

## ORIGINAL RESEARCH

# The Value of Urine NAG, NGAL Combined with Serum Cys-C in Early Diagnosis of Neonatal Hyperbilirubinemia-related Acute Kidney Injury

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## Abstract

**Objective:** To explore the early diagnostic value of urinary NAG, NGAL and serum Cys-C detection for neonatal hyperbilirubinemia-related acute kidney injury (AKI) in full-term neonates with hyperbilirubinemia. **Methodology:** One hundred and ninety-six full-term jaundiced newborns were categorized as mild (n = 65), moderate (n = 69) or severe hyperbilirubinemia (n = 62). The severe group was divided into a non-AKI group (n = 35) and an AKI group (n = 27). Sixty-five full-term newborns with normal serum bilirubin and renal function were analyzed as a normal control group. Urine NAG, urine NGAL and serum Cys-C were measured. The value of urinary NAG, NGAL combined with serum Cys-C in early diagnosis of neonatal hyperbilirubinemia-related AKI was evaluated by Receiver Operating Characteristic Curve (ROC). **Result:** Levels of urine NAG, NGAL and serum Cys-C in the mild, moderate and severe groups were higher than those in the normal control group ( $p < 0.001$ ), the above indexes levels in the severe group were higher than those in the mild and moderate groups ( $p < 0.001$ ), and the above indexes levels in the moderate group were higher than those in the mild group ( $p < 0.001$ ). Pearson correlation analysis showed that serum Cys-C was positively correlated with urinary NAG and urinary NGAL in AKI group ( $r = 0.805$ ,  $p < 0.001$ ;  $r = 0.864$ ,  $p < 0.001$ ); there was a positive correlation between urinary NAG and urinary NGAL in AKI group ( $r = 0.948$ ,  $p < 0.001$ ). AUC of urinary NAG, urinary NGAL combined with serum Cys-C in diagnosing neonatal hyperbilirubinemia-related AKI is 0.900 (95% CI: 0.824-0.976), which is higher than that of urinary NAG, urinary NGAL and serum Cys-C alone. **Conclusion:** Combined measurement of urine NAG, NGAL and serum Cys-C is helpful for early diagnosis of neonatal hyperbilirubinemia-related AKI.

## Keywords

N-Acetyl- $\beta$ -D-glucosaminidase (NAG), Neutrophil gelatinase-associated lipid carrier protein (NGAL), Cysteine protease inhibitor C (Cys-C), Hyperbilirubinemia, Acute kidney injury (AKI), Newborn

## 1. Introduction

Neonatal hyperbilirubinemia is one of the common diseases of the newborn period [1]. Previous studies have shown that severe hyperbilirubinemia can induce bilirubin encephalopathy and lead to permanent damage to the nervous system [2, 3]. One study has found that bilirubin is a potential cause of acute renal tubular necrosis [4]. Some studies have also found that acute kidney injury (AKI) is closely related to hyperbilirubinemia [5]. AKI can induce neonatal death [6]. Clinicians often use oliguria, blood urea nitrogen and creatinine levels as detection methods for kidney injury. Due to the lack of accuracy in the assessment of urine volume in the newborn period, strong renal compensatory ability and low sensitivity in renal function detection, the increase of blood urea nitrogen and creatinine can only be identified when renal

function is already seriously damaged, and early detection of renal injury is not easy [7].

Acetyl- $\beta$ -D-glucosaminidase (NAG) widely exists in lysosomes of various tissues, organs, body fluids and blood cells, and is one of the most sensitive indicators of renal tubular function [8]. NAG in urine increases significantly when renal tubules are damaged [9]. Some studies have shown that urine NAG may be an appropriate indicator to predict acute renal damage caused by hyperbilirubinemia in full-term newborns [10].

Neutrophil gelatinase-associated lipid carrier protein (NGAL) belongs to the human apolipoprotein superfamily, and directly reflects urinary system epithelial injury. When renal tubular epithelial cells are stimulated by injury, NGAL is significantly induced and highly expressed [11]. The detection

method of urinary NGAL has the advantages of simplicity, rapidity and non-invasiveness. Some studies have shown that urinary NGAL is helpful for diagnosis of AKI [12].

Cysteine protease inhibitor C (Cys-C) is an endogenous indicator reflecting glomerular filtration rate [13]. Studies have confirmed that serum Cys-C could be used as a diagnostic tool for AKI [14, 15].

The etiology, pathogenesis and pathophysiological process of AKI inpatients with severe hyperbilirubinemia are complex, and a single marker cannot comprehensively and accurately reflect the overall changes of AKI. Up to now, there is no specific marker that can be used as an ideal indicator for early diagnosis of neonatal hyperbilirubinemia-related AKI. Therefore, finding a combination of markers that can comprehensively reflect the pathophysiological changes of AKI has become a focus of AKI research related to neonatal hyperbilirubinemia. At present, there is little research on the value of urinary NAG, NGAL combined with serum Cys-C in early the diagnosis of neonatal hyperbilirubinemia-related AKI.

