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Diagnostic Indicators of Superimposed Preeclampsia in Women With CKD

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Introduction: Diagnosis of superimposed preeclampsia in women with chronic kidney disease (CKD) is complicated by the presence of hypertension and proteinuria due to renal disease. The aims of this study were to determine mechanistic links between superimposed preeclampsia and renin-angiotensin system activation, endothelial pathology, complement dysfunction, and tubular injury, and to explore the role of diagnostic indicators of superimposed preeclampsia.

Methods: Plasma and urinary biomarkers derived from the renin-angiotensin system (active renin, angiotensinogen), endothelial glycocalyx (hyaluronan, intercellular adhesion molecule, vascular cell adhesion molecule [VCAM], P-selectin, E-selectin), complement activation (C3a, C5a, complement factor H, C5b-9), and tubular injury (kidney injury molecule-1, urinary lipocalin-2) were quantified in 60 pregnant women with CKD including 15 women at the time of superimposed preeclampsia diagnosis and 45 women who did not develop superimposed preeclampsia, 18 women with preeclampsia, and 20 normal pregnancies. Correlation with placental growth factor was assessed.

Results: Plasma concentrations of hyaluronan (67.5 ng/ml vs. 27.5 ng/ml, $P = 0.0017$, receiver operating characteristic area 0.80) and VCAM (1132 ng/ml vs. 659 ng/ml, $P < 0.0001$, receiver operating characteristic area 0.86) distinguished women with CKD and superimposed preeclampsia from those without superimposed preeclampsia, and correlated with placental growth factor concentration. The diagnostic discrimination of markers of the renin-angiotensin system was reduced by adjustment for chronic hypertension, antihypertensive drug use, and black ethnicity. Other markers offered limited or no diagnostic discrimination for superimposed preeclampsia.

Conclusion: This study suggests that endothelial dysfunction contributes to the pathophysiology of superimposed preeclampsia and a diagnostic role for plasma hyaluronan and VCAM is hypothesized.

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It is estimated that up to 3% of women who become pregnant have underlying CKD,[1] which is associated with adverse pregnancy outcomes including preeclampsia, fetal growth restriction, preterm delivery, and decline in maternal renal function.

A diagnosis of preeclampsia is made on the basis of *de novo* hypertension developing after 20 weeks' gestation in conjunction with either new proteinuria or evidence of maternal organ or uteroplacental dysfunction.[2] In women with CKD, the diagnosis of superimposed preeclampsia is complicated by coexisting chronic hypertension and/or proteinuria, which are estimated to be present in one-half and one-third of pregnancies, respectively,[3,4] thereby rendering standard diagnostic criteria redundant. In the absence of formal diagnostic criteria for superimposed preeclampsia, meta-analysis estimates a 10-fold increased overall risk for the development of superimposed preeclampsia in women with CKD compared with women without CKD,[5] with preeclampsia affecting 20% to 87% of pregnancies in women with CKD, depending on prepregnancy disease stage and the diagnostic criteria used.[3,6]

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Preeclampsia remains a key determinant of adverse pregnancy outcome, contributing to preterm delivery and maternal and neonatal morbidity in both the general obstetric population[7] and in women with CKD.[4,8] The disease trajectory and associated morbidity of preeclampsia require distinction from gestational change in CKD, yet unless systemic or fetal complications of preeclampsia arise, discrimination of superimposed preeclampsia from CKD remains challenging. Although used,[3,4,8] the diagnostic value of relative changes in both blood pressure and proteinuria in preeclampsia remain unclear.[2] Diagnostic utility has however been demonstrated in the use of angiogenic (placenta growth factor [PlGF]) and antiangiogenic (soluble fms-like tyrosine kinase-1) biomarkers in preeclampsia in the general population,[9–11] with emerging data on their use as a diagnostic adjunct in pregnant women with CKD.[4,12,13]

The pathophysiological processes by which CKD confers an increased risk of superimposed preeclampsia remain poorly understood. Putative mechanisms, which offer a mechanistic link between renal disease and preeclampsia, include endothelial dysfunction,[14–16] renin-angiotensin system (RAS) activation,[17] complement dysregulation,[18–20] and kidney injury.[21,22]

This aim of this study was to explore mechanistic links and investigate potential diagnostic indicators in superimposed preeclampsia linked to pathophysiology, including markers of the RAS (active renin, angiotensinogen), endothelial glycocalyx dysfunction (hyaluronan, intercellular adhesion molecule, VCAM, P-selectin, E-selectin), complement activation (C3a, C5a, complement factor H, C5b-9), and kidney injury (kidney injury molecule-1, urinary lipocalin-2). Given the inherent complexity in diagnosing superimposed preeclampsia in women with CKD and the emerging diagnostic role of PlGF, a, correlation between the novel markers and PlGF concentration was examined.

METHODS

Pregnant women with and without CKD, and nonpregnant women with CKD were recruited at 3 London centers (Guy's and St. Thomas' NHS Foundation Trust, Imperial College Healthcare NHS Trust, King's College Hospital NHS Trust) between 2009 and 2015. Approval was provided by the Research Ethics Service and the Health Research Authority (11/LO/1776 and 15/WA/0009). Inclusion criteria were women of reproductive age with a known diagnosis of CKD based on Kidney Disease: Improving Global Outcomes criteria,[23,24] in addition to pregnant women with a presumed diagnosis of CKD based on either a raised creatinine (>85 μmol/l) in the absence of risk factors for acute kidney injury, or persistent proteinuria (urinary protein:creatinine ratio >30 mg/mmol) before 20 weeks' gestation.

Pregnant women were enrolled prospectively. Plasma (EDTA) and urine samples were collected at routine outpatient attendance. Samples were stored on ice before being centrifuged at 1500*g* for 10 minutes at 4 °C. The separated supernatant was aliquoted and stored at −80 °C.

