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Original Article

Elevated KLF2 and YKL-40 with reduced vitamin A as predictive biomarkers for severity and prognosis in neonatal respiratory distress syndrome

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Abstract: Objective: This study aimed to investigate the relationships between Krüppel-like factor 2 (KLF2), YKL-40, and vitamin A levels, as well as their association with the severity and prognosis of Neonatal Respiratory Distress Syndrome (NRDS), with the goal of improving predictive accuracy and clinical management. Methods: A retrospective cohort study was conducted at Bozhou Municipal People's Hospital between January 2019 and January 2022. A total of 128 neonates diagnosed with NRDS were included, along with 128 healthy neonates as controls. Blood samples were collected within one hour of admission. Levels of KLF2, YKL-40, and vitamin A were measured using enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC). Neonates were grouped based on NRDS severity, and the relationships between biomarker levels and clinical outcomes were analyzed. Results: Significant differences in KLF2, YKL-40, and vitamin A levels were observed between the NRDS group and control group ($P < 0.001$). Elevated KLF2 and YKL-40 levels were strongly correlated with more severe disease, while lower vitamin A levels were associated with worse outcomes. Multivariate logistic regression identified KLF2, YKL-40, and vitamin A levels as independent predictors of NRDS severity ($P < 0.05$). The nomogram achieved a concordance index (C-index) of 0.93 (95% CI: 0.89-0.97), indicating excellent discrimination of different disease severity. Receiver's operating characteristic (ROC) curve analysis showed that combining these biomarkers improved the prediction of NRDS severity, with the joint model achieving an AUC of 0.95, sensitivity of 91.2%, and specificity of 90.1%. Conclusion: KLF2, YKL-40, and vitamin A are significant biomarkers for predicting the severity and prognosis of NRDS. Combining these biomarkers enhances predictive accuracy, aiding in the early identification and personalized management of neonates with NRDS.

Keywords: Neonatal respiratory distress syndrome, KLF2, YKL-40, vitamin A, biomarkers, severity prediction, prognosis

Introduction

Neonatal Respiratory Distress Syndrome (NRDS) remains a leading cause of morbidity and mortality in premature infants worldwide, characterized by insufficient pulmonary surfactant production, resulting in impaired gas exchange and respiratory failure [1, 2]. Epidemiological studies indicate that NRDS predominantly affects preterm infants, with its incidence higher in those with lower birth weight and younger gestational age. Recent advancements in neonatal care, such as the use of antenatal corticosteroids and surfactant therapy, have reduced its incidence in preterm infants. However, its occurrence in full-term infants has risen, potentially due to factors such as persistent pulmonary hypertension [3, 4].

In NRDS clinical presentation, there is growing recognition of the role of biomarkers in predicting the severity and prognosis of NRDS. Vitamin A, a key nutrient for lung development, has emerged as a potential biomarker for NRDS severity. Lower levels of vitamin A correlate with increased disease severity and poor prognosis in neonates, as it is essential for lung tissue growth and surfactant production [5, 6]. Moreover, YKL-40, a protein involved in inflam-

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mation and tissue remodeling, has been studied as a potential marker for neonatal inflammatory conditions, including NRDS. Elevated YKL-40 levels are associated with more severe disease outcomes, providing insights into the inflammatory processes that underlie the syndrome [7]. Studies suggest that KLF2 may influence lung tissue development and the inflammatory response in respiratory diseases, potentially offering a target for therapeutic interventions [8].

NRDS remains a major contributor to neonatal morbidity and mortality, despite advances in current therapies. The combination of biomarkers such as KLF2, YKL-40, and Vitamin A introduces an innovative approach to understanding the complex pathophysiology of NRDS. This study is the first to systematically investigate the relationships between these biomarkers in neonates and assess how their levels correlate with the severity and prognosis of NRDS. By integrating these novel biomarkers with traditional clinical assessments, our research not only enhances early diagnostic precision but also paves the way for the development of targeted therapeutic strategies. The clinical significance of this study lies in its potential to enable personalized intervention plans, ultimately improving patient outcomes and reducing long-term complications associated with NRDS.

Methods

Research design

This study is a single-center, retrospective cohort study conducted at Neonatology Department of Bozhou People's Hospital, a tertiary Grade A academic medical center. The research protocol was reviewed and approved by the hospital's Ethics Committee (Approval Number: 20190215003). Due to the retrospective design and minimal risk to patients, informed consent from patients was waived.

Study subjects and case selection

This study included neonates diagnosed with NRDS who were hospitalized in the Neonatology Department of Bozhou People's Hospital from January 2019 to January 2022. Based on the clinical manifestations and imaging findings of NRDS, cases were grouped according to their severity and compared with a healthy neonate control group.

Inclusion Criteria: (1) Gestational age ≥ 28 weeks; (2) Diagnosed with NRDS based on clinical symptoms (e.g., respiratory distress, hypoxemia) and imaging findings (e.g., chest X-ray showing alveolar collapse, pulmonary consolidation); (3) Blood samples collected within one hour of admission; (4) Complete clinical and laboratory data available in the Electronic Medical Record System (EMS).

