Abstract. Aim: Pre-eclampsia is a syndrome characterized by endothelium dysfunction, systemic inflammation, and kidney injury that could be associated with increased levels of neutrophil gelatinase-associated lipocalin (NGAL). We investigated whether serum and urinary NGAL may have a clinical value in defining the severity of pre-eclampsia.

Patients and Methods: This cross-sectional case–control study enrolled 18 women with pre-eclampsia matched for gestational age with 22 uncomplicated pregnancies. We evaluated the correlation between NGAL levels and blood pressure and 24-hour proteinuria values by linear regression.

Results: Linear regression disclosed a positive and significant correlation between urinary NGAL and 24-hour proteinuria. Serum NGAL appeared to be higher, but not significantly different, in severe pre-eclampsia. Conclusion: These preliminary data indicate that NGAL may correlate with an inflammatory renal involvement in severe pre-eclampsia. Further studies would be useful to better estimate the clinical value of an NGAL increase for evaluating the possibility of delivery induction.

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa glycoprotein, firstly identified as a matrix protein of specific granules of human neutrophils (1). It is well-known that NGAL expression is up-regulated in the case of injured epithelial cells, such as those of the lung, colon and, in particular, the kidney. For this reason, NGAL appears to be a promising biomarker for early diagnosis of acute kidney injury (AKI) (2). Recent studies have demonstrated that NGAL may be predictive of AKI in a broad range of patients, including pediatric and adult patients undergoing cardiac surgery (3, 4), adults admitted to hospital via emergency department (5) and children and adults undergoing renal transplantation (6-8). A systematic review of NGAL as a biomarker for diagnosis, prediction, prevention and prognosis of non-AKI diseases, including chronic kidney diseases, vascular disorders, cancer, infection and pre-eclampsia, concluded that it is hard to recommend clinical use of NGAL because few studies and of limited sample size are available and no standardized or validated analytical methods have been used (9).

Although the role of NGAL in pregnancy was unknown until recently, the characteristics of this molecule, which increases in hypertensive non-pregnant women and in individuals with renal damage and inflammation, has brought recent research to focus on hypertensive disorders in pregnancy, in particularly on its role as a potential diagnostic marker of pre-eclampsia (10-13). Pre-eclampsia is a syndrome characterized by endothelium dysfunction, systemic inflammation, hypertension and initial kidney injury, a condition that might be associated with increased levels of NGAL (14). However, few data are currently available on the usefulness of NGAL for the diagnosis and definition of pre-eclampsia severity (15). In the present study, we compared serum and urinary NGAL levels in women with pre-eclampsia and those with uncomplicated pregnancies to identify the potential association of these concentrations with the severity of the disease.

Patients and Methods

A one-year cross-sectional case–control study was performed at the Department of Obstetrics and Gynecology (University of Bologna) in collaboration with the Nephrology, Dialysis and Transplantation Unit (University of Bologna). The study was carried-out following the ethical rules of St. Orsola-Malpighi
General Hospital, Bologna, Italy. All participants involved in the study gave their written consent and the study was approved by the Ethical Committee (n. approval 127/2011/U/Sper). Women affected by pre-eclampsia and uncomplicated pregnancy were enrolled at the time of admission to hospital and were matched for gestational age.

The inclusion criterion was a diagnosis of pre-eclampsia, whether mild-to-moderate, severe, or atypical PE, at the time of admission to hospital. Mild pre-eclampsia was defined as the development of hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on two occasions at least six hours apart) and proteinuria (>0.3 g/day) after 20 weeks of gestation. Severe pre-eclampsia was defined by one or more of the following evaluation criteria: severe hypertension (systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg on two occasions at least 6h apart during bed rest), severe proteinuria (≥5 g in a 24-hour urine specimen in two random urine specimens collected at least 4 hours apart), intra-uterine growth retardation or clinical signs/laboratory findings of severe organ dysfunction. Atypical pre-eclampsia was defined as the presence of gestational hypertension or proteinuria associated with signs/symptoms of organ damage (16,17).

Blood test and urine collection were performed at time of pre-eclampsia diagnosis. NGAL assay was performed with ELISA technique. Specimens were kept refrigerated at 4°C until they were sent to laboratories. Blood samples were centrifuged at 500 xg for 15 min and those of urine at 150 g for 10 min, then serum and urine supernatants were frozen at −20°C until analysis. For NGAL determination in serum and urine supernatant, NGAL ELISA kit (Thermo Fisher Scientific Inc., Rockford, IL, USA) pre-eclampsia was used according to manufacturer’s instructions. NGAL values were expressed as ng/ml.

Statistical analysis was performed using SAS software (SAS, version 9.1; Sas, Cary, NC, USA).

All data were explored with descriptive statistics for the whole population and for the sub-populations with and without pre-eclampsia. In the pre-eclampsia group, linear regression was used to evaluate the correlation between NGAL and blood pressure, uric acid, creatinine and 24-hour proteinuria. Results were considered statistically significant at a p-value of less than 0.05.

### Results

Between November 2009 and June 2010, a cross-sectional case–control study of 40 pregnancies was performed. Eighteen women affected by pre-eclampsia and 22 women with uncomplicated pregnancy were enrolled. As shown in Table I, the two groups were similar in terms of maternal age, frequency of first and multiple pregnancies, Body Mass Index (BMI), number of smokers, and number of thrombophilic individuals. A total of 44.4% (n=8) of patients who were affected by pre-eclampsia had severe disease that in all cases arose at or before 34 weeks of gestation; 33.3% (n=6) of patients had mild to moderate pre-eclampsia with an early onset (at/ before 34th week) in four cases; 22.2% (n=4) of patients had atypical pre-eclampsia with early onset in one case.

