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Original Article

Serum galectin-3 level as a marker for diagnosis and prognosis of neonatal necrotising enterocolitis: a cohort study

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Abstract: Objective: Neonatal necrotising enterocolitis (NEC) is a leading factor in neonatal mortality. Diagnosing NEC is difficult since it presents with various clinical appearances with divergent symptoms. This study determines the value of galectin-3 (GAL-3) for diagnosing NEC. Methods: Seventy-two newborn patients with NEC and 64 preterm infants with jaundice (control group) were prospectively enrolled. The levels of white blood cells (WBC), C-reactive protein (CRP), procalcitonin (PCT), intestinal fatty acid binding protein (I-FABP), serum cytosolic β-glycosidase (CBG), and GAL-3 in the serum were measured. In addition, the diagnostic values of GAL-3 for diagnosing early and severe NEC were analysed by a receiver operating characteristic curve. Results: WBC, CRP, PCT, I-FABP, CBG, and GAL-3 showed an increasing trend in the control, NEC I, and NEC II+III groups. Moreover, in the diagnosis of early and severe NEC, GAL-3 had a higher sensitivity and specificity than WBC, CRP, PCT, I-FABP, and CBG. The results also suggest that the GAL-3 level is an independent prognostic measure to indicate poor prognosis in NEC. Conclusion: GAL-3 is a useful marker for diagnosing and prognosis of neonatal necrotising enterocolitis.

Keywords: Galectin-3, neonatal necrotising enterocolitis, diagnosis, prognosis, early, severe

Introduction

Neonatal necrotising enterocolitis (NEC) is a leading factor in neonatal mortality, with a mortality rate of 20-30% [1, 2]. Premature preterm and low birth weight infants are at high risk of developing NEC [3, 4]. Diagnosing NEC is difficult since it presents with various clinical presentations that have divergent symptoms [5]. It is therefore of great significance to identify novel markers with high sensitivity and specificity to diagnose NEC. Several serum markers that reflect the state of systemic inflammation such as white blood cell (WBC) count, C-reactive protein (CRP), and procalcitonin (PCT) levels have been proposed as markers for diagnosing NEC, yet these markers provide poor sensitivity and specificity [6, 7]. Other markers that reflect the state of intestinal inflammation such as serum cytosolic β-glycosidase (CBG), and intestinal fatty acid binding protein (I-FABP) levels have unsatisfactory sensitivity and specificity in NEC screening tests [8-10]. Thus, there is an urgent need to identify new molecular markers for early diagnosis and NEC screening.

Galectin-3 (GAL-3) is an α-galactoside-binding lectin that can mediate cell-to-cell and cell-to-extracellular matrix interactions, and also acts as a novel chemoattractant for monocytes and macrophages [11]. GAL-3 controls a variety of signalling pathways that include cell adhesion, proliferation, differentiation, apoptosis, and secretion of inflammatory factors [12]. Previous studies indicate that GAL-3 is a novel marker that indicates the pro-inflammatory status during heart failure, atrial fibrillation, and chronic kidney disease [13-15]. The present study is on the use of GAL-3 as a marker for NEC.

Methods

Patients and serum samples

The patient flow diagram is shown in **Figure 1**. A total of 72 newborn patients with NEC (45 pre-

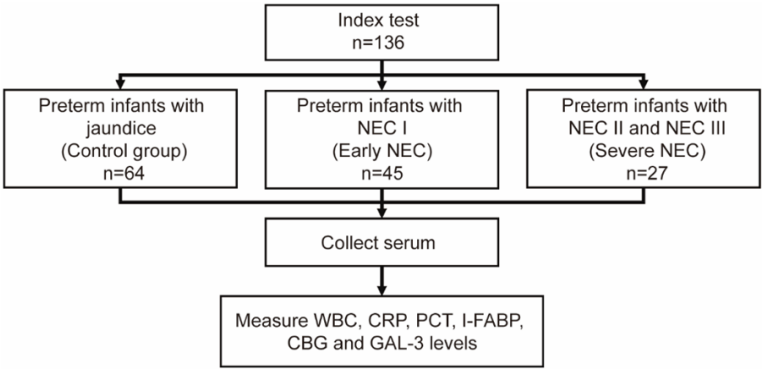

Figure 1. Patient flow diagram. NEC: neonatal necrotising enterocolitis.