Exclusion Criteria: (1) Gestational age < 28 weeks; (2) Congenital anomalies (e.g., congenital heart disease, chromosomal abnormalities); (3) Severe comorbidities (e.g., sepsis, liver/kidney failure); (4) Missing critical data (e.g., incomplete biomarker measurements or clinical outcomes).

Grouping Criteria: Patients were grouped based on the severity of NRDS using the chest X-ray grading method. Following the American Academy of Pediatrics (AAP) Neonatal Resuscitation Guidelines [9], the patients were divided into three groups:

(1) Mild Group (n = 43): Chest X-ray showed reduced lung markings, diffuse punctate granulation shadows, and no obvious bronchial inflation signs, indicating mild NRDS; (2) Moderate Group (n = 43): Chest X-ray showed denser granular shadows, clear bronchial inflation signs, and reduced lung field transparency, indicating moderate NRDS; (3) Severe Group (n = 42): Chest X-ray showed large consolidation shadows with bronchial inflation signs, significant lung volume reduction, bilateral ground-glass opacity, and blurred cardiac and diaphragmatic outlines, indicating severe NRDS.

After applying the inclusion and exclusion criteria, a total of 128 neonates were included in the NRDS group. To ensure the study's control and reliability, 128 healthy neonates were selected as the control group. These healthy neonates had no respiratory or other major diseases and did not meet the clinical or imaging criteria for NRDS.

Data collection

Laboratory data included: 1) Blood gas analysis (pH, PaO_2, $PaCO_2$, lactate) to assess respiratory

and metabolic status. 2) Complete blood count (CBC) to evaluate infection and anemia. 3) Blood cultures to rule out sepsis, as neonatal infections can mimic or exacerbate NRDS. 4) Biomarker assays: KLF2 and YKL-40 (ELISA), vitamin A (HPLC).

Explanation for blood cultures: Blood cultures were routinely performed to exclude sepsis, a common differential diagnosis in neonates with respiratory distress. This ensured accurate NRDS diagnosis and appropriate management.

Patient information: Demographic and clinical data were extracted from the EMS, including basic information such as gestational age, birth weight, gender, and mode of delivery. Clinical evaluations, including 1-minute and 5-minute Apgar scores, oxygen requirement, and respiratory support methods, were also recorded. These data were collected within 24 hours of patient admission.

Biomarker testing: KLF2 and YKL-40 were measured using enzyme-linked immunosorbent assay (ELISA) kits provided by Shanghai Yun Biotechnology Co., Ltd. (Batch Number: BYK-20210901), strictly following the manufacturer's instructions. Absorbance was measured at 450 nm using a Beckman Coulter microplate reader, and concentrations were calculated using a standard curve. Vitamin A levels were measured by high-performance liquid chromatography (HPLC) using a Waters e2695 separation module and a Waters 2489 UV/Vis detector. The analysis was performed with a C18 column (4.6 mm × 150 mm, 5 μm, Agilent Technologies) and a mobile phase of methanol: water (95:5, v/v) at a flow rate of 1.0 mL/min, with a detection wavelength of 325 nm. Samples were collected on the second day after neonatal admission.

Clinical data collection: Oxygen Requirement (%) is defined as the percentage of oxygen concentration required during oxygen therapy relative to the total gas concentration. The maximum oxygen requirement percentage during the neonate's hospital stay was recorded. The method of respiratory support was documented, specifying the type of respiratory support provided (e.g., CPAP or mechanical ventilation). The total hospital stay duration was recorded for each neonate.

Data management: All collected data were anonymized before being entered into the database to ensure data security and privacy. Data cleaning and preprocessing were performed by two independent researchers to ensure data accuracy and consistency. Laboratory quality control measures included the use of quality control samples for each test batch to ensure the reliability and reproducibility of the results.

Statistical analysis

Statistical analysis was performed using SPSS 26.0. Continuous variables were assessed for normality using Shapiro-Wilk tests. Normally distributed data were expressed as mean ± standard deviation (SD) and compared using one-way ANOVA with Bonferroni post-hoc correction. Non-normally distributed data were expressed as median (interquartile range, IQR) and analyzed using the Kruskal-Wallis test. Categorical variables were compared using chi-square tests. Multivariate logistic regression (forward stepwise method) was applied to identify independent predictors of NRDS severity, adjusting for gestational age, birth weight, and Apgar scores. Variables with $P < 0.05$ in univariate analysis were included. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Prior to multivariate logistic regression, the linearity assumption between continuous predictors and the logit of the outcome was assessed using the Box-Tidwell test. Variables with a non-linear relationship ($P < 0.05$) would require transformation or categorization; however, all continuous variables in this study met the linearity assumption ($P > 0.05$). Receiver's operating characteristic (ROC) curves assessed biomarker performance. All tests were two-tailed, with $P < 0.05$ deemed significant.

