The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89 (2), Page 6895- 6901

Value of Serum NGAL Combined with Serum Cystatin C for Predicting Acute Kidney Injury in Preterm Neonates with Respiratory Distress Syndrome

Aliaa M. Diab[1], Ghada S. Abdelmotaleb[1], Mosad Fatouh Rashed[3], Waled Abd Elateef[2], Fatma S. Elshaarawy[1], Wesam E. Affi[1]

Departments of [1]Pediatrics and [2]Clinical Pathology, Faculty of Medicine, Benha University, Egypt

Department of [3]Public Health, Faculty of Medicine, Al-Azhar University, Egypt

***Corresponding author:** Wesam E. Affi, **Mobile:** (+20) 01064218197, **E-Mail:** Dr.elmenshawy2007@gmail.com

ABSTRACT

Background: The most frequent cause of respiratory failure in preterm newborns, as well as the most frequent cause of mortality and long-term morbidity associated with prematurity, is respiratory distress syndrome (RDS). RDS affects half of newborns with birth weights (BW) under 1.5 kg, and a sizable portion of those newborns also experience acute kidney injury (AKI).

Objective: The purpose of this study was to identify useful biomarkers (serum cystatin C and NGAL) to predict AKI in premature infant with RDS.

Patients and Methods: This was a prospective (case-control) study was conducted on 90 preterm neonates between 28 and 36 gestational weeks (GW) from the neonatal intensive care unit (NICU) of Benha University hospitals. Serum creatinine, Blood urea nitrogen Levels, Serum cystatin C and Serum NGAL were measured for all included neonates.

Results: Serum creatinine showed no significant difference between the studied groups at day 3 (P = 0.273). At day 5 and day 7, it was significantly higher in group I (1.4 & 1.8 mg/dl, respectively) than groups II (0.7 & 0.6 mg/dl, respectively) and III (0.6 & 0.5 mg/dl, respectively). Also, it was significantly higher in group II (0.7 & 0.6 mg/dl, respectively) than group III (0.6 & 0.5 mg/dl, respectively). Serum cystatin C showed an overall significant difference between the studied groups at day 3 (P < 0.001). In post hoc analyses, it was significantly higher in group I (1.7 mg/l) than groups II (1.1 mg/l) and III (01 mg/l).

Conclusion: NGAL and sCys C levels were found to have a statistically significant association with development of AKI in preterm neonates with RDS and they were elevated earlier than sCr which makes NGAL and sCys C a good predictive marker for AKI in preterm neonates better than sCr.

Keywords: Serum NGAL, Serum Cystatin C, Acute kidney injury, Respiratory Distress Syndrome.

INTRODUCTION

Hyaline membrane disease, also referred to as neonatal respiratory distress syndrome (NRDS), is primarily brought on by a deficiency in pulmonary surfactant (PS), which raises the alveolar wall surface tension and decreases pulmonary compliance. As a result, the newborn experiences dyspnea soon after birth and may even develop clinical respiratory failure syndromes. Infants born prematurely frequently develop NRDS, particularly those born before 34 weeks. Up to 80% of premature infants with gestational ages within 28 weeks have a chance of developing NRDS [(1)].

Preterm births account for about 11% of all births worldwide, and as overall survival rates rise within this patient population, the best possible early care for these newborns is anticipated to have positive effects on their long-term health [(2)].

The illness known as acute kidney injury (AKI), formerly known as acute renal failure (ARF), affects the structure and function of the kidneys. Currently, the term "AKI" refers to a decline in kidney function, including renal failure and a decreased glomerular filtration rate (GFR) [(3)]. AKI affects 12% to 40% of premature newborns and is linked to poor outcomes [(4)].

AKI is currently diagnosed using a higher serum creatinine (Cr) level and/or a decreased urine output. Neonatal blood Cr levels cannot be used to evaluate kidney function in premature newborns because they mimic maternal levels during the early postnatal period and decline over days to weeks rather than remaining constant. On the other hand, because non-oliguric AKI frequently occurs in preterm infants, oliguria is an insensitive diagnostic for the early identification of kidney injury [(5)].