The objective of this study was to evaluate the early diagnostic value of urinary NAG, NGAL and serum Cys-C for neonatal hyperbilirubinemia-related AKI in full-term neonates with hyperbilirubinemia, so as to provide reference values for the early diagnosis and disease assessment of neonatal hyperbilirubinemia-related AKI.

## 2. Methodology

This study was conducted in Suzhou integrated hospital of traditional Chinese and Western Medicine, from September 2017 to March 2020. This study was approved by the ethics committee of Suzhou integrated hospital of traditional Chinese and Western Medicine. One hundred and ninety-six full-term jaundiced newborns were selected as the research subjects. Inclusion criteria were full-term age  $\leq 7$  days, no history of asphyxia, infection, dehydration. Exclusion criteria were gestational age  $< 37$  weeks; hereditary nephropathy, chromosome all disorder maternal systemic lupus erythematosus and other diseases; history of infection, hypoxia and asphyxia; intracranial hemorrhage; newborns receiving nephrotoxic drug therapy and whose mothers have a history of nephropathy. The diagnostic criteria of AKI in KDIGO clinical practice guidelines included rapid decline of renal function within 48 hours and the absolute value of the elevated level of serum creatinine  $\geq 26.5 \mu\text{mol/L}$ ; or the absolute value of serum creatinine is elevated  $\geq 50\%$  (1.5 times) as high as the baseline value; or the urine volume is  $< 0.5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{hour}^{-1}$  for 6 hours [16].

According to the jaundice intervention standard of Chinese medical association [17], 196 neonates with term jaundice were divided into mild (65 cases) (bilirubin peak value did not meet the phototherapy standard, namely serum total bilirubin  $< 291 \mu\text{mol/L}$ ), moderate (69 cases) (bilirubin peak value reached the phototherapy standard but did not meet the exchange blood standard, namely serum total bilirubin  $\geq 291 \mu\text{mol/L}$ , and serum total bilirubin  $< 376 \mu\text{mol/L}$ ) and severe (62 cases) (bilirubin peak value meet the exchange blood standard, namely serum total bilirubin  $\geq 376 \mu\text{mol/L}$ , and serum total bilirubin  $< 428 \mu\text{mol/L}$ ). The severe group

was divided into non-AKI group ( $n = 35$ ) and AKI group ( $n = 27$ ) according to whether the definition of AKI was met. Sixty-five full-term newborns with normal serum bilirubin and renal function were analyzed as normal control group.

All subjects had blood and urine samples drawn within 24 hours after admission. A 4mL urine sample was collected by neonatal aseptic urine bag and placed in aseptic test tube for inspection. The 1-2 mL venous blood was collected and sent to dry vacuum tube for inspection. Urine samples and blood samples were tested on the day of sampling.

Urine NAG level was detected by colorimetry, the kit was provided by Beijing Jiuqiang Biotechnology Co., Ltd.. Urine NGAL level was detected by ELISA, the kit was provided by Chongqing Novegent Biotechnology Co., Ltd. Serum Cys-C level was determined by latex immunoturbidimetry, the kit was provided by Beijing Jiuqiang Biotechnology Co., Ltd..

Data were analyzed using SPSS 25.0. Kolmogorov-Smirnov test or Shapiro-Wilk test was used to test the normality of all measurement data. Measurement data were expressed as mean  $\pm$  SD. One-way ANOVA was used to compare the measurement data among groups, and LSD-t test was used to compare the two groups. Measurement data between the two groups were compared by independent sample t test. Pearson correlation analysis was used to analyze the correlation between measurement data conforming to normal distribution. The value of urinary NAG, NGAL combined with serum Cys-C in early diagnosis of neonatal hyperbilirubinemia-related AKI was evaluated by Receiver Operating Characteristic Curve (ROC). A p value less than 0.05 was regarded as statistically significant.

## 3. Results

In the mild group, 31 cases were male (47.69%) and 34 cases were female (52.31%). The mean gestational age was  $39.72 \pm 1.35$  weeks; the mean age was  $5.36 \pm 1.18$  days; The mean birth weight was  $3269.25 \pm 381.43$  g; Caesarean section was performed in 29 cases (44.62%) and vaginal delivery in 36 cases (55.38%); mean serum total bilirubin was  $216.72 \pm 5.49 \mu\text{mol/L}$ .