Outcomes were based on predetermined criteria. In the absence of prepregnancy hypertension and proteinuria, standard diagnostic criteria were used for the diagnosis of superimposed preeclampsia.[2] For women with preexisting hypertension and proteinuria, in whom isolated rises in blood pressure and proteinuria are insufficient for a diagnosis of superimposed preeclampsia,[2] predefined diagnostic criteria for the purposes of this study included either the development of severe hypertension (>160/110 mm Hg) or an increment in antihypertensive treatment to maintain blood pressure <160/110 mm Hg, a doubling of proteinuria above the pathological threshold for pregnancy (300 mg/24 hours or urinary protein:creatinine ratio >30 mg/mmol), or clinical features of preeclampsia including liver involvement (alanine transaminase >71 U/l, right upper quadrant or epigastric pain), platelet count <100,000/μl, pulmonary edema, new-onset cerebral or visual disturbance, and fetal growth restriction.[4] In addition, all complex cases were reviewed and a diagnosis confirmed by 2 senior clinical staff with expertise in renal disease in pregnancy (KB, LCC, LL) assessing independently, and without access to study results.

Obstetric outcomes included mode of delivery, gestational age, preterm delivery defined as <37 and <34 weeks' gestation, neonatal unit admission, birthweight, and birthweight centile assessed as a customized birth weight percentile calculated using the Gestation Related Optimal Weight method by freely available software (www.pi.nhs.uk/download/graw/GRAWCentv1.xls[25]). Small for gestational age was reported as <10th and <3rd centile.

A nested case-control group was retrospectively selected based on time of disease (preeclampsia/superimposed preeclampsia). This included all women with CKD who had biological samples taken at the time of a diagnosis of superimposed preeclampsia. These women were matched for CKD stage and week of gestation with pregnant women with CKD who did not develop superimposed preeclampsia, and for week of gestation

with pregnant women without CKD who did, and did not, develop preeclampsia. Additional analysis of discriminatory plasma biomarkers was carried out in nonpregnant women of reproductive age with advanced CKD to assess the effect of reduced renal clearance on biomarker concentration.

Given that chronic hypertension is known to be an independent modifier of RAS,[26–28] the case-control group was extended to facilitate additional RAS analysis in women with, and without, chronic hypertension. This extended group included biological samples at gestations that were unmatched between study subgroups.

Biomarker Analysis

Plasma and urine samples were tested with masking of clinical outcomes. The selection of specific analytes was based on hypothesized mechanistic links between renal disease and preeclampsia, feasibility in the context of sampling methodology and long-term storage of biological samples at −80°C, and the authors' experience of biomarker research in pregnancy.[4,9,29] Hyaluronan, active renin, angiotensinogen, and complement components (C3a, C5a, C5b-9, complement factor H) were quantified using specific enzyme-linked immunosorbent assay kits according to manufacturers' protocols. Although the MIcroVue SC5b-9 enzyme-linked immunosorbent assay is not validated for urine, quantification of urinary C5b-9 has been achieved.[30,31] Predilution of urine was not required to achieve quantification of urinary C5b-9 within the detectable range, and plate shaking at 80 revolutions per minute was added to the manufacturer's protocol during both conjugate and substrate incubations to achieve satisfactory assay performance in urine, as previously described.[32] Extraction of C5a in urine was insufficiently sensitive and analysis was not possible. Plasma concentrations of intracellular adhesion molecule, VCAM, P-selectin, E-selectin, and Kidney Injury Molecule-1, and urinary concentrations of lipocalin-2 (NGAL) were quantified simultaneously using a Luminex (Austin, TX) Performance Assay multiplex kit according to the manufacturer's protocol and read by a Luminex FlexMap3D analyzer system. Urine creatinine was quantified to report urinary biomarker concentrations as a normalized ratio to urinary creatinine concentration. Plasma PlGF was quantified using the Triage PlGF Test by Alere (Waltham, MA).[4,33] Manufacturers' details and measures of precision are given in Table 1.

Statistical Analysis

Categorical data were examined by the use of Fisher exact test. For continuous data, a Mann-Whitney test was used. As the biomarkers had log-normal distributions, *t*-tests of log-transformed data were used to generate geometric mean ratios of biomarker concentration, including interval regression as appropriate for concentrations censored at the upper or lower limit of detection. Although the study subgroups were matched for prepregnancy CKD stage and sample gestation, interval regression was also used to examine for any significant effect due to these variables.[34] Nonparametric receiver operating characteristic curve analyses examined the capacity of each biomarker to discriminate preeclampsia from normal pregnancy, both in women with and without CKD. Optimal cut-points were determined for each discriminatory biomarker through the examination of the Youden Index[35] in women with and without CKD. To correct for gestational variation, plasma PlGF concentration between 20 and 37 weeks was transformed into a PlGF centile calculated using data from 1366 samples from 247 women without preeclampsia.[33] Correlation between the biomarkers of interest and PlGF centile was measured using nonparametric Spearman correlation.

Table 1. Biomarker assay details

Analyte	Assay kit	Detectable range	Intra-assay CV (%)	Inter-assay CV (%)
Active renin	IBL GMBH RE53321	0.81–128 pg/ml	4.0	11.6
Angiotensinogen	IBL GMBH REJP27412	0.31–20 ng/ml	Plasma 4.2 Urine 4.8	Plasma 14.1 Urine 11.6
Hyaluronan	R&D DHYALO	0.625–40 ng/ml	4.2	14.9
C3a	Hycult Biotech HK354	31.3–2000 pg/ml	8.1	13.4
C5a	Hycult Biotech HK349	0.3–20 ng/ml	6.4	12.0
Complement factor H	Microvue SC5–9 Plus EIA	8.8 ng/ml	8.1	11.8
C5b-9	Hycult Biotech HK342	3.9–250 ng/ml	3.4	6.9
Intercellular adhesion molecule	R&D Human Adhesion Molecules LKT007	52.5–18716 pg/ml	4.6	12.3
Vascular cell adhesion molcule		10–9233 pg/ml	5.0	9.0
P-selectin		135–23246 pg/ml	5.8	17.7
E-selectin		123–23978 pg/ml	5.0	11.7
Kidney Injury Molecule -1	R&D Kidney Biomarker Premixed Kit FCSTM16	159–116,000 pg/ml	10.1	10.5
Lipocalin 2 (NGAL)		50–36,100 pg/ml	3.8	8.8
Urine creatinine	Roche Diagnostics enzymatic creatinine method		0.8	2.1

Statistical analysis was performed using GraphPad (La Jolla, CA) Prism 7 XML and Stata 15.1 (StataCorp, College Station, TX).

