Improved diagnosis of preeclampsia with severe features and end organ injury using complement activation measurement in urine and plasma

Catalina M. Valencia, **Richard M. Burwick**, Jesús A. Velásquez, Jaime L. Silva, Jorge Gutiérrez-Marín, Francisco Edna-Estrada, Juliana Trujillo-Otálvaro, Yamile Bernal, Alvaro Quintero, Ana M. Gómez, Nataly González, Carlos Cabas, Mónica Rincón, Viviana Lenis-Ballesteros, Jorge E. Tolosa

Disclosures

- Speakers Bureau, Alexion Pharmaceuticals
- Funding, Preeclampsia Foundation

Preeclampsia

- Leading cause of maternal mortality
- Hypertension, systemic inflammation and endothelial injury
- Severe disease defined by end-organ injury
- Biomarkers may be used to help diagnose subtypes of disease

REF: ACOG Task Force 2013; Redman et al. 1999; Roberts et al. 1989

Complement System

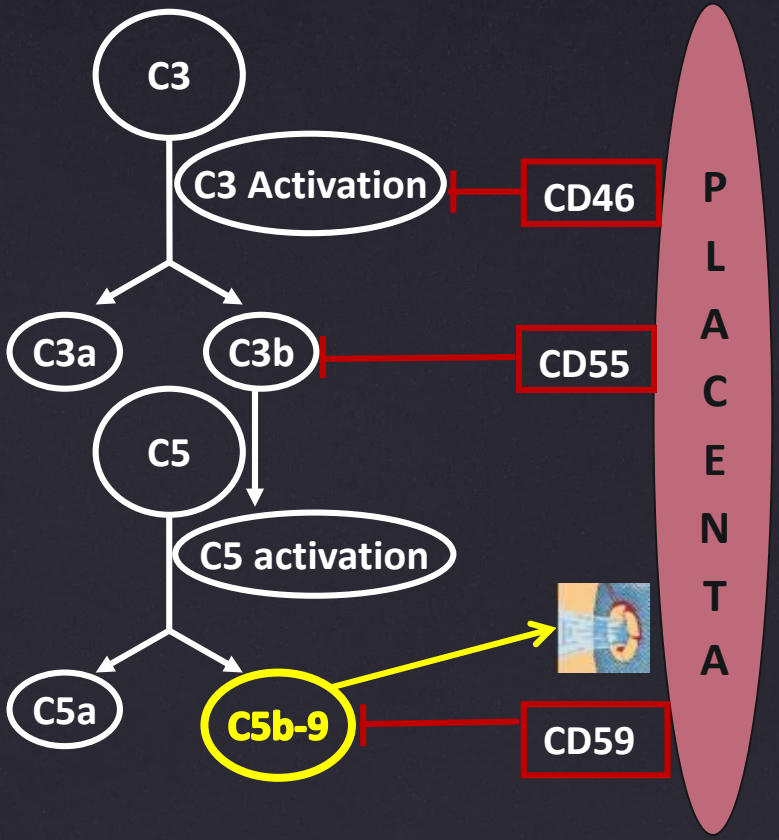
- Complement system is critical for host defense / innate immunity
- Complement proteins activated following recognition of:

  - Foreign cells or pathogens
  - Apoptotic debris, DNA
  - Immune complexes

REF: Feinberg 2006; Regal et al. 2015

Complement System

- Complement activation increases in normal pregnancy
- Activation of terminal pathway generates C5b-9
- Balance between activation and regulation



REF: Richani et al. 2005; Burwick et al. 2013

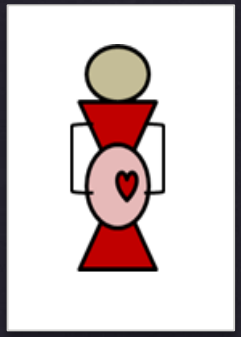
C5b-9 (membrane attack complex)

- Membrane bound C5b-9 mediates cell lysis
- Soluble C5b-9 is a marker of inflammation and cellular injury
- C5b-9 levels increased in preeclampsia
- Association between C5b-9 and preeclampsia disease severity is unknown