Results

Basic characteristics of patients

The basic characteristics of the two groups of neonates are shown in **Table 1**. In terms of basic characteristics, there were no significant differences in gender and delivery mode between the NRDS group and the control group ($P > 0.05$). However, significant differences were observed in gestational age, birth weight, 1-minute and 5-minute Apgar scores, oxygen requirement, and levels of KLF2, YKL-40, and vitamin A ($P < 0.05$). Specifically, the NRDS

Table 1. Comparison of basic characteristics between NRDS and Control groups

Variable	NRDS Group (n = 128)	Control Group (n = 128)	χ^2/t	*P-value*
Gestational Age (weeks)	32.4 ± 2.5	34.7 ± 2.2	t = -5.21	< 0.001
Birth Weight (g)	1802 ± 345	2125 ± 320	t = -3.78	0.013
Gender (Male/Female)	75/53	70/58	χ^2 = 0.12	0.728
Delivery Mode (Vaginal/C-Section)	60/68	55/73	χ^2 = 0.23	0.634
1-minute Apgar Score	5.8 ± 1.2	7.9 ± 0.8	t = -8.56	< 0.001
5-minute Apgar Score	7.2 ± 1.1	8.7 ± 0.6	t = -6.89	< 0.001
Oxygen Requirement (%)	45.6 ± 15.4	21.3 ± 5.7	t = 7.23	< 0.001
Respiratory Support (%)				
CPAP	58.6	0	χ^2 = 70.15	< 0.001
Mechanical Ventilation	41.4	0	χ^2 = 52.14	< 0.001
KLF2 (ng/mL)	2.8 ± 0.9	1.4 ± 0.6	t = 6.78	< 0.001
YKL-40 (ng/mL)	5.6 ± 1.7	2.9 ± 0.8	t = 7.15	< 0.001
Vitamin A (μg/dL)	28.4 ± 6.2	42.1 ± 5.9	t = -5.64	< 0.001

Notes: NRDS, Neonatal Respiratory Distress Syndrome; CPAP, Continuous Positive Airway Pressure; KLF2, Kruppel-like Factor 2; YKL-40, Chitinase-3-like Protein 1.

Table 2. Validation of linearity assumption via Box-Tidwell test in logistic regression analysis

Variable	Box-Tidwell Test *P*-value	Linearity Assumption Valid
Gestational Age	0.18	Yes (P > 0.05)
Birth Weight	0.27	Yes (P > 0.05)
1-minute Apgar Score	0.15	Yes (P > 0.05)
5-minute Apgar Score	0.22	Yes (P > 0.05)
Oxygen Requirement (%)	0.31	Yes (P > 0.05)
KLF2 (ng/mL)	0.12	Yes (P > 0.05)
YKL-40 (ng/mL)	0.21	Yes (P > 0.05)
Vitamin A (μg/dL)	0.34	Yes (P > 0.05)

Notes: KLF2, Kruppel-like Factor 2; YKL-40, Chitinase-3-like Protein 1.

group had significantly higher levels of KLF2, YKL-40, and vitamin A compared to the control group. The distribution of respiratory support methods also differed significantly between the two groups, with a higher proportion of NRDS patients requiring mechanical ventilation and CPAP ($P < 0.001$).

Multivariate analysis

To identify variables associated with the occurrence of NRDS, a multivariate logistic regression analysis was conducted. The Box-Tidwell test demonstrated no significant deviations from linearity for any continuous variables (e.g., KLF2: $P = 0.12$; YKL-40: $P = 0.21$; Vitamin A: $P = 0.34$), confirming that these variables could be treated as linear predictors in the logistic regression model (**Table 2**). The variables selected for multivariate analysis were those that showed significant differences in the univariate analysis. In **Table 3**, continuous variables (e.g., gestational age, vitamin A) were included as continuous predictors. Apgar scores and oxygen requirements were treated as continuous variables. For biomarkers (KLF2, YKL-40, vitamin A), raw concentrations were directly entered into the model without transformation. The multivariate analysis identified gestational age, birth weight, 1-minute and 5-minute Apgar scores, oxygen requirement, KLF2, YKL-40, and vitamin A levels as independent risk factors for NRDS.

Comparison of basic characteristics and laboratory indicators among neonates with different disease severity

To further understand the impact of NRDS severity on various variables, patients were divided into mild, moderate, and severe groups, and a univariate analysis was conducted (**Table 4**). The results revealed that 1-minute and 5-minute Apgar scores, oxygen requirement, KLF2, YKL-40, and vitamin A levels differed significantly among the three groups. Specifically, the Mild Group had the lowest levels of KLF2, YKL-40, and 1-minute and 5-minute Apgar scores, while oxygen requirements and vitamin

Table 3. Multivariate logistic regression analysis results with variable assignment