RESULTS

Study Cohorts

The cases in the case-control group were 15 women with CKD with superimposed preeclampsia. The controls were (i) 45 women with CKD but without superimposed preeclampsia matched for gestation and prepregnancy CKD stage, (ii) 18 women without CKD but with preeclampsia matched for gestation, and (iii) 20 women without CKD or preeclampsia matched for gestation. In addition, quantification of discriminatory biomarkers was carried out in 22 nonpregnant women with CKD stages 2 to 4 (median estimated glomerular filtration rate 35 ml/min per 1.73 m^2, range 23–48 ml/min per 1.73 m^2). Standard diagnostic criteria were used for the diagnosis or exclusion of superimposed preeclampsia in 25% (15 of 60) of the women with CKD. Demographic (Table 2) and outcome data (Table 3) are shown.

The extended group used in the analysis of RAS biomarkers was an extension of the case-control group and included an additional 3 women with superimposed preeclampsia, 20 women with CKD but no superimposed preeclampsia, 11 women with preeclampsia, 23 women with chronic hypertension in the absence of both CKD and preeclampsia, and 45 additional healthy pregnant controls. Demographic and

Table 2. Nested case-control cohort demographics

Diagnosis	Superimposed preeclampsia	CKD	Preeclampsia	Normal pregnancy	Nonpregnant
n	15	45	18	20	22
Gestation of sample	33.0	32.0	33.3	33.1	N/A
Median (IQR)	(30.6–34.7)	(30.3–33.7)	(31.1–34.3)	(27.6–33.7)	N/A
Age (yr)	31.0	34.0	31	31	35
Median (IQR)	(25.5–34.5)	(28.9–36.1)	(27.8–35.6)	(29.0–33.3)	(30.3–38.5)
BMI (kg/m^2)	26.0	24.0	27.0	23.2	N/A
Median (IQR)	(23.8–32.8)	(22.3–29.8)	(24.2–31.1)	(21.1–28.5)	
Ethnicity					
• White (%)	5 (33)	26 (58)	5 (28)	17 (85)	10 (45)
• Black (%)	7 (47)	10 (22)	11 (61)	2 (10)	6 (27)
• Asian (%)	0 (0)	4 (16)	2 (11)	1 (5)	5 (23)
• Other (%)	3 (20)	5 (11)	0 (0)	0 (0)	1 (5)
Nulliparous (%)	10 (67)	21 (47)	10 (56)	15 (75)	N/A
SBP[a]	119	115	120	109	126
Median (IQR)	(110–122)	(110–122)	(111–128)	(101–110)	(120–136)
DBP[a]	74	73	75	62	75
Median (IQR)	(70–83)	(66–80)	(70–85)	(60–70)	(70–82)
Chronic hypertension (%)	8 (53)	16 (36)	5 (28)	0 (0)	14 (64)
Treatment for BP <20 weeks/nonpregnant (%)	5 (33)	10 (22)	2 (11)	0 (0)	13 (59)
CKD stage					
1 (%)	8 (53)	32 (71)	0 (0)	0 (0)	0 (0)
2 (%)	3 (20)	6 (13)	0 (0)	0 (0)	1 (5)
3 (%)	2 (13)	7 (15)	0 (0)	0 (0)	14 (64)
4 (%)	0 (0)	0 (0)	0 (0)	0 (0)	5 (23)
5 (%)	0 (0)	0 (0)	0 (0)	0 (0)	2 (9)
Unknown	2 (13)	0 (0)	0 (0)	0 (0)	0 (0)
Renal diagnosis (%)					
Lupus	3 (20)	15 (33)	N/A	N/A	2 (9)
Non-lupus GN	2 (13)	10 (22)	N/A	N/A	9 (41)
Diabetic nephropathy	4 (27)	2 (4)	N/A	N/A	1 (5)
Reflux/CAKUT	2 (13)	4 (9)	N/A	N/A	5 (23)
Other	1 (7)	4 (9)	N/A	N/A	1 (5)
Unknown	3 (20)	10 (22)	N/A	N/A	4 (18)
Transplant (%)	2 (13)	3 (7)	0 (0)	0 (0)	9 (41)
≥2+ Proteinuria at booking (%)	6 (40)	15 (33)	0 (0)	0 (0)	N/A
Nonpregnant PCR[b] >50 mg/mmol	N/A	N/A	N/A	N/A	5 (36)
Prepregnancy DM (%)	4 (27)	3 (7)	0 (0)	0 (0)	1 (5)

BMI, body mass index; CAKUT, congenital abnormalities of kidney and urinary tract; CKD, chronic kidney disease (without preeclampsia); DBP, diastolic blood pressure; DM, diabetes mellitus; GN, glomerulonephritis; N/A, no relevant data, i.e., gestation; PCR, protein:creatinine ratio; SBP, systolic blood pressure.
[a]Blood pressure was the first value recorded in pregnancy or a nonpregnant value for nonpregnant controls
[b]Data available for 14 women.

