

Incremental Predictive Value of Cord Blood Oxidative Stress Biomarkers for Neonatal Acute Respiratory Distress Syndrome After Meconium-Stained Amniotic Fluid Exposure

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Abstract

Neonatal acute respiratory distress syndrome (NARDS) develops in only a subset of neonates exposed to meconium-stained amniotic fluid (MSAF), and birth-time biological markers may improve early risk assessment. We evaluated whether umbilical cord blood oxidative stress biomarkers provide incremental predictive information beyond clinical variables in a single-center retrospective cohort. Of 182 neonates with intrapartum MSAF screened between January 2020 and January 2026, 168 were included and 50 developed NARDS within 7 days. Cord blood malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), and superoxide dismutase (SOD) were measured by ELISA. A clinical logistic model was developed using LASSO-selected predictors, and the three biomarkers were added as a prespecified panel. Addition of MDA, 4-HNE, and SOD increased the AUC from 0.901 to 0.969 (difference 0.068, 95% CI 0.019–0.117; $P = 0.006$) and reduced the Brier score from 0.106 to 0.055. Optimism-corrected AUCs were 0.890 and 0.955 for the clinical and enhanced models, respectively, and the enhanced model showed greater net benefit across clinically relevant thresholds. Cord blood oxidative stress biomarkers provided incremental predictive information beyond birth-time clinical variables; external validation is required before individual risk estimation.

Introduction

Meconium-stained amniotic fluid (MSAF) is readily recognized at delivery, yet the subsequent respiratory course remains difficult to anticipate.^[1] Most exposed neonates do not develop severe lung injury, whereas a subset progresses to neonatal acute respiratory distress syndrome (NARDS), often within a narrow postnatal window.^[1, 2] More than 90% of perinatal NARDS cases are diagnosed within the first 3 days of life, placing the clinically relevant prediction window at or near birth, before respiratory failure is fully established.^[3] Clinical prediction based on perinatal characteristics and routine laboratory data is feasible, but such information mainly reflects clinical susceptibility and may not capture the biological response linking meconium exposure to subsequent lung injury.^[4, 5]

Meconium-induced lung injury is not solely a consequence of airway obstruction. Meconium activates innate immune and inflammatory pathways, promotes neutrophil and macrophage activation, and enhances reactive oxygen species generation, lipid peroxidation, and disruption of antioxidant defenses.^[6–8] Oxidative stress therefore lies within the pathophysiological pathway connecting meconium exposure with inflammatory lung injury rather than representing a nonspecific laboratory abnormality. Consistent with this biological framework, redox imbalance has been detected in umbilical cord blood from neonates exposed to MSAF, indicating that this response is already measurable at birth.^[9, 10] Whether such early biological signals provide predictive information beyond routinely available clinical data, however, remains unclear.

Umbilical cord blood provides a particularly suitable window for addressing this question. It is available immediately after delivery without additional neonatal venipuncture and precedes most cases of NARDS by only hours to a few days, allowing biological assessment before postnatal disease progression and

treatment may modify circulating biomarkers.^[11, 12] We therefore used an at-birth clinical model as a reference framework and evaluated whether a prespecified cord-blood oxidative stress panel comprising malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), and superoxide dismutase (SOD) provided incremental predictive information for subsequent NARDS among neonates exposed to MSAF.

Materials and methods

Study design and participants

This single-center retrospective cohort study included neonates admitted to the neonatal intensive care unit (NICU) of the First Affiliated Hospital (Southwest Hospital), Army Medical University, between January 1, 2020, and January 1, 2026. All consecutive neonates with meconium-stained amniotic fluid (MSAF) documented at delivery were screened.

For inclusion in the final analysis, clinical records had to be sufficient to determine whether NARDS developed within the first 7 postnatal days, written parental or legal-guardian consent for cord-blood collection and biobanking had to have been obtained, and a corresponding archived cord-blood specimen of adequate quality had to be available for the planned biomarker analyses. Neonates were excluded if they had major congenital or chromosomal abnormalities that could independently affect respiratory function, including complex congenital heart disease, major pulmonary developmental abnormalities, congenital diaphragmatic hernia, other major structural abnormalities affecting respiration, or severe chromosomal disorders. Additional exclusion criteria were substantial hemolysis or inappropriate processing or storage rendering the archived specimen unsuitable for biomarker measurement, and inability to ascertain the 7-day outcome. Death before completion of the 7-day assessment period was considered an exclusion criterion only when NARDS status could not be determined.

All eligible neonates during the predefined study period were included. Because the study was retrospective and used all available eligible cases, no a priori sample-size calculation was performed and the cohort was not randomly divided into development and validation subsets.

This retrospective study was approved by the Ethics Committee of the First Affiliated Hospital of Army Medical University, PLA (approval No. (B) KY2025266; December 3, 2025), which waived the requirement for additional study-specific informed consent. The cord-blood specimens used in the present study were not collected specifically for this investigation but had been archived through an ongoing institutional obstetric cord-blood biobank program. Under this program, cord blood was collected after written parental or legal-guardian consent permitting specimen collection, storage, and subsequent use for medical research. After eligible neonates were identified from the predefined clinical cohort, the corresponding archived cord-blood specimens were retrieved from the biobank for study-specific biomarker assays. Study-specific data extraction, specimen retrieval, oxidative-stress biomarker assays, and statistical analyses were conducted under the above ethics approval. All clinical data were de-

identified before analysis and handled confidentially. The study was conducted in accordance with the Declaration of Helsinki and its later amendments.