In the moderate group, 33 cases were male (47.83%) and 36 cases were female (52.18%). Mean gestational age was  $39.51 \pm 1.62$  weeks; The age was  $5.29 \pm 1.23$  days; The mean birth weight was  $3301.46 \pm 406.57$  g; Caesarean section was performed in 30 cases (43.48%) and vaginal delivery in 39 cases (56.52%); mean serum total bilirubin was  $338.04 \pm 9.21 \mu\text{mol/L}$ .

In the severe group, 30 cases were male (48.39%) and 32 cases were female (51.61%). The mean gestational age was  $39.16 \pm 1.48$  weeks; The age was  $5.22 \pm 1.35$  days; The mean birth weight was  $3288.46 \pm 517.29$  g; Caesarean section was performed in 27 cases (43.55%) and vaginal delivery in 35 cases (56.45%); mean serum total bilirubin was  $397.35 \pm 7.82 \mu\text{mol/L}$ .

There were 32 males (49.23%) and 33 females (50.77%) in the normal control group. The gestational age was  $39.68 \pm 1.37$  weeks; The age was  $5.32 \pm 1.14$  days; The birth weight was  $3297.85 \pm 408.54$  g; Caesarean section was performed in 30 cases (46.15%) and normal delivery in 35 cases (53.85%);

**TABLE 1. Comparison of urinary NAG, NGAL and serum Cys-C in 4 groups.**

Parameter	Normal control group (n = 65)	Mild group (n = 65)	Moderate group (n = 69)	Severe group (n = 62)	p value
Urine NAG (U/L)	8.63 ± 0.83	10.17 ± 1.54	12.01 ± 1.77	14.44 ± 2.12	< 0.001
Urine NGAL (µg/L)	2.50 ± 0.48	4.76 ± 0.75	7.02 ± 1.30	8.47 ± 2.70	< 0.001
Serum Cys-C (mg/L)	0.78 ± 0.09	1.07 ± 0.14	1.25 ± 0.18	1.52 ± 0.24	< 0.001

**TABLE 2. Comparison of urinary NAG, NGAL and serum Cys-C between AKI group and non-AKI group.**

Parameter	Non-AKI group (n = 35)	AKI group (n = 27)	p value
Urine NAG (U/L)	13.57 ± 1.74	15.58 ± 2.06	< 0.001
Urine NGAL (µg/L)	6.49 ± 1.13	12.03 ± 1.28	< 0.001
Serum Cys-C (mg/L)	1.40 ± 0.26	1.70 ± 0.14	< 0.001

serum total bilirubin was 24.31 ± 6.02 µmol/L.

The levels of urine NAG, NGAL and serum Cys-C in the mild group, moderate group and severe group were higher than those in the normal control group (p < 0.001), the levels of all indices in the severe group were higher than those in mild group and moderate group (p < 0.001), and the levels in the moderate group were higher than those in mild group (p < 0.001), as shown in Table 1.

Urine NAG, NGAL and serum Cys-C levels in AKI group were higher than those in non-AKI group (p < 0.001), as shown in Table 2.

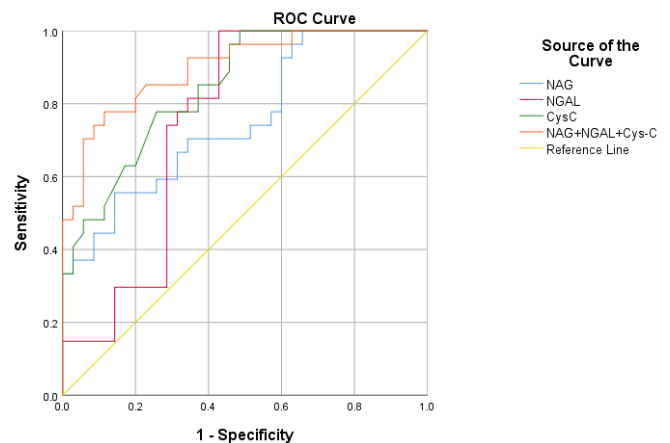
Pearson correlation analysis showed that serum Cys-C was positively correlated with urinary NAG and urinary NGAL in AKI group (r = 0.805, p < 0.001; there was a positive correlation between urinary NAG and urinary NGAL in AKI group (r = 0.948, p < 0.001).