Table 3. Nested case-control pregnancy outcomes

Outcome	Superimposed preeclampsia (SPE) (n = 15[a])	CKD (n = 45)	Preeclampsia (n = 18)	Normal pregnancy (n = 20[b])
Clinical features of preeclampsia				
Highest SBP	172[i]	130	170[p]	138
Median (IQR)	(162–175)	(120–146)	(158–189)	(130–140)
Highest DBP	97[e]	90	104[p]	83
Median (IQR)	(91–106)	(75–100)	(97–108)	(78–91)
Severe hypertension (SBP>160 or DBP>110 mm Hg)	11 (85)[j]	7 (16)	11 (61)[p]	0 (0)
Antihypertensives, i.v. (%)	3 (33)[g]	0 (0)	3 (17)	0 (0)
Magnesium sulphate, i.v. (%)	2 (13)	0 (0)	5 (28)[j]	0 (0)
Highest urine PCR	126	105	55	6[c]
Median (IQR)	(113–662)	(33–222)	(45–82)	
Doubling of proteinuria >30 mg/mmol (%)	10 (77)[i]	1 (2)	1 (6)[q]	0 (0)
ALT>70 IU/l (%)	0 (0)	3 (7)	3 (17)	0 (0)
Platelet count <100 × 10^9/l (%)	0 (0)	1 (2)	1 (6)	0 (0)
Delivery outcomes				
Vaginal delivery (%)	1 (7)[g]	21 (47)	6 (33)[m]	16 (80)
Cesarean delivery (%)	14 (93)[g]	24 (53)	12 (67)[m]	4 (20)
Emergency cesarean delivery (%)	9 (60)[d]	14 (31)	8 (44)	3 (15)
Neonatal outcomes				
Gestational age	34.0[i]	38.0	35.0[k]	40.6
Median (IQR)	(32.4–36.6)	(37.3–39.6)	(32.6–35.4)	(39.9–41.0)
Preterm <37 weeks (%)	11 (73)[i]	6 (13)	15 (83)[p]	0 (0)
Preterm <34 weeks (%)	5 (33)[f]	2 (4)	6 (33)[l]	0 (0)
NNU admission (%)	7 (47)[h]	2 (4)	9 (50)[o]	1 (5)
Birthweight, g	2050[i]	3132	1985[p]	3393
Median (IQR)	(1284–2655)	(2640–3220)	(1588–2313)	(3203–3715)
Birthweight <10th centile (%)	7 (47)	11 (24)	10 (55)[n]	2 (10)
Birthweight <3rd centile (%)	5 (33)[f]	2 (4)	6 (33)[l]	0 (0)

ALT, alanine aminotransferase; CKD, chronic kidney disease; DBP, diastolic blood pressure; NICU, neonatal intensive care unit; NNU, neonatal unit admission; PCR, protein:creatinine ratio; SBP, systolic blood pressure.
[a]Outcome data for 11–15 women.
[b]Outcome data for 16–20 women.
[c]PCR quantified in a single healthy pregnant control.
Comparison between superimposed preeclampsia and CKD: [d]$P = 0.047$, [e]$P = 0.026$, [f]$P = 0.008$, [g]$P = 0.004$, [h]$P = 0.0004$, [i]$P < 0.0001$.
Comparison between preeclampsia and normal pregnancy: [j]$P = 0.017$, [k]$P = 0.014$, [l]$P = 0.007$, [m]$P = 0.005$, [n]$P = 0.003$, [o]$P = 0.002$, [p]$P < 0.0001$.
Comparison between superimposed preeclampsia and preeclampsia: [q]$P = 0.0004$.

outcome data from this longitudinal group are shown in Supplementary Table S1.

There were no significant baseline differences in women who developed superimposed preeclampsia compared with those with CKD who did not develop superimposed preeclampsia including age, body mass index, black/nonblack ethnicity, parity, prevalence of chronic hypertension, and booking levels of blood pressure and proteinuria. Higher systolic and diastolic blood pressures during pregnancy and a greater relative increase in proteinuria in women with superimposed preeclampsia were consistent with the criteria used for diagnosis. Women with superimposed preeclampsia delivered at an earlier gestation compared with women with CKD who did not develop superimposed preeclampsia. The infants of women with superimposed preeclampsia were also more likely to be born both preterm (<37 weeks) and very preterm (<34 weeks), be small for gestational age, and require neonatal unit admission. These differences were also evident in women with preeclampsia in the absence of CKD compared with women with normal pregnancies. There were no significant differences in obstetric outcomes between women with superimposed preeclampsia compared with those with preeclampsia in the absence of CKD, with the exception of proteinuria, which was higher in women with superimposed preeclampsia consistent with underlying CKD. The nonpregnant controls were selected to allow biomarker quantification at higher stages of CKD; these women had an increased prevalence of chronic hypertension and proportion of women with renal transplants compared with pregnant CKD groups. However, absolute levels of blood pressure and rates of clinically significant proteinuria were comparable across all CKD study groups.

Discriminatory Biomarkers: Plasma Hyaluronan, Plasma VCAM

Plasma hyaluronan and plasma VCAM concentrations were significantly higher in women with superimposed preeclampsia compared with women with CKD in the absence of superimposed preeclampsia, and in women

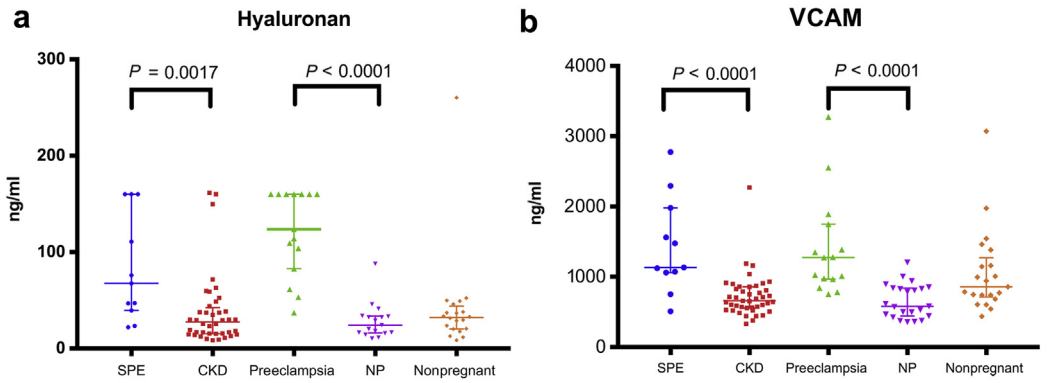

Figure 1. Plasma hyaluronan (a) and plasma vascular cell adhesion molecule (VCAM) (b) concentrations in superimposed preeclampsia (SPE), women with chronic kidney disease (CKD) who do not develop preeclampsia, women with preeclampsia (in the absence of CKD), normal pregnancy (NP), and in nonpregnant controls with CKD stages 2–4 (bars at median and interquartile range).

with preeclampsia compared with those with normal pregnancies (Figure 1, Table 4). Area under the curve estimations for prediction of superimposed preeclampsia for hyaluronan were 0.98 (95% confidence interval [CI]: 0.94–1.00) for women without CKD and 0.80 (95% CI: 0.65–0.94) for women with CKD. Equivalent values for VCAM were 0.91 (95% CI: 0.82–1.00) for women without CKD and 0.86 (95% CI: 0.71–1.00) for women with CKD (Figure 2, Table 4). Sensitivity, specificity, positive and negative predictive values are given in Table 5. Both plasma hyaluronan ($P < 0.0001$) and plasma VCAM ($P < 0.0001$) showed a significant correlation with PlGF, with an increase in both biomarkers when PlGF was <10th centile for gestation (Figure 3).