REF: Burwick et al. 2013. Kerjaschki et al. 1989; Morita et al. 2000

Objective

To determine if complement activation, as measured by soluble C5b-9 in plasma and urine, is increased in association with disease severity in preeclampsia



Project COPA:

COmplement and Preeclampsia in the Americas

Multicenter, prospective case-control study of complement biomarkers in hypertensive disorders of pregnancy

Study Sites: 3 cities, 6 centers

Hospital	City
Hospital Universitario de San Vicente Fundacion	**Medellín**
Clínica Universitaria Bolivariana	**Medellín**
Hospital General de Medellín	**Medellín**
E.S.E. Clínica de Maternidad Rafael Calvo	**Cartagena**
Hospital Universitario San Ignacio	**Bogotá**
Clínica Reina Sofía, SANITAS	**Bogotá**

Project COPA: Nov ’15 – July ‘16

- Inclusion criteria (≥ 24 wks, singleton gestation)
  - Healthy
  - Chronic hypertension (CHTN)
  - Gestational hypertension (GHTN)
  - Preeclampsia (PE)
  - Preeclampsia with severe features (PE-SF)
- Exclusion criteria
  - Multiple gestation, fetal demise, lupus, active infection, diabetes, kidney disease

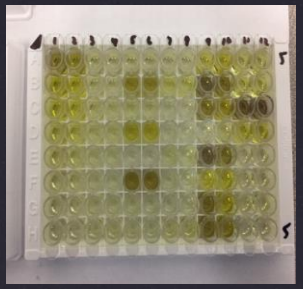
Study Enrollment

- IRB approval at each site; Enrollment in blocks by gestational age and diagnosis
- Ca
- Co
- Target:
  - 100 cases
  - 200 controls (n=50/group)

Site	City	N	PE-SF	Controls
1	Medellín	85	36	49
2	Medellín	59	13	45
3	Medellín	47	20	27
4	Cartagena	53	16	37
5	Bogotá	49	8	41
6	Bogotá	60	8	52
Total		352	101	251

Sample Collection and Assays

- Blood/urine at enrollment
- Plasma and urine aliquots stored at -80°C
- Samples sent to central lab in Bogotá (ColSanitas)
- Automated 4-plate ELISA (Dynex Technologies) utilized for C5b-9 assays (BD Biosciences)