Variable	Assignment Method	Odds Ratio (95% CI)	*P*-value
Gestational Age	Per week decrease (continuous)	1.23 (1.05-1.43)	0.012
Birth Weight	Per gram decrease (continuous)	1.56 (1.21-2.01)	< 0.001
1-minute Apgar Score	Per minute decrease (continuous)	1.78 (1.32-2.39)	< 0.001
5-minute Apgar Score	Per minute decrease (continuous)	1.45 (1.12-1.87)	0.004
Oxygen Requirement (%)	Per percentage point increase (continuous)	1.02 (1.01-1.03)	< 0.001
KLF2 (ng/mL)	Per ng/mL increase (continuous)	2.15 (1.68-2.91)	< 0.001
YKL-40 (ng/mL)	Per ng/mL increase (continuous)	1.89 (1.44-2.63)	< 0.001
Vitamin A (μg/dL)	Per μg/dL decrease (continuous)	1.03 (1.01-1.05)	0.006

Notes: CI, Confidence Interval; KLF2, Kruppel-like Factor 2; YKL-40, Chitinase-3-like Protein 1.

Table 4. Comparison of basic characteristics and laboratory indicators among three severity groups

Variable	Mild Group (n = 43)	Moderate Group (n = 43)	Severe Group (n = 42)	*P*-value
Gestational Age (weeks)	32.8 ± 2.3	32.2 ± 2.5	31.9 ± 2.6	0.124
Birth Weight (g)	1842 ± 341	1792 ± 348	1765 ± 355	0.083
Gender (Male/Female)	23/20	21/22	20/22	0.891
Delivery Mode (Vaginal/C-Section)	18/25	20/23	19/23	0.845
1-minute Apgar Score	6.2 ± 1.1	5.7 ± 1.2	5.3 ± 1.3	< 0.001
5-minute Apgar Score	7.5 ± 0.9	7.1 ± 1.0	6.8 ± 1.1	< 0.001
Oxygen Requirement (%)	38.4 ± 12.1	46.2 ± 14.5	53.4 ± 16.2	< 0.001
Respiratory Support (%)				
CPAP	51.2%	48.8%	45.2%	0.632
Mechanical Ventilation	30.2%	35.1%	40.5%	0.235
KLF2 (ng/mL)	2.3 ± 0.7	2.7 ± 0.8	3.1 ± 0.9	< 0.001
YKL-40 (ng/mL)	4.8 ± 1.4	5.4 ± 1.6	6.2 ± 1.7	< 0.001
Vitamin A (μg/dL)	30.4 ± 5.8	28.1 ± 5.9	26.5 ± 6.1	< 0.001

Notes: NRDS, Neonatal Respiratory Distress Syndrome; CPAP, Continuous Positive Airway Pressure; KLF2, Kruppel-like Factor 2; YKL-40, Chitinase-3-like Protein 1.

A levels were the highest. KLF2 and YKL-40 levels increased sequentially across the Mild, Moderate, and Severe groups ($P < 0.001$), while 1-minute and 5-minute Apgar scores, oxygen requirements, and vitamin A levels decreased sequentially ($P < 0.001$).

Multivariate regression analysis grouped by severity

Multivariate logistic regression analysis (**Table 5**) revealed that lower Apgar scores (1-minute: OR = 0.75, P < 0.001; 5-minute: OR = 0.71, P < 0.001) and higher oxygen requirements (OR = 1.02, P < 0.001) were significantly associated with NRDS severity. Elevated KLF2 (OR = 1.17, P = 0.001) and YKL-40 (OR = 1.23, P < 0.001) levels were independent predictors of severe NRDS, while lower vitamin A levels (OR = 0.95, P = 0.039) were associated with worse outcomes. These findings underscore the combined predictive value of clinical and biomarker data in assessing NRDS severity.

Nomogram construction and performance validation

To visually represent the combined predictive value of KLF2, YKL-40, and vitamin A for NRDS severity, a nomogram was developed using the multivariate logistic regression model. Each biomarker and clinical variable was assigned points based on their regression coefficients, enabling clinicians to estimate the probability of severe NRDS by summing the points from individual predictors.

The nomogram achieved a concordance index (C-index) of 0.93 (95% CI: 0.89-0.97), demonstrating excellent discrimination between se-

Table 5. The impact of various variables on the severity of NRDS in multivariate analysis

Variable	β Coefficient	Standard Error	*P*-value	OR Value	95% CI
1-minute Apgar Score	-0.28	0.08	< 0.001	0.75	(0.63, 0.89)
5-minute Apgar Score	-0.34	0.09	< 0.001	0.71	(0.59, 0.85)
Oxygen Requirement (%)	0.02	0.006	< 0.001	1.02	(1.01, 1.04)
KLF2 (ng/mL)	0.16	0.04	0.001	1.17	(1.08, 1.27)
YKL-40 (ng/mL)	0.21	0.05	< 0.001	1.23	(1.12, 1.35)
Vitamin A (μg/dL)	-0.05	0.02	0.039	0.95	(0.90, 1.01)

Notes: NRDS, Neonatal Respiratory Distress Syndrome; KLF2, Kruppel-like Factor 2; YKL-40, Chitinase-3-like Protein 1.