Plasma hyaluronan ($P < 0.0001$; geometric mean ratio 4.93; 95% CI: 3.01–8.108) and plasma VCAM concentrations ($P < 0.0001$; geometric mean ratio 1.88; 95% CI: 1.49–2.36) were also higher in preeclampsia (in the absence of CKD) compared with CKD (in the absence of preeclampsia) facilitating diagnostic distinction between previously unknown or new CKD in pregnancy, from preeclampsia.

There was no detectable difference in hyaluronan concentration in nonpregnant women with CKD stage 2–4 (median 32.2 ng/ml, interquartile range [IQR] 20.3–44.0 ng/ml) compared with both normal pregnancy (geometric mean ratio 0.79; 95% CI: 0.50–1.26; $P = 0.23$) and CKD in the absence of preeclampsia (geometric mean ratio 0.98; 95% CI: 0.66–1.46; $P = 0.52$). However, plasma VCAM was increased in nonpregnant women with CKD stage 2–4 (median 857 ng/ml; IQR 711–1270 ng/ml) compared with both normal pregnancy (geometric mean ratio 1.54; 95% CI: 1.22–1.94; $P = 0.005$) and CKD in the absence of superimposed preeclampsia (geometric mean ratio 1.38; 95% CI: 1.12–1.70; $P = 0.03$), although this was not explained by the higher number of women with renal transplants in the nonpregnant group. No detectable difference in VCAM concentration was detected in nonpregnant women with renal transplants (median 857 ng/ml; IQR 670–1151 ng/ml) compared with those without (median 883 ng/ml; IQR 698–1442 ng/ml), and differences in plasma VCAM concentration in superimposed preeclampsia (median 1476 ng/ml; IQR 911–2136 ng/ml) compared with CKD (median 688 ng/ml; IQR 559–858 ng/ml) remained after exclusion of women with renal transplants ($P < 0.0001$).

Prepregnancy estimated glomerular filtration rate and gestation were not significant modifiers of either hyaluronan or VCAM concentration (see Supplementary Table S2).

Variable Discrimination: Plasma Active Renin, Urinary Angiotensinogen:Creatinine

Plasma active renin was lower in superimposed preeclampsia compared with CKD (geometric mean ratio 0.27; 95% CI: 0.15–0.49) and in preeclampsia compared with normal pregnancy (geometric mean ratio 0.18; 95% CI: 0.13–0.27) (Table 4). However, active renin concentrations were also lower in women with chronic hypertension compared with normotensive pregnant women (median 8.2 pg/ml; IQR 3.5–15.7) versus 17.2 pg/ml (IQR 12.2–25.0; $P < 0.0001$; geometric mean ratio 0.40; 95% CI: 0.30–0.53) and the detected correlation between active renin and PlGF in normotensive women ($r = 0.33$; 95% CI: 0.17–0.48; $P < 0.0001$) was not evident in women with chronic hypertension ($r = 0.06$; 95% CI: −0.14 to 0.26; $P = 0.55$). Urinary angiotensinogen:creatinine was higher in superimposed preeclampsia compared with CKD (geometric mean ratio 8.72; 95% CI: 3.07–24.76), and in preeclampsia compared with normal pregnancy (geometric mean ratio 3.65; 95% CI: 1.32–10.07) (Table 4), although there was no measurable correlation between urinary angiotensinogen:creatinine and PlGF centile ($r = -0.14$; 95%

Table 4. Quantification of biomarkers in superimposed preeclampsia, CKD (without preeclampsia), preeclampsia (without CKD), and in normal pregnancy