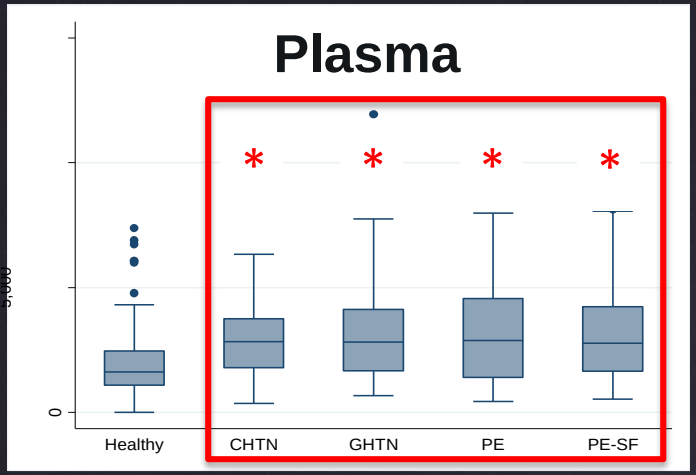


Baseline Characteristics of Study Population

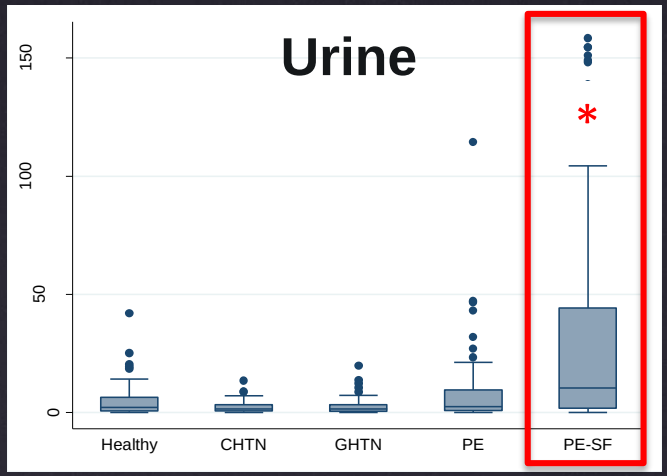
	Healthy (n=59)	CHTN (n=42)	GHTN (n=92)	PE (n=58)	PE-SF (n=101)
Enrollment GA (wks)	34.4 ± 4.1	33.8 ± 4.3	35.5 ± 4.1	35.4 ± 3.7	33.2 ± 4.2
Age (years)	30.3 ± 6.3	29.9 ± 6.4	26.0 ± 6.2	25.9 ± 6.8	25.9 ± 6.5
BMI (kg/m^2)	24.1 ± 3.9	28.4 ± 5.5	25.5 ± 4.6	25.6 ± 5.0	24.7 ±4.3
Systolic BP (mm Hg)	116 ± 13	141 ± 12	142 ± 11	141 ± 11	149 ± 17
Diastolic BP (mm Hg)	68.1 ± 9.9	85.9 ± 12	89.9 ± 10	88.5 ± 10	95.0 ± 12
Nulliparous (%)	59.7	52.4	68.2	79.3	64.0
African descent (%)	3.7	9.8	21.6	15.8	19.0

Data are mean ± SD, unless otherwise stated
BP, blood pressure at enrollment
GA, gestational age

C5b-9 in Plasma and Urine



* P<0.02



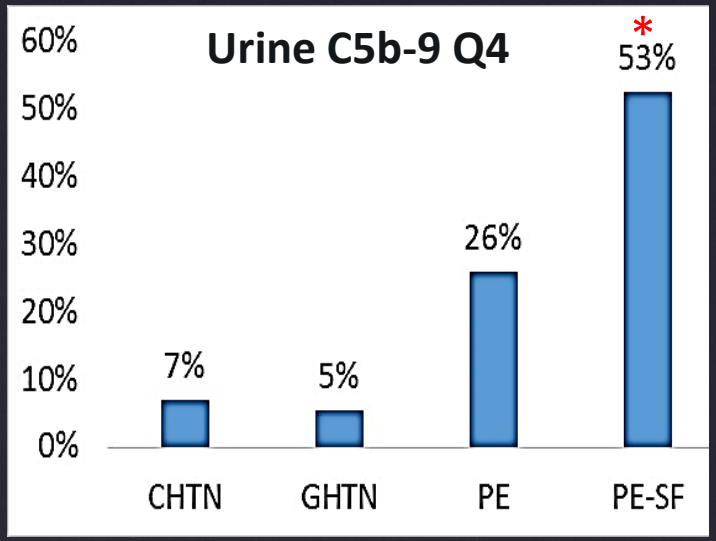
* P<0.001

- Plasma C5b-9 is significantly increased in CHTN, GHTN, PE, PE-SF
- Urine C5b-9 is significantly increased in PE-SF

Urine C5b-9 Quartiles in Hypertensive Disorders

C5b-9 (ng/ml) Quartiles (Q1-4)

Q1: <0.69
Q2: 0.7-2.3
Q3: 2.4-8.4
Q4: ≥ 8.5



* p<0.01

- Subjects with PE-SF more likely to have urine C5b-9 levels in upper quartile

Urine C5b-9 Quartiles and PE-SF: Logistic Regression

Variables	PE-SF (OR)	95% CI	P-value
C5b-9 Quartile 4 (Unadjusted)	6.8	4.0-11.6	<0.001
C5b-9 Quartile 4 (Adjusted for age, race, parity, BMI for systolic and diastolic BP, urine protein)	4.0	2.1-7.9	<0.001