It is advised to employ alternative indicators that do not fluctuate with changes in muscle mass or tubular secretion and reabsorption. As biomarkers for determining the location and extent of renal damage, certain proteins that are secreted in the urine following harm to particular nephron segments can be identified. Many specialists and academics have suggested various biomarkers for the early detection of AKI in both adults and children, including Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Cystatin C. However, there has been very little published studies on premature infants. NGAL, a 25-kDa protein that is a member of the lipocalin group and is abundantly expressed in proximal tubular epithelial cells, plays a role in the growth and repair of renal tubular epithelial cells. It is expressed in neutrophils and in trace amounts in the kidney, prostate, and

Received: 16/08/2022
Accepted: 17/10/2022

gastrointestinal and respiratory tract epithelia. Tubular epithelial cells are harmed during AKI, and NGAL is highly expressed in both blood and urine [(6)].

Nucleated cells produce cystatin C, a 13-kDa cysteine proteinase inhibitor that can freely pass through glomeruli before being reabsorbed and broken down by tubular cells. It is less affected by factors like age, sex, race, muscle mass, steroid medication, infection, liver disease, and inflammation. Cystatin C has a shorter distribution range and is only distributed in the extracellular fluid volume, therefore it responds to a decrease in GFR more quickly than creatinine does. Consequently, serum cystatin C is a more accurate and timely GFR decrease predictor than sCr [(7)].

AIM OF THE STUDY

The purpose of this study is to identify useful biomarkers (serum cystatin C and NGAL) to predict AKI in premature infant with RDS.

PATIENTS AND METHODS

This was a prospective (case-control) study carried out from February 2019 to January 2022. The study was conducted on 90 preterm neonates between 28 and 36 gestational weeks (GW) from the neonatal intensive care unit (NICU) of Benha university hospitals.

The study group was subdivided into:

- Group (I): (RDS-AKI) which included 18 preterm neonates who developed AKI, they were 10 males and 8 females. Their mean gestational age (GA) was 30±1 weeks.
- Group (II): (RDS- no AKI (Which included 42 preterm neonates without AKI, they were 21 males and 21 females. Their mean GA was 32±1 weeks.
- Group (III): (control group (Which included 30 healthy preterm neonates. They were 15 male and 15 females, their mean GA was 33±1 weeks.

Table (1): AKI definitions [(8)]:

	KDIGO
Diagnosis	Increase in sCr by ≥26.5 µmol/l within 48 hours or increase in sCr to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7 days
Stage 1	sCr increase to 1.5 to 1.9 times baseline or increase in sCr by ≥26.5 µmol/l
Stage 2	sCr increase to 2.0 to 2.9 times baseline
Stage 3	sCr increase to 3.0 times baseline or sCr increase by ≥353.6 µmol/l or initiation of RRT

Inclusion criteria:

Preterm neonates between 28 and 36 gestational weeks of both sexes. Preterm neonates with diagnosis of RDS. A healthy control group of 30 newborns of the same gestational age (GA) and sex who had no RDS or AKI.

Exclusion criteria:

Neonates with major congenital defects, such as congenital heart disease or kidney abnormalities. Hypoxic ischemic encephalopathy in newborns. History of the mother (gestational diabetes, chorioamnionitis, urinary tract infection, treatment with nephrotoxic drugs).

Methods:

All studied neonates were subjected to full history taking, complete clinical examination and laboratory assessment as;

- Complete blood count, C reactive protein, blood cultures, serum electrolytes (Na, K & Ca) and capillary blood gases.
- Serum creatinine, Blood urea nitrogen Levels were measured on day 3 & again on day 5, 7 for all included neonates.
- Serum cystatin C on day 3 by ELIZA technique.
- Serum NGAL on day 3 by ELIZA technique.

Imaging:

- Chest X-ray to detect RDS**,** chest X-ray findings were graded according to severity of the cases.

