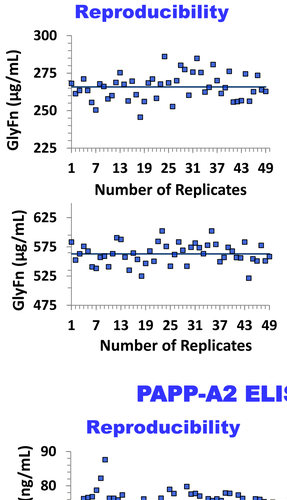
Study Cohorts (n = number of subjects)	Controls (n)	PE (n)	PIH (n)	Renal (n)	Undiagnosed (n)	Total (n)
Verification Set	50	9	14	3	22	98
Validation Set	116	68	73	4	186	447

METHODS AND RESULTS

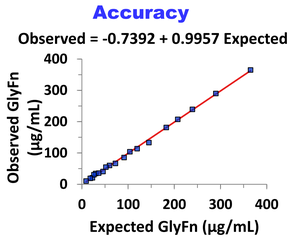


GlyFibronectin ELISA Validation

Reproducibility



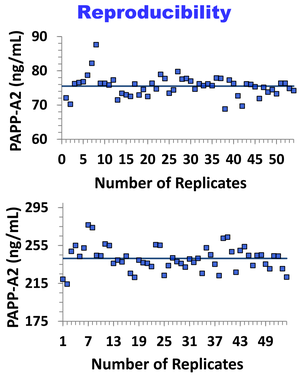
Accuracy



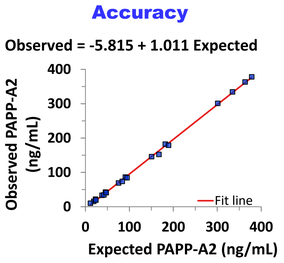
Imprecision	N	Mean GlyFn (µg/mL)	% CV
C-I	49	265.8	3.3
C-II	49	563.0	3.2

PAPP-A2 ELISA Validation

Reproducibility

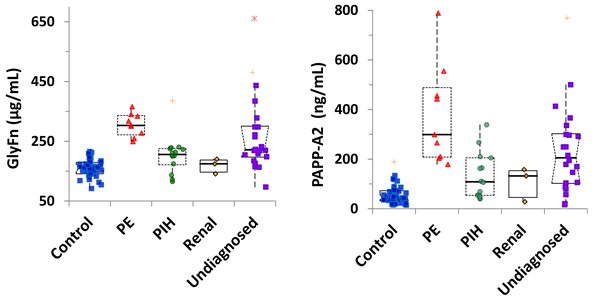


Accuracy

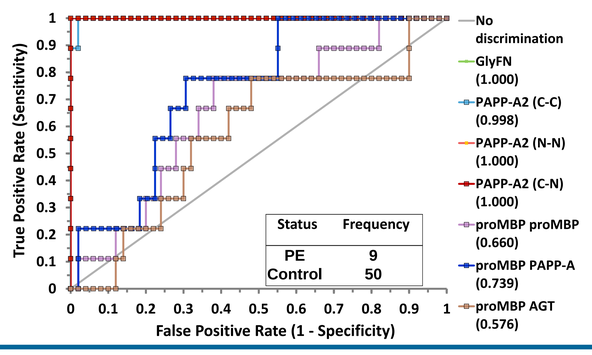


Imprecision	N	Mean PAPP-A2 (ng/mL)	% CV
C-I	54	75.5	4.0
C-II	54	241.3	5.4

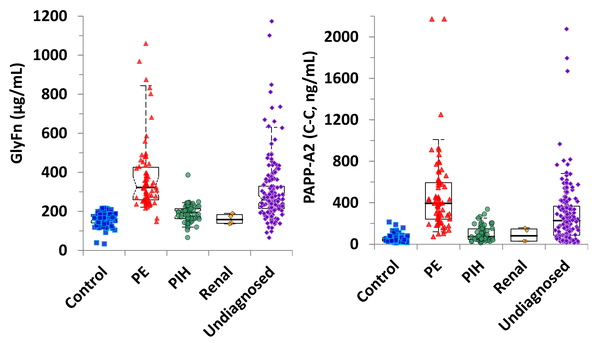
GlyFn and PAPP-A2 Serum Levels (Verification Set)