- **Association between urine C5b-9 and PE-SF is independent of urine protein**

C5b-9 and End-Organ Injury

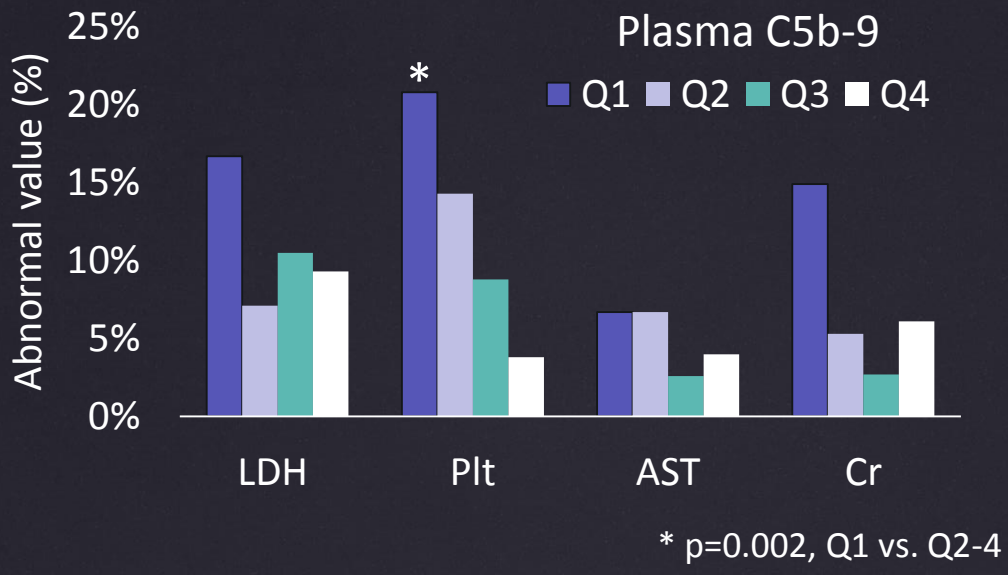
- Next, we evaluated association between C5b-9 and end-organ injury
- Hemolysis, thrombocytopenia, liver dysfunction, kidney injury
- Healthy controls excluded (no lab data)

Criteria for End-Organ Injury

Lab	Criteria*	Abnormal Value
Lactate dehydrogenase (LDH)	>90th %ile	≥500 U/L
Platelet count (Plt)	<10th %ile	<150,000 /µl
Aspartate transaminase (AST)	>90th %ile	≥70 U/L
Creatinine (Cr)	>90th %ile	≥1.0 mg/dl

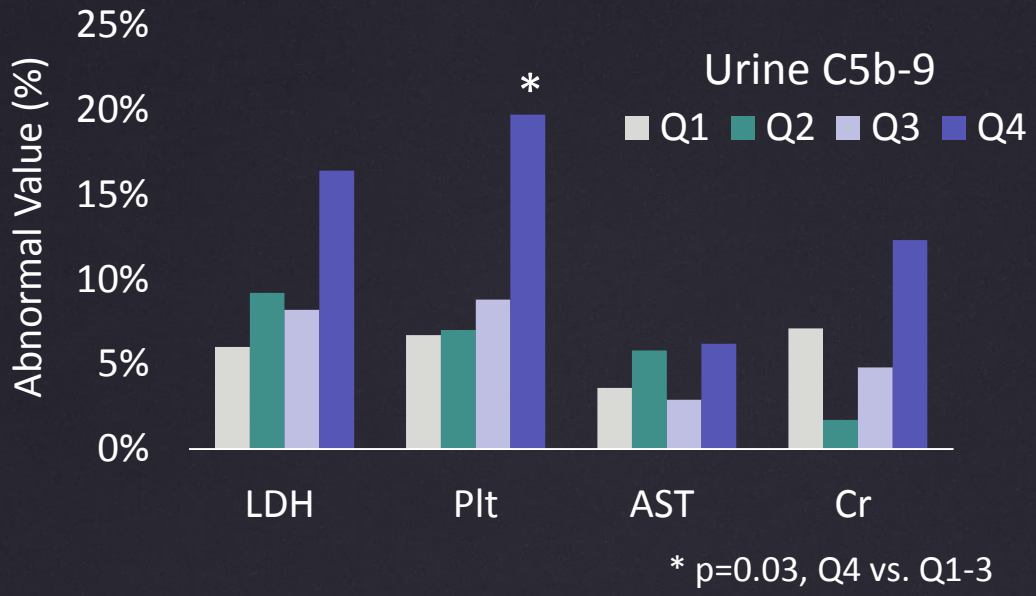
* Distribution determined from COPA study cohort