Because the accrual periods partially overlapped, 19 of the 168 neonates (11.3%) included in the present study had also been included in a previously published cohort from our group that developed a clinical nomogram for ARDS among neonates exposed to MSAF.^[5] The previous study used conventional perinatal and admission laboratory variables and did not include the cord-blood MDA, 4-HNE, or SOD measurements or any of the incremental biomarker analyses reported here.

Clinical data and outcome definition

Maternal, obstetric, and neonatal data were obtained from the electronic medical records. Recorded maternal and obstetric variables included premature rupture of membranes (PROM), gestational hypertension, gestational diabetes mellitus, intrahepatic cholestasis of pregnancy, and placental abnormalities. Neonatal variables included gestational age, birth weight, sex, 1- and 5-min Apgar scores, white blood cell count (WBC), platelet count (PLT), absolute neutrophil count (ANC), and absolute lymphocyte count (ALC). All variables considered for prediction were available at birth.

The primary outcome was NARDS diagnosed within the first 7 postnatal days according to the Montreux definition.^[13] Diagnosis required acute respiratory deterioration within 7 days of a known or suspected clinical insult, bilateral diffuse pulmonary opacities or infiltrates, pulmonary edema not primarily attributable to congenital heart disease or cardiac failure, and respiratory failure not better explained by neonatal respiratory distress syndrome, transient tachypnea of the newborn, or congenital abnormalities. Oxygenation impairment was assessed using the oxygenation index (OI), calculated as $\text{FiO}_2 \times \text{mean airway pressure} \times 100 / \text{PaO}_2$; an $\text{OI} \geq 4$ fulfilled the oxygenation criterion for NARDS. Neonates meeting the Montreux criteria during the 7-day period were classified as the NARDS group, and the remaining eligible neonates as the non-NARDS group. Age at NARDS diagnosis was recorded for descriptive analysis only and was not used as a predictor.

Umbilical cord blood collection and biomarker assays

As part of the ongoing institutional obstetric cord-blood biobank program, umbilical venous blood had been collected from the placental side of the cord immediately after delivery and cord clamping. A 1-mL aliquot designated for serum biobanking was kept at 4°C for 60 min and centrifuged at 2000 ×g for 20 min. The clear serum supernatant was aliquoted and stored at – 80°C. Routine hematological indices were measured shortly after collection from a separate fresh cord-blood aliquot by the hospital clinical laboratory. For the present study, archived serum specimens corresponding to eligible neonates were retrieved from the biobank and analyzed during the same analytical campaign. Retrieval of archived specimens and all study-specific oxidative-stress biomarker assays were performed after ethics approval. Each archived serum sample underwent a single freeze–thaw cycle.

Serum malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), and superoxide dismutase (SOD) were measured using commercially available ELISA kits (Nanjing BYabs Science Technology Co., Ltd., Nanjing, Jiangsu, China; MDA, BY-WJZF0118; 4-HNE, BY-WJZF1594; SOD, BY-EH112140) according to the manufacturers' instructions. MDA and 4-HNE were measured by competitive ELISA and SOD by double-antibody sandwich ELISA. Accordingly, the SOD measurement represents circulating SOD protein concentration rather than enzymatic activity. All samples were assayed in duplicate. Optical density was measured at 450 nm, and concentrations were derived from plate-specific calibration curves using four-parameter logistic fitting. Samples exceeding the upper calibration limit were diluted and reassayed, with final concentrations corrected for the dilution factor.

The analytical ranges were 0.5–8 nmol/mL for MDA, 3–48 μ mol/L for 4-HNE, and 7.5–240 ng/mL for SOD; the corresponding lower limits of detection were < 0.1 nmol/mL, < 0.1 μ mol/L, and < 1.0 ng/mL. Manufacturer-reported intra-assay and inter-assay coefficients of variation were < 10% and < 15%, respectively. Laboratory personnel performing the assays were blinded to NARDS status. The study-specific oxidative-stress biomarker results were not available during the infants' original clinical course and therefore could not influence clinical diagnosis or management.

Predictor selection and model development

The prediction time point was defined as birth. Candidate clinical predictors were restricted to information available at that time. To reduce redundancy among closely related variables, gestational age was retained instead of birth weight, ANC instead of WBC, and the 1-min Apgar score instead of the 5-min score, which showed limited variability. The clinical candidate set therefore comprised PROM, gestational hypertension, gestational diabetes mellitus, intrahepatic cholestasis of pregnancy, placental abnormalities, gestational age, sex, 1-min Apgar score, PLT, ANC, and ALC.

Least absolute shrinkage and selection operator (LASSO) logistic regression with fivefold cross-validation was used for clinical predictor selection.^[14] At the selected penalty ($\lambda = 0.0651$), variables with non-zero coefficients were entered into a multivariable logistic regression model, designated the clinical model (Model 1).

To assess the additional predictive information provided by oxidative stress, the structure of Model 1 was retained and MDA, 4-HNE, and SOD were added simultaneously to form the oxidative stress-enhanced model (Model 2). These biomarkers were specified as a panel and were not subjected to further variable selection. Both models were fitted to the complete analysis cohort.

Odds ratios (ORs) are reported with 95% confidence intervals (CIs). For continuous predictors, ORs were expressed per 1-week increase in gestational age, per 10×10^9 /L increase in PLT, per 1×10^9 /L increase in ANC, per 1 nmol/mL increase in MDA, per 5 μ mol/L increase in 4-HNE, and per 10 ng/mL increase in SOD.