Sampling measurements:

On postnatal day 3 (PND), peripheral blood samples were obtained for biochemical markers like blood urea nitrogen (BUN), Cr, sCysC, and sNGAL. At day 5, day 7, and Cr levels, we also took measurements.

Ethical consent:

The Research Ethics Committee of the Faculty of Medicine at Benha University gave its approval to the study. Informed written consents were taken from parents of the included neonates before starting the study. The Declaration of Helsinki, the World Medical Association's code of ethics for studies involving humans, guided the conduct of this work.

Statistical methods

Using SPSS version 25, data management and statistical analysis were conducted (IBM, Armonk, New York, United States). The Shapiro-Wilk test and methods for direct data visualisation were used to determine the normality of quantitative data. Numerical data were summarised using means, standard deviations, or medians and ranges in accordance with normality. Numbers and percentages were used to represent a categorical set of data.

Utilizing the one-way ANOVA or the Kruskal Wallis test for numerical variables with normally distributed or non-normal distribution, respectively, quantitative data were compared between the research groups. The Fisher's exact test or the Chi-square test

was used to compare categorical data. AKI was predicted using ROC analysis for serum creatinine, cystatin C, and NEGAL.

The optimal cut-off point, diagnostic indices, and Area Under Curve (AUC) with a 95% confidence interval were calculated. Spearman's correlation was used to conduct correlation analysis. Each and every statistical test had two sides. P values under 0.05 were regarded as significant.

RESULTS

General characteristics:

As shown in **table 2**, no significant differences were noted between the studied groups regarding age (P = 0.915) and gender (P = 0.364). Gestational age showed an overall significant difference between the three groups (P < 0.001). Post-hoc analyses revealed that it was significantly lower in group I (30 weeks) than groups II (32 weeks) and III (33 weeks). Apgar scores at 1 and 5 minutes showed an overall significant difference between the studied groups (P < 0.001).

Post hoc analyses showed that they were significantly lower in group I (4 and 6, respectively) than groups II (6 and 8, respectively) and III (8 & 9, respectively).

Also, they were significantly lower in group II (6 and 8, respectively) than group III (8 & 9, respectively). Birth weight showed an overall significant difference between the studied groups (P <0.001). In post-hoc analyses, it was significantly lower in group I (1.4 kg) than groups II and III (1.5 kg & 1.6 kg, respectively).

RDS grade showed a significant difference between groups I & II (P < 0.001); RDS grade IV was higher in group I (22.2%) than group II (0.0%), and RDS grade I was higher in group II (38.1) than group I (0.0%). The use of mechanical ventilators and inotropes were significantly higher in group I (88.9% and 38.9%, respectively) than group II (50% and 26.2%, respectively); P values were <0.001 and 0.002, respectively. Also, oliguria was significantly higher in group I (27.8%) than group II (0.0%) (P < 0.001). In contrast, sepsis was significantly higher in group II (40.5%) than group I (33.3%).

Table (2): General characteristics of the studied groups

		Group I (n = 18)	Group II (n = 42)	Group III (n = 30)	P-value
Gender	Males n (%)	10 (55.6)	21 (50.0)	15 (50.0)	0.915
	Females n (%)	8 (44.4)	21 (50.0)	15 (50.0)	
Mode of delivery	CS n (%)	9 (50.0)	14 (33.3)	14 (46.7)	0.364
	NVD n (%)	9 (50.0)	28 (66.7)	16 (53.3)	
Gestational age (wk)	Mean ±SD	30 ±1	32 ±1	33 ±1	<0.001
APGAR 1 minute	Median (range)	4 (3 - 5)	6 (5 - 7)	8 (6 - 8)	<0.001
APGAR 5 minutes	Median (range)	6 (6 - 6)	8 (7 - 9)	9 (8 - 10)	<0.001
Birth weight (Kg)	Mean ±SD	1.4 ±0.16	1.5 ±0.14	1.6 ±0.15	<0.001
RDS Grade	I n (%)	0 (0.0)	16 (38.1)	-	<0.001
	II n (%)	2 (11.1)	25 (59.5)	-	
	III n (%)	12 (66.7)	1 (2.4)	-	
	IV n (%)	4 (22.2)	0 (0.0)	-	
Mechanical ventilator	n (%)	16 (88.9)	21 (50.0)	-	<0.001
Inotropes	n (%)	7 (38.9)	11 (26.2)	-	0.002
Sepsis	n (%)	6 (33.3)	17 (40.5)	-	<0.001
Oliguria	n (%)	5 (27.8)	0 (0.0)	-	<0.001