Plasma C5b-9 Quartiles and End-Organ Injury



- Low platelet count more common in plasma C5b-9 Quartile 1

Urine C5b-9 Quartiles and End-Organ Injury



- Low platelet count more common in urine C5b-9 Quartile 4

Plasma and Urine C5b-9 Quartiles and End-Organ Injury

Diagnosis or lab feature	Plasma Q2-4 + Urine Q1-3 (n=177)	Plasma Q1 + Urine Q4 (n=20)	p-value
Preeclampsia with SF	23.2%	70%	<0.001
End-organ injury (≥1 of below labs)	14.7%	35%	0.02
Creatinine ≥1.0 mg/dl	2.7%	18.8%	0.003
Platelet <150,000/µl	6.0%	31.3%	<0.001
LDH ≥500 U/L	7.6%	23.1%	0.056
AST ≥70 U/L	4.4%	14.3%	0.11

- Low Plasma C5b-9 + High Urine C5b-9 associated with PE-SF and End-organ Injury

Conclusions

- Complement activation is increased in hypertensive disorders
- Excess complement activation, assessed by urine C5b-9, is present in >50% of PE-SF cases
- We describe for the first time a pattern of ***Low Plasma C5b-9*** and ***High Urine C5b-9*** that is significantly associated with PE-SF and end-organ injury

Acknowledgements

TEAM COPA:
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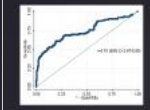
CEDARS-SINAI MEDICAL CENTER

Additional slides

ROC Curve:
Urine C5b-9 and PE-SF

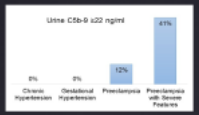


Optimal cut-point for PE-SF:

Urinary C5b-9 level ≥22ng/ml

- 96% specificity
- LR+ 11.3

ROC: Urine C5b-9 by Group

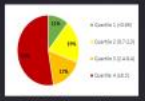


P<0.001,
PE-SF vs. CHTN, GHTN, PE

ROC Regression:
Urine C5b-9 and PE-SF

Variable	PE-SF Odds Ratio	95% CI	p-value
Urine C5b-9 ≥22 ng/ml (unadjusted)	18.4	8.5-39.9	<0.001
Urine C5b-9 ≥22 ng/ml (adjusted for age, BMI, race, parity, gestational age)	16.4	6.9-39.0	<0.001
Urine C5b-9 ≥22 ng/ml (additional adjustment for urine protein and creatinine)	13.1	4.8-36.1	<0.001

PE-SF by Urine C5b-9 Quartile



53% of PE-SF cases with urine C5b-9 ≥8.5 ng/ml

Adverse maternal and neonatal outcomes by plasma C5b-9 quartiles

	Q1 <1443 ng/ml	Q2 1444-2558 ng/ml	Q3 2559-4074 ng/ml	Q4 >4075 ng/ml
Composite Adverse Maternal Outcome* (%)	18.3	12.9	8.4	10.0
Composite Adverse Neonatal Outcome† (%)	55.0	54.3	38.6	40.0

* Composite maternal (any of the following): eclampsia, pulmonary edema, acute kidney injury (Cr ≥1.0 mg/dl) or liver dysfunction (AST/ALT ≥70 U/L)

† Composite neonatal (any of the following): preterm birth <37wks, 5-minute Apgar <7, NICU admission or respiratory distress syndrome

Adverse maternal and neonatal outcomes by urine C5b-9 quartiles

	Q1 <0.69 ng/ml	Q2 0.70-2.34 ng/ml	Q3 2.35-8.48 ng/ml	Q4 >8.49 ng/ml
Composite Adverse Maternal Outcome* (%)	8.7	7.9	13.9	17.1
Composite Adverse Neonatal Outcome† (%)	43.5	42.1	41.7	56.6