term infants with NEC I and 27 preterm infants with severe NEC, including NEC II and NEC III) and 64 preterm infants with jaundice (control group) were prospectively enrolled between January 2018 and December 2019 from the Second Affiliated Hospital of Zhengzhou University. In this study, all the guardians of the participants signed informed consent forms. All experiments were approved by the Ethics Committee of the Second Affiliated Hospital of Zhengzhou University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The sex, gestational age, age of sampling (days), and birth weight (g) in the control group, NEC I group, and NEC II+III group were not significantly different ($P > 0.05$). Five millilitres of fasting venous blood was extracted from each newborn patient using an ordinary biochemical tube without anticoagulants. After 30 minutes, the blood was centrifuged at 3000 rpm for 10 minutes and the serum was then used for subsequent testing.

Measurement of levels of WBC, CRP, PCT, I-FABP, CBG, and GAL-3

WBC count was carried out by routine laboratory procedures using the ABX Pentra 120 (Impedance & Optical, Minnesota, USA). CRP levels were measured by a Roche Cobas 8000 fully automatic biochemical analyser and reagents by immunoturbidimetry (Roche, Basle, Switzerland). PCT levels were measured by the Roche Cobas E601 fully automatic electrochemiluminescence immunoassay analyser and reagents by electrochemiluminescence (Roche). I-FABP, CBG, and GAL-3 levels were measured with the Human I-FABP Quantikine enzyme-linked immunosorbent assay (ELISA) Kit (R&D Systems, Minneapolis, MN, USA), Human CBG ELISA Kit (CUSABIO, Wuhan, Hubei, China), and human galectin-3 Quantikine ELISA kit (R&D Systems) in accordance with manufacturer's instructions.

Statistical analyses

SPSS 19.0 software (IBM, Armonk, NY, USA) was used to perform all analyses. The Shapiro-Wilk test was used to assess the normal distribution of the data. Data that did not conform to a normal distribution are described using the median and the 25th and 75th percentiles. The differences among control, NEC I, and NEC II+III groups were evaluated using a nonparametric test followed by post hoc Dunn's multiple comparisons test. The Mann-Whitney U test was also applied as a nonparametric significance test between the two groups. Diagnostic value was analysed by the receiver operating characteristic (ROC) curve to calculate the best cutoff, as well as by the area under the curve (AUC), Youden index, sensitivity, and specificity. In all analyses, a $P < 0.05$ was considered significant.

Results

Presence of WBC, CRP, PCT, I-FABP, CBG, and GAL-3 in serum

The levels of WBC, CRP, PCT, I-FABP, CBG, and GAL-3 in the serum are shown in **Figure 2**. The results show that the levels of WBC, CRP, PCT, I-FABP, CBG, and GAL-3 were significantly higher in the NEC I and NEC II+III groups, compared to the control group. Compared with the NEC I group, the levels of WBC, CRP, PCT, I-FABP, CBG, and GAL-3 were significantly higher in the NEC II+III group.

