# Subclinical First Trimester Renal Abnormalities Are Associated With Preeclampsia in Normoalbuminuric Women With Type 1 Diabetes

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## OBJECTIVE

**This study was conducted to determine the utility of tubular (urinary/plasma neutrophil gelatinase-associated lipocalin [NGAL] and urinary kidney injury molecule 1 [KIM-1]) and glomerular (estimated glomerular filtration rate [eGFR]) biomarkers in predicting preeclampsia (PE) in pregnant women with type 1 diabetes mellitus (T1DM) who were free of microalbuminuria and hypertension at the first trimester.**

## RESEARCH DESIGN AND METHODS

**This was a prospective study of T1DM pregnancy. Maternal urinary and plasma NGAL, urinary KIM-1 (ELISA of frozen samples), and eGFR (Chronic Kidney Disease Epidemiology Collaboration equation) were determined at three study visits (V1: 12.4 ± 1.8; V2: 21.7 ± 1.4; V3: 31.4 ± 1.5 weeks' gestation [mean ± SD]) in 23 women with T1DM with subsequent PE (DM+PE+), 24 who remained normotensive (DM +PE−), and, for reference, in 19 normotensive pregnant women without diabetes (DM−). The groups with diabetes were matched for age, diabetes duration, and parity. All subjects were normotensive and free of microalbuminuria or albuminuria at V1. All study visits preceded the onset of PE.**

## RESULTS

**Urinary creatinine-corrected NGAL (uNGALcc, ng/mg) was significantly elevated at V1 in DM+PE+ vs. DM+PE− women ($P$ = 0.01); this remained significant after exclusion of leukocyte-positive samples (5 DM+PE+ and 2 DM+PE−) ($P$ = 0.02). Accounting for BMI, $HbA_{1c}$, and total daily insulin dose, a doubling of uNGALcc at V1 conferred a sevenfold increase in risk for PE ($P$ = 0.026). In contrast, neither plasma NGAL nor urinary KIM-1 predicted PE. Also at V1, eGFR was elevated in DM+PE+ vs. DM+PE− ($P$ = 0.04).**

## CONCLUSIONS

**Early tubular and glomerular dysfunction may predict PE in first trimester women with T1DM, even if free of microalbuminuria. These data suggest that subclinical renal tubular and glomerular injury, if present early in pregnancy, may predispose women with T1DM to PE.**


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Preeclampsia (PE) is a major cause of morbidity and mortality in pregnant women and infants. It is defined as new-onset hypertension accompanied by proteinuria (or other end-organ dysfunction) occurring after 20 weeks' gestation in a previously normotensive woman (1). Its prevalence in the general population is 4–6% (2), but in women with pregestational type 1 diabetes mellitus (T1DM), the rate of PE is three to four times higher (3,4), even in those free of microalbuminuria early in pregnancy (5). PE is associated with maternal renal disease later in life (6,7), and in the presence of diabetes, shared features of renal disease and PE (8,9) make it difficult to decipher whether PE predisposes to renal disease or vice versa (10–13). Thus in women with T1DM, subclinical renal dysfunction, even before the onset of microalbuminuria, could predispose to PE. Biomarkers to detect such early renal damage, either tubular or glomerular, could have great clinical utility.

Human neutrophil gelatinase-associated lipocalin (NGAL) was first described as a product of activated neutrophils (14) but is now known to be widely expressed, including by infected, inflamed, or ischemic epithelia and other tissues (15–17). Its role as an early and sensitive biomarker for acute and chronic kidney disease was first described by Mishra et al. (18). Subsequent work showed NGAL is protective against ischemic and nephrotoxic injuries (19) and established its utility as a "real-time indicator of active kidney damage" (20,21); however, prospective studies to test the associations between NGAL and PE are sparse. Kidney injury molecule 1 (KIM-1) is considered a marker of renal proximal tubular damage (21) but may have less predictive power than NGAL (22,23).

Diabetes-induced changes to the glomerular vasculature play important roles in the development of albuminuria and proteinuria, and a transient elevation of the glomerular filtration rate (GFR) occurs early in the evolution of nephropathy (24). Glomerular hyperfiltration is also one the earliest renal changes during normal pregnancy (25), but the extent of hyperfiltration, reflected by GFR early in T1DM pregnancy, might provide an early marker for PE. We investigated whether urinary NGAL (uNGAL) and/or plasma NGAL (pNGAL), urinary KIM-1, and estimated GFR (eGFR) predict subsequent PE in pregnant women with T1DM.