Laboratory findings:

BUN D showed an overall significant difference between the studied groups at day 3 (P = 0.031), day 5 (P < 0.001), and day 7 (P < 0.001).

Post hoc analyses showed that at day 3, it was significantly lower in group I (13.9 mg/dl) than group II (15.1 mg/dl). On day 5 and day 7, it was significantly higher in group I (17.6 & 20.8 mg/dl, respectively) than group II (14.3 & 13.9 mg/dl, respectively) and III (13.1 & 11.9 mg/dl, respectively).

Also, it was significantly higher in group II (14.3 & 13.9 mg/dl, respectively) than group III (13.1 & 11.9 mg/dl, respectively) (Table **3**).

Serum creatinine showed no significant difference between the studied groups at day 3 (P = 0.273). At day 5 and day 7, it was significantly higher in group I (1.4 & 1.8 mg/dl, respectively) than groups II (0.7 & 0.6 mg/dl, respectively) and III (0.6 & 0.5 mg/dl, respectively). Also, it was significantly higher in group II (0.7 & 0.6 mg/dl, respectively) than group III (0.6 & 0.5 mg/dl, respectively) (Table **3**).

Day 3's analysis of serum cystatin C revealed a significant overall difference between the groups under study (P 0.001). It was substantially greater in group I (1.7 mg/l) than in groups II (1.1 mg/l) and III (01 mg/l) in post hoc analyses. Additionally, group II had a substantially greater level of it (1.1 mg/l) than group III (1 mg/l). (Table **3**).

Serum NEGAL showed an overall significant difference between the studied groups at day 3 (P < 0.001). Post hoc analyses showed that it was significantly higher in group I (244 mg/ml) than groups II (32 mg/ml) and III (36 mg/ml) **(**Table **3)**.

Table (4): Laboratory findings in the studied groups

	Group I (n = 18)	Group II (n = 42)	Group III (n = 30)	P-value
BUN D (mg/dl)				
Day 3	13.9 ±1.4	15.1 ±1.6 [b]	14.6 ±1.9	0.031
Day 5	17.6 ±1.8	14.3 ±1.5 [b]	13.1 ±1.7	<0.001
Day 7	20.8 ±2.1	13.9 ±1.6 [b]	11.9 ±1.4	<0.001
Serum creatinine (mg/dl)				
Day 3	0.83 ±0.1	0.78 ±0.11	0.79 ±0.12	0.273
Day 5	1.4 ±0.2	0.7 ±0.1	0.6 ±0.1	<0.001
Day 7	1.8 ±0.2	0.6 ±0.1	0.5 ±0.1	<0.001
Serum cystatin C (mg/l)				
Day 3	1.7 ±0.1	1.1 ±0.1	1 ±0.2	<0.001
Serum NEGAL (mg/ml)				
Day 3	244 ±60.1	32 ±8	36 ±8.5	<0.001

ROC analysis for AKI prediction in neonates with RDS: ROC analyses were done for serum creatinine, serum cystatin, and serum NEGAL at day 3 for prediction of AKI in RDS neonates. Serum creatinine showed a non-significant AUC of 0.653 (P = 0.062). In contrast, serum cystatin C and NEGAL showed a significant AUC of 1 (P < 0.001 for each). The best cut-off points were > 1.25 mg/l for serum cystatin and > 64 mg/ml for serum NEGAL, at which sensitivity and specificity were 100% for both (Figure **1).**

Correlation between different markers at day 3 and RDS grade in neonates with AKI: At day 3, serum cystatin C showed a significant positive correlation with RDS grade (r = 0.601 & P = 0.008). In contrast, serum creatinine and serum NEGAL showed non-significant correlations with RDS grade (Table **4)**.