* Composite maternal (any of the following): eclampsia, pulmonary edema, acute kidney injury (Cr ≥1.0 mg/dl) or liver dysfunction (AST/ALT ≥70 U/L)

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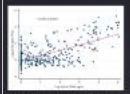
C5b-9 and urine protein/creatinine

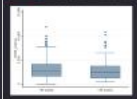


Positive correlation between Urine C5b9 and spot pr/cr

Group	Urine C5b9 Spot (+)	Urine C5b9 Spot (+)	P-value
GHTN	1.61 (0.5-3.4)	4.6 (3.9-16)	0.70
PE	1.51 (0.4-2.6)	5.1 (1.7-13)	0.01
PE-SF	1.3 (0.4-2.3)	22.2 (4.1-47)	<0.001

Urine C5b9 higher in preeclampsia groups with + spot pr/cr >0.3

Plasma C5b-9 by Enrollment GA



Spearman correlation C5b-9 and GA:
rho = 0.15, p = 0.005

- Preterm vs. term gestations:
  2870 vs. 3572 ng/ml, p=0.006

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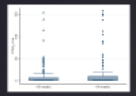


Spearman correlation C5b-9 and GA

Spearman's rho = -0.03
Prob > |t| = 0.5767

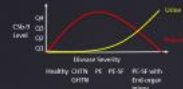
ACOG severe criteria-
Plasma C5b9 and End Organ Injury

Characteristic	Plasma C5b-9 Q1 (n=216)	Urine C5b-9 Q2-4 (n=74)	P-value
Platelet count <100,000/µl	0.9%	4.3%	0.09
Creatinine > 1.1 mg/dl	0	4.3%	0.004
Headache or visual changes	52%	64%	0.10
Right upper quadrant pain	26%	24%	0.74

ACOG severe criteria-
Urine C5b9 and End Organ Injury

Characteristic	Urine C5b-9 Q1-3 (n=216)	Urine C5b-9 Q4 (n=74)	P-value
Platelet count <100,000/µl	1.0%	2.8%	0.28
Creatinine > 1.1 mg/dl	0.6%	1.5%	0.45
Headache or visual changes	56%	49%	0.27
Right upper quadrant pain	26%	24%	0.73

Concept Figure: C5b-9 in Plasma and Urine



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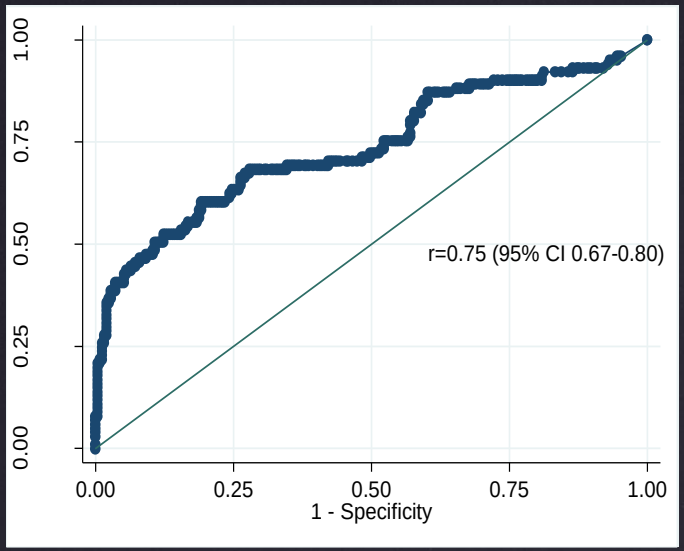