## RESEARCH DESIGN AND METHODS

### Study Design and Participants

The study was approved by the Institutional Review Boards of all participating institutions, was conducted according to the principles of the Declaration of Helsinki, and was approved by the School of Medicine, Dentistry and Biomedical Sciences Ethics Committee, Queen's University, Belfast. Written informed consent was obtained from all study participants.

Design, participants, and inclusion/exclusion criteria have been described previously (5,26,27). Briefly, 151 pregnant women with established T1DM and 24 pregnant women without diabetes were enrolled in the first trimester and monitored throughout pregnancy. Most important, all eligible enrollees were normotensive and free of microalbuminuria or overt proteinuria (i.e., urinary albumin-to-creatinine ratios were $<$30 mg/g at the first study visit [gestational age 9–16 weeks]). Clinical data and blood and urine samples were collected at three study visits: late first trimester (V1: gestation 12.4 $\pm$ 1.8 weeks [mean $\pm$ SD]), mid-second trimester (V2: 21.7 $\pm$ 1.4 weeks), and early third trimester (V3: 31.4 $\pm$ 1.5 weeks). Urine aliquots were stored frozen at $-$80°C. Serum and plasma were obtained from fasting blood samples and stored at $-$80°C.

PE was defined as new-onset hypertension ($>$140/90 mmHg) and proteinuria ($>$300 mg/24 h) after 20 weeks of gestation in a previously normotensive woman. For the current report, we analyzed available samples from an original total of 26 women with T1DM who developed PE (DM+PE+, $n$ = 23; 3 lost as a result of sample attrition), from a matched group of 26 women with T1DM who remained normotensive (DM+PE$-$; $n$ = 24 after attrition), and from 19 normotensive women without diabetes (DM$-$) to obtain reference values. The two groups with diabetes were matched as closely as possible by age, diabetes duration, $HbA_{1c}$, and parity. V1 urine samples from an additional 27 unmatched DM+PE$-$ women were subsequently added for secondary analysis to check the robustness of a logistic regression model.

Medication usage was recorded at each study visit. All patients with diabetes were taking insulin. At V1 and thereafter, most patients were taking vitamin supplements and folic acid, and their use did not differ by PE outcome or presence of diabetes. No patient was taking nonsteroidal anti-inflammatory drugs or any other potentially nephrotoxic agent. Two were taking thyroid hormone replacement. Six patients took one of the following: low-dose aspirin, cephalexin, metformin, carbamazepine, albuterol, or ondansetron.

### Laboratory Measures

uNGAL and pNGAL (ng/mL) were measured using Human Lipocalin-2/NGAL Quantikine ELISA Kits (R&D Systems, Minneapolis, MN), according to the manufacturer's protocols. uNGAL was creatinine corrected (uNGALcc, ng/mg). Urinary KIM-1 (ng/mL) was also measured by Quantikine ELISA (R&D Systems) and then creatinine-corrected (ng/mg). Before assay, samples were maintained frozen at $-$80°C from collection, and NGAL and KIM-1 detection were unaffected by freeze/thaw cycles during assay validation. The intra- and interassay coefficients of variation were 2.2% and 10.2%, respectively. Urinary creatinine was measured at University of Oklahoma Health Sciences Center Clinical Chemistry Laboratory, as previously described (5). Urinary leukocyte status was defined as positive (i.e., trace, +, ++, or +++) or negative by Multistix 10SG urinalysis reagent strips (Siemens, Munich, Germany).

Serum creatinine was measured at the Department of Clinical Biochemistry, Royal Victoria Hospital, Belfast, Northern Ireland. eGFR was calculated at each study visit from the serum creatinine concentration, using the Chronic Kidney Disease Epidemiology Collaboration equation (28). All women had an eGFR $\geq$60 mL/min/1.73 $m^2$ and thus were classified as having normal renal function.