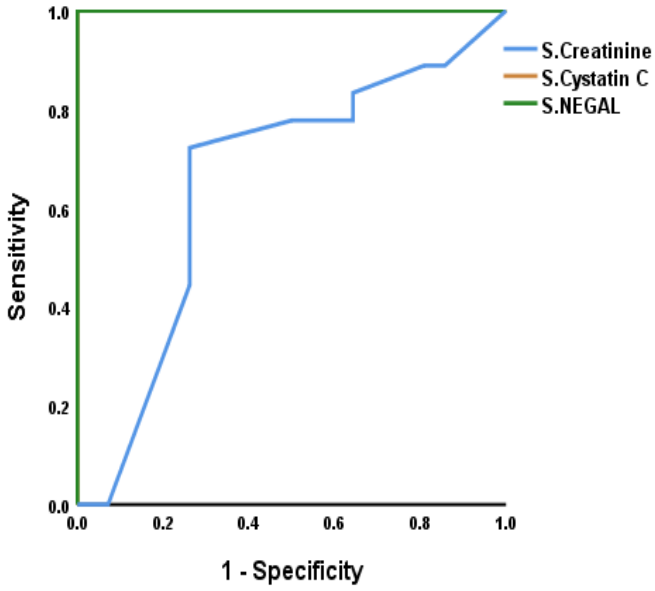

Figure (1): ROC analysis of serum creatinine, cystatin C, and NEGAL in predicting AKI

Table (4): Correlation between different markers at day 3 and RDS grade in neonates with AKI

	S. Creatinine (mg/dl)		S. Cystatin C (mg/l)		S.NEGAL (ng/ml)	
	r	P	r	P	r	P
RDS Grade	-0.197	0.433	.601*	**0.008**	0.322	0.192

Spearman's correlation was used, r: Correlation coefficient

DISCUSSION

AKI was 20% of the time in our unit. This is similar with the study by **Koralkar *et al.*** [9] which found that 18% of 229 VLBW infants had AKI using modified KDIGO criteria.

Earlier research either utilised urine output or serum creatinine as the criteria, or just serum creatinine. AKI was present in 20% and 6.3% of the patients, respectively, highlighting the need of having AKI defined consistently [10, 11]. The wide variability of incidence of AKI in the available data from different units can be attributed to demographic characteristics of population studied, and secondly no consensus definition of AKI was used.

In the current study, birth weight showed an overall significant difference between the studied groups (P <0.001). In post-hoc analyses, it was significantly lower in group I (1.4 kg) than groups II and III (1.5 kg & 1.6 kg, respectively).

Similar to this, a prior Indian study revealed that the proportion of infants in the AKI group with birth weights under 2500 g was higher than that of healthy newborns [12]. This was also supported by **Ahmed *et al.*** [13], who found that neonates in the RDS-AKI subgroup had significantly lower mean birth weights than controls (p=0.025).

In the current study, gestational age showed an overall significant difference between the three groups (P < 0.001). Post-hoc analyses revealed that it was significantly lower in group I (30 weeks) than groups II (32 weeks) and III (33 weeks).

This is consistent with the findings of **Ahmed *et al.*** [13], who discovered that neonates with AKI had considerably lower gestational ages than non-AK neonates (27.6 vs. 30.3 weeks, p0.001). According to a study by **Stojanovi *et al.*** [14] newborns with AKI had considerably lower GA than neonates without AKI, which is consistent with the findings of this investigation.

In current study, there is a male predominance (55.6%) in Group I.

This was in line with the findings of **Bansal *et al.*** [15], who noted that the AKI group consisted primarily of male neonates (n=61; 82.4%). This runs counter to a different NICU study which found a higher rate of AKI among girls [16].