ROC Analysis (PE *vs* Controls Verification Set)

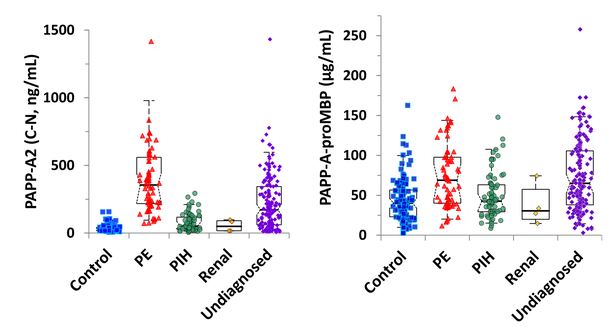


GlyFn and PAPP-A2 Serum Levels (Validation Set)



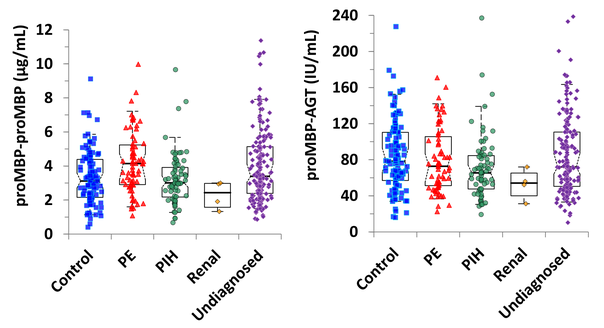
Subjects	N	Mean GlyFn (µg/mL)	Mean PAPP-A2 (ng/mL)
Control	116	162.1	51.5
PE	68	375.4	477.3
PIH	73	188.9	98.5
Renal	4	160.5	87.2
Undiagnosed	186	298.2	280.9

PAPP-A2 and PAPP-A-proMBP Levels (Validation Set)



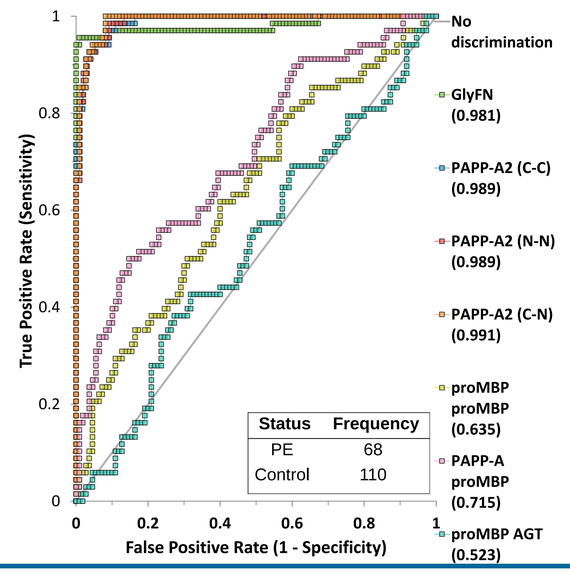
Subjects	N	Mean PAPP-A-proMBP (µg/mL)	Mean PAPP-A2 (ng/mL)
Control	115	44.2	33.4
PE	68	73.8	445.1
PIH	72	49.5	77.5
Renal	4	37.5	53.3
Undiagnosed	186	72.6	235.1

proMBP, proMBP-AGT Serum Levels (Validation Set)