Model performance and conditional internal validation

Discrimination was evaluated using receiver operating characteristic curves and the area under the curve (AUC) with 95% CIs. Calibration was assessed by calibration plots and calibration slopes, and overall prediction error by the Brier score. Decision curve analysis was used to evaluate the net benefit of each model across a range of clinically relevant threshold probabilities.^[15] Model comparisons focused on threshold probabilities from 0.05 to 0.60.

Because Model 2 was nested within Model 1, the contribution of MDA, 4-HNE, and SOD was evaluated using a likelihood-ratio test. AUCs were compared using the paired DeLong test, and the difference in AUC was reported with its 95% CI.^[16] The change in Brier score was evaluated using paired bootstrap resampling. Integrated discrimination improvement (IDI) and continuous net reclassification improvement (NRI) were calculated as exploratory measures of incremental prediction. Classification performance at probability thresholds of 0.20, 0.30, 0.40, and 0.50 was examined as a supplementary analysis.

Conditional internal validation of the finalized model structures was performed without splitting the cohort. Optimism-corrected AUC, calibration slope, and Brier score were estimated using 1000 bootstrap resamples.^[17] Predictive stability of the locked model structures was additionally assessed using stratified 10-fold cross-validation repeated 20 times. Because LASSO variable selection was not repeated within each resample, these analyses quantify uncertainty in the fitted final model structures but do not incorporate the additional uncertainty introduced by the clinical predictor-selection step.

Sensitivity analyses

Variance inflation factors and Spearman correlation coefficients were used to assess multicollinearity. Possible non-linear associations between continuous predictors and the log odds of NARDS were examined using four-knot restricted cubic splines and likelihood-ratio tests.

Alternative specifications of the oxidative stress biomarkers, including logarithmic transformations and restricted cubic splines, were examined in sensitivity analyses. Because Model 2 contained seven predictors relative to the number of NARDS events, ridge-penalized logistic regression was additionally used to assess the effect of coefficient shrinkage. Continuous predictors were standardized, and the penalty strength was selected by repeated stratified 10-fold cross-validation according to mean log loss. Cross-validated AUC, Brier score, and calibration slope were used to assess model performance after penalization.

Statistical analysis

Continuous variables are presented as median (interquartile range), and categorical variables as number (percentage). Differences between the NARDS and non-NARDS groups were assessed using the Mann–

Whitney U test for continuous variables and Fisher's exact test for categorical variables. Group comparisons were descriptive and were not used as the sole criterion for predictor selection.

Univariable logistic regression was used to describe associations between individual predictors and NARDS, followed by the multivariable modeling procedures described above. No imputation was performed because the final analysis cohort had complete predictor and outcome data. All tests were two-sided, with $P < 0.05$ considered statistically significant. Analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria; packages including glmnet, rms, pROC, and rmda) and Python (Python Software Foundation). This study is reported in accordance with the STROBE guideline for cohort studies and the TRIPOD + AI guideline for prediction model studies.

Code availability

The analysis code used to generate the results reported in this study is available from the corresponding author on reasonable request. No custom software package or proprietary algorithm was developed for this study.

Results

Study population and baseline characteristics

- A total of 182 neonates with intrapartum MSAF were screened. Fourteen were excluded: five because parental consent for cord blood collection and biobanking had not been obtained, four because cord blood samples were unsuitable for ELISA owing to hemolysis, two with pulmonary sequestration, two with congenital diaphragmatic hernia, and one who died before completion of 7-day outcome ascertainment. The final cohort comprised 168 neonates, including 50 (29.8%) who developed NARDS and 118 who did not (Fig. 1). Among neonates with NARDS, the median age at diagnosis was 3 days (IQR 2.25–4; range 2–5 days).
- The clinical characteristics of the two groups are shown in Table 1. PROM was more frequent among neonates who developed NARDS (22.0% vs 8.5%, $P = 0.022$), and gestational age was lower [35.2 (34.2–36.5) vs 36.5 (35.2–38.4) weeks, $P < 0.001$]. The NARDS group had higher cord blood WBC and ANC and lower PLT and ALC. Cord blood MDA and 4-HNE concentrations were higher in the NARDS group [4.78 (4.12–6.11) vs 4.38 (3.87–4.96) nmol/mL, $P = 0.002$; and 23.3 (16.1–31.7) vs 18.7 (15.3–22.5) $\mu\text{mol/L}$, $P = 0.003$, respectively], whereas SOD was lower [205 (192–227) vs 245 (231–256) ng/mL, $P < 0.001$]. The remaining maternal, obstetric, and neonatal characteristics did not differ significantly between groups.

Table 1
Maternal, perinatal, and cord blood characteristics of the study population