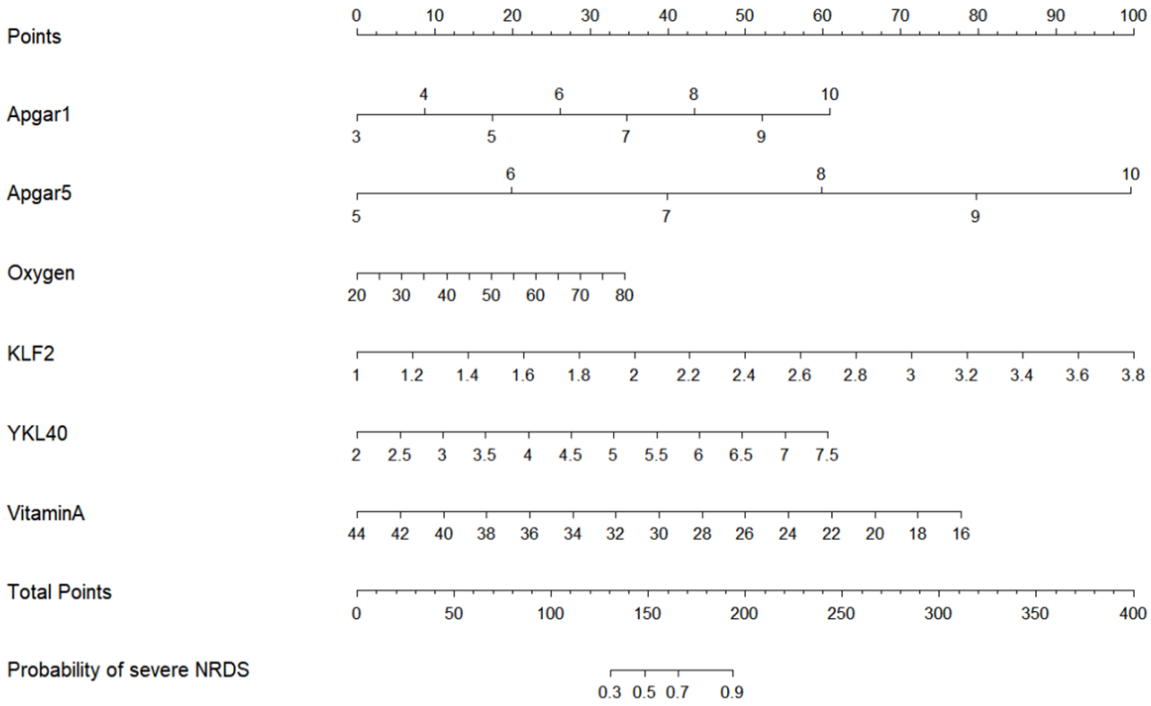

Figure 1. Nomogram for predicting NRDS severity. NRDS, Neonatal Respiratory Distress Syndrome; KLF2, Kruppel-like Factor 2; YKL-40, Chitinase-3-like Protein 1.

verity groups (**Figure 1**). The calibration curve (**Figure 2**) demonstrated strong agreement between predicted and observed probabilities of severe NRDS, with a mean absolute error of 0.021, suggesting the model's low prediction error across different risk intervals.

ROC curve analysis

The predictive performance of KLF2, YKL-40, Vitamin A, and their combination in assessing NRDS severity were evaluated using ROC curve analysis. Vitamin A demonstrated excellent predictive performance, with an AUC of 0.91, sensitivity of 89.5%, and specificity of 86.3%, indicating strong accuracy in distinguishing NRDS severity levels. YKL-40 showed a high AUC of 0.89, with sensitivity of 85.2% and specificity of 82.7%, reflecting reliable predictive ability for NRDS severity. KLF2 displayed moderate predictive performance with an AUC of 0.86, sensitivity of 83.0%, and specificity of 79.4%, suggesting it provides useful insights into NRDS severity. The combined predictive factors yielded the best overall performance, with an AUC of 0.95, a sensitivity of 91.2%, and a specificity of 90.1%, highlighting the high accuracy and reliability of this multi-factor model in predicting NRDS severity (**Figure 3**).

Comparison of hospital stay among neonates with different disease severity

The duration of hospital stay varied significantly across the three NRDS severity groups (**Table**

