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MULTI-DOMAIN LABORATORY BIOCHEMICAL PROFILING OF PULMONARY SURFACTANT REPLACEMENT THERAPY IN SERUM, CEREBROSPINAL FLUID, AND BRONCHOALVEOLAR LAVAGE FLUID OF PRETERM INFANTS WITH NEONATAL RESPIRATORY DISTRESS SYNDROME: A RETROSPECTIVE COHORT STUDY

VIŠEDOMENSKO LABORATORIJSKO BIOHEMIJSKO PROFILISANJE SUPSTITUCIONE TERAPIJE SURFAKTANTOM U SERUMU, CEREBROSPINALNOJ TEČNOSTI I BRONHOALVEOLARNOM LAVATU KOD PREVREMENO ROĐENE DECE SA NEONATALNIM RESPIRATORNIM DISTRES SINDROMOM: RETROSPEKTIVNA KOHORTNA STUDIJA

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Summary

Background: Neonatal respiratory distress syndrome (NRDS) remains a major cause of early morbidity in preterm infants. Although exogenous pulmonary surfactant (PS) replacement therapy has improved short-term respiratory outcomes, the laboratory biochemical alterations accompanying PS treatment across different body fluid compartments have not been fully characterised. This study aimed to describe multi-domain biochemical changes associated with PS replacement therapy in preterm infants with NRDS.
Methods: A total of 99 preterm infants with NRDS admitted to our hospital from January 1 to December 31, 2025, were included and divided into the PS treatment group (n=47) and the control group (n=52) according to treatment strategy. Fifty biochemical indicators involving nine core biochemical pathways were measured in serum, cerebrospinal fluid, and bronchoalveolar lavage fluid before treatment, 72 h after treatment, and 7 d after treatment. These pathways included bronchial development, intracranial injury, oxidative stress, inflammatory response, coagulation-fibrinolysis, vascular endothelial function, energy metabolism, hepatic function, and pulmonary surfactant function.
Results: Before treatment, no significant differences were found in any biochemical indicators between the two groups (P>0.05). At 72 h and 7 d after treatment, the

Kratak sadržaj

Uvod: Neonatalni respiratorni distres sindrom (NRDS) i dalje predstavlja jedan od glavnih uzroka ranog morbiditeta kod prevremeno rođene dece. Iako je terapija egzogenim plućnim surfaktantom (PS) značajno poboljšala kratkoročne respiratorne ishode, biohemijske promene u različitim telesnim tečnostima tokom ove terapije još uvek nisu u potpunosti opisane. Cilj ove studije je bio da prikaže višedomenske biohemijske promene povezane sa terapijom nadoknade surfaktanta kod prevremeno rođene dece sa NRDS.
Metode: U studiju je uključeno ukupno 99 prevremeno rođene dece sa NRDS, koja su hospitalizovana u periodu od 1. januara do 31. decembra 2025. godine, i podeljena u grupu koja je primala PS terapiju (n=47) i kontrolnu grupu (n=52) prema terapijskoj strategiji. Mereno je 50 biohemijskih pokazatelja koji obuhvataju devet ključnih biohemijskih puteva u serumu, cerebrospinalnoj tečnosti i bronhoalveolarnom lavatu, pre terapije, 72 sata nakon terapije i 7 dana nakon terapije. Ovi putevi uključivali su razvoj bronhija, intracerebralno oštećenje, oksidativni stres, inflamatorni odgovor, koagulaciono-fibrinolitički sistem, funkciju vaskularnog endotela, energetski metabolizam, funkciju jetre i funkciju plućnog surfaktanta.
Rezultati: Pre terapije nije bilo značajnih razlika između grupa u bilo kojim biohemijskim parametrima (P>0,05). Nakon 72 sata i 7 dana, nivoi markera povezanih sa

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levels of markers related to bronchial epithelial injury, intracranial injury, oxidative injury, inflammatory response, coagulation-fibrinolytic disorder, and vascular endothelial injury were lower in the PS treatment group than in the control group (P<0.05). In contrast, indicators related to endogenous antioxidant capacity, pulmonary surfactant function, mitochondrial energy metabolism, and hepatic synthetic function were higher in the PS treatment group than in the control group (P<0.05). No significant changes were observed in the cerebrospinal fluid/serum S100β ratio or serum pyruvate level at any time point in either group (P>0.05). These negative findings suggest that PS treatment was not associated with laboratory evidence of blood-brain barrier disruption and that pyruvate-related glycolytic homeostasis remained broadly stable in both groups.

Conclusion: In this retrospective cohort, PS replacement therapy was associated with coordinated improvements in several biochemical domains in preterm infants with NRDS, including pulmonary surfactant function, oxidative-inflammatory status, coagulation-fibrinolytic balance, vascular endothelial markers, energy metabolism, and hepatic synthetic function. These findings provide exploratory laboratory evidence for multi-domain biochemical changes after PS treatment.

Keywords: neonatal respiratory distress syndrome, pulmonary surfactant replacement therapy, preterm infants, laboratory biochemistry, multimatrix biomarkers, bronchoalveolar lavage fluid

oštećenjem bronhijalnog epitela, intracerebralnim ošteć enjem, oksidativnim stresom, inflamacijom, poremećajem koagulacije i fibrinolize, kao i oštećenjem vaskularnog endotela bili su niži u grupi koja je primala PS terapiju nego u kontrolnoj grupi (P<0,05). Nasuprot tome, pokazatelji povezani sa endogenim antioksidativnim kapacitetom, funkcijom plućnog surfaktanta, mitohondrijalnim energetskim metabolizmom i sintezom u jetri bili su viši u PS grupi nego u kontrolnoj (P<0,05). Nisu uočene značajne promene u odnosu S100β u cerebrospinalnoj tečnosti i serumu, niti u nivou piruvata u serumu u bilo kom vremenskom trenutku (P>0,05). Ovi negativni nalazi ukazuju da terapija surfaktantom nije bila povezana sa laboratorijskim dokazima narušavanja krvno-moždane barijere, kao ni sa poremećajem stabilnosti glikolitičke homeostaze.

Zaključak: U ovoj retrospektivnoj kohortnoj studiji, terapija nadoknade surfaktanta bila je povezana sa koordinisanim poboljšanjima više biohemijskih domena kod prevremeno rođene dece sa NRDS, uključujući funkciju plućnog surfaktanta, oksidativno-inflamatorni status, koagulaciono-fibrinolitičku ravnotežu, markere vaskularnog endotela, energetski metabolizam i funkciju jetre. Ovi rezultati pružaju preliminarne laboratorijske dokaze o višedomenskim biohemijskim promenama nakon primene PS terapije.