NGAL samples were taken at a median gestational age of 32 weeks (range=27-39 weeks). We considered serum and urinary NGAL distribution according to pre-eclampsia: women with pre-eclampsia and normotensive controls did not differ significantly in terms of serum and urinary NGAL levels at 32 weeks of gestation (p-value=0.787 and 0.989, respectively), with higher levels in case of severe pre-eclampsia (Figure 2). Linear regression performed to investigate the correlation between NGAL and blood pressure, creatinine, uric acid and 24-hour proteinuria in the pre-eclampsia group revealed a positive and significant correlation between urinary NGAL and 24-hour proteinuria, as shown in Figure 3 (p-value=0.001).

### Discussion

In this cross-sectional case–control study, we investigated the usefulness of serum and urinary NGAL levels in diagnosis and definition of severity of pre-eclampsia. To the best of our knowledge, this is the first study to investigate both serum and urinary NGAL simultaneously in patients with pre-eclampsia. Concerning serum NGAL evaluation, we identified a positive trend for increasing NGAL values according to the severity of pre-eclampsia, even if we were not able to detect a statistically significant difference by NGAL among cases of mild and severe forms of PE. Urinary NGAL did not differ in pre-eclamptic and normotensive women, as previously described (16). However, we observed a positive and significant correlation between urinary NGAL and 24-hour proteinuria in the pre-eclampsia group. It is well-known that in cases of pre-eclampsia, kidney injury is associated with urinary excretion of non-specific and small proteins. This mechanism might explain our data considering that NGAL is a small protein of 25 kDa. It is feasible that the positive correlation between urinary NGAL and proteinuria could be due to a direct production of NGAL by damaged tubular cells with a defensive intent. Indeed, urinary NGAL derives from local synthesis in the loop of Henle and collecting ducts according to its ability to induce re-epithelialization (17).
To better clarify this phenomenon in pre-eclamptic patients, it could be useful to evaluate the specific increase of urinary NGAL in relation to another protein of similar molecular weight (e.g. β-2-microglobulin).

Although the pathogenic mechanism of pre-eclampsia remains to be elucidated, several factors implicated in inflammatory activation are thought to play a critical role in the development of disease (e.g. endothelial cell dysfunction, immune maladaptation, inadequate placental development and trophoblast invasion, placental ischemia, oxidative stress, thrombosis) (18). In pre-eclamptic women, endothelium activation stimulates leukocytes, including monocytes and granulocytes, and vice versa (19), pro-inflammatory cytokines are released into the circulation, and superoxide generation is increased (20). As a direct consequence of these events, syncytiotrophoblast apoptosis occurs, leading to placental ischemia and reperfusion, with dissemination of apoptotic cells into the maternal circulation (21, 22). It has been demonstrated in vitro that NGAL has immunomodulatory properties by up-regulating HLA-G expression and expansion of T-regulatory cells (23). Thus, the positive correlation between NGAL and proteinuria further supports the evidence that NGAL may be implicated in an inflammatory process.

![Figure 1](image1.png)

**Figure 1.** (A) Serum Neutrophil gelatinase-associated lipocalin (NGAL) concentration was determined in pre-eclamptic and in the control groups (p=0.787, Mann Whitney test). (B) Urinary NGAL concentration was determined in pre-eclamptic and in the control groups (p=0.989, Mann Whitney test).

![Figure 2](image2.png)

**Figure 2.** Distribution of Serum Neutrophil gelatinase-associated lipocalin (NGAL) (A) and urinary (B) NGAL levels in patients with mild pre-eclampsia, severe pre-eclampsia and in the control group. According to Kruskal Wallis test, p-values were 0.447 and 0.971, respectively.
Based on this background and the scarcity of existing studies on the potential diagnostic markers of pre-eclampsia, the interest in NGAL is particularly intriguing. A growing body of evidence has shown that NGAL is a promising stand-alone structural biomarker in plasma and urine for the early diagnosis or prediction of AKI and several other conditions, defined as non-AKI diseases, including chronic kidney diseases, vascular disorders, cancer, and infection (9). Recent studies by D’Anna et al. investigated the use of serum NGAL as a pre-diagnostic marker of pre-eclampsia in each gestational trimester and reported higher serum NGAL concentrations in pre-eclamptic women compared to women with normotensive uncomplicated pregnancies, with significant differences in each trimester (10-12).

The main limitation of the current study is its small sample size. This could reasonably explain why we found only a trend between serum NGAL and severity of pre-eclampsia, without demonstrating a significant correlation.

These preliminary data indicate that NGAL correlates with inflammatory renal involvement in cases of severe pre-eclampsia. Further studies on a larger patient population should be useful for better estimating the clinical value of NGAL as a biomarker of severe pre-eclampsia, to minimize the effects of potential bias (e.g. chronic hypertension) and potentially help in the evaluation of delivery induction.

References


Figure 3. Correlation between urinary Neutrophil gelatinase-associated lipocalin (NGAL) and 24-hour proteinuria. NGAL and 24-hour proteinuria were significantly correlated (R=0.47, p-value=0.001).


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