The AUC of urinary NAG, urinary NGAL and serum Cys-C in diagnosing neonatal hyperbilirubinemia-related AKI were 0.754 (95% CI: 0.634-0.875), 0.748 (95% CI: 0.623-0.873) and 0.844 (95% CI: 0.751-0.938), respectively. When the critical value of urinary NAG was 15.26 U/L, the sensitivity and specificity were 55.60% and 85.70%, respectively. When the critical value of urinary NGAL was 8.14 g/L, its diagnostic sensitivity was 74.10% and specificity 71.40%. When the critical value of serum Cys-C was 1.55 mg/L, its diagnostic sensitivity was 85.20% and specificity 62.90%. The AUC of urinary NAG, urinary NGAL combined with serum Cys-C in diagnosing neonatal hyperbilirubinemia-related AKI is 0.900 (95% CI: 0.824-0.976), which is higher than that of urinary NAG, urinary NGAL and serum Cys-C alone. Its diagnostic sensitivity is 77.80%, and its specificity is 88.60%, as shown in Fig. 1.

#### 4. Discussion

Hyperbilirubinemia may cause damage to neonatal kidneys due to the increase of serum free bilirubin [18]. Free bilirubin is fat soluble and can penetrate through cell membrane and enter renal cells, which can interfere with renal cell metabolism and cause renal function changes [19].

Some studies have shown that serum SCr and BUN have poor sensitivity and low diagnostic efficiency in predicting neonatal hyperbilirubinemia-related AKI [20]. A study has



**FIGURE 1. ROC curve analysis results.**

shown that serum Cys-C is more sensitive to renal function damage than SCr, and is an ideal homologous marker reflecting changes in glomerular filtration rate [21].

NAG widely exists in lysosomes of various tissues, organs, body fluids and blood cells, and is one of the most sensitive indicators of renal tubular function. NAG in urine increased significantly when renal tubules were damaged [22]. NAG is a sensitive indicator of renal tubular injury [23]. Katagiri et al. confirmed combined detection of urinary L-type fatty acid-binding protein and NAG could detect adult post-cardiac surgery AKI [24]. The results of this study showed that the urine NAG levels in the mild, moderate and severe groups were higher than those in normal control group, the urine NAG levels in severe group were higher than those in mild group, and the urine NAG levels in moderate group were higher than those in mild group. Urine NAG level in AKI group was higher than that in non-AKI group. It is suggested that urinary NAG can be used to diagnose AKI of full-term newborns.

Some studies have shown that the specificity of urinary NAG in diagnosing diabetic renal damage is not as good as that of urinary NGAL [25]. The biological function of NGAL has not been clearly defined. NGAL is an acute phase reactive protein, which may participate in chemotaxis, immune response and reduce tissue damage caused by oxidative stress. Studies have found that renal proximal convoluted tubule cells can highly express NGAL after injury [26]. Some studies have

also found that exogenous NGAL protein plays a protective role in ischemia/reperfusion injury, providing a new way for the prevention and treatment of renal ischemia/reperfusion injury [27]. Kari et al. demonstrated that urinary NGAL and serum Cys-C could predict AKI early in children admitted to an intensive care unit [28]. The results of our study showed that the urine NGAL level of the mild group, moderate group and severe group was higher than that of normal control group, the urine NGAL level of severe group was higher than that of mild group, and the urine NGAL level of moderate group was higher than that of mild group. Urine NGAL level in AKI group was higher than that in non-AKI group. It is suggested that urinary NGAL can be used to diagnose AKI in full-term newborns. Some studies have also found that urinary NGAL is significantly related to birth weight and gestational age of premature infants [29]. Urine NGAL as a biomarker of AKI in premature infants deserves further study.

Correlation analysis showed that urinary NAG, NGAL and serum Cys-C were positively correlated, which further shows that urinary NAG, NGAL and serum Cys-C were sensitive indicators reflecting early renal function damage.

Combined marker monitoring is helpful to improve the efficiency of predicting AKI in severe patients. However, it is still unclear which markers are the most ideal combination for joint detection. Using ROC curve analysis, the AUC of urinary NAG, NGAL combined with serum Cys-C in predicting neonatal hyperbilirubinemia-related AKI was higher than that of urinary NAG, urinary NGAL and serum Cys-C alone, and the sensitivity and specificity were higher, which was a reliable index for predicting neonatal hyperbilirubinemia complicated with AKI. However, the sample size of this study was small, and a large sample and multi-center clinical study are needed to further verify the clinical value of the combined detection of the above three markers. Different from adults and children, newborns' kidney is not yet mature and their renal function is influenced by a number of factors, such as gestational age and birth weight. Therefore, the results of this study may be affected by these confounding factors, such as gestational age and birth weight. In future, the influence brought by the above confounding factors should be examined via multiple logistic regression analysis, and whether urinary NAG, NGAL and serum Cys-C are an independent predictive index of AKI associated with neonatal hyperbilirubinemia shall be determined.

## 5. Conclusion

Combined detection of urine NAG, NGAL and serum Cys-C is helpful for early diagnosis of neonatal hyperbilirubinemia-related AKI.

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## CONFLICT OF INTEREST

The authors have no conflicts of interests to declare.

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