Ključne reči: neonatalni respiratorni distres sindrom, terapija surfaktantom, prevremeno rođena deca, laboratorijska biohemija, multimarkeri, bronhoalveolarni lavat

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Introduction

Neonatal respiratory distress syndrome (NRDS) is the most common respiratory emergency in preterm infants. The disorder is primarily caused by insufficient synthesis or functional impairment of pulmonary surfactant (PS), largely due to the immaturity of type II alveolar epithelial cells (1). Epidemiological data show that the incidence of NRDS is approximately 60% among preterm infants with a gestational age of less than 32 weeks, and exceeds 80% in extremely preterm infants with a gestational age of less than 28 weeks. For this reason, NRDS remains a major contributor to early mortality and long-term multiorgan dysfunction in this population (2, 3). The widespread application of exogenous PS replacement therapy has markedly reduced acute mortality in NRDS. Recent meta-analytic data in very-low-birth-weight or very-low-gestational-age neonates reported pooled BPD prevalences of 35% when defined by oxygen or positive-pressure support at 28 days and 21% when defined at 36 weeks' postmenstrual age. In a recent Chinese multicentre cohort of preterm infants with a respiratory score >4, BPD occurred in 54.0% of infants. For severe intraventricular haemorrhage, recent Chinese Neonatal Network data reported a rate of 5.1% among very preterm infants (4, 5). The potential biochemical mechanisms by which PS treatment affects these two complications have not yet been systematically elucidated.

Most studies have focused on the short-term improvement in pulmonary ventilation after PS replacement therapy, while the biochemical pathways through which PS may influence bronchial development and intracranial haemorrhage risk have received comparatively less attention (6). Meanwhile, existing biochemical studies are often limited to detecting a single marker or a small number of markers (7, 8) and have not established a comprehensive biochemical marker profile that covers the relevant pathophysiological processes. For example, some studies have focused on a limited set of inflammatory biomarkers, such as IL-6, TNF-α, or CRP, whereas others have used isolated neural injury markers, such as S100β or NSE, to evaluate disease severity or treatment response. More importantly, most available studies have mainly examined local markers in blood, urine, BALF, or a single body fluid. Systematic evidence regarding the dynamic associations among markers in multiple body fluids, such as serum, cerebrospinal fluid (CSF), and bronchoalveolar lavage fluid (BALF), remains limited (9, 10). The selection of these three matrices was based on their complementary biological information: serum reflects systemic biochemical status, CSF more directly indicates central nervous

system injury, and BALF provides a local assessment of the pulmonary microenvironment. This multi-matrix combination may therefore offer a more comprehensive laboratory perspective on pulmonary and extrapulmonary biochemical changes associated with PS treatment. As a result, the regulatory effects of PS treatment on the systemic multi-organ biochemical microenvironment have not been fully characterised.

To address this gap, the present study systematically evaluated the effects of PS replacement therapy on 50 biochemical indicators across nine core biochemical pathways in preterm infants with NRDS. Given the exploratory laboratory design and the number of biochemical indicators examined, the present findings should be considered hypothesis-generating and require validation in independent cohorts. The pathways covered bronchial epithelial injury and repair, central nervous system injury, oxidative stress and antioxidant defence, inflammatory response, pulmonary surfactant system function, coagulation-fibrinolytic balance, vascular endothelial function, energy metabolism and mitochondrial function, and hepatic synthetic and metabolic function. This panel was designed to cover the major pathophysiological processes involved in NRDS progression and its pulmonary, neurological, inflammatory, coagulation-endothelial, metabolic, and hepatic complications. To our knowledge, systematic multi-pathway biochemical profiling of PS treatment across these body-fluid compartments has not been fully reported. These findings may help clarify how PS replacement therapy regulates multiorgan biochemical status in preterm infants. They may also support the identification of biochemical marker combinations for early prediction of abnormal bronchial development and intracranial haemorrhage risk, and provide a biochemical perspective on the multiorgan protective effects of PS treatment. In addition, the results may offer a laboratory basis for optimising treatment strategies for preterm infants with NRDS and reducing the incidence of long-term complications.

Materials and Methods

Ethics statement

This was a single-centre, retrospective cohort study approved by our hospital's Medical Ethics Committee. Written informed consent for the use of clinical data and residual biological specimens was obtained from the legal guardians of all enrolled infants. All study procedures were conducted in accordance with the Declaration of Helsinki.

Study population

This study included 99 preterm infants with neonatal respiratory distress syndrome (NRDS) who were admitted to the neonatal intensive care unit of our hospital from January 1 to December 31, 2025. This was an observational retrospective cohort study, and treatment allocation was not randomised. The inclusion criteria were as follows: gestational age of 24~31^{+6} weeks; admission within 6 h after birth with completion of the first full set of laboratory tests; fulfilment of the 2023 NRDS diagnostic criteria issued by the European Association of Perinatal Medicine, namely diffuse ground-glass opacity with air bronchograms on chest radiography; available residual serum, CSF, and BALF specimens from three key time points within 7 d after admission; and complete, traceable electronic medical records and original laboratory data.

The exclusion criteria were as follows: congenital pulmonary malformation, chromosomal karyotype abnormality, or confirmed inherited metabolic disease; death or discharge against medical advice within 72 h after birth; maternal chorioamnionitis, severe preeclampsia, or gestational diabetes melli-tus during pregnancy; grade III~IV intracranial haemorrhage confirmed by cranial ultrasound at admission; antenatal maternal glucocorticoid therapy for foetal lung maturation; or use of exogenous pulmonary surfactant (PS) before admission.

Grouping

The infants were classified into a PS treatment group (research group, n=47) and a non-PS control group (contraindications or family refusal, n=52) according to the treatment actually received after admission. Infants in the PS treatment group received intratracheal instillation of porcine pulmonary phospholipid injection within 2~6 h after birth, at a routine clinical dose of 100 mg/kg. The control group received only conventional supportive treatment for NRDS, including nasal continuous positive airway pressure or mechanical ventilation, intravenous nutritional support, and empirical anti-infective therapy. Ventilator parameter adjustment, fluid management, and anti-infective regimens in both groups followed the unified diagnostic and therapeutic protocols of our hospital and were implemented by the same medical and nursing team. There were no statistically significant differences between the two groups in gestational age, birth weight, sex distribution, delivery mode, or initial blood gas parameters at admission ($P>0.05$, *Table I*).

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Table I Baseline clinical characteristics and admission blood gas parameters of preterm infants with neonatal respiratory distress syndrome.