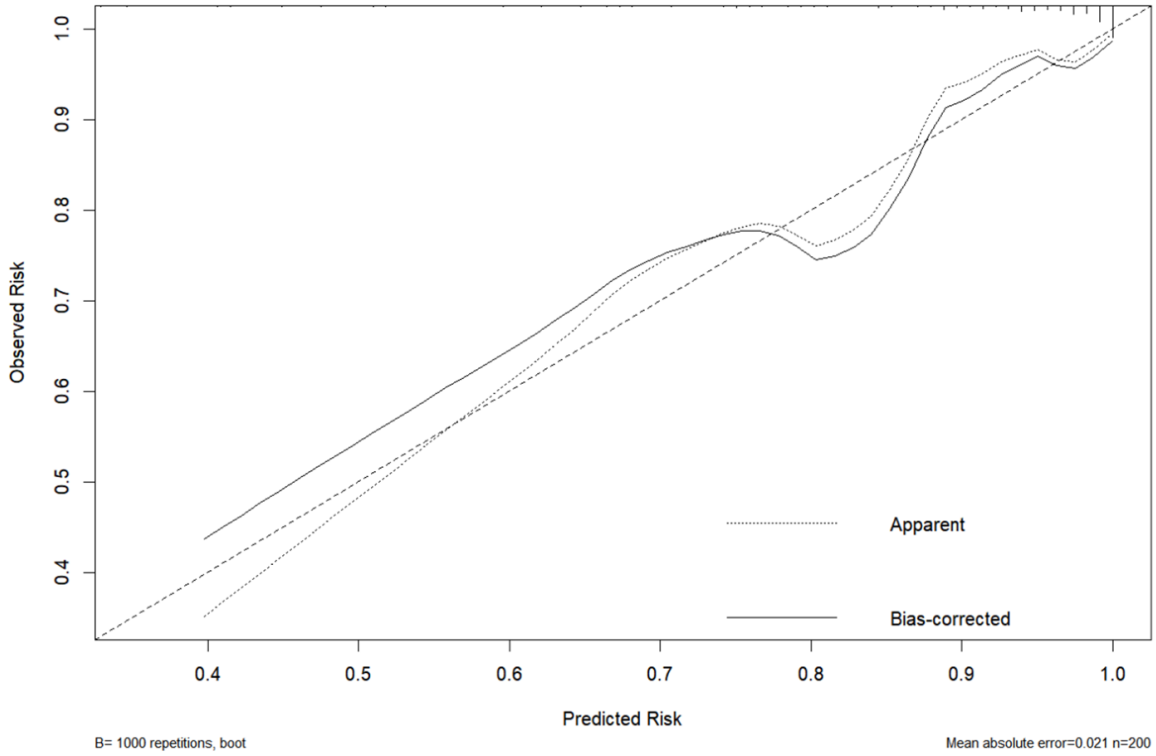

Figure 2. Calibration curve for the prediction model. The calibration curve evaluates the agreement between predicted and observed probabilities of severe NRDS. The orange line represents the calibrated model, showing the observed probabilities for different prediction intervals. The blue line represents the prediction model's curve, while the dashed black line represents perfect calibration (ideal agreement between predicted and observed probabilities). The close alignment of the calibrated model and prediction model curves with the reference line indicates strong calibration and predictive accuracy of the nomogram.

6; **Figure 4**). The mean hospital stays were 6.2 ± 1.1 days, 10.5 ± 2.3 days, and 18.7 ± 4.5 days for Mild, Moderate, and Severe groups, respectively ($P < 0.001$).

Correlation analysis of KLF2, YKL-40, and vitamin A with hospital stay duration

As shown in **Table 7**, the levels of KLF2 and YKL-40 were significantly positively correlated with the duration of hospital stay (KLF2: r = 0.65, $P < 0.001$; YKL-40: r = 0.52, $P = 0.003$). This suggests that higher levels of KLF2 and YKL-40 are associated with longer hospital stays, potentially reflecting their roles in inflammation and disease severity. In contrast, Vitamin A levels showed a significant negative correlation with hospital stay duration (r = -0.48, $P = 0.007$), suggesting that higher levels of vitamin A may be associated with shorter hospital stays, potentially indicating a protective role in reducing the length of hospital stay.

Discussion

This study aimed to explore the relationships between KLF2, YKL-40, and vitamin A levels and their association with the severity and prognosis of NRDS. The findings revealed that elevated levels of KLF2 and YKL-40 were significantly correlated with increased NRDS severity, while lower levels of vitamin A were associated with worse outcomes. Specifically, the study demonstrated that higher concentrations of KLF2 and YKL-40 were observed in neonates with more severe NRDS, and their levels were linked to prolonged hospital stays. Conversely, lower vitamin A levels were associated with more severe disease and a longer duration of hospitalization. These biomarkers, therefore, show promise as predictive indicators for NRDS severity and prognosis.

KLF2, a transcription factor involved in vascular homeostasis and inflammation, plays a crucial role in neonatal lung development. In this study,