Characteristic	Non-NARDS (n = 118)	NARDS (n = 50)	P value
Maternal and obstetric characteristics			
PROM, n (%)	10 (8.5)	11 (22.0)	0.022
Gestational hypertension, n (%)	17 (14.4)	4 (8.0)	0.314
Gestational diabetes mellitus, n (%)	30 (25.4)	17 (34.0)	0.265
Intrahepatic cholestasis of pregnancy, n (%)	18 (15.3)	5 (10.0)	0.466
Placental abnormalities, n (%)	19 (16.1)	9 (18.0)	0.822
Neonatal characteristics			
Gestational age, weeks	36.5 (35.2–38.4)	35.2 (34.2–36.5)	< 0.001
Birth weight, kg	2.69 (2.26–3.19)	2.55 (2.24–2.86)	0.168
Male sex, n (%)	69 (58.5)	35 (70.0)	0.170
1-min Apgar score	10 (10–10)	10 (9–10)	0.123
5-min Apgar score	10 (10–10)	10 (10–10)	0.061
Cord blood hematological indices			
WBC, ×10 ⁹ /L	10.4 (7.64–15.3)	16.4 (12.9–22.3)	< 0.001
PLT, ×10 ⁹ /L	252 (212–292)	236 (200–282)	0.043
ANC, ×10 ⁹ /L	6.42 (3.96–9.65)	12.1 (8.78–17.6)	< 0.001
ALC, ×10 ⁹ /L	2.90 (2.27–3.61)	2.40 (1.85–3.25)	0.021
Cord blood oxidative stress biomarkers			
MDA, nmol/mL	4.38 (3.87–4.96)	4.78 (4.12–6.11)	0.002
4-HNE, μmol/L	18.7 (15.3–22.5)	23.3 (16.1–31.7)	0.003
SOD, ng/mL	245 (231–256)	205 (192–227)	< 0.001
Data are median (interquartile range) unless otherwise indicated. P values were calculated using the Mann–Whitney U test for continuous variables and Fisher’s exact test for categorical variables. ALC, absolute lymphocyte count; ANC, absolute neutrophil count; 4-HNE, 4-hydroxynonenal; MDA, malondialdehyde; NARDS, neonatal acute respiratory distress syndrome; PLT, platelet count; PROM, premature rupture of membranes; SOD, superoxide dismutase; WBC, white blood cell count.			

Development of the prediction models

- LASSO regression was applied to the 11 candidate clinical predictors. At the cross-validated penalty parameter ($\lambda = 0.0651$), PROM, gestational age, PLT, and ANC retained non-zero coefficients and were used to construct the clinical model (Supplementary Fig. S1). In this model, gestational age was inversely associated with NARDS (OR 0.533 per week, 95% CI 0.406–0.698; $P < 0.001$), whereas ANC was positively associated with NARDS (OR 1.416 per $1 \times 10^9/L$, 95% CI 1.252–1.601; $P < 0.001$). The associations for PROM and PLT did not reach statistical significance (Table 2).
- The oxidative stress-enhanced model retained the same four clinical predictors and additionally included MDA, 4-HNE, and SOD. Higher MDA (OR 2.478 per 1 nmol/mL, 95% CI 1.141–5.382; $P = 0.022$) and 4-HNE (OR 1.901 per 5 $\mu\text{mol/L}$, 95% CI 1.188–3.040; $P = 0.007$) were associated with higher odds of NARDS, whereas higher SOD was associated with lower odds (OR 0.475 per 10 ng/mL, 95% CI 0.340–0.666; $P < 0.001$). Gestational age and ANC remained associated with the outcome after addition of the biomarker panel (Table 2). Univariable associations and the discrimination of individual predictors are reported in Supplementary Table S1.

Table 2
Multivariable logistic regression models for NARDS prediction

Predictor	Clinical model OR (95% CI)	P value	Oxidative stress-enhanced model OR (95% CI)	P value
PROM, yes vs no	2.433 (0.668–8.866)	0.178	1.582 (0.202–12.372)	0.662
Gestational age, per 1 week	0.533 (0.406–0.698)	< 0.001	0.514 (0.352–0.751)	< 0.001
PLT, per $10 \times 10^9/L$	0.929 (0.861–1.002)	0.057	0.911 (0.808–1.028)	0.129
ANC, per $1 \times 10^9/L$	1.416 (1.252–1.601)	< 0.001	1.502 (1.271–1.775)	< 0.001
MDA, per 1 nmol/mL	—	—	2.478 (1.141–5.382)	0.022
4-HNE, per 5 $\mu\text{mol/L}$	—	—	1.901 (1.188–3.040)	0.007
SOD, per 10 ng/mL	—	—	0.475 (0.340–0.666)	< 0.001
The clinical model included PROM, gestational age, PLT, and ANC. The oxidative stress-enhanced model retained the same clinical predictors and added MDA, 4-HNE, and SOD as a prespecified biomarker panel. CI, confidence interval; OR, odds ratio. Other abbreviations are defined in Table 1.				

Predictive performance and incremental value of the oxidative stress panel

- The clinical model had an apparent AUC of 0.901 (95% CI 0.847–0.955), compared with 0.969 (95% CI 0.942–0.995) for the oxidative stress-enhanced model (Fig. 2a). Addition of MDA, 4-HNE, and SOD improved overall model fit (likelihood-ratio $\chi^2 = 58.861$, $df = 3$; $P < 0.001$). The increase in AUC

was 0.068 (95% CI 0.019–0.117; paired DeLong $P = 0.006$). The Brier score decreased from 0.106 to 0.055, corresponding to a difference of -0.051 (95% bootstrap CI -0.092 to -0.025) (Table 3).

Table 3

Predictive performance, incremental value, and conditional internal validation of the clinical and oxidative stress-enhanced models