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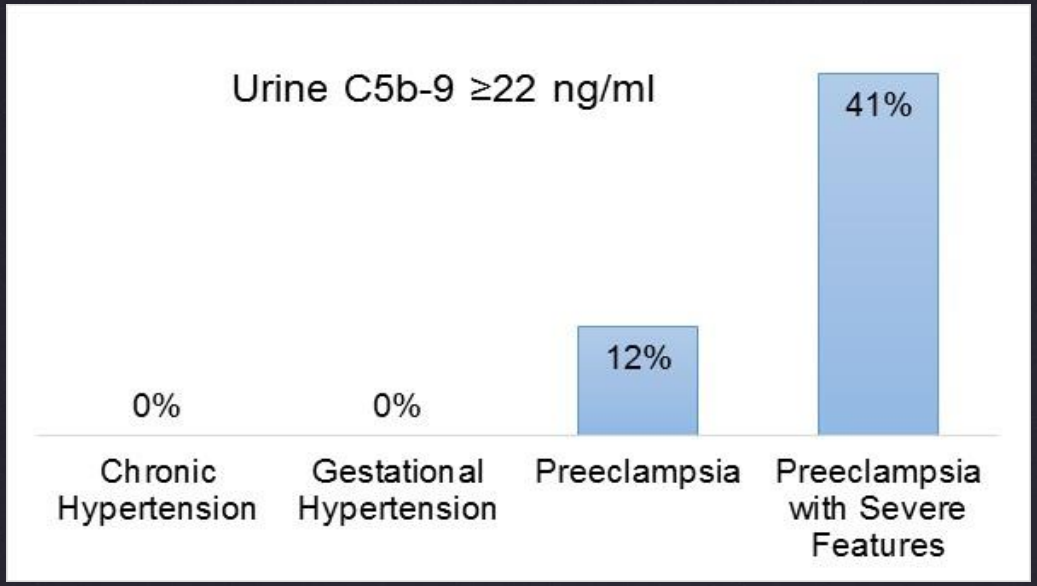
- LR+ 11.3

Additional slides

ROC: Urine C5b-9 by Group



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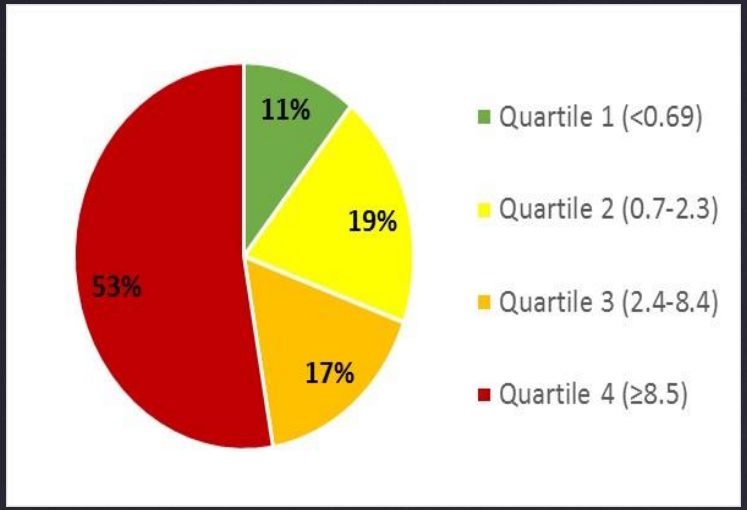
Additional slides

PE-SF by Urine C5b-9 Quartile



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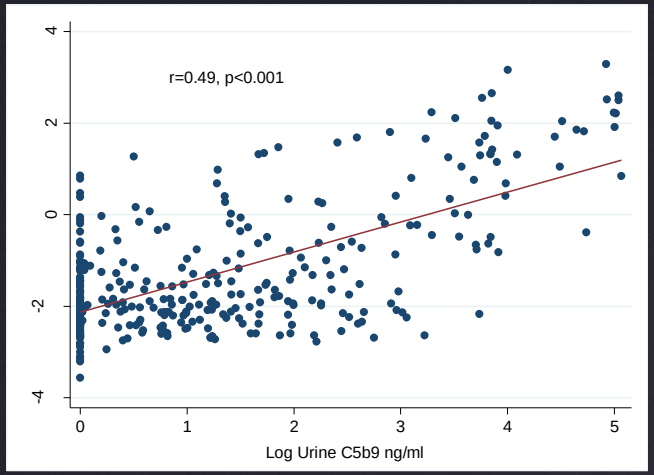
Additional slides

C5b-9 and urine protein/creatinine



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PE	1.51 (0.4-2.9)	5.1 (1.7-17)	0.01
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Urine C5b9 higher in preeclampsia groups with + spot pr/cr >0.3