GAL-3 as a marker for diagnosis of early NEC

The control group was considered a negative group and the NEC I group was the positive group, while the diagnostic values of WBC, CRP, PCT, I-FABP, CBG, and GAL-3 for the early diag-

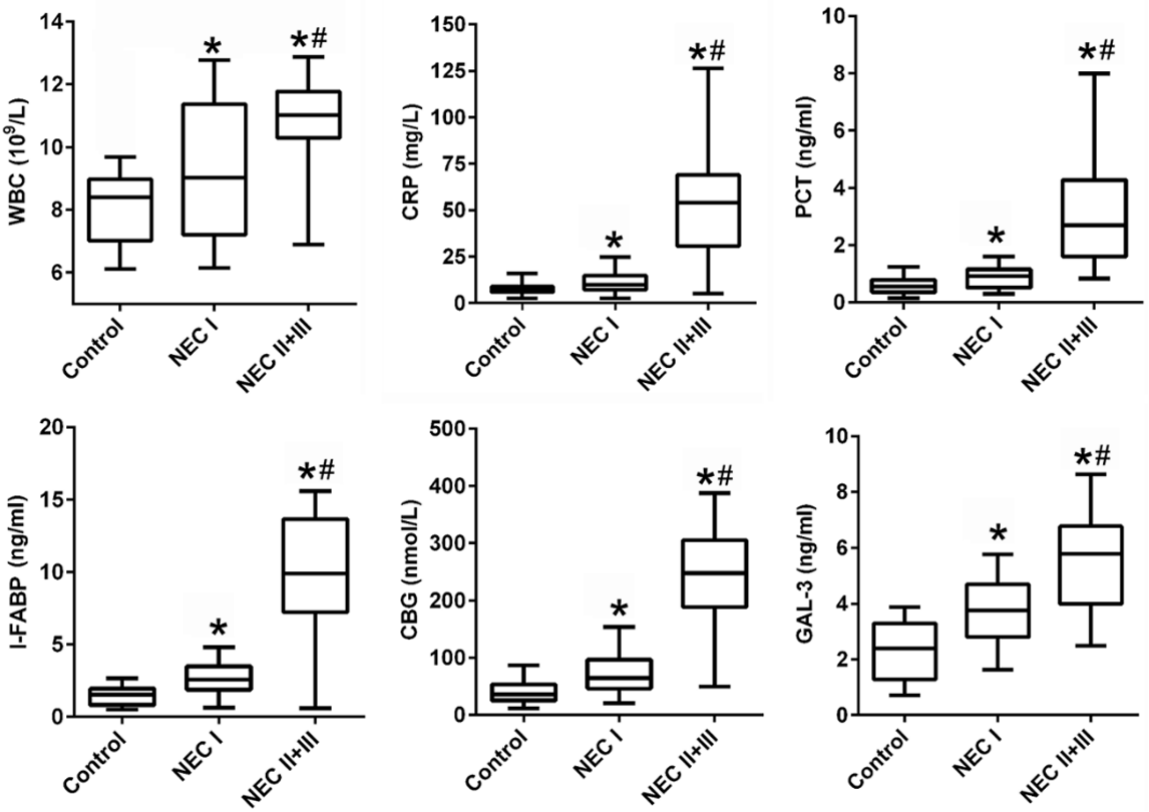

Figure 2. Levels of WBC, CRP, PCT, I-FABP, CBG, and GAL-3 were higher in early and severe NEC. Levels of white blood cells (WBC), C-reactive protein (CRP), procalcitonin (PCT), intestinal fatty acid binding protein (I-FABP), serum cytosolic β-glycosidase (CBG), and galectin-3 (GAL-3) in the control, NEC I, and NEC II+III groups were measured. *P < 0.05, NEC I, and NEC II+III groups compared with the control group; #P < 0.05, NEC II+III group compared with NEC I group. NEC: neonatal necrotising enterocolitis.

nosis of NEC were analysed with a ROC curve. These results are shown in **Figure 3A**, and the diagnostic characteristics of these markers for the early stages of NEC are shown in **Table 1**. According to these results, GAL-3 had higher sensitivity and specificity than the other markers. At the optimal cutoff value (GAL-3 > 2.615 mg/ml), the sensitivity and specificity were 59.38% and 88.89%, respectively, with an AUC of 0.828 (0.739-0.917).