Metric	Clinical model	Oxidative stress-enhanced model	Comparison / difference
Apparent performance and incremental comparison			
AUC	0.901	0.969	$\Delta\text{AUC} = 0.068$ (95% CI 0.019–0.117); $P = 0.006$
Brier score	0.106	0.055	$\Delta = -0.051$ (95% bootstrap CI -0.092 to -0.025)
Likelihood-ratio test	—	—	$\chi^2 = 58.861$; $df = 3$; $P < 0.001$
Bootstrap validation of locked model structures (1000 resamples)			
Optimism-corrected AUC	0.890	0.955	—
Optimism-corrected Brier score	0.114	0.071	—
Optimism-corrected calibration slope	0.919	0.764	—
Repeated stratified 10-fold cross-validation of locked model structures (20 repeats)			
AUC, mean $\pm$ SD	0.885 $\pm$ 0.005	0.942 $\pm$ 0.008	—
Brier score, mean $\pm$ SD	0.114 $\pm$ 0.002	0.072 $\pm$ 0.003	—
Calibration slope, mean $\pm$ SD	0.844 $\pm$ 0.038	0.716 $\pm$ 0.050	—
AUC differences were tested using the paired DeLong method. The Brier-score difference was estimated using paired bootstrap resampling. Bootstrap and cross-validation procedures evaluated the locked final model structures; Bootstrap and cross-validation procedures evaluated the locked final model structures; LASSO variable selection was not repeated within resamples. Accordingly, these estimates do not incorporate uncertainty arising from the clinical predictor-selection step. AUC, area under the receiver operating characteristic curve; CI, confidence interval; SD, standard deviation.			

- Additional measures of model fit and classification at the Youden-optimal thresholds are provided in Supplementary Table S2. The enhanced model also showed consistently higher sensitivity, specificity, and overall classification accuracy across the prespecified probability thresholds of 0.20, 0.30, 0.40, and 0.50 (Supplementary Table S3). Exploratory bootstrap analyses yielded an IDI of

0.260 (95% CI 0.136–0.457) and a continuous NRI of 1.351 (95% CI 1.005–1.839), consistent with the primary model comparisons (Supplementary Table S4).

Conditional internal validation, calibration, and decision curve analysis

- Conditional bootstrap validation of the locked model structures showed an optimism-corrected AUC of 0.890 for the clinical model and 0.955 for the oxidative stress-enhanced model. The corresponding optimism-corrected Brier scores were 0.114 and 0.071. Calibration slopes were 0.919 for the clinical model and 0.764 for the enhanced model (Table 3).
- The pattern of higher discrimination and lower prediction error for the enhanced model was also observed in repeated stratified 10-fold cross-validation. Mean AUCs were 0.885 ± 0.005 for the clinical model and 0.942 ± 0.008 for the enhanced model, with corresponding Brier scores of 0.114 ± 0.002 and 0.072 ± 0.003 . Mean calibration slopes were 0.844 ± 0.038 and 0.716 ± 0.050 , respectively (Table 3).
- Decision curve analysis showed positive net benefit for both models across the clinically relevant threshold range. The oxidative stress-enhanced model yielded greater net benefit than the clinical model across the prespecified comparison range of 0.05–0.60 and remained superior to the treat-all and treat-none strategies (Fig. 2b). Bootstrap calibration curves for the clinical and enhanced models are shown in Fig. 2c and d, respectively.

Sensitivity analyses

- No evidence of problematic multicollinearity was found. Variance inflation factors ranged from 1.017 to 1.059 in the clinical model and from 1.035 to 1.326 in the enhanced model. The largest absolute pairwise Spearman correlation among continuous predictors in the enhanced model was 0.287. Restricted cubic spline analyses indicated evidence of nonlinearity for ANC in the clinical model ($P = 0.031$) and for MDA ($P = 0.029$), 4-HNE ($P < 0.001$), and SOD ($P = 0.003$) in the enhanced model (Supplementary Table S5a–b).
- Alternative biomarker specifications did not improve cross-validated performance (Supplementary Table S6). Models using logarithmic transformations of MDA and 4-HNE, with or without log transformation of SOD, had repeated cross-validated AUCs of 0.938 and 0.937, respectively, compared with 0.942 for the primary linear enhanced model. A model using four-knot restricted cubic splines for all three biomarkers achieved an apparent AUC of 0.994 but a repeated cross-validated AUC of 0.911 ± 0.013 and a calibration slope of 0.182 ± 0.022 .
- With ridge penalization, the enhanced model retained a cross-validated AUC of 0.949 ± 0.004 and a Brier score of 0.068 ± 0.002 , while the calibration slope was 1.042 ± 0.041 (Supplementary Table S6).

Discussion

The respiratory course after MSAF exposure is highly heterogeneous.^[1, 18] A visible perinatal exposure therefore does not, by itself, explain which infants will progress to NARDS. In this cohort, part of that divergence was already apparent in cord blood at birth. Infants who later developed NARDS showed a pattern consistent with greater lipid peroxidation and lower circulating SOD concentrations, and this information was not fully captured by perinatal characteristics and routine hematology. The findings place redox imbalance early in the pathway from meconium exposure to inflammatory lung injury and provide a biological basis for variation in outcome among infants with the same clinically recognizable exposure.^[18, 19]

This pattern is biologically plausible. Meconium-related lung injury involves more than airway obstruction and surfactant inactivation. Meconium can activate complement and Toll-like receptor pathways, stimulate macrophages and neutrophils, and promote release of inflammatory mediators and reactive oxygen species.^[6–8, 20, 21] Oxidative stress can then amplify inflammation through lipid peroxidation, membrane injury, and disruption of epithelial and endothelial barrier integrity. The direction of the three biomarkers fits this sequence. MDA and 4-HNE are products of lipid peroxidation, whereas SOD is a major enzymatic defense against superoxide. Higher MDA and 4-HNE together with lower circulating SOD concentrations therefore represent complementary markers of a disturbed redox state rather than unrelated laboratory abnormalities. Lower gestational age and higher ANC remained associated with NARDS in both multivariable models, adding further biological coherence. Antioxidant defenses are less mature at lower gestational ages, while neutrophil activation is closely coupled to oxidant generation.^[22, 23] The clinical and biochemical signals thus converge on an inflammatory–oxidative phenotype, although circulating biomarkers cannot identify the lung as their sole source. Meconium-induced activation of complement, innate immune signaling, and neutrophil oxidative responses is well established experimentally and is regarded as an important component of meconium-associated lung injury.^[7, 21]