Biomarker	Plasma/ urine	Superimposed preeclampsia	CKD	P[a]	Geometric mean ratio[a] (95% CI)	ROC AUC[a] (95% CI)	Preeclampsia	Normal pregnancy	P[b]	Geometric mean ratio[b] (95% CI)	ROC AUC[b] (95% CI)
Discriminatory											
Hyaluronan (ng/ml)	Plasma	67.5 (39.5–160)	27.5 (15.4–42.5)	0.0017	2.47 (1.49–4.07)	0.80 (0.65–0.94)	123.7 (82.9–160)	32.2 (20.3–44.0)	<0.0001	6.12 (3.53–10.62)	0.98 (0.94–1.00)
VCAM (ng/ml)	Plasma	1132 (1060–1979)	659 (556–857)	<0.0001	1.89 (1.46–2.44)	0.86 (0.71–1.00)	1275 (966–1748)	579 (442–840)	<0.0001	2.09 (1.62–2.69)	0.91 (0.82–1.00)
PlGF centile[c]	Plasma	0 (0–4)	18 (8–40)	0.002			0 (0–1)				
Variable discrimination											
Active renin (pg/ml)	Plasma	2.87 (1.12–6.15)	11.18 (6.48–19.01)	0.02	0.27 (0.15–0.49)	0.89 (0.77–1.00)	5.56 (0.81–9.58)	17.19 (12.22–24.94)	<0.0001	0.18 (0.13–0.27)	0.92 (0.86–0.98)
Angiotensinogen:creatinine (ng/micromol)	Urine	127.5 (61.0–166.6)	9.7 (3.8–27.6)	<0.001	8.72 (3.07–24.76)	0.89 (0.78–1.00)	18.6 (6.8–85.9)	7.1 (2.7–15.6)	<0.01	3.65 (1.32–10.07)	0.76 (0.63–0.89)
CFH (mcg/ml)	Plasma	1053 (616–1228)	1060 (886–1194)	0.69	0.87 (0.72–1.06)	0.54 (0.31–0.77)	672 (567–805)	1116 (1006–1171)	<0.0001	0.62 (0.50–0.78)	0.91 (0.80–1.00)
Nondiscriminatory											
Angiotensinogen (ng/ml)	Plasma	121 (87–137)	111 (86–131)	0.53	1.22 (0.60–2.48)	0.57 (0.33–0.81)	107 (70–131)	114 (89–150)	0.07	0.59 (0.40–0.87)	0.39 (0.28–0.50)
C3a (ng/ml)	Plasma	63.5 (48.0–74.5)	49.8 (41.1–64.3)	0.15	1.16 (0.91–1.49)	0.64 (0.46–0.83)	43.7 (29.6–60.2)	34.2 (30.2–50.3)	0.48	1.13 (0.85–1.50)	0.59 (0.35–0.82)
C3a:creatinine (ng/micromol)	Urine	45.2 (10.2–236.3)	46.7 (11.4–233)	0.78	0.84 (0.26–2.77)	0.47 (0.24–0.69)	14.2 (11.4–30.5)	4.2 (4.2–24.2)	0.53	2.45 (0.33–18.23)	0.64 (0.22–1.00)
C5a (ng/ml)	Plasma	1.25 (1.25–1.28)	1.25 (1.25–1.65)	0.49	0.45 (0.07–2.81)	0.44 (0.289–0.59)	1.25 (1.25–2.17)	1.45 (1.25–3.37)	0.19	0.27 (0.05–1.61)	0.37 (0.19–0.55)
C5b-9 (ng/ml)	Plasma	367 (297–509)	339 (249–443)	0.29	1.24 (0.93–1.65)	0.61 (0.43–0.79)	294 (225–429)	238 (193–360)	0.30	1.13 (0.84–1.51)	0.61 (0.40–0.82)
C5b-9:creatinine (ng/micromol)	Urine	3.02 (0.30–4.80)	0.60 (0.33–1.69)	0.25	2.04 (0.74–5.67)	0.62 (0.40–0.84)	0.73 (0.43–0.98)	0.41 (0.41–0.73)	0.31	1.08 (0.34–3.42)	0.62 (0.35–0.89)
E-selectin (ng/ml)	Plasma	34.1 (21.0–49.4)	39.6 (27.7–43.7)	0.80	1.01 (0.76–1.35)	0.53 (0.31–0.75)	46.7 (24.2–55.8)	35.2 (26.6–43.2)	0.15	1.24 (0.93–1.64)	0.64 (0.44–0.84)
ICAM (ng/ml)	Plasma	121.5 (87.7–152.1)	147.0 (115.5–171.4)	0.13	0.67 (0.47–0.97)	0.35 (0.14–0.56)	137.7 (102.9–182.2)	131.2 (105.1–154.5)	0.64	1.11 (0.78–1.60)	0.55 (0.34–0.75)
KIM-1 (pg/ml)	Plasma	352 (313–402)	313 (273–402)	0.42	1.07 (0.85–1.35)	0.66 (0.40–0.91)	293 (253–303)	263 (253–290)	0.47	1.05 (0.81–1.35)	0.43 (0.00–0.88)
Lipocalin-2:creatinine (ng/micromol)	Urine	10.3 (3.8–25.6)	11.1 (5.8–32.9)	0.60	1.53 (1.09–2.14)	0.44 (0.23–0.65)	7.9 (4.8–12.8)	1.7 (1.0–6.2)	0.05	0.76 (0.52–1.10)	0.73 (0.51–0.95)
P-selectin (ng/ml)	Plasma	55.2 (39.3–66.4)	52.4 (40.4–62.6)	0.82	1.04 (0.84–1.28)	0.52 (0.33–0.72)	53.3 (41.0–68.8)	57.7 (44.7–71.1)	0.37	0.87 (0.71–1.07)	0.41 (0.22–0.60)

CFH, complement factor H; CI, confidence interval; CKD, chronic kidney disease; ICAM, intracellular adhesion molecule; KIM-1, Kidney Injury Molecule-1; PlGF, placenta growth factor; ROC AUC, area under the receiver operating curve; VCAM, vascular cell adhesion molecule.
[a]Comparison between CKD and superimposed preeclampsia.
[b]Comparison between preeclampsia and normal pregnancy.
[c]PlGF centile not derived >37 weeks' gestation.
Values are median (interquartile range).

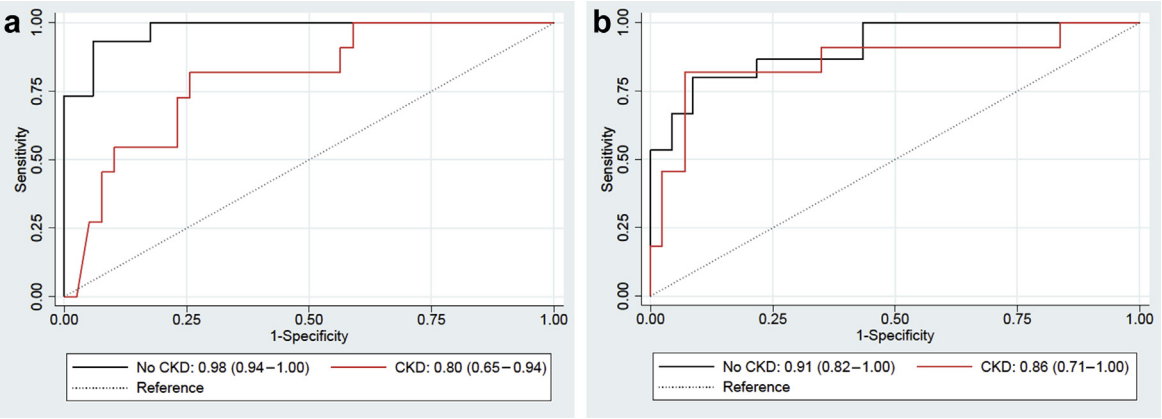

Figure 2. Receiver operating characteristic (ROC) curve of plasma hyaluronan (a) and plasma vascular cell adhesion molecule (VCAM) (b) in the diagnosis of preeclampsia in women with (red) and without (black) chronic kidney disease (CKD). Values are area under the curve with 95% confidence intervals.

CI: −0.28 to 0.11; $P = 0.06$). The diagnostic discrimination of both plasma active renin and urinary angiotensinogen:creatinine was reduced in women with chronic hypertension, women requiring antihypertensive treatment before 20 weeks' gestation and in black ethnicity (Table 6).