NEC I group was used as a negative group while the NEC II+III group was used as the positive group, and the diagnostic values of WBC, CRP, PCT, I-FABP, CBG, and GAL-3 for the detection of severe NEC were analysed with an ROC curve. These results are shown in **Figure 3B** and the diagnostic characteristics of these markers for the detection of severe NEC are listed in **Table 2**. This indicates that these markers have a clear diagnostic value for diagnosing severe NEC. The results show that GAL-3 has a definite value for diagnosing severe NEC. The GAL-3 had higher sensitivity and specificity than the other markers. At the optimal cutoff value (GAL-3 > 5.15 mg/ml), the sensitivity and specificity were 93.33% and 88.89%, respectively, with an AUC of 0.934 (0.861-1.000).

Correlation between GAL-3 and prognosis in patients with NEC

To evaluate the correlation between GAL-3 and prognosis in patients with NEC, we found 72 patients with NEC, including 11 patients who died (four preterm infants with NEC I and seven preterm infants with severe NEC, including NEC II and NEC III). Univariate and multivariate analysis were utilised to evaluate whether GAL-3 level and various clinicopathologic values were independent prognostic parameters of patient outcome. The results are shown in **Table 3**. The results suggest that GAL-3 level is an independent prognostic factor for poor prognosis in NEC.

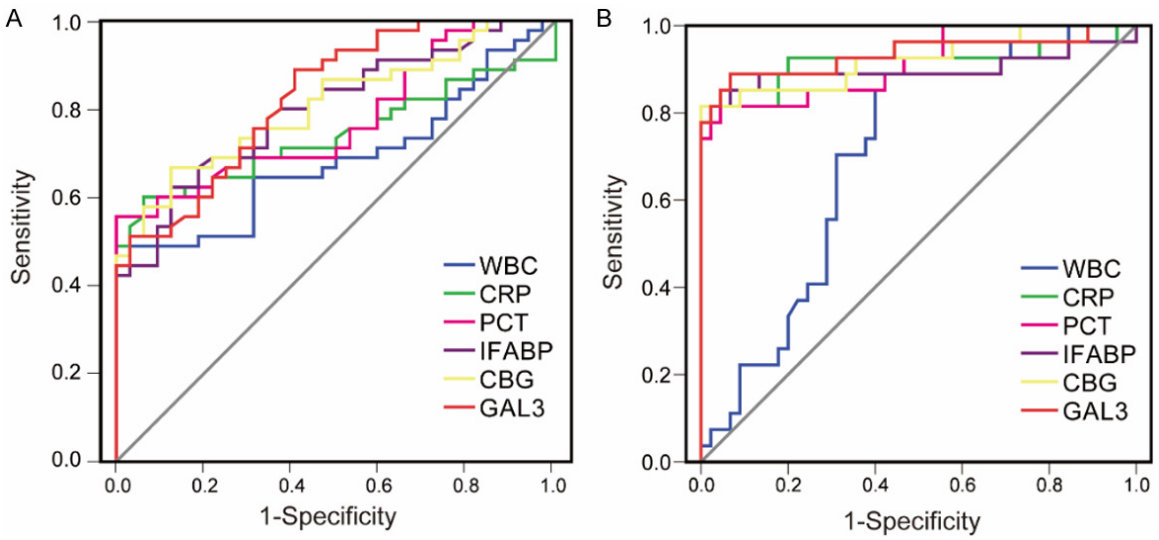

Figure 3. GAL-3 as a biomarker for the diagnosis of early and severe NEC. GAL-3 had diagnostic value for early and severe stages of neonatal necrotising enterocolitis. A. The diagnostic values of white blood cells (WBC), C-reactive protein (CRP), procalcitonin (PCT), intestinal fatty acid binding protein (I-FABP), serum cytosolic β-glycosidase (CBG), and galectin-3 (GAL-3) for the early stages of neonatal necrotising enterocolitis were analysed by receiver operating characteristic curve. B. The diagnostic values of white blood cells (WBC), C-reactive protein (CRP), procalcitonin (PCT), intestinal fatty acid binding protein (I-FABP), serum cytosolic β-glycosidase (CBG), and galectin-3 (GAL-3) for severe neonatal necrotising enterocolitis were analysed by receiver operating characteristic curve. NEC: neonatal necrotising enterocolitis.