Earlier cord-blood studies also support oxidative stress in the setting of MSAF. Higher MDA, oxidative DNA damage, and 8-isoprostanes have been reported when infants born through MSAF were compared with those born through clear amniotic fluid.^[9, 10] Those observations established that oxidative disturbance can accompany meconium exposure. The clinically relevant question once MSAF has been identified, however, is whether biological variation within the exposed population helps explain the subsequent respiratory course. In the present cohort, the comparison was made entirely within MSAF-exposed neonates. This distinction matters because MSAF is an exposure state, not a diagnosis of meconium aspiration syndrome, and most exposed infants do not develop severe respiratory disease. A more pronounced redox disturbance at birth may therefore reflect variation in the fetal or immediate perinatal response to a shared exposure and greater susceptibility to later inflammatory lung injury.

The incremental value of the biomarker panel should be interpreted against an already informative clinical background. Previous NARDS prediction models have achieved good discrimination using conventional clinical and laboratory variables.^[4, 5] Earlier work from our group developed an MSAF-

specific clinical nomogram based on 1-min Apgar score, WBC, PLT, red cell distribution width, and serum calcium.^[5] That study relied on conventional perinatal and admission laboratory measurements and did not assess cord-blood oxidative-stress biomarkers. Although a limited proportion of participants was shared between the two cohorts because their study periods partially overlapped, the present study addresses a distinct question: whether biological information already measurable in cord blood at birth adds predictive information beyond birth-time clinical variables. In the present analysis, the prespecified oxidative-stress panel was added without further biomarker selection to the clinical reference model developed in the present cohort. Model fit improved, discrimination increased, and the Brier score decreased. The direction of benefit remained after conditional bootstrap correction and repeated cross-validation of the locked model structures, and decision-curve analysis favored the enhanced model across the prespecified threshold range. These findings suggest that the redox panel captures a component of NARDS risk that is only partly represented by gestational age, PROM, platelet count, and neutrophil count.

The birth-time structure of the model has a separate practical implication. The clinical component was limited to maternal and neonatal characteristics available at delivery and hematological indices measured from cord blood. Gestational age was used rather than birth weight to represent developmental maturity, and ANC rather than total WBC to represent the leukocyte signal, reducing closely related information in a modest-sized dataset. This structure also limits additional neonatal phlebotomy. Paired-sample studies have shown useful agreement between cord and early neonatal blood for WBC, platelet, and neutrophil measurements, although absolute values are not always interchangeable and cord-specific reference ranges remain important.^[11, 12] For preterm and critically ill neonates, reducing diagnostic blood loss is clinically relevant, and recent data also support cord-blood collection as a means of reducing early iatrogenic blood loss in very-low-birth-weight infants.^[24] Routine postnatal inflammatory tests such as CRP and PCT are dynamic and depend on sampling time and indication; incorporating them would shift the assessment away from a fixed birth-time window and generally require additional neonatal sampling.^[25] By contrast, the present framework combines delivery characteristics, cord-blood hematology, and oxidative biomarkers obtained from the same early biological window. This may be particularly useful when the aim is to estimate risk before respiratory deterioration and treatment begin to modify the circulating profile.

High discrimination should not be equated with fully reliable individual risk estimates. The enhanced logistic model retained strong discrimination in the resampling analyses, but its calibration slope fell below 1, indicating predictions that were too extreme.^[26] With 50 NARDS events and seven predictors, some coefficient overfitting is plausible.^[27] The ridge sensitivity analysis is informative in this respect. Shrinkage preserved cross-validated discrimination while bringing the calibration slope close to unity, suggesting that the predictive signal was not dependent on the magnitude of the unpenalized coefficients.^[28] The analyses of functional form led to a similar conclusion. ANC showed evidence of nonlinearity in the clinical model, while MDA, 4-HNE, and SOD showed evidence of nonlinearity in the enhanced model. Nevertheless, fitting restricted cubic splines to all three biomarkers produced very high

apparent discrimination and poor cross-validated calibration. The data therefore support the presence of biological signal more strongly than precise estimation of complex dose–response shapes. With the present sample size, the simpler linear specification offers a more stable representation. Larger cohorts may allow nonlinear effects to be estimated without the same loss of reproducibility.

The most immediate clinical implication is risk stratification rather than a stand-alone diagnostic tool. MSAF is recognized at delivery, while NARDS in this cohort emerged over the following days. A model available at birth could help identify infants who warrant closer respiratory observation before overt deterioration occurs. Cord blood is attractive for this purpose because it is available at the relevant time point without an additional neonatal venipuncture.^[11, 12, 24] The oxidative panel, however, currently relies on ELISA-based assays that are not part of routine neonatal testing. Turnaround time, assay standardization, and transportability across laboratories will determine whether these markers can move beyond research use. The present coefficients should therefore not be converted directly into a bedside score. External validation should first establish whether the incremental value persists across centers and whether shrinkage or recalibration is required for individual risk estimation.