Our study showed that, sepsis was significantly higher in group II (40.5%) than group I (33.3%). In numerous research conducted across the world, sepsis has repeatedly been linked to an increased chance of developing AKI, accounting for as many as 78% of cases in some studies of newborns [16]. According to a research by **Mathur *et al.*** [12] from India, 26% of 200 neonates with sepsis went on to develop AKI. The study came to the conclusion that people with AKI had lower birth weights and were more susceptible to septic shock, meningitis, and disseminated intravascular coagulation. Due to secondary hypotension brought on by sepsis and a direct negative impact on the renal microvasculature, neonates with sepsis are thought to be prone to developing acute kidney injury (AKI).

In this study, Apgar scores at 1 and 5 minutes showed an overall significant difference between the studied groups (P < 0.001). Post hoc analyses showed that they were significantly lower in group I (4 and 6, respectively) than groups II (6 and 8, respectively) and III (8 & 9, respectively). Also, they were significantly lower in group II (6 and 8, respectively) than group III (8 & 9, respectively). This was consistent with the results of **Abdelaal *et al.*** [17], who investigated the potential utility of serum cystatin C (sCysC) as a marker for acute kidney injury (AKI) in preterm neonates with respiratory distress syndrome (RDS). They showed that compared to neonates with RDS but no AKI, neonates with RDS-AKI had significantly lower Apgar scores at 1 and 5 minutes.

This is in line with the research of **Bansal *et al.*** [15], who found a significant relationship between AKI and low APGAR scores at one and five minutes.

Our study revealed that group I patients were substantially more likely than group II patients to require mechanical ventilators and inotropes (88.9% vs. 38.9% vs. 50% vs. 26.2%, respectively); the corresponding P values were 0.001 and 0.002, respectively.

This was in line with the observation made by **Bansal *et al.*** [15] that AKI and the need for mechanical ventilation were related to death.

This contradicted the findings of **Abdelaal *et al.*** [17], who discovered no statistically significant difference in mechanical ventilation between newborns with and without AKI; nonetheless, the AKI group required more mechanical ventilation and spent more time in the NICU.

Our study revealed that, oliguria was significantly higher in group I (27.8%) than group II (0.0%) (P < 0.001).

This was in line with research by **Youssef *et al.*** [18], who observed that oliguria was present in 29.6% of AKI cases, and **Girish *et al.*** [19], who discovered that oliguria was present in 37.5% of asphyxiated newborns with AKI.

Our study revealed that, serum creatinine showed no significant difference between the studied groups at day 3 (P = 0.273). At day 5 and day 7, it was significantly higher in group I (1.4 & 1.8 mg/dl, respectively) than groups II (0.7 & 0.6 mg/dl, respectively) and III (0.6 & 0.5 mg/dl, respectively). Also, it was significantly higher in group II (0.7 & 0.6 mg/dl, respectively) than group III (0.6 & 0.5 mg/dl, respectively).

This was in agreement with **Abdelaal *et al.*** [17], who said that even in individuals with decreasing renal function indicated by AKI classification, we could not discover any significant change in sCr in, or between, the research groups and subgroups at the time points days of life (DOL)-1 and DOL-3. The mean sCr values among newborns with AKI were substantially greater only at DOL-7.

This is in line with what **Youssef *et al.*** [18] discovered, who identified no differences in sCr levels or eGFR between neonates with RDS and controls at DOL-1 or between neonates in the RDS group at DOL-1 and DOL-3.

In line with our study, **Elmas *et al.*** [20] showed no differences in sCr between RDS with AKI, RDS without AKI, and controls at DOL- 3 and DOL-30.

Our research found that, on day 3, there was a statistically significant difference between the analysed groups in terms of serum cystatin C (P 0.001). It was substantially greater in group I (1.7 mg/l) than in

groups II (1.1 mg/l) and III (01 mg/l) in post hoc analyses. Additionally, group II had a substantially greater level of it (1.1 mg/l) than group III (1 mg/l).