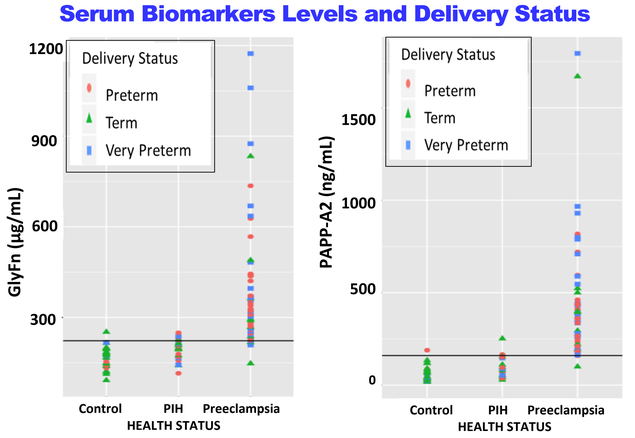


Subjects	N	Mean proMBP (ug/mL)	Mean proMBP-AGT (IU/mL)
Control	116	3.32	83.3
PE	68	4.21	79.5
PIH	73	3.22	71.0
Renal	4	2.30	52.9
Undiagnosed	186	3.99	83.2

(PE *vs* Controls, Validation Set, ROC Analysis)



Serum Biomarkers Levels and Delivery Status



SUGGESTED LABORATORY TESTING TO EVALUATE PE

Standard Testing

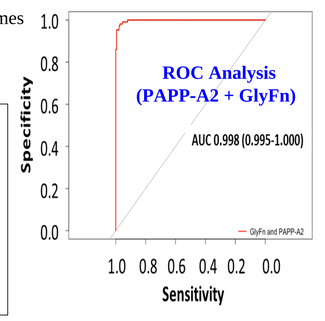
- ❑ Urinary protein
- ❑ Uric acid
- ❑ Doppler ultrasound
- ❑ Liver enzymes
- ❑ Platelets

PE Diagnosis

Positive : GlyFn & PAPP-A2 (High Risk)

Negative : GlyFn & PAPP-A2 (Optimal)

Intermediate Results Only GlyFn or PAPP-A2 is elevated. Other evaluations are required



Markers	Optimal	Elevated	High Risk
PAPP-A2	< 189 (ng/mL)		> 250 (ng/mL)
GlyFn	< 216 (µg/mL)		> 260 (µg/mL)
Uric Acid	< 6.0 (mg/dL)		> 7.5 (mg/dL)
Body Mass Index	< 25 (kg/m²)		> 30 (kg/m²)
Blood Pressure	< 120/80 mm Hg		> 140/90 mm Hg

PREECLAMPSIA MANAGEMENT

Preeclampsia: Who and When to Test?

PE test is recommended for pregnant patients who may have one or more of the following:

- ❑ *Clinical assessment of increased risk for PE*
- ❑ *Nulliparous*
- ❑ *Family history of or previous hypertension*
- ❑ *Family history of PE*
- ❑ *Pre-existing type-1 or type-2 diabetes*
- ❑ *Clinically evaluated obesity*

Initial testing: *Recommended between **17 – 36 weeks***

Follow up testing: *Recommended between **20 – 36 weeks***

Suspected PE Diagnosis: *Mild and stable PE subjects may be managed as outpatients with weekly monitoring, including BP checks, non-stress tests, amniotic fluid checks, and labs.*

Hospital Admission and Monitoring: *Severe PE patients may require hospitalization with daily monitoring of maternal BP, urine output, and fetal monitoring.*

CONCLUSIONS

1) GlyFn and PAPP-A2 serum measurements suggest that these proteins play a critical role in preeclampsia.
2) PAPP-A-proMBP, proMBP-proMBP, and proMBP-AGT serum levels may not play a significant role in preeclampsia diagnosis.
3) **The unique combination of placental (PAPP-A2) and hepatic (GlyFn) protein biomarkers increases the sensitivity and specificity of PE diagnosis.**
4) The tests should be used in assessment of PE subjects with borderline blood pressure and proteinuria changes. **The test is positive 2-4 weeks before the onset of symptom.**
5) **Early prediction of preeclampsia** will help manage pregnancy and will significantly **reduce the hospitalization cost** incurred by patient or the care provider.
6) GlyFn and PAPP-A2 tests in combination with other clinical information **provides biochemical confirmation of PE** and should be recommended for incorporation in ACOG guidelines for PE diagnosis.