Additional slides

Plasma C5b-9 by Enrollment GA

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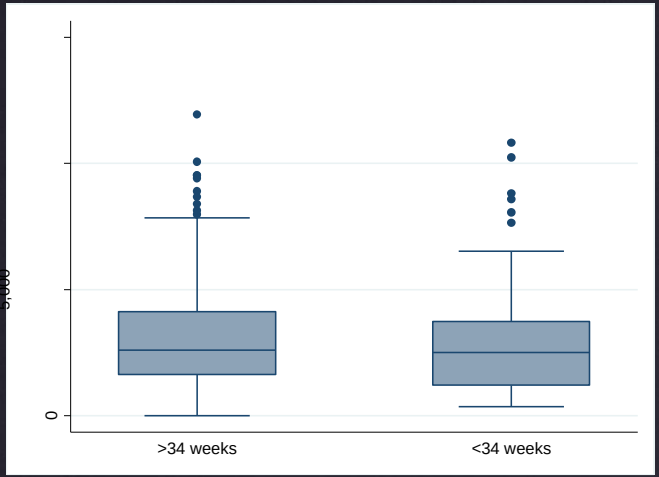

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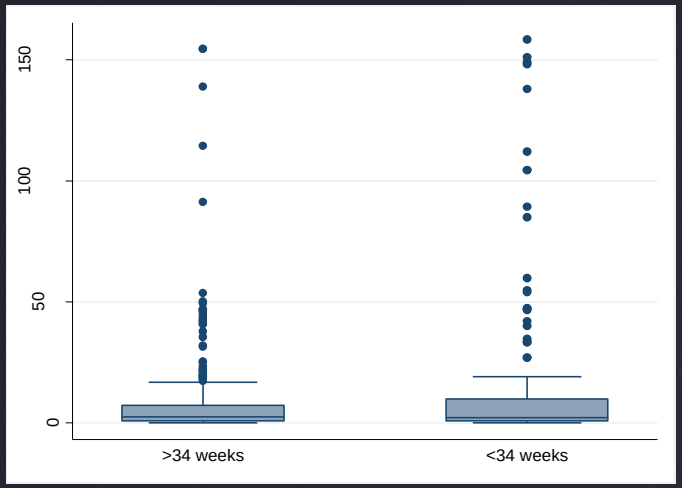

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Creatinine > 1.1 mg/dl	0.6%	1.5%	0.45
Headache or visual changes	56%	49%	0.27
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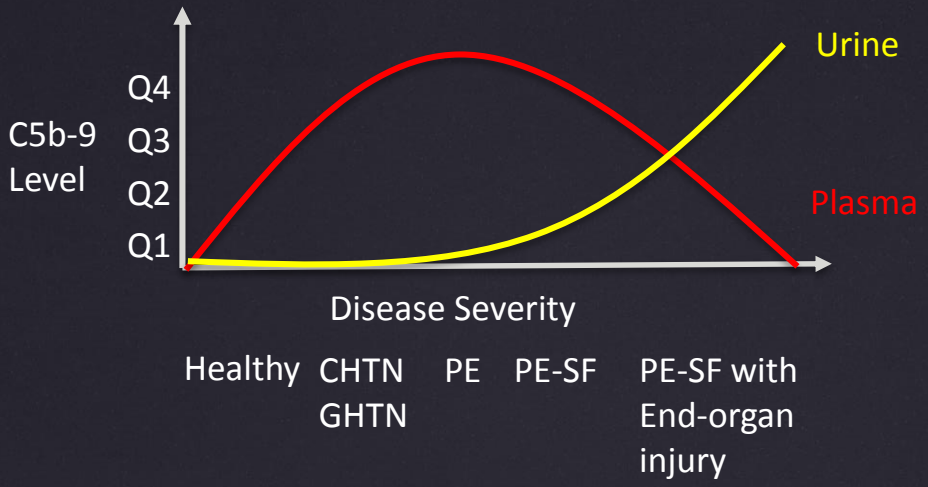
Additional slides

Concept Figure: C5b-9 in Plasma and Urine



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