Plasma complement factor H was lower in preeclampsia compared with normal pregnancy. However, no detectable differences were seen in superimposed preeclampsia where diagnostic distinction was poor (Table 4).

Nondiscriminatory Markers

Quantification of plasma angiotensinogen, C3a, C5a, C5b-9, E-selectin, intercellular adhesion molecule, Kidney Injury Molecule-1, and P-selectin, and urinary lipocalin-2 failed to discriminate women with superimposed preeclampsia from CKD in the absence of preeclampsia, and preeclampsia from normal pregnancy.

DISCUSSION

Diagnosis of superimposed preeclampsia remains challenging in women with CKD, and underlying pathophysiology predisposing to increased risk is poorly understood. This study demonstrates that quantification of plasma hyaluronan and plasma VCAM have the potential to distinguish superimposed preeclampsia in pregnant women with CKD. Correlation of both hyaluronan and VCAM with serum PlGF concentration supports the diagnostic criteria used in this study to define superimposed preeclampsia. Suggested cutoffs of for the diagnosis of preeclampsia of >39 ng/ml for plasma hyaluronan and >950 ng/ml for plasma VCAM have a sensitivity of 82% and a negative predictive value of 94% to 95% in women with CKD. All other markers tested showed variable discrimination or failed to discriminate preeclampsia in women with and without CKD.

Plasma hyaluronan and VCAM are components of the endothelial cell glycocalyx, which is a negatively charged protective layer that sits between the endothelial cell surface and the flow of blood. It is thought to play a physiological role in vascular protection, modulation, and hemostasis.[36] An increase in circulating concentrations of hyaluronan and VCAM is hypothesized to represent damage to the endothelial glycocalyx. The model of preeclampsia as an endothelial disorder driven by placentally derived antiangiogenic factors is supported by both the systemic nature of disease and its resolution following delivery.[14] Endothelial dysfunction in CKD is manifest by accelerated vascular disease, as well as proteinuria, which is a clinical manifestation of intrarenal endothelial disease.[15] Thus, endothelial glycocalyx dysfunction provides a mechanistic link between CKD and preeclampsia, whereby damage to the endothelial glycocalyx in CKD confers vulnerability to additional

Table 5. Sensitivity, specificity, positive and negative predictive values (%) with 95% confidence intervals for the prediction of preeclampsia in women with and without CKD

	Sensitivity		Specificity		Negative predictive value		Positive predictive value	
Biomarker	CKD	No CKD	CKD	No CKD	CKD	No CKD	CKD	No CKD
Hyaluronan >39 ng/ml	82 (47–97)	93 (66–97)	74 (58–86)	82 (56–95)	94 (77–99)	93 (66–100)	47 (25–71)	84 (56–95)
VCAM >950 ng/ml	82 (48–97)	80 (51–95)	91 (77–97)	91 (70–98)	95 (82–99)	88 (66–97)	69 (39–90)	86 (56–97)

CKD, chronic kidney disease; VCAM, vascular cell adhesion molecule.

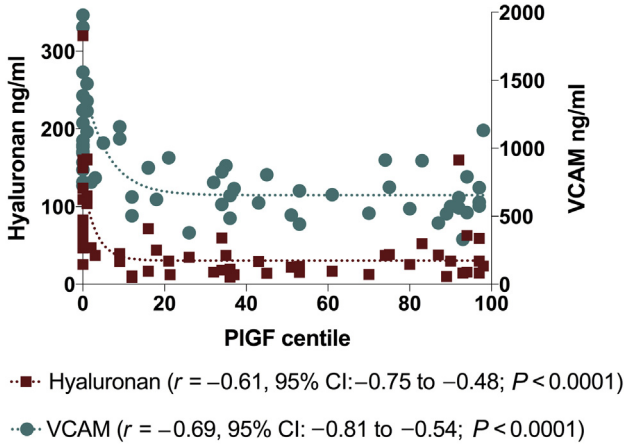

Figure 3. Correlation of plasma hyaluronan and plasma vascular cell adhesion molecule (VCAM) with placenta growth factor (PIGF) centile measured by Spearman's rank correlation coefficient.

injury in preeclampsia, offering a potential explanation for the increased rates of superimposed preeclampsia seen in women with CKD.[3,5] Although increases in both plasma hyaluronan and VCAM have been similarly shown in small cohort studies of pregnant women without underlying CKD,[37–41] including women with chronic hypertension,[42] this is the first report showing utility in pregnant women with CKD. This study also demonstrates the potential capacity of these markers to distinguish preeclampsia from a new diagnosis of CKD in pregnancy; a phenomenon that represents up to one-third of CKD in pregnancy.[43] Receiver operating curve areas of 0.80–0.86 are higher than those for clinical variables including systolic and diastolic blood pressure and the detection of *de novo* proteinuria on urine dipstick testing, which may not distinguish the need for delivery due to preeclampsia even in the absence of complicating CKD.[9]

Biomarkers of the RAS evaluated in this study included plasma active renin, plasma angiotensinogen, and urinary angiotensinogen:creatinine. Concentrations of plasma active renin were significantly lower in both preeclampsia and superimposed preeclampsia consistent with previously published data,[44] and the consensus hypothesis that hypertension in preeclampsia is due to an exaggerated pressor response to RAS, rather than a measurable increase in RAS components.[45,46] The finding of an increase in urinary angiotensinogen in preeclampsia without a measured increase in plasma levels raises the possibility of intrarenal synthesis of angiotensinogen in preeclampsia, with excretion into urine where it can be measured. This is substantiated by reports of angiotensinogen gene expression in renal biopsy tissue,[47] and the use of urinary angiotensinogen:creatinine as a marker of intrarenal RAS activation in both hypertension[48,49] and CKD.[47,50,51] However, diagnostic discrimination of both plasma active renin and urinary angiotensinogen:creatinine was reduced in the context of known modifiers of RAS, including chronic hypertension,[26–28] hypertensive drug use,[52] and ethnicity.[28] Such modifiers are important, as their prevalence in CKD cohorts is high. In this study, 40% (24/60) of women with CKD were hypertensive before pregnancy, which is comparable to other published cohorts,[3,4] 25% (15/60) were using antihypertensive medication before 20 weeks' gestation, and 28% (17/60) of women were of black ethnicity. In addition, gestational changes in angiotensinogen are described, including an altered ratio of oxidized to reduced forms in preeclampsia,[53] polymorphic conformational change, and pregnancy-specific high molecular weight forms.[54,55] Such factors may contribute to the inconsistency in published data with reports both supporting[56] and refuting[57] an association between high urinary angiotensinogen:creatinine and complicated pregnancy including preeclampsia. In the context of multiple confounders, the use of RAS biomarkers in the diagnosis of superimposed preeclampsia is likely to be complex, with limited clinical utility.