### Statistical Analysis

Symmetrically distributed variables were summarized using mean and SD; those that were positively skewed were expressed as median (interquartile range) or geometric mean (95% CI), as appropriate. Group comparisons for categorical variables used $\chi^2$ tests and independent samples $t$ tests or Mann-Whitney tests, as appropriate, for continuous variables. Repeated measures analyses were conducted using the Friedman test. Logistic regression analysis was used to estimate the probability of women with diabetes developing PE based on clinical characteristics and biomarker values. These probabilities were used to generate receiver operating characteristic (ROC) curves, and the improvement in the area

under the curve (AUC) when biomarkers were added to clinical characteristics was assessed by the method of DeLong et al. (29). To quantify the improvement in prediction by the addition of a new biomarker, two additional novel methods were applied: the category-free net reclassification improvement (NRI) and the integrated discrimination improvement (IDI). NRI quantifies correct reclassification by counting the upward movement of predicted probabilities for patients with PE and the downward movement of predicted probabilities for patients without PE caused by the addition of a new marker to the logistic model containing only established risk factors (30). IDI compares the actual change of calculated risk by adding the average increase in predicted risk in women with PE to the average decrease in predicted risk in women without PE after the addition of a new marker (31). All tests were two-tailed, with $P < 0.05$ considered significant. Statistical analyses were performed using SPSS 22 software (IBM Corp., Armonk, NY), Stata 13 software (StataCorp, College Station, TX), and R software (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

### Clinical Characteristics

Table 1 reports the baseline clinical characteristics of the three groups. Between DM+PE+ and DM+PE− groups, there were no significant differences in age, alcohol use, smoking, pregnancy outcomes (i.e., gravida, parity, abortus), age of onset and duration of T1DM, systolic and diastolic blood pressure, mean arterial pressure, total cholesterol, LDL cholesterol, triacylglycerol, microalbumin-to-creatinine ratio, and gestational age. At V1, however, BMI, $HbA_{1c}$, and total daily insulin were significantly higher in DM+PE+ than in DM+PE−, and HDL cholesterol was significantly lower. There were no significant differences between DM+PE− and DM− groups at V1, except, as expected, $HbA_{1c}$ was higher in women with diabetes.

### uNGAL

After logarithmic transformation (base 2), the primary analysis of uNGALcc showed higher levels (geometric mean [95% CI]) in DM+PE+ vs. DM+PE− at V1 (26.2 [20.0–34.4] vs. 16.7 [13.3–21.1], $P$ = 0.011) (Fig. 1*A*). uNGALcc did not differ between DM+PE− (16.7 [13.3–21.1]) and DM− (19.2 [13.8–26.7]) ($P$ = 0.467). There was a sustained significant temporal increase in uNGALcc during pregnancy in each group (Friedman test): DM+PE+ ($P$ = 0.002), DM+PE− ($P < 0.001$), and DM− ($P$ = 0.006). When the analyses were restricted to leukocyte-negative