In addition, **Elmas *et al.*** [20] observed that the sCys C levels on DOL-3 in the RDS-AKI subgroup were significantly higher than those in the RDS-no AKI subgroup and the control group, and they proposed that an elevated sCys C level might function as a reliable early marker of renal function in neonates with RDS.

According to the current study, by day 3 there was a statistically significant difference between the analysed groups' serum NEGAL levels (P 0.001). It was substantially greater in group I (244 mg/ml) compared to groups II (32 mg/ml) and III (36 mg/ml), according to post hoc analyses.

According to **Baumert *et al.*** [21]**,** NGAL concentrations in the umbilical cord and venous blood were significantly different in asphyxiated babies with and without AKI after 24 hours of life.

ROC analyses were done for serum creatinine, serum cystatin, and serum NEGAL at day 3 for prediction of AKI in RDS neonates. Serum creatinine showed a non-significant AUC of 0.653 (P = 0.062). In contrast, serum cystatin C and NEGAL showed a significant AUC of 1 (P < 0.001 for each). The best cut-off points were > 1.25 mg/l for serum cystatin and > 64 mg/ml for serum NEGAL, at which sensitivity and specificity were 100% for both.

In a cross-sectional observational cohort of preterm newborns, **Abitbol *et al.*** [22] demonstrated that sCys C level is a superior biomarker to sCr in the assessment of GFR. Our findings are consistent with their findings.

Serum NGAL concentration, at a cut-off value of 140.7 mg/dL after 24 h, was able to predict the onset of AKI with high sensitivity (88.9%) and specificity (95.0%), while umbilical NGAL concentration was characterised by low sensitivity (55.6%) but high specificity (89.5%) for the prediction of AKI, according to **Baumert *et al.*** [21]**'s** analysis of the ROC curve.

In a manner similar to **Abdelaal *et al.*** [17]**,** who recommended using Cys C to diagnose AKI starting on the first day of life because they discovered that Cys C measured on the third day of life was superior to SCr in detecting AKI in critically ill neonates and that its level rises 48 hours before the rise of creatinine level.

Our research supports the findings of **Abitbol *et al.*** [22] who demonstrated that s CysC level is a superior biomarker to sCr for determining GFR in a cross-sectional observational cohort of premature infants.

In a recent study, **Zhang *et al.*** [23] compared the sensitivity and specificity of serum creatinine and new biomarkers for AKI in neonates and discovered that there were significant differences between the AKI and nonAKI groups in terms of serum Cys C, 2-MG, urinary NGAL, and 1-MG. This finding provides strong support for the conclusion they reached (p 0.05).

CONCLUSION

NGAL and sCys C levels were found to have a statistically significant association with development of AKI in preterm neonates with RDS and they are elevated earlier than sCr which makes NGAL and sCys C a good predictive marker for AKI in preterm neonates better than sCr.

Funding: No fund

Conflicts of Interest: Regarding the publishing of this paper, the authors state that they have no conflicts of interest.