This multicenter study is novel in examining a well-defined cohort of pregnant women with CKD, rather than a mixed cohort of high-risk women; and in specifically examining for differences between women with CKD who do, and do not develop superimposed

Table 6. Area under the receiver operating curve values (SE) for the diagnosis of preeclampsia using plasma active renin and urinary angiotensinogen:creatinine in women with and without underlying CKD, adjusted for CHT, antihypertensive drug use before 20 weeks' gestation, and black/nonblack ethnicity

	Plasma active renin		Urinary angiotensinogen:creatinine	
	CKD	No CKD	CKD	No CKD
All extended cohort	0.80 (0.12)	0.88 (0.03)	0.82 (0.08)	0.80 (0.04)
Women without CHT	0.92 (0.04)	0.92 (0.04)	0.90 (0.06)	0.86 (0.04)
Women with CHT	0.61 (0.38)	0.76 (0.09)	0.68 (0.26)	0.61 (0.14)
No antihypertensive use <20 wk	0.92 (0.04)	0.92 (0.03)	0.88 (0.07)	0.83 (0.05)
Antihypertensive use <20 wk	0.61 (0.39)	0.73 (0.14)	0.67 (0.27)	0.65 (0.17)
Nonblack ethnicity	0.90 (0.05)	0.92 (0.04)	0.89 (0.08)	0.85 (0.05)
Black ethnicity	0.71 (0.26)	0.76 (0.08)	0.69 (0.17)	0.64 (0.12)

CHT, chronic hypertension; CKD, chronic kidney disease.

preeclampsia to identify diagnostic indicators and explore mechanistic pathways. The diagnostic criteria for preeclampsia superimposed on CKD are ambiguous[2,58]; however, this study used predefined blood pressure and proteinuria thresholds and expert diagnostic consensus masked to biomarker concentrations for all complex cases. In addition, correlation with PlGF was used as a confirmatory diagnostic adjunct. This was done on the basis of supporting data in women with a combination of chronic hypertension and/or CKD that demonstrate a capacity to predict the need for delivery, a low false-positive rate (3/161), and independence from serum creatinine.[4] Although circulating s-Flt1 levels were not assessed in this study, no difference has been demonstrated between the diagnostic performance of PlGF centile when compared with s-Flt1 concentration or s-Flt-1:PlGF ratio in women with CKD and chronic hypertension.[4,59]

This study is limited by the sample size. Despite matching for prepregnancy CKD stage, the possibility that reduced renal clearance impacts biomarker quantification also warrants further assessment. Although concentrations of hyaluronan in nonpregnant women with CKD stages 2 to 4 were comparable to that in normal pregnancy, concentrations of VCAM were increased in both superimposed preeclampsia and nonpregnant CKD. Although increased calcineurin inhibitors have been associated with increased VCAM messenger RNA synthesis,[60] exclusion of women with renal transplants from the study data had no impact on the results, suggesting the possibility of confounding by reduced glomerular filtration. There are limited data showing increases in both hyaluronan[61] and VCAM[15] with reduced renal function and validation of the diagnostic capacity of these markers in pregnant women with advanced CKD is warranted. The urinary markers in this study used a ratio to urinary creatinine to correct for urinary flow rate and concentration. This was based on an assumption that urinary creatinine excretion is constant, and that biomarker excretion has a linear relationship with creatinine excretion,[62] which does not explicitly address ethnic differences in creatinine production and excretion, or false amplification due to acute kidney injury, which may exist in the context of both preeclampsia and superimposed preeclampsia. Recruitment to this study was pragmatic, with samples taken from participants at their convenience, coordinated with other hospital attendances. In the absence of a standardized gestation over which longitudinal changes in biomarker concentration could be examined, analysis was restricted to concentrations at the time of disease (preeclampsia/superimposed preeclampsia) when biomarker changes were anticipated to be most exaggerated. Whether longitudinal changes in biomarker concentration are predictive or diagnostic of superimposed preeclampsia remains unknown.

The findings of this exploratory study suggest that quantification of plasma hyaluronan and VCAM have the potential to aid in the diagnosis of superimposed preeclampsia. Current standard diagnostic criteria can be used only in women who do not have preexisting hypertension and proteinuria, excluding 75% of women with CKD in this cohort. Test performances for plasma hyaluronan and VCAM are comparable to that of soluble fms-like tyrosine kinase-1:PlGF ratio >85,[4] which is based on an antiangiogenic, placental driven model of preeclampsia. However, a placental model of disease may be an inadequate representation of the pathophysiology of superimposed preeclampsia in women with CKD. The consistent association between CKD and preeclampsia, including an increment in the risk of preeclampsia with increasing CKD severity, suggests a significant maternal contribution to the disease process in superimposed preeclampsia. Yet, mechanisms by which maternal CKD influences the pathology of preeclampsia remain unknown, and maternal drivers of disease are unmeasured with the isolated use of placental biomarkers. Quantification of plasma hyaluronan and VCAM therefore offers a potential measure of "maternal disease" in the prediction, diagnosis, and long-term prognosis of superimposed preeclampsia in women with CKD. The findings of this study warrant validation in a larger, prospective cohort including pregnant women with advanced stage CKD, and multivariable analysis to assess the diagnostic value of these novel biomarkers over existing clinical parameters including PlGF and soluble fms-like tyrosine kinase-1.

DISCLOSURE

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SUPPLEMENTARY MATERIAL

Table S1. Extended cohort characteristics.

Table S2. Interval regression showing the impact of matched variables on biomarker concentration.

Supplementary material is linked to the online version of the paper at www.kireports.org.

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