**Table 1—Clinical characteristics at study entry**

| Clinical characteristics | DM+PE+ ($n$ = 23) | $P$ value* | DM+PE− ($n$ = 24) | $P$ value† | DM− ($n$ = 19) |
|---|---|---|---|---|---|
| Age (years) | 28.5 ± 5.6 | 0.31 | 29.9 ± 3.8 | 0.25 | 31.4 ± 4.5 |
| BMI (kg/m$^2$) | 27.9 ± 5.9 | **0.03** | 24.6 ± 4.1 | 0.50 | 23.8 ± 3.8 |
| Alcohol use (%)‡ | | | | | |
| None | 18 | 0.39 | 25 | 0.55 | 11 |
| None during pregnancy | 68 | | 58 | | 68 |
| Smoking (%)‡ | | | | | |
| No | 91 | 0.69 | 88 | 0.55 | 100 |
| Stopped in pregnancy | 5 | | 4 | | 0 |
| Pregnancy outcome | | | | | |
| Gravida ($n$) | 1.3 ± 0.7 | 1.00 | 1.3 ± 0.7 | 0.19 | 1.7 ± 1.0 |
| Para ($n$) | 0.2 ± 0.5 | 0.91 | 0.2 ± 0.5 | 0.13 | 0.5 ± 1.0 |
| Abortus ($n$) | 0.1 ± 0.4 | 0.91 | 0.1 ± 0.3 | 0.81 | 0.2 ± 0.4 |
| Age at onset of T1DM (years) | 11.5 ± 5.5 | 0.07 | 15.2 ± 7.5 | — | — |
| Duration of T1DM (years) | 16.8 ± 6.8 | 0.32 | 14.8 ± 7.0 | — | — |
| $HbA_{1c}$ (%) | 7.4 ± 1.2 | **0.05** | 6.7 ± 1.0 | **<0.0001** | 5.3 ± 0.3 |
| $HbA_{1c}$ (mmol/mol) | 57 ± 14 | **0.05** | 50 ± 11 | **<0.0001** | 35 ± 3 |
| Blood pressure (mmHg) | | | | | |
| Systolic | 113.1 ± 12.4 | 0.26 | 109.4 ± 9.6 | 0.23 | 113.3 ± 8.7 |
| Diastolic | 66.6 ± 9.0 | 0.27 | 63.8 ± 8.1 | 0.24 | 66.9 ± 7.6 |
| Mean arterial | 82.1 ± 9.0 | 0.21 | 79.0 ± 7.7 | 0.14 | 82.7 ± 6.2 |
| Total daily insulin (IU/day) | 62.2 ± 19.7 | **0.01** | 47.9 ± 14.2 | — | — |
| Cholesterol (mmol/L) | | | | | |
| Total | 4.7 ± 0.7 | 0.53 | 4.5 ± 0.9 | 0.18 | 4.9 ± 0.7 |
| HDL cholesterol | 1.9 ± 0.4 | **0.03** | 2.2 ± 0.5 | 0.71 | 2.1 ± 0.6 |
| LDL cholesterol | 2.4 ± 0.7 | 0.08 | 2.0 ± 0.7 | 0.18 | 2.3 ± 0.8 |
| Triacylglycerol (mmol/L) | 1.0 ± 0.3 | 0.27 | 0.8 ± 0.3 | 0.09 | 1.1 ± 0.4 |
| Microalbumin-to-creatinine ratio (mg/g) | 5.9 (4.1, 8.7) | 0.99 | 6.3 (3.7, 8.8) | 0.40 | 7.4 (5.4, 8.7) |
| Gestational age (weeks) | | | | | |
| V1 | 12.3 ± 2.1 | 0.94 | 12.3 ± 1.7 | 0.49 | 12.6 ± 1.7 |
| V2 | 22.1 ± 1.6 | 0.18 | 21.5 ± 1.3 | 0.95 | 21.5 ± 1.3 |
| V3 | 31.7 ± 1.7 | 0.39 | 31.3 ± 1.5 | 0.82 | 31.2 ± 1.1 |

Data are presented as mean ± SD or median (interquartile range). Measurements refer to V1 unless otherwise indicated. Independent samples $t$ tests, Mann-Whitney tests, and $\chi^2$ tests were used as appropriate. $P$ values <0.05 (statistically significant) are highlighted in bold. *$P$ value, DM+PE+ vs. DM+PE−. †$P$ value, DM+PE− vs. DM−. ‡$P$ values refer to combined percentage (i.e., "none" and "stopped during pregnancy" or "no" and "quit because of pregnancy").

samples (i.e., 70% of diabetic and 72% of nondiabetic samples), uNGALcc remained significantly higher in DM+PE+ vs. DM +PE− at V1 (22.0 [16.3–29.6] vs. 15.0 [12.6–17.9], $P = 0.02$); and again, there was no difference between uNGALcc in DM+PE− (15.0 [12.6–17.9]) and DM− (15.5 [11.0–21.8]) ($P = 0.852$) (Fig. 1*B*). Furthermore, with leukocyte-negative urine samples, the temporal increase in uNGALcc remained significant in the DM +PE− group ($P = 0.002$), close to significant in the DM− group ($P = 0.097$), but was not significant for the DM+PE+ group ($P = 0.135$, Friedman test). None of the groups differed at V2 or V3.

### pNGAL

pNGAL did not differ at any visit between any of the three study groups (Fig. 1*C*). A significant temporal increase in pNGAL with gestation was observed only in DM +PE+ women ($P = 0.015$).

### Urinary KIM-1

Creatinine-corrected urinary KIM-1 did not differ at any visit between any of the three study groups (data not shown) (exclusion of leukocyte-positive samples is not relevant). Significant temporal