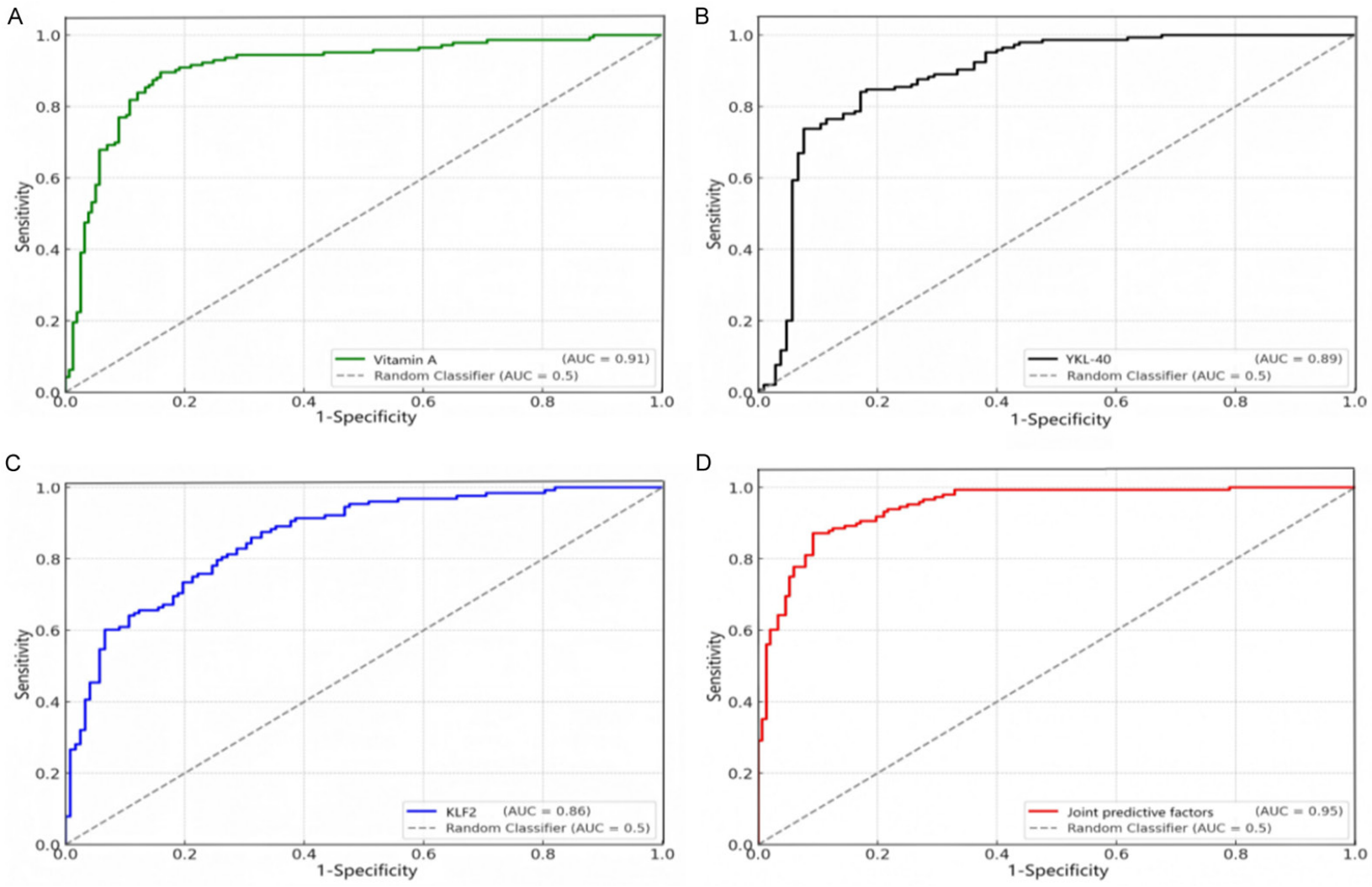

Figure 3. ROC curve and AUC comparison of predictive factors for NRDS severity. A: ROC curve for vitamin A in assessing NRDS, with an AUC of 0.91; B: ROC curve for YKL-40 in assessing NRDS, with an AUC of 0.89; C: ROC curve for KLF2 in assessing NRDS, with an AUC of 0.86; D: ROC curve for combined detection of vitamin A, YKL-40, and KLF2 in assessing NRDS, with an AUC of 0.95. ROC, Receiver's operating characteristic; KLF2, Kruppel-like Factor 2; YKL-40, Chitinase-3-like Protein 1.

Table 6. Comparison of hospital stay duration among three groups

Variable	Mild Group (n = 43)	Moderate Group (n = 43)	Severe Group (n = 42)	*P*-value
Hospital Stay (days)	6.2 ± 1.1	10.5 ± 2.3	18.7 ± 4.5	< 0.001

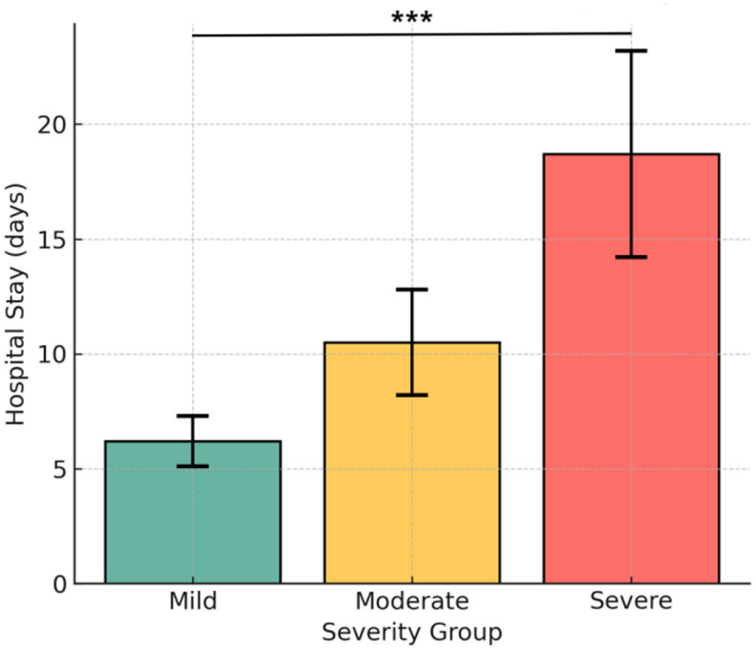

Figure 4. Comparison of hospital stay duration among three groups. ***$P < 0.001$.