REFERENCES

1. **Yadav S, Lee B, Kamity R (2021):** Neonatal Respiratory Distress Syndrome. StatPearls. Treasure Island (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK560779/
2. **Course C, Chakraborty M (2020):** Management of respiratory distress syndrome in preterm infants in wales: a full audit cycle of a quality improvement project. Sci Rep., 10(1):3536. doi: 10.1038/s41598-020-60091-6.
3. **Makris K, Spanou L (2016):** Acute Kidney Injury: Definition, Pathophysiology and Clinical Phenotypes. Clin Biochem Rev., 37(2):85-98.
4. **Ahn Y, Lee J, Chun J *et al.* (2020):** Urine biomarkers for monitoring acute kidney injury in premature infants. Kidney Res Clin Pract., 39(3):284-294.
5. **Nada A, Bonachea E, Askenazi D (2017):** Acute kidney injury in the fetus and neonate. Semin Fetal Neonatal Med., 22(2):90-97.
6. **Tramonti G, Kanwar Y (2013):** Review and discussion of tubular biomarkers in the diagnosis and management of diabetic nephropathy. Endocrine, 43(3):494-503.
7. **He Y, Deng Y, Zhuang K *et al.* (2020):** Predictive value of cystatin C and neutrophil gelatinase-associated lipocalin in contrast-induced nephropathy: A meta-analysis. PLoS One, 15(4):e0230934. https://doi.org/10.1371/journal.pone.0230934
8. **Mehta R, Burdmann E, Cerdá J *et al.* (2016):** Recognition and management of acute kidney injury in the International Society of Nephrology by 25 Global Snapshot: a multinational cross-sectional study. Lancet, 387(10032):2017–25.
9. **Koralkar R, Ambalavanan N, Levitan E *et al.* (2011):** Acute kidney injury reduces survival in very low birth weight infants. Pediatr Res., 69:354-58.
10. **Bezerra C, Vaz Cunha L *et al.* (2013):** Defining reduced urine output in neonatal ICU: importance for mortality and acute kidney injury classification. Nephrol Dial Transplant., 28:901-09.
11. **Vachvanichsanong P, McNeil E, Dissaneevate S *et al.* (2012):** Neonatal acute kidney injury in a tertiary center in a developing country. Nephrol Dial Transplant., 27:973-77.
12. **Mathur N, Agarwal H, Maria A (2006):** Acute renal failure in neonatal sepsis. India J Pediatr., 73:499-502.

13. **Ahmed P, Ashraf M, Riyaz A (2019):** Role of Serum Cystatin C Levels in Preterm Neonates with Respiratory Distress Syndrome in Diagnosing Neonatal AKI. J Clin Diag Res., 13(5): 7-10.
14. **Stojanovic V, Bari sic N, Milanovic B *et al.* (2014):** Acute kidney injury in preterm infants admitted to a neonatal intensive care unit. Pediatr Nephrol., 29: 2213–2220.
15. **Bansal S, Nimbalkar A, Kungwani A *et al.* (2014):** Clinical profile and outcome of newborns with acute kidney injury in a level 3 neonatal unit in Western India. J Clin Diag Res., 11(3): 01-04.
16. **Momtaz H, Sabzehei M, Rasuli B *et al.* (2014):** The main etiologies of acute kidney injury in the newborns hospitalized in the neonatal intensive care unit. J Clin Neonatol., 3:99-102.
17. **Abdelaal N, Shalaby S, Khashana A *et al.* (2017):** Serum cystatin C as an earlier predictor of acute kidney injury than serum creatinine in preterm neonates with respiratory distress syndrome. Saudi J Kidney Dis Transpl., 28:1003-14.
18. **Youssef D, Abd-Elrahman H, Shehab M *et al.* (2015):** Incidence of acute kidney injury in the neonatal intensive care unit. Saudi J Kidney Dis Transpl., 26:67–72.
19. **Girish G (2014):** Acute kidney injury (AKI) in perinatal asphyxia. India J Pharm Biol Res., 2:60–65.
20. **Elmas A, Tabel Y, Elmas O (2013):** Serum cystatin C predicts acute kidney injury in preterm neonates with respiratory distress syndrome. Pediatr Nephrol., 28:477-84.
21. **Baumert M, Surmiak P *et al.* (2017):** Serum NGAL and copeptin levels as predictors of acute kidney injury in asphyxiated neonates. Clin Exp Nephrol., 21: 658–664.
22. **Abitbol C, Seeherunvong W, Galarza M *et al.* (2014):** Neonatal kidney size and function in pre-term infants: What is a true estimate of glomerular filtration rate? J Pediatr., 164: 1026-31.
23. **Zhang Y, Zhang B, Wang D *et al.* (2020):** Evaluation of Novel Biomarkers for Early Diagnosis of Acute Kidney Injury in Asphyxiated Full-Term Newborns. Med Princ Pract., 29(3):285-291.