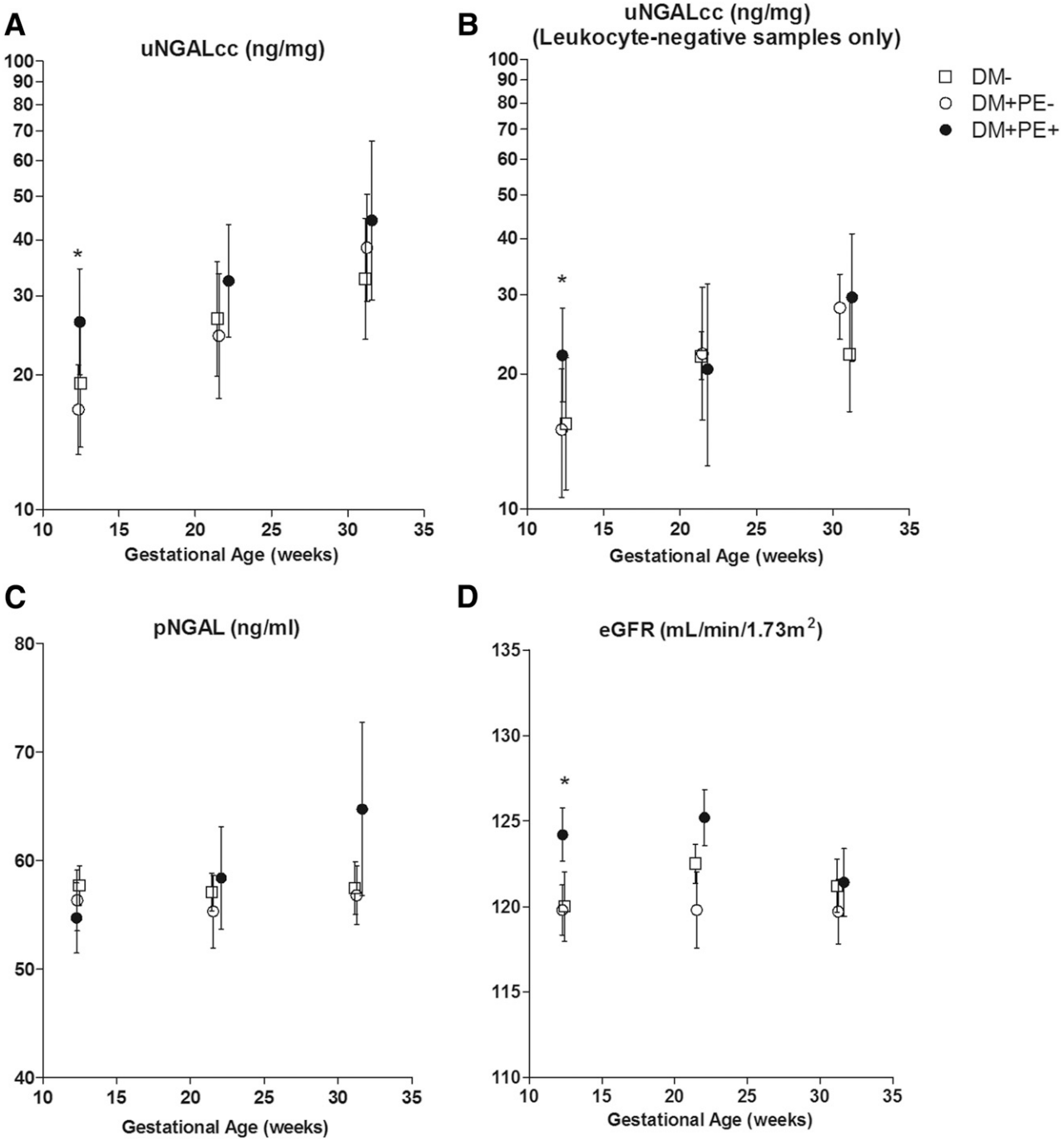


**Figure 1—**Levels of uNGALcc and pNGAL and eGFR before PE diagnosis. Longitudinal changes of uNGALcc (*A*), uNGALcc in leukocyte-negative samples (*B*), pNGAL (*C*), and eGFR (*D*) before clinical onset of PE in a prospective cohort of pregnant women. Values in *A–C* are the geometric mean ± 95% CI; values in *D* are the mean ± SEM plotted against the average gestational age at three visits. *$P < 0.05$ DM+PE+ vs. DM+PE−.