Several limitations should be acknowledged. First, this retrospective single-center study included only NICU-admitted neonates with MSAF and required availability of an adequate archived cord-blood specimen, which may limit generalizability and introduce selection bias. Second, specimens were collected over several years, resulting in variable durations of frozen storage; although samples were processed under a common biobank protocol and biomarkers were measured during the same analytical campaign, storage-related effects cannot be completely excluded. Third, MDA, 4-HNE, and SOD are systemic redox markers and may also reflect placental inflammation, fetal hypoxia, infection, or other perinatal stresses; therefore, the observed associations do not establish a lung-specific or causal pathway, and residual confounding or diagnostic overlap remains possible. Finally, internal validation was conditional on the locked model structures because LASSO predictor selection was not repeated within each resample, and decision-curve analysis was performed in the development cohort. The reported performance may therefore remain optimistic. External multicenter validation with standardized sampling and appropriate shrinkage or recalibration is required before clinical use.

Conclusions

Umbilical cord blood MDA, 4-HNE, and SOD provided incremental predictive information beyond a birth-time model based on perinatal characteristics and cord-blood hematology in neonates exposed to MSAF. The findings identify redox imbalance as an early biological correlate of subsequent NARDS and support further evaluation of cord-blood biomarkers for risk stratification at birth. External multicenter validation, with appropriate attention to coefficient shrinkage and recalibration, is required before individual risk estimates are used clinically.

Abbreviations

HNE
4-hydroxynonenal
ALC
absolute lymphocyte count
ANC
absolute neutrophil count
AUC
area under the receiver operating characteristic curve
CI
confidence interval
CRP
C-reactive protein
ELISA
enzyme-linked immunosorbent assay
 FiO_2
fraction of inspired oxygen
IDI
integrated discrimination improvement
IQR
interquartile range
LASSO
least absolute shrinkage and selection operator
MDA
malondialdehyde
MSAF
meconium-stained amniotic fluid
NARDS
neonatal acute respiratory distress syndrome
NICU
neonatal intensive care unit
NRI
net reclassification improvement
OI
oxygenation index
OR
odds ratio
 PaO_2
arterial partial pressure of oxygen
PCT
procalcitonin

PLT
platelet count
PROM
premature rupture of membranes
SOD
superoxide dismutase
WBC
white blood cell count.

Declarations

Ethics Approval and Informed Consent

This retrospective study was approved by the Ethics Committee of the First Affiliated Hospital of Army Medical University, PLA (approval No. (B) KY2025266; December 3, 2025), which waived the requirement for additional study-specific informed consent. Cord-blood specimens used in this study had been collected independently of the present investigation through an ongoing institutional obstetric cord-blood biobank program under written parental or legal-guardian consent permitting specimen collection, storage, and subsequent medical research use. After eligible neonates were identified, the corresponding archived specimens were retrieved for study-specific biomarker assays under the above ethics approval. All clinical data were de-identified before analysis and handled confidentially. The study was conducted in accordance with the Declaration of Helsinki and its later amendments.

Consent for Publication

Not applicable. The manuscript contains no individually identifiable patient information, images, or other material requiring consent for publication.

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Author contributions

Xiaodong Sun: Investigation, Writing—original draft. Liping Yang: Investigation, Data curation, Writing—original draft. Ruixue Zheng: Formal analysis. Leilei Shen: Conceptualization, Methodology, Funding acquisition, Supervision, Project administration, Writing—review and editing. Shuhua Yi: Investigation, Resources. All authors contributed to drafting, revising, or critically reviewing the manuscript; approved the final version for publication; agreed on the journal to which the article is being submitted; and agree to be accountable for all aspects of the work.

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Competing interests

The authors declare no competing interests.

Data Availability

The de-identified datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request, subject to institutional and ethical approval requirements.