Table 1. Diagnostic characteristics of white blood cells (WBC), C-reactive protein (CRP), procalcitonin (PCT), intestinal fatty acid binding protein (I-FABP), serum cytosolic β-glycosidase (CBG), and galectin-3 (GAL-3) for early stages of neonatal necrotising enterocolitis

Cutoff	AUC (95% CI)	Specificity	Sensitivity
WBC > 9.74×10^9/L	0.685 (0.567-0.803)	100.00% (32/32)	46.67% (21/45)
CRP > 10.54 mg/L	0.740 (0.628-0.851)	93.75% (30/32)	60.00% (27/45)
PCT > 0.831 ng/ml	0.775 (0.672-0.877)	100.00% (32/32)	55.56% (25/45)
I-FABP > 2.195 ng/ml	0.800 (0.703-0.896)	87.50% (28/32)	62.22% (28/45)
CBG > 54.63 nmol/L	0.808 (0.713-0.902)	87.50% (28/32)	66.67% (30/45)
GAL-3 > 2.615 ng/ml	0.828 (0.739-0.917)	59.38% (19/32)	88.89% (40/45)

Table 2. Diagnostic characteristics of white blood cells (WBC), C-reactive protein (CRP), procalcitonin (PCT), intestinal fatty acid binding protein (I-FABP), serum cytosolic β-glycosidase (CBG), and galectin-3 (GAL-3) for severe neonatal necrotising enterocolitis

Cutoff	AUC (95% CI)	Specificity	Sensitivity
WBC > 9.98×10^9/L	0.711 (0.591-0.830)	57.78% (26/45)	88.89% (24/27)
CRP > 27.56 mg/L	0.915 (0.827-1.000)	100.00% (45/45)	81.48% (22/27)
PCT > 1.42 ng/ml	0.914 (0.841-0.988)	95.56% (43/45)	81.48% (22/27)
I-FABP > 5.60 ng/ml	0.899 (0.797-1.000)	100.00% (45/45)	81.48% (22/27)
CBG > 167.70 nmol/L	0.923 (0.849-0.997)	100.00% (45/45)	81.48% (22/27)
GAL-3 > 5.15 ng/ml	0.934 (0.861-1.000)	93.33% (42/45)	88.89% (24/27)

Discussion

Clinical features of NEC are complex and there is a lack specific markers corresponding to the development of the disease. It is therefore important to identify biomarkers that can be used to diagnose early NEC and to assess disease severity. In this study, we found that levels

Table 3. Results of univariate and multivariate analyses of different measures in patients with NEC by Cox regression

Measure	Univariate analysis			Multivariate analysis		
	Risk ratio	95% CI	P	Risk ratio	95% CI	P
WBC	1.280	0.896-1.830	0.175			
CRP	1.028	1006-1.051	0.011*	0.985	0.933-1.040	0.583
PCT	2.435	1.463-4.051	0.001*	2.002	0.758-1.040	0.161
I-FABP	1.346	1.154-1.570	< 0.001*	1.235	0.860-1.774	0.253
CBG	1.013	1.006-1.021	< 0.001*	0.983	0.958-1.008	0.175
GAL-3	5.877	2.268-15.231	< 0.001*	6.834	1.274-36.657	0.025*

CI: confidence interval; WHO: World Health Organization. *: P < 0.05 was considered significant.

of WBC, CRP, PCT, I-FABP, CBG, and GAL-3 were significantly higher in patients with NEC (including NEC I, II, and III) compared to the control group. Compared with the NEC I group, the levels of WBC, CRP, PCT, I-FABP, CBG, and GAL-3 were significantly higher in the NEC II+III group. Moreover, it was found that GAL-3 had a clear diagnostic value for diagnosing early and severe NEC. To diagnose early and severe NEC, use of the GAL-3 marker had higher sensitivity and specificity than the use of other markers such as WBC, CRP, PCT, I-FABP, and CBG. These results suggest that GAL-3 can be used to diagnose early NEC and to assess the severity of NEC in the patient.