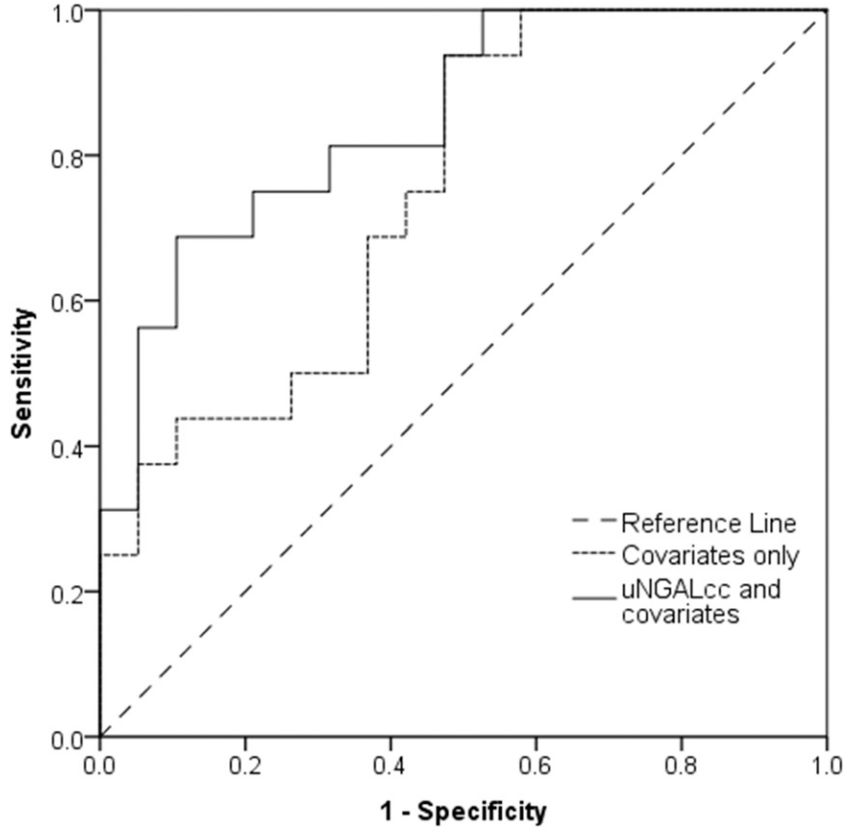


**Figure 2**—ROC curve for a predictive model of PE at V1 (<15 weeks' gestation) with and without covariates. ROC curve comparing a model with and without uNGALcc, using only leukocyte-negative samples. Dashed line: reference line indicating AUC = 0.5. Dotted line: AUC for model including clinical covariates only. Continuous line: AUC for model including clinical covariates and uNGALcc.

increases in creatinine-corrected urinary KIM-1 with gestation were observed in all three groups ($P < 0.01$).

### eGFR

eGFR was significantly elevated at V1 in DM+PE+ vs. DM+PE− (124.2 ± 1.6 vs. 119.8 ± 1.5 mL/min/1.73 $m^2$ [mean ± SEM], $P = 0.04$) but did not differ at any other time point (Fig. 1*D*). A significant temporal decrease in eGFR with gestation was observed only in DM+PE+ women ($P = 0.002$). The presence of urinary leukocytes did not affect the eGFR results.

### A New Model to Predict PE

To explore the significance of first trimester uNGALcc and eGFR in pregnant women with T1DM, we developed a mathematical model to predict PE risk before 15 weeks of gestation using readily available clinical information. To prevent confounding by NGAL derived from leukocytes, only participants with leukocyte-negative urine were included. A fixed group of covariates was selected for the model, considering group comparisons at baseline, known risk factors, and availability (i.e., clinical convenience): BMI, $HbA_{1c}$, and daily total insulin dose (all continuous). After adjustment for these factors, we found that the odds of developing PE were increased sevenfold for every twofold increase in first trimester uNGALcc.

ROC curve analysis was used to assess the utility of uNGALcc to improve PE prediction based on the three clinical factors at V1 (Fig. 2 and Table 2). A model with only the clinical risk factors performed better than one using uNGALcc alone (AUC = 0.75 vs. 0.714, respectively). However, the addition of uNGALcc to the clinical risk factor model improved the predictive value (AUC = 0.849). Although this improvement did not reach statistical significance ($P = 0.157$), IDI and NRI results did indicate significant utility: adding uNGALcc improved discrimination between ($P = 0.016$) and reclassification of ($P = 0.046$) of women according to subsequent PE status. The final model resulted in a sensitivity of 75% and specificity of 79%. Adjusting to account for the overall cohort prevalence (~21%), the positive predicted value was 60% and negative predictive value was 90%. In contrast, pNGAL had no predictive value (AUC = 0.546)

To test our model, we studied 27 other DM+PE− patients from our cohort (no additional DM+PE+ patients were available) who had leukocyte-negative urine samples and no evidence of urinary tract infection. uNGALcc remained significantly elevated