Table 7. Correlation analysis

Variable	Correlation Coefficient (r)	95% CI	*P-value*
KLF2 (ng/mL)	0.65	(0.45, 0.80)	< 0.001
YKL-40 (μg/mL)	0.52	(0.24, 0.70)	0.003
Vitamin A (μg/dL)	-0.48	(-0.72, -0.20)	0.007

Notes: KLF2, Kruppel-like Factor 2; YKL-40, Chitinase-3-like Protein 1.

elevated levels of KLF2 correlated with more severe NRDS, suggesting its potential as a biomarker for disease severity. This finding is consistent with previous studies indicating that KLF2 is involved in lung inflammation and tissue remodeling in various neonatal respiratory diseases [10, 11]. Furthermore, KLF2's role in regulating endothelial function and inflammatory responses has been highlighted as an essential factor in the pathophysiology of NRDS [12]. These findings further support the use of KLF2 as a biomarker for predicting the severity of NRDS and potentially guiding early intervention strategies.

YKL-40, a glycoprotein associated with inflammation and tissue remodeling, has been identified as a promising biomarker in various diseases, including NRDS. This study found that higher levels of YKL-40 were associated with increased disease severity and longer hospital stays, aligning with previous research. Steletou et al. demonstrated that elevated YKL-40 levels are indicative of an intense inflammatory response, a key characteristic of NRDS [13]. Similarly, YKL-40's involvement in fibrosis and airway remodeling has been well documented, suggesting that it could serve as an important marker for monitoring inflammatory processes in NRDS [14-16]. These findings strengthen the argument that YKL-40 could be integrated into clinical practice as a prognostic tool for NRDS.

Vitamin A, essential for lung development and surfactant production, has long been considered a protective factor in neonatal respiratory health. In this study, lower vitamin A levels were associated with more severe cases of NRDS, supporting the hypothesis that vitamin A deficiency exacerbates respiratory failure. Elfarargy et al. [17] also highlighted the importance of vitamin A in lung tissue growth and surfactant synthesis, indicating that insufficient vitamin A levels contribute to the pathogenesis of NRDS. Moreover, studies have shown that vitamin A supplementation may improve outcomes in neonates with respiratory distress, making it a potential target for therapeutic interventions [18-20]. These results reinforce vitamin A's role as a protective factor in neonatal respiratory health and underscore its potential as a therapeutic agent in NRDS management.

The integration of KLF2, YKL-40, and vitamin A as biomarkers could significantly enhance the

clinical management of NRDS. The ability to predict disease severity and outcomes using these biomarkers could inform treatment decisions, leading to more personalized and effective care for neonates. The ROC analysis conducted in this study demonstrated high sensitivity and specificity for these biomarkers, particularly when used in combination. This finding is consistent with previous studies, which emphasize the reliability of these biomarkers in assessing disease severity and prognosis [21-24]. However, while the results are promising, further longitudinal studies are needed to confirm the utility of these biomarkers in clinical practice, particularly in large-scale, multicenter trials, to validate their predictive accuracy and therapeutic potential.

Despite the promising results, this study has several limitations that should be considered. First, the retrospective nature of the study may introduce bias, particularly in terms of patient selection and data collection. While the sample size was adequate for preliminary analysis, it remains relatively small, and the results may not be fully generalizable to larger or more diverse populations. Additionally, while this study focuses on the relationship between these biomarkers and NRDS severity, it does not address the potential effects of interventions, such as vitamin A supplementation or targeted therapies for KLF2 and YKL-40, on patient outcomes. Further randomized controlled trials are necessary to evaluate the therapeutic potential of targeting these biomarkers. Finally, the cross-sectional design limits the ability to draw conclusions about the long-term prognostic value of these biomarkers beyond the immediate neonatal period.

Conclusion

KLF2, YKL-40, and vitamin A are identified as robust biomarkers for predicting NRDS severity and prognosis. Elevated KLF2 and YKL-40 levels reflect inflammatory and tissue-remodeling pathways, while vitamin A deficiency exacerbates disease progression. The integration of these biomarkers into clinical practice could improve early risk stratification and personalized management, potentially reducing morbidity and shortening hospitalization duration. Future prospective studies are needed to confirm these findings and explore therapeutic interventions targeting these biomarkers.

Disclosure of conflict of interest

None.

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