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Figures

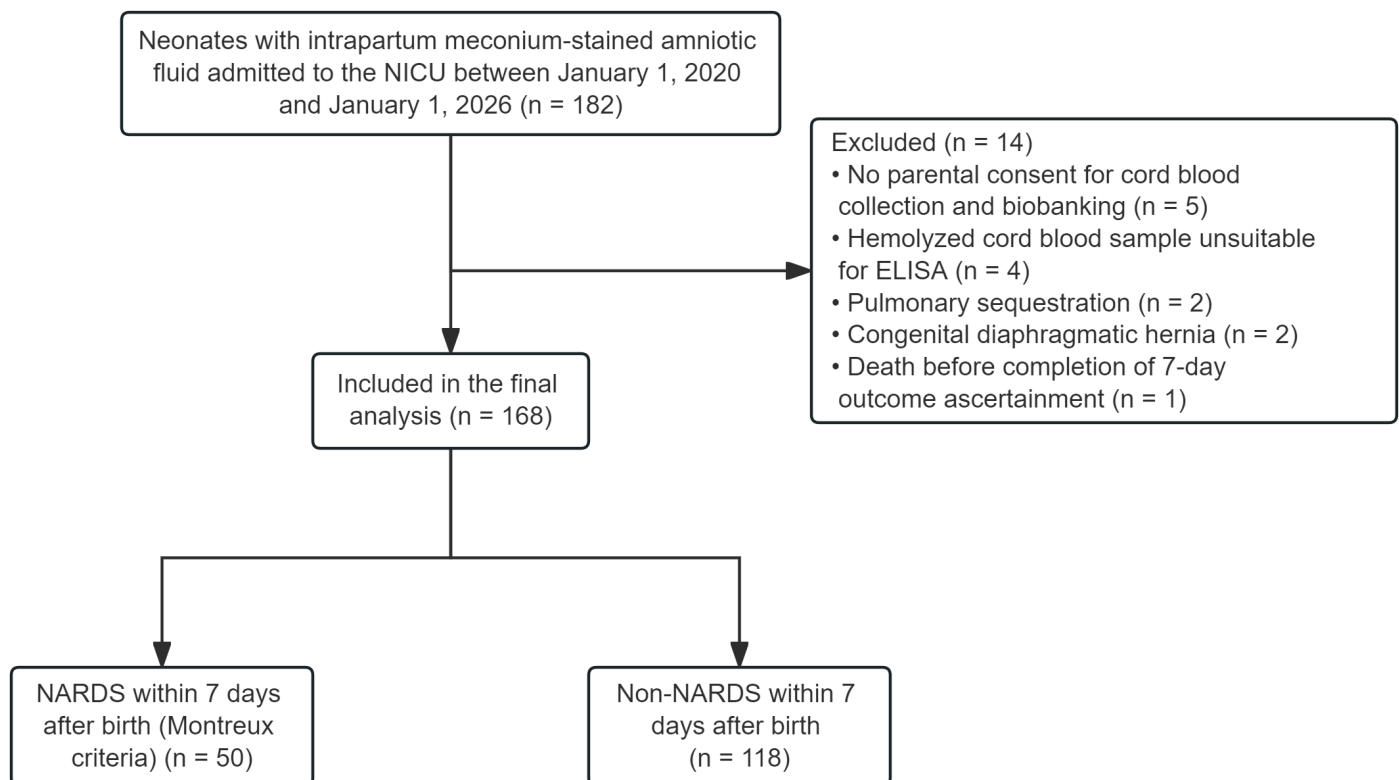


Figure 1

Flow diagram of participant selection and grouping in the study cohort. NICU, neonatal intensive care unit; NARDS, neonatal acute respiratory distress syndrome; ELISA, enzyme-linked immunosorbent assay.

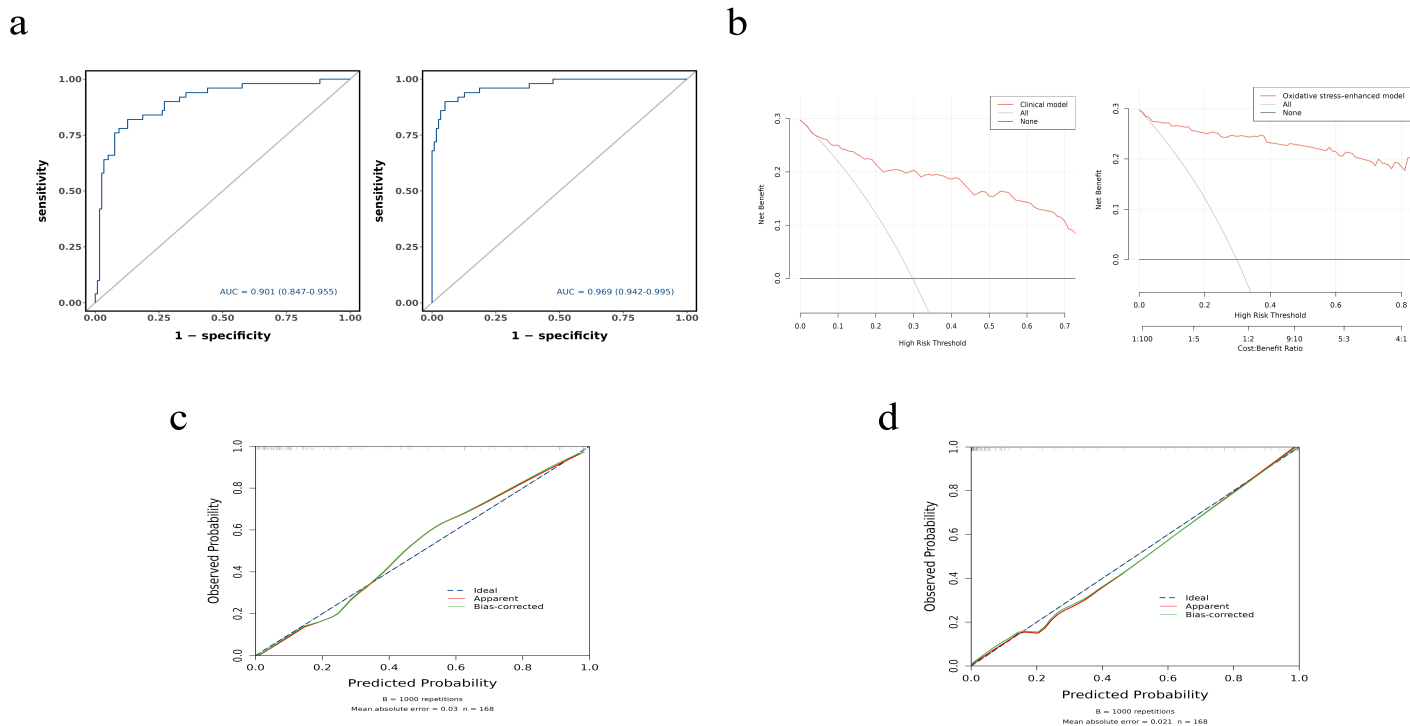


Figure 2

Discrimination, clinical utility, and calibration of the prediction models. **a** Receiver operating characteristic curves for the clinical model (left) and oxidative stress-enhanced model (right), with apparent AUCs of 0.901 (95% CI 0.847-0.955) and 0.969 (95% CI 0.942-0.995), respectively. **b** Decision curve analyses for the clinical model (left) and oxidative stress-enhanced model (right). **c** Bootstrap calibration of the clinical model. **d** Bootstrap calibration of the oxidative stress-enhanced model. Calibration plots show the ideal, apparent, and bias-corrected curves based on 1000 bootstrap resamples. AUC, area under the receiver operating characteristic curve

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