**Table 2—AUC (ROC Curve), IDI, and NRI for logarithmically transformed uNGAL creatinine corrected [$log_2$(uNGALcc)] and pNGAL**

| Variables per model | AUC (ROC curve) | *P* value | IDI score | *P* value | NRI score | *P* value |
|---|---|---|---|---|---|---|
| No variables | 0.5 | — | — | — | — | — |
| uNGALcc only (leukocyte-negative) | 0.714 | **0.027*** | — | — | — | — |
| pNGAL only | 0.546 | 0.590† | — | — | — | — |
| Covariates only | 0.744 | — | — | — | — | — |
| Covariates only (leukocyte-negative) | 0.750 | — | — | — | — | — |
| uNGALcc + covariates (leukocyte-negative) | 0.849 | 0.157‡ | 0.157 | **0.016**§ | 0.638 | **0.046**‖ |
| pNGAL + covariates | 0.751 | 0.419¶ | −0.002 | 0.496 | −0.190 | 0.523 |

Established risk factors (clinical covariates) were BMI, $HbA_{1c}$, and total daily insulin. Bold *P* values are statistically significant. *AUC of "uNGALcc-only" model (leukocyte-negative) is significantly different to AUC = 0.5. †AUC of "pNGAL-only" model is not significantly different to AUC = 0.5. ‡uNGALcc (leukocyte-negative) improved the AUC relative to AUC of 0.750 for a logistic model containing only the covariates, although this was not significant ($P = 0.157$). ¶Relative to AUC of 0.744 for a logistic model containing only the covariates. §Statistically significant improvement ($P = 0.016$) in predicted risk of PE after the addition of uNGALcc to model with covariates only. ‖Statistically significant improvement ($P = 0.046$) in the reclassification of preeclampsia risk after the addition of uNGALcc to model with covariates only.

at V1 in DM+PE+ ($n$ = 16) vs. DM+PE− ($n$ = 46): 22.0 (16.3–29.6) vs. 16.2 (14.4–18.3), geometric mean (95% CI), $P$ = 0.02. The final model, with the larger subset, resulted in a sensitivity of 75% and specificity of 70%. Adjusting to take account the overall cohort prevalence (~21%), the positive predictive value was 32% and negative predictive value was 93%.

At the first trimester, eGFR considered alone was significantly higher in women with than in those without subsequent PE ($P < 0.05$); however, the significant association between eGFR and PE was lost when covariates were considered. Combining eGFR in the previously defined model with uNGALcc and maternal characteristics (BMI, $HbA_{1c}$, daily total insulin) did not improve prediction of PE, whereas uNGALcc remained independently associated with PE ($P < 0.05$).

## CONCLUSIONS

We investigated whether uNGAL and/or pNGAL, urinary KIM-1, and eGFR early in pregnancy were associated with subsequent PE in women with T1DM who were free of hypertension and microalbuminuria at study entry. This is the first simultaneous analysis of these biomarkers longitudinally in pregnancy, irrespective of diabetes or PE status. We observed a significant elevation in uNGALcc in the first trimester in DM+PE+ women compared with DM+PE− or with pregnant control subjects without diabetes. This significance was maintained when analysis was restricted to leukocyte-negative urine samples. When combined with other readily available first trimester data (BMI, $HbA_{1c}$, total daily insulin dose) and in the absence of urinary leukocytes, uNGALcc remained predictive and enabled a model that predicted PE in women with T1DM (AUC = 0.849). Urinary KIM-1, in contrast, was not predictive of PE. The addition of eGFR did not alter the association of first trimester uNGALcc with PE. Nevertheless, when considered alone and at the first trimester, eGFR was positively associated with subsequent PE; however, this association was lost when covariates were also considered. In contrast to urinary levels, pNGAL levels did not show significant differences between subject groups and did not contribute to the model. It is important to note that our study was prospective and that we did not compare women with and without extant PE.

If our findings are confirmed, two very important points emerge, one of practical and the other of mechanistic value. First, uNGALcc combined with readily available clinical data may improve the early prediction of PE in women with T1DM. The ability to define which women with T1DM are at highest risk early in pregnancy would enable patient stratification for testing new interventions. Further, if one could reliably predict PE early in pregnancy, the current paradigm for pregnancy monitoring could be significantly transformed.