WBC, CRP, and PCT are non-specific inflammatory markers that are elevated in febrile diseases, various inflammation-induced diseases, and trauma. A previous study has shown that in intestinal stricture-a severe and common complication of NEC-levels of CRP, WBC, and PCT were elevated and maintained until the stricture was healed [6]. Another study found that WBC, CRP, and PCT levels were increased in infants with NEC stages II and III compared to those with NEC stage I [16]. These studies suggested that CRP and PCT are of limited value for the early diagnosis of NEC, yet these markers are important for NEC monitoring and prognosis. As expected, this study found that CRP, WBC, and PCT levels showed an increasing trend in the control, NEC I, and NEC II+III groups, and these markers had a lower sensitivity and specificity for diagnosing early NEC; while CRP and PCT had high sensitivity and specificity for diagnosing severe NEC. The results suggest that CRP and PCT act as markers for diagnosing severe NEC.

I-FABP, CBG, and GAL-3 are novel inflammatory markers. I-FABP is a small soluble protein in intestinal mucosal epithelial cells, and is a sensitive biomarker for early intestinal mucosal injury due to mesenteric ischemia [17]. I-FABP levels were significantly higher in patients with NEC compared to healthy preterm infants, indicating that they can be used as a specific measure for the early diagnosis, monitoring, and prognosis of NEC [18-20]. CBG is present in several organs but mostly in the small intestine. A previous study found that CBG levels were high in patients with NEC, and that serum CBG may be an early marker for diagnosing NEC [9]. GAL-3 is a novel marker reflecting pro-inflammatory status, since GAL-3 levels rapidly increase when inflammation occurs. In this study, it was found that I-FABP, CBG, and GAL-3 levels showed an increasing trend in the control, NEC I, and NEC II+III groups. I-FABP, CBG, and GAL-3 had higher sensitivity and specificity than CRP, WBC, and PCT for diagnosing early NEC. Moreover, GAL-3 had higher sensitivity and specificity than I-FABP and CBG for diagnosing early NEC. Furthermore, I-FABP, CBG, and GAL-3 had clear diagnostic value for diagnosing severe NEC. In particular, GAL-3 had higher sensitivity and specificity than WBC, CRP, PCT, I-FABP, and CBG for diagnosing severe NEC. Overall, the results of the present study suggest that GAL-3 can be used as a marker for diagnosis of early NEC and severe NEC.

Nonetheless, the present study had several limitations, one of which was the small sample size. Additionally, this study only measured levels of markers just after patients were admitted to hospital and no longitudinal data and changes were obtained. The diagnostic value of these markers for diagnosing NEC must therefore be further evaluated. Furthermore, correlations between mortality, antibiotic treatment,

and marker levels were not evaluated. Finally, GAL-3 has an unsatisfactory sensitivity (59.38%) and specificity (88.89%) for diagnosing early NEC. A set of markers, encompassing multiple markers such as I-FABP, CBG, and GAL-3 could be used to diagnose NEC in a timely fashion.

Conclusion

GAL-3 is a suitable marker for the diagnosis and prognosis of early and severe NEC and provides higher sensitivity and specificity than WBC, CRP, PCT, I-FABP, or CBG.

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Disclosure of conflict of interest

None.

Abbreviations

NEC, neonatal necrotising enterocolitis; GAL-3, galectin-3; WBC, white blood cell; CRP, Creactive protein; PCT, procalcitonin; I-FABP, intestinal fatty acid binding protein; CBG, serum cytosolic β-glycosidase.

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