Variable	Research group (n=47)	Control group (n=52)	Statistical value	P
Gestational age (weeks)	28.53±2.24	29.15±3.21	1.105	0.272
Birth weight (g)	1243.51±327.73	1175.17±280.84	1.117	0.267
Sex distribution			0.518	0.472
Male infants [n (%)]	26 (55.32)	25 (48.08)		
Female infants [n (%)]	21 (44.68)	27 (51.92)		
Mode of delivery			0.234	0.629
Vaginal delivery [n (%)]	15 (31.91)	19 (36.54)		
Caesarean delivery [n (%)]	32 (68.09)	33 (63.46)		
Admission pH	7.22±0.07	7.22±0.10	0.3007	0.994
Admission PaO_2 (mmHg)	47.75±7.59	48.32±6.55	0.402	0.689
Admission $PaCO_2$ (mmHg)	55.55±8.30	57.51±10.11	1.05	0.296

Data extraction

All residual specimens in the specimen bank were processed within 1 h of collection and aliquoted into 2 mL cryovials with unique QR code identifiers, with 200 μL in each tube. The specimens were transported under a continuous cold chain and stored in a -80 °C ultra-low-temperature freezer (Haier). During the study period, the specimen bank experienced no power interruption, and the freezing chain was maintained for all specimens. This study used only residual specimens collected from each infant at three key time points: baseline (before PS administration), 72 h after treatment, and 7 d after treatment. These time points were selected to reflect pretreatment baseline status, early post-treatment biochemical response, and the early postnatal window during which respiratory stabilisation, evolving lung injury, and IVH-related changes are clinically relevant in very preterm infants. Specifically, 72 h after treatment was used as the early response window after PS-associated improvement in pulmonary function, whereas 7 d after treatment was selected to capture early biochemical trajectories during the first-week risk window for IVH and evolving lung injury related to later BPD. Each tube of specimen underwent only one freeze-thaw cycle.

Specimen processing and storage

Serum specimens: Serum specimens were obtained from 3 mL of fasting venous blood, collected into vacuum tubes without anticoagulant (BD). The samples were allowed to stand at room temperature (22±2 °C) for 30 min until natural coagulation occurred. They were then centrifuged at 3000×g for 10 min at 4 °C. The upper serum layer was carefully aspirated with a pipette, avoiding contact with the erythrocyte layer, aliquoted, and immediately transferred to a -80 °C freezer for storage.

CSF specimens: All CSF specimens were collected by lumbar puncture performed by the same group of senior physicians. A total of 2 mL of CSF was drawn and placed in sterile enzyme-free tubes. After collection, the specimens were immediately placed in an ice box and transported to the laboratory. The samples were centrifuged at 3000×g for 15 min at 4 °C, and the supernatant was aliquoted and stored at -80 °C. Grossly bloody CSF specimens were excluded to avoid interference caused by erythrocyte rupture.

BALF specimens: Sterile normal saline prewarmed to 37 °C was slowly instilled through the tracheal tube at 3 mL/kg, and the lavage fluid was recovered by negative-pressure suction at <100 mmHg. A recovery rate of ≥40% was considered

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acceptable according to paediatric bronchoscopy recommendations (11). The recovered fluid was filtered through four layers of sterile gauze to remove mucus and cellular debris, centrifuged at 2000×g for 10 min at 4 °C, and the supernatant was aliquoted and stored at -80 °C.

Biochemical indicator detection

All assays were performed in our hospital's clinical biochemistry laboratory by the same senior technician with more than 10 years of relevant experience. All procedures were strictly followed according to the instructions for the instruments and assay kits.

Biochemical markers related to bronchial development

For bronchial development-related markers, serum CC16, MMP-9, and TIMP-1 were detected by chemiluminescent immunoassay using a cobas e 602 fully automated chemiluminescence immunoassay analyser (Roche). Serum SP-A, SP-D, and TGF-β1 were measured using double-antibody sandwich ELISA kits purchased from R&D Systems. The assay procedures were strictly followed: incubation at 37 °C for 2 h, washing 5 times, addition of the chromogenic substrate, light-protected reaction for 15 min, and absorbance measurement at 450 nm with 630 nm as the reference wavelength.

Biochemical markers specific to intracranial haemorrhage

For intracranial haemorrhage-related markers, S100β protein and NSE were measured in both CSF and serum by electrochemiluminescence using a cobas e 801 fully automated electrochemiluminescence immunoassay analyser (Roche). The calibrators were traceable to the international reference material NIBSC 93/662, ensuring the traceability of test results. The laboratory information system automatically generated the CSF/serum S100β concentration ratio.

Biochemical markers of oxidative stress and the antioxidant system

Oxidative stress and antioxidant indicators were assessed using a combination of biochemical, immunological, and chromatographic methods. Serum MDA, SOD, GSH-Px, and T-AOC were measured by the thiobarbituric acid colourimetric method (532 nm), xanthine oxidase method (550 nm), colourimetric method (412 nm), and ABTS method (734 nm), respectively, using a Hitachi 7600 fully automated biochemical analyser. The kits were purchased from Nanjing Jiancheng. Serum 8-OHdG was measured using an ELISA kit from R&D Systems. Serum MPO was detected on a Siemens ADVIA Centaur XP fully automated chemiluminescence immunoassay analyser. The serum GSH/GSSG ratio was determined by high-performance liquid chromatography using an Agilent 1260 Infinity II system equipped with a C18 column (4.6 mm×250 mm, 5 μm). The mobile phase was methanol-water (5:95, v/v), the flow rate was 1.0 mL/min, and the detection wavelength was 210 nm.

Inflammatory cytokines and chemokines

Serum IL-6, TNF-α, and IL-1β were measured by electrochemiluminescence on Roche instruments. Serum IL-8 and MCP-1 were measured by chemiluminescence on a Siemens ADVIA Centaur XP analyser. Serum CRP was determined by immunoturbidimetry on a Hitachi 7600 biochemical analyser, with a detection wavelength of 570 nm.

Biochemical indicators related to pulmonary surfactant system function

Total phospholipid concentration in BALF was measured by phosphomolybdic acid colourimetry on a Hitachi 7600 biochemical analyser. The BALF lecithin/sphingomyelin (L/S) ratio was determined by thin-layer chromatography, using chloroform-methanol-water (65:25:4, v/v/v) as the developing solvent. After iodine vapour staining, quantification was performed using a thin-layer scanner. The BALF saturated phosphatidylcholine (Sat-PC) concentration was measured using an Agilent 1260 Infinity II high-performance liquid chromatography system. BALF SP-B was measured using an ELISA kit from R&D Systems.

Biochemical markers of coagulation and fibrinolysis

For coagulation and fibrinolytic system indicators, plasma D-dimer and fibrinogen were measured by immunoturbidimetry on a Sysmex CS-5100 fully automated coagulation analyser. Plasma TAT and PAP were detected using ELISA kits from Shanghai Enzyme-linked Biotechnology. The plasma t-PA/PAI-1 ratio was determined using an ELISA kit from R&D Systems.

Biochemical markers related to energy metabolism and mitochondrial function

Energy metabolism-related indicators were measured using enzymatic, immunological, luminescence-based, and molecular methods. Serum

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lactate and pyruvate were measured by enzymatic colourimetry on a Hitachi 7600 biochemical analyser. Serum CK-MB was measured by the immunoinhibition method on a Roche cobas e 602 analyser. Serum ATP was detected using a luciferase assay kit from Promega on a GloMax 20/20 luminometer. Serum mitochondrial DNA (mtDNA) copy number was measured by real-time quantitative PCR using an ABI 7500 real-time PCR system. The reaction volume was 20 μL, and the amplification conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

Core biochemical markers of hepatic synthetic and metabolic function

Indicators of hepatic synthetic and metabolic function were measured using a Roche cobas c 702 fully automated biochemical analyser. These included total protein (biuret method, 540 nm), albumin (bromocresol green method, 630 nm), prealbumin (immunoturbidimetry, 570 nm), alanine aminotransferase (rate method, 340 nm), aspartate aminotransferase (rate method, 340 nm), total bilirubin, and direct bilirubin (diazo method, 540 nm).

Biochemical markers related to vascular endothelial injury and permeability

Among vascular endothelial injury markers, serum vWF was measured using an ELISA kit from Shanghai Enzyme-linked Biotechnology. Serum sICAM-1 and sVCAM-1 were measured by chemiluminescence on a Beckman UniCel DxI 800 fully automated chemiluminescence immunoassay analyser. Serum ET-1 was determined by radioimmunoassay using a kit purchased from the North Institute of Biotechnology, and radioactivity was measured with a γ-counter.

Laboratory quality control

All tests strictly followed the Clinical and Laboratory Standards Institute (CLSI) EP5-A3 guideline for precision evaluation. Three levels of internal quality control materials at low, medium, and high concentrations were included in each batch of tests. Internal quality control was performed according to Westgard multirules ($1_2s/2_2s/R_4s/4_1s/10_x$). Once an out-of-control result occurred, testing was immediately suspended, the cause was investigated, and quality control materials were retested after correction. Clinical specimens were tested and reported only after quality control results returned to an in-control state. All test items in our laboratory participated in the 2025 national external quality assessment program, achieving a 100% pass rate. The same batch of reagents and calibrators was used, and all calibrators were traceable to the corresponding international reference materials. Preliminary stability testing of frozen specimens showed that, within 6 months of storage at -80 °C, intra-assay and inter-assay CVs were calculated separately using low-, medium-, and high-concentration quality control materials for each indicator. The intra-assay CVs of all tested indicators were <5%, and the inter-assay CVs were <8%, meeting the requirements for clinical laboratory testing. Because this was a retrospective study using archived residual specimens, not all specimens collected at the three time points could be confirmed to have been analysed within the same analytical run. To reduce analytical variation, the same lot of reagents and calibrators was used whenever possible, and high- and low-concentration internal quality control materials were included in each batch.

Statistical analysis

SPSS 26.0 statistical software was used for data analysis. Propensity score matching and multivariable covariate adjustment were not performed because of the limited sample size and the exploratory biomarker-profiling purpose of this study. Baseline clinical characteristics and admission blood gas parameters were compared between groups before biomarker analyses, and potential residual confounding was addressed in the limitations. The normality of all continuous variables was assessed using the Shapiro-Wilk test. Normally distributed measurement data were expressed as mean ± standard deviation ($\bar{\chi}$±s), whereas non-normally distributed data were expressed as median and interquartile range. Comparisons between two groups were performed using the independent-samples t test, and comparisons among multiple time points were performed using repeated-measures analysis of variance. Mauchly's test of sphericity was performed first; when the sphericity assumption was not met, the Greenhouse-Geisser correction was applied. Bonferroni correction was used for comparisons within the same group across time points and between groups at the same time point. $P<0.05$ was considered statistically significant.

Results

Changes in core biochemical markers related to bronchial development

At baseline, serum levels of bronchial development-related markers were above the reference ranges for preterm infants in both groups, with no significant between-group differences

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(P>0.05). At 72 h after PS treatment, all six indicators in the research group had decreased from pretreatment levels (P<0.05) and were lower than those in the control group at the same time point (P<0.05). By 7 days after treatment, CC16 in the PS treatment group had decreased to (12.02±2.51) ng/mL, whereas the corresponding value in the non-PS control group was (19.19±3.19) ng/mL; the difference between groups was statistically significant (P<0.05). Although some indicators also decreased in the control group, the overall magnitude of reduction was smaller than that in the research group (*Figure 1*).

Changes in biochemical markers specific to intracranial haemorrhage

The trends in intracranial haemorrhage-related markers were similar in CSF and serum. Before treatment, S100β protein and NSE levels in both groups were above reference values, with no significant differences between groups (P>0.05). At 72 h after PS treatment, CSF S100β in the research group decreased from (0.81±0.14) μg/L to (0.30±0.05) μg/L (P<0.05), while serum S100β decreased in parallel to (0.48±0.08) μg/L (P<0.05). Both levels were lower than those in the control group at the same time point (P<0.05). At 7 days after treatment, both indicators in the research group had returned to the reference range, whereas they remained elevated in the control group (P<0.05). NSE changed in a similar direction to S100β. Notably, although absolute CSF and serum S100β levels decreased after treatment, the CSF/serum S100β ratio remained stable at all time points in both groups (P>0.05, *Figure 2*). This stable ratio suggests that PS treatment was not associated with laboratory evidence of blood-brain barrier disruption during the observation period.

Changes in oxidative stress and antioxidant system markers

Before treatment, both groups had evident oxidative imbalance, reflected by elevated oxidative injury markers and reduced antioxidant capacity. At 72 h after PS treatment, the oxidative injury marker MDA decreased in the research group (P<0.05), while the antioxidant indicator GSH/GSSG ratio increased from (6.03±1.07) to (11.18±1.90) (P<0.05). By 7 days after treatment, the GSH/GSSG ratio in the research group had further increased to (14.99±2.15), which was higher than that in the control group [(10.98±2.03), P<0.05]. Other oxidative injury and antioxidant indicators showed similar trends, and the degree of improvement was greater in the research group than in the control group (P<0.05, *Figure 3*).

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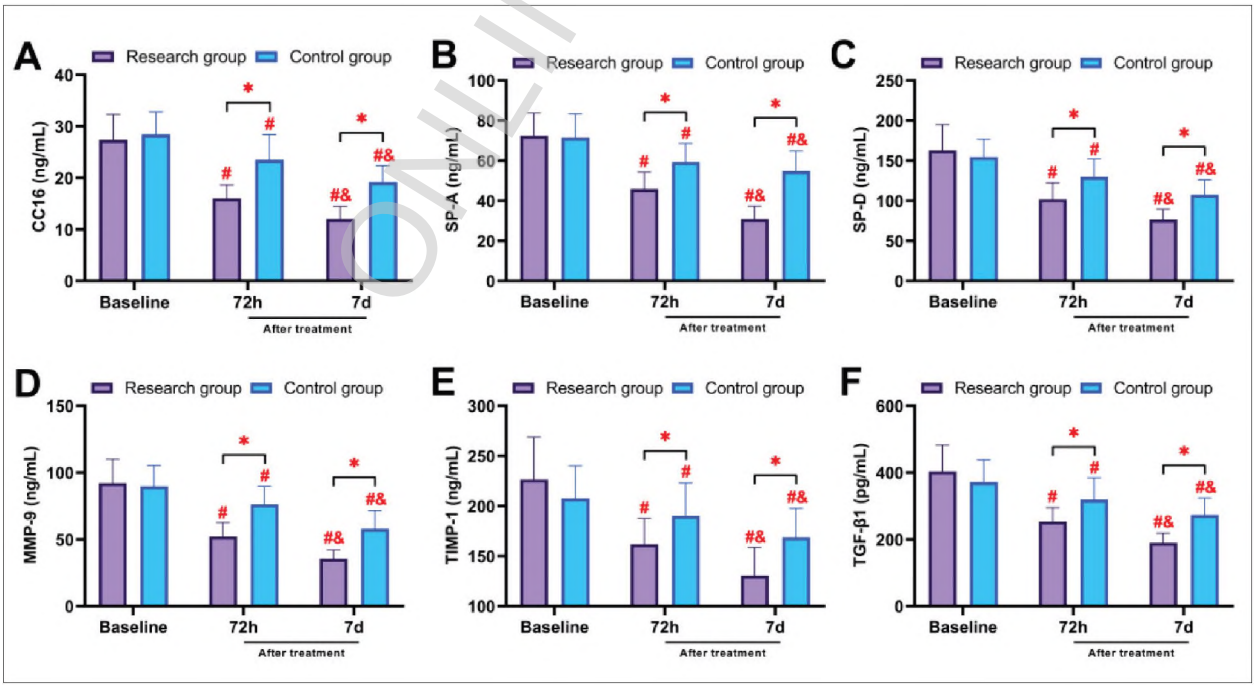

Figure 1 Changes in serum biochemical markers related to bronchial epithelial injury and tissue remodelling.

(A) Serum CC16; (B) serum SP-A; (C) serum SP-D; (D) serum MMP-9; (E) serum TIMP-1; (F) serum TGF-β1. # indicates P<0.05 versus baseline within the same group; & indicates P<0.05 versus 72 h after treatment within the same group; * indicates P<0.05 for the research group compared with the control group at the same time point.

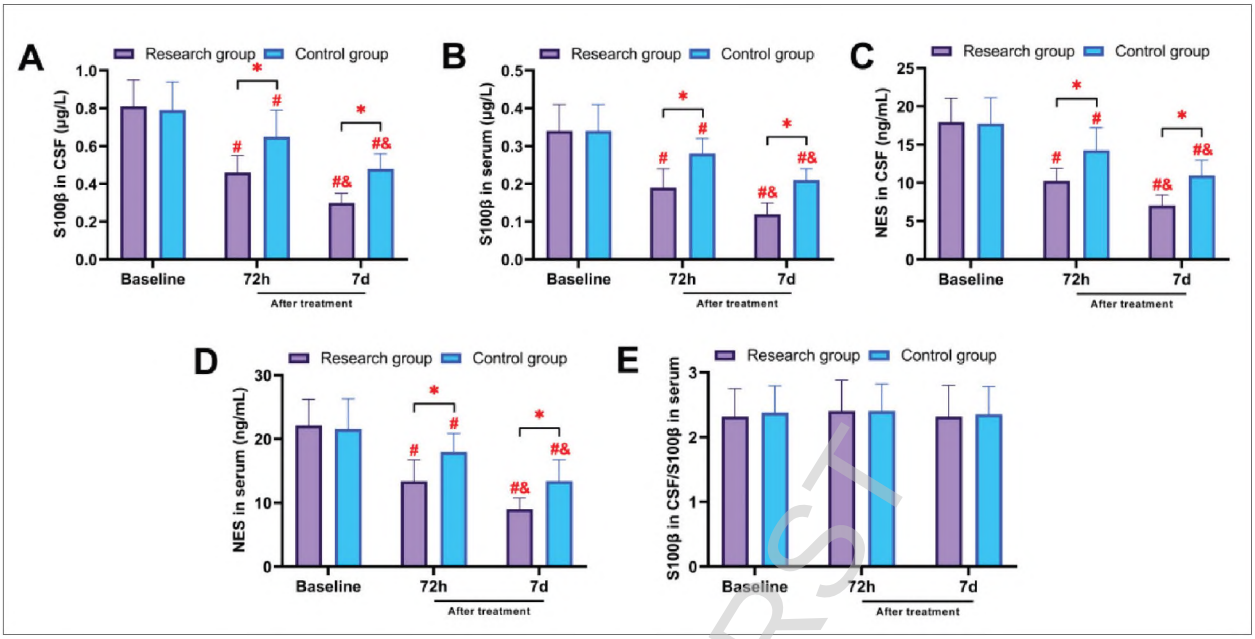

Figure 2 Changes in cerebrospinal fluid and serum biochemical markers related to intracranial and neuronal injury.

(A) CSF S100β; (B) serum S100β; (C) CSF NSE; (D) serum NSE; (E) CSF/serum S100β ratio. # indicates P<0.05 versus baseline within the same group; & indicates P<0.05 versus 72 h after treatment within the same group; * indicates P<0.05 for the research group compared with the control group at the same time point.

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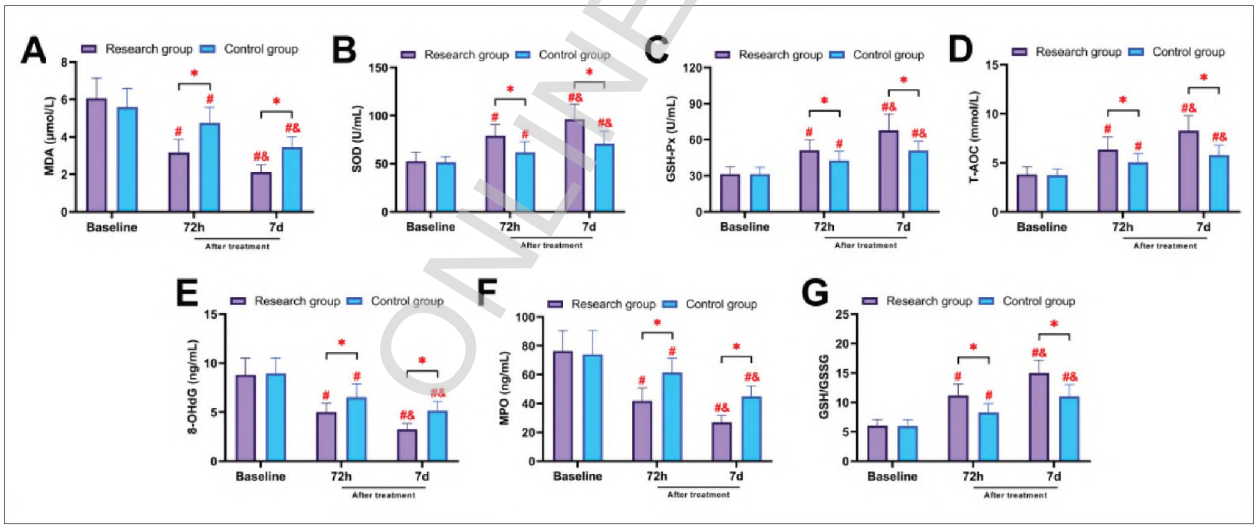

Figure 3 Dynamic changes in oxidative stress and antioxidant defence biomarkers after pulmonary surfactant replacement therapy.

(A) Serum MDA; (B) serum SOD; (C) serum GSH-Px; (D) serum T-AOC; (E) serum 8-OHdG; (F) serum MPO; (G) serum GSH/GSSG ratio. # indicates P<0.05 versus baseline within the same group; & indicates P<0.05 versus 72 h after treatment within the same group; * indicates P<0.05 for the research group compared with the control group at the same time point.

Changes in inflammatory cytokines and chemokines

Before treatment, serum inflammatory indicators were elevated in both groups. At 72 h after PS treatment, inflammatory indicators in the research group showed a general decline, and IL-6 decreased by 57% (P<0.05). At 7 days after treatment, IL-6 in the research group had fallen to (9.59±2.17) pg/mL, returning to the reference range, whereas the corresponding level in the control group was (21.12±4.92) pg/mL (P<0.05). The decline in

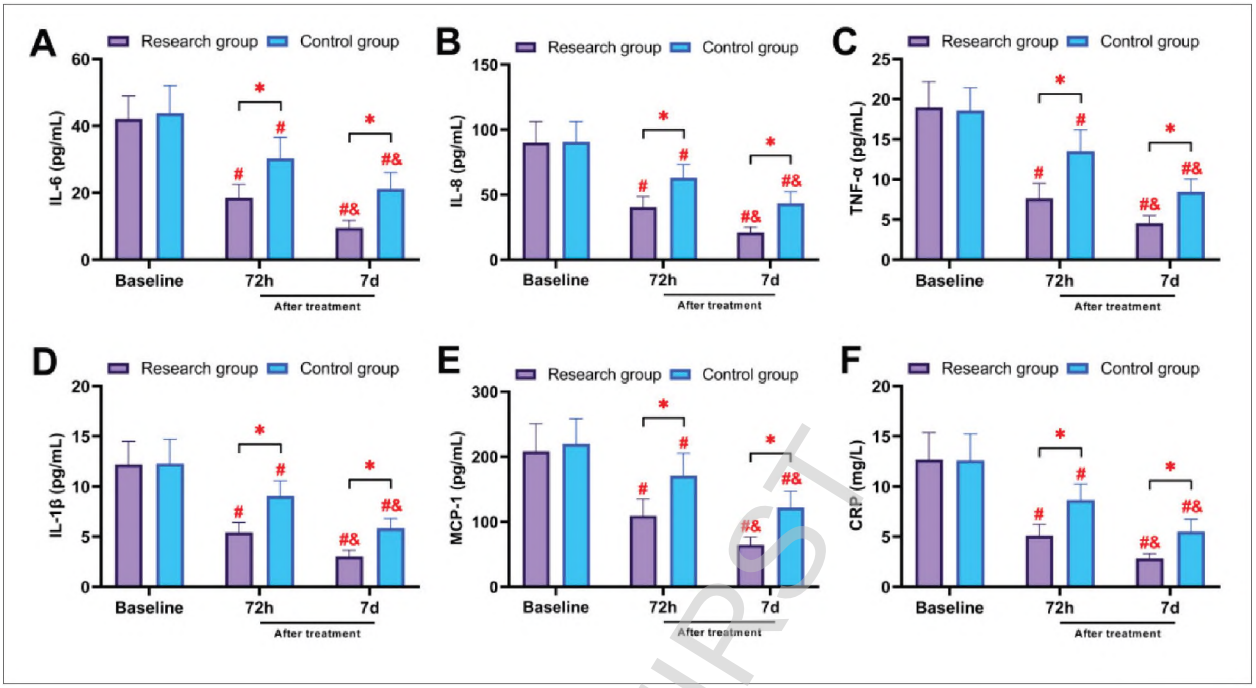

Figure 4 Changes in inflammatory cytokines and chemokines after pulmonary surfactant replacement therapy.

(A) Serum IL-6; (B) serum TNF-α; (C) serum IL-1β; (D) serum IL-8; (E) serum MCP-1; (F) serum CRP. # indicates P<0.05 versus baseline within the same group; & indicates P<0.05 versus 72 h after treatment within the same group; * indicates P<0.05 for the research group compared with the control group at the same time point.

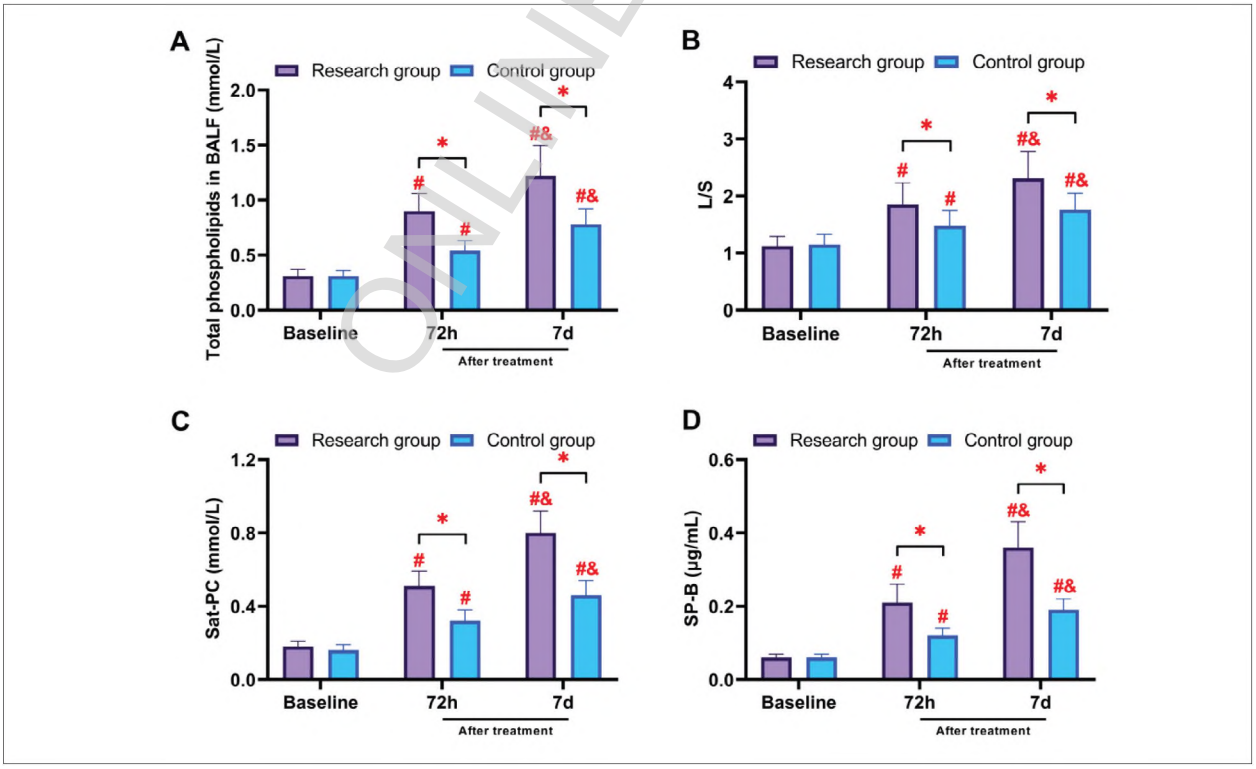

Figure 5 Changes in bronchoalveolar lavage fluid biochemical indicators of pulmonary surfactant system function.

(A) BALF total phospholipid concentration; (B) BALF lecithin/sphingomyelin ratio; (C) BALF saturated phosphatidylcholine concentration; (D) BALF SP-B. # indicates P<0.05 versus baseline within the same group; & indicates P<0.05 versus 72 h after treatment within the same group; * indicates P<0.05 for the research group compared with the control group at the same time point.

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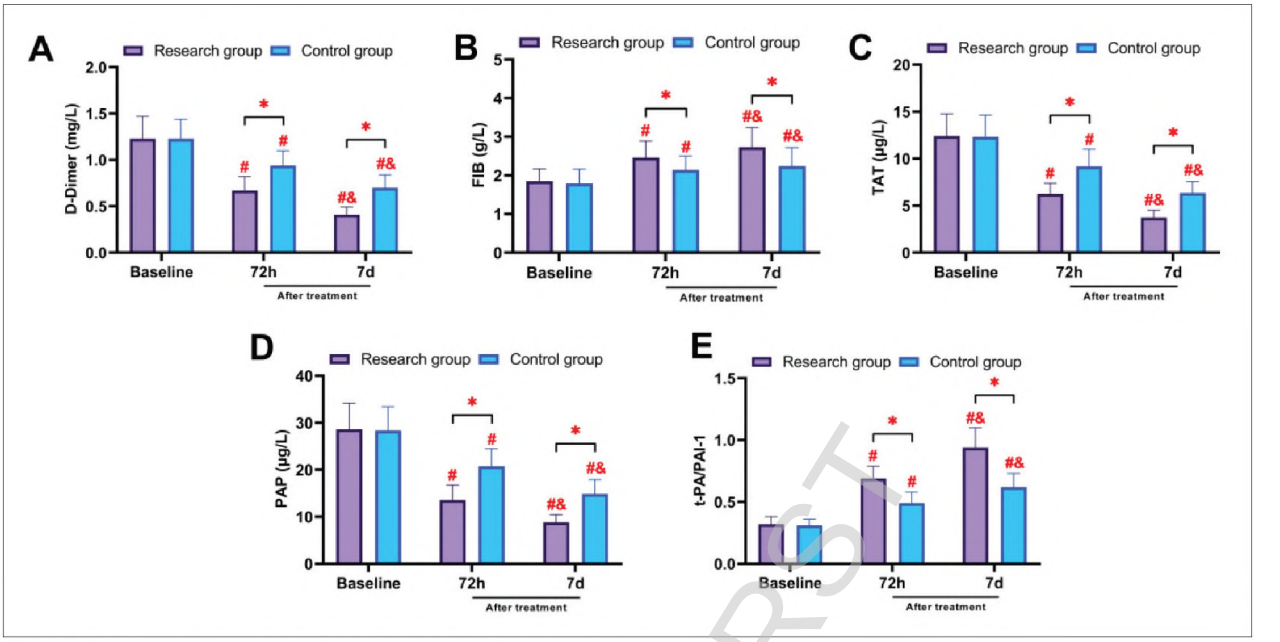

Figure 6 Changes in coagulation and fibrinolysis-related laboratory biomarkers.

(A) Plasma D-dimer; (B) plasma fibrinogen; (C) plasma thrombin-antithrombin complex; (D) plasma plasmin-α2-antiplasmin complex; (E) plasma t-PA/PAI-1 ratio. # indicates P<0.05 versus baseline within the same group; & indicates P<0.05 versus 72 h after treatment within the same group; * indicates P<0.05 for the research group compared with the control group at the same time point.

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inflammatory indicators was slower in the control group, and several markers remained above the reference range at 7 d after treatment (*Figure 4*).

Changes in biochemical indicators related to pulmonary surfactant system function

Before treatment, pulmonary surfactant-related indicators in BALF were below the reference values in both groups, indicating marked endogenous surfactant insufficiency. At 72 h after PS treatment, total phospholipid concentration and the L/S ratio increased in the research group compared with pretreatment levels (P<0.05). By 7 days after treatment, the L/S ratio in the research group had increased to (2.31±0.47), exceeding the threshold for lung maturity (>2.0), whereas the value in the control group was only (1.76±0.29), still below the maturity threshold (P<0.05). Sat-PC and SP-B levels also increased in parallel, and both were higher in the research group than in the control group (P<0.05, *Figure 5*).

Changes in coagulation and fibrinolytic system markers

Before treatment, both groups showed coagulation-fibrinolytic dysfunction. At 72 h after PS treatment, D-dimer in the research group decreased from (1.23±0.24) mg/L to (0.41±0.08) mg/L (P<0.05). At 7 days after treatment, the t-PA/PAI-1 ratio in the research group had recovered to (0.94±0.16), reaching the normal reference range, whereas the corresponding value in the control group was (0.62±0.11) (P<0.05). FIB, TAT, PAP, and other indicators also improved in parallel, with greater recovery in the research group than in the control group (P<0.05, *Figure 6*).

Changes in biochemical markers related to energy metabolism and mitochondrial function

Before treatment, both groups showed energy metabolism disorders and mitochondrial injury. At 72 h after PS treatment, serum lactate in the research group decreased from (3.19±0.54) mmol/L to (1.20±0.21) mmol/L (P<0.05), while ATP increased from (0.32±0.05) μmol/L to (0.64±0.09) μmol/L (P<0.05). At 7 days after treatment, mtDNA copy number in the research group had returned to the reference range. Notably, serum pyruvate levels showed no significant fluctuation throughout the observation period in either group, and no statistically significant differences were observed between or within groups (P>0.05, *Figure 7*). This finding indicates that pyruvate-related glycolytic pathway homeostasis was not markedly affected in either group during the early observation period.

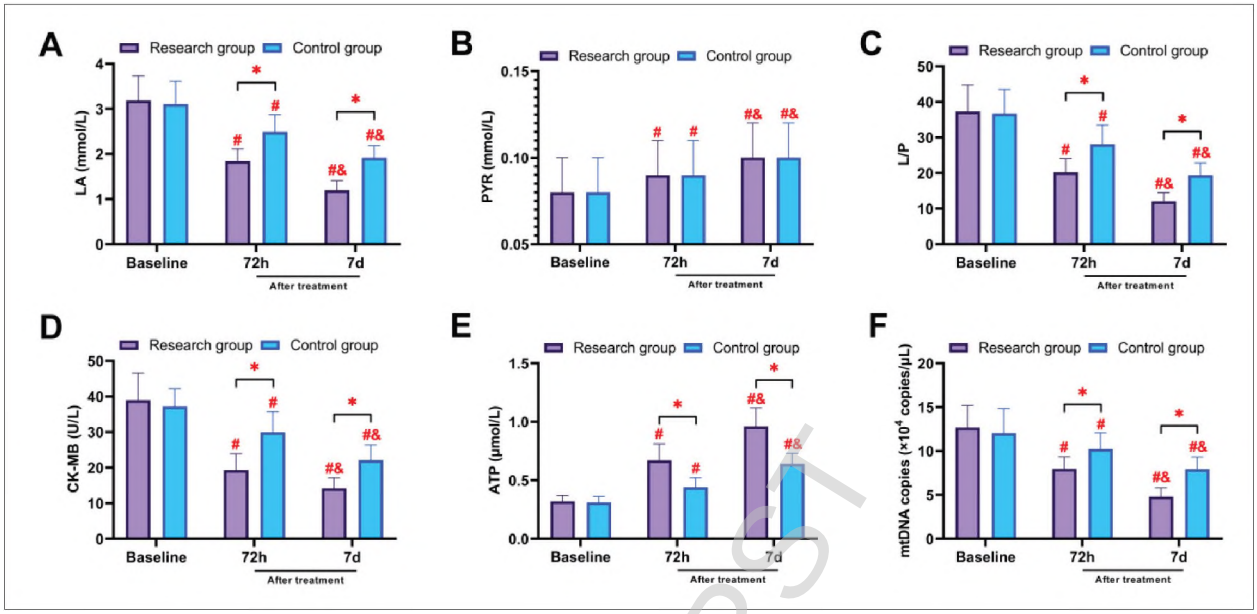

Figure 7 Changes in biochemical markers related to energy metabolism and mitochondrial injury.

(A) Serum lactate; (B) serum pyruvate; (C) serum CK-MB; (D) serum ATP; (E) serum mitochondrial DNA copy number. # indicates P<0.05 versus baseline within the same group; & indicates P<0.05 versus 72 h after treatment within the same group; * indicates P<0.05 for the research group compared with the control group at the same time point.

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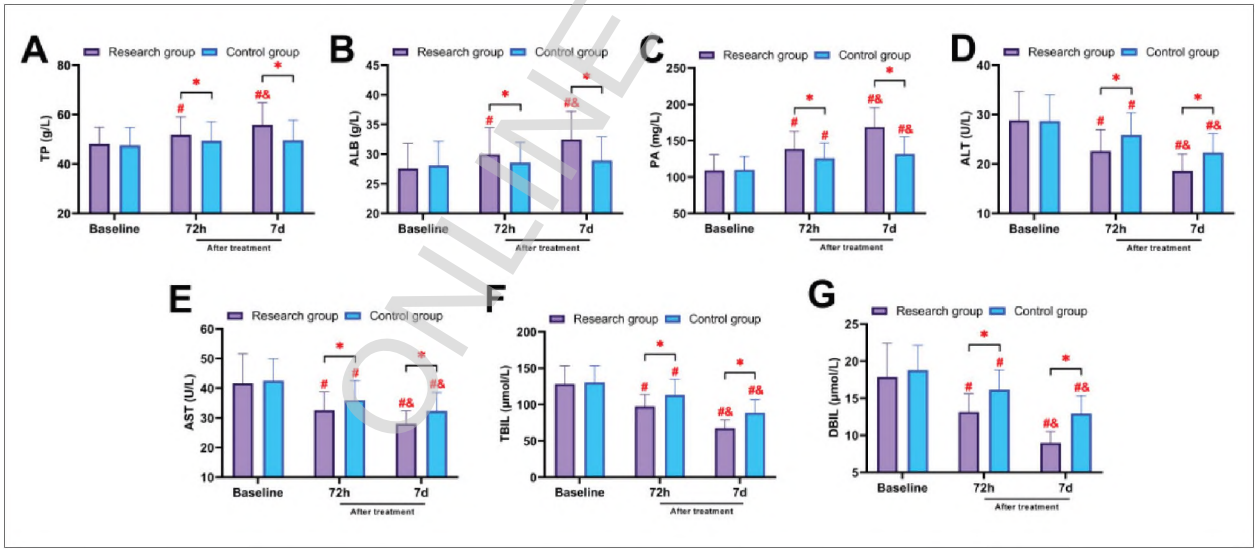

Figure 8 Changes in core biochemical markers of hepatic synthetic and metabolic function.

(A) Serum total protein; (B) serum albumin; (C) serum prealbumin; (D) serum alanine aminotransferase; (E) serum aspartate aminotransferase; (F) serum total bilirubin; (G) serum direct bilirubin. # indicates P<0.05 versus baseline within the same group; & indicates P<0.05 versus 72 h after treatment within the same group; * indicates P<0.05 for the research group compared with the control group at the same time point.

Changes in core biochemical markers of hepatic synthetic and metabolic function

Before treatment, both groups showed mildly reduced hepatic synthetic function and mildly elevated hepatocellular injury markers. At 72 h after PS treatment, hepatic synthetic indicators and injury markers improved in the research group (P<0.05). By 7 days after treatment, prealbumin in the research group had increased from (109.31±21.46) mg/L to (168.70±26.74) mg/L (P<0.05), while total bilirubin had decreased to (67.64±11.03) μmol/L (P<0.05). Both values had returned to the normal reference range. TP, ALB, ALT, AST, and other