Second, and mechanistically, our data suggest that even before the appearance of microalbuminuria, women with T1DM who subsequently develop PE already have subclinical renal abnormalities, both tubular and glomerular (with stronger evidence for the former). Unfortunately, no prepregnancy urine samples were available to us, but it is perhaps likely that the highest-risk women had elevated uNGALcc before conception. It is also likely that as early as the first trimester, any biomarker for PE is of maternal, not placental, origin and that uNGALcc and eGFR, as early markers of maternal renal dysfunction, fall into this category.

Previous studies of uNGAL in women without diabetes, mainly reporting time points late in pregnancy, have shown no association (32,33) or a decrease (34) of uNGAL in the presence of PE. There are several possible sources for uNGAL, all potentially enhanced by diabetes: increased glomerular filtration of the small (25-kDa) protein, decreased reabsorption, or increased secretion by metabolically stressed, ischemic, or inflamed tubules, and release from neutrophils (18,19). Given the good health of our subjects with diabetes and the persistence of the first trimester association of uNGALcc with PE after exclusion of leukocyte-positive samples, we conclude that elevated urinary levels may reflect subclinical renal injury. An explanation based on glomerular leakage of a low-molecular-weight protein in the presence of early renal tubular stress is consistent with the absence of clear-cut changes in pNGAL. It is also consistent with observations (by others) that elevated uNGAL precedes microalbuminuria in developing nephropathy in nonpregnant patients with diabetes (35–38) and with the finding that proximal tubule injury precedes other conventional clinical markers of disease (39,40). These considerations provide clues to the mechanisms of PE in T1DM, and this knowledge may facilitate rational development of preventive measures in the future.

Recent studies suggest that KIM-1 has only weak prognostic value compared with NGAL to detect progression of renal disease (22,23). Our results, in the setting of PE in women with T1DM, are in concert with this conclusion. Two other studies addressed urinary KIM-1 in relation to PE. In one, KIM-1 showed no predictive ability for PE when measured at the second trimester in a general population cohort (41). In the other, KIM-1 was measured at term and was increased in women with versus without PE, but its predictive value was not addressed (42).

Pregnancy imposes a considerable burden on all aspects of renal physiology. GFR and renal blood flow both increase during gestation (43). Our finding that relatively elevated first trimester eGFR is associated with PE in the third trimester in women with T1DM is biologically plausible but will need confirmation. Increased renal ultrafiltration during pregnancy may cause tubular reabsorption overload, wherein tubules become unable to manage the filtered protein load (25). The increased eGFR may therefore contribute directly to elevated uNGAL. The data implicate both tubular and glomerular subclinical injury as harbingers of PE.

pNGAL has been studied by others in relation to PE in women without diabetes. Only two studies had longitudinal case-control designs (44,45), and these reported progressive increases in pNGAL in PE cases, with one finding a similar temporal change in normotensive pregnancy (45). In contrast to our findings, both observed elevated pNGAL throughout pregnancy in women who subsequently developed PE compared with those who did not. The discrepancy may relate to baseline differences (blood pressure and proteinuria were not clearly defined) and/or in the severity of PE. Most prior studies of pNGAL and/or uNGAL in kidney disease have addressed severe ischemic or nephrotoxic renal injury (18) and generally suggest that uNGAL is a more sensitive biomarker of renal insult than pNGAL, as reviewed by Chakraborty et al. (17). It follows that in the evolution of renal disease, changes might be expected to appear first in urine.

Our study has strengths and limitations. It is the first to longitudinally investigate uNGAL, pNGAL, urinary KIM-1, and eGFR in pregnancy complicated by PE. It

used a standardized urine collection protocol. It focuses on women with T1DM, and although this means the findings may be specific to diabetes, the high case yield may also enable efficient elucidation of markers and mechanisms for PE in the general population (women with diabetes are often excluded from studies of PE). The exclusion of women with microalbuminuria is both a strength and a limitation: it reduced the heterogeneity of an inevitably small study cohort, and in this study, enabled a focus on preclinical renal disease. The significance of the findings may extend to more slowly developing complications of diabetes, including nephropathy, where uNGALcc may also have prognostic potential. The main limitation is the small sample size. This problem has afflicted many studies of PE, and we fully endorse recent efforts to establish large international collaborations to address the disease more effectively (46).

In conclusion, uNGALcc and eGFR were elevated at the first trimester in women with T1DM who later developed PE versus those who remained normotensive. When combined with other readily available clinical data, uNGALcc enabled a model that improves the prediction of PE well before the onset of microalbuminuria or other clinical signs or symptoms. uNGALcc thus holds promise as a new marker for PE in T1DM and may enable improved management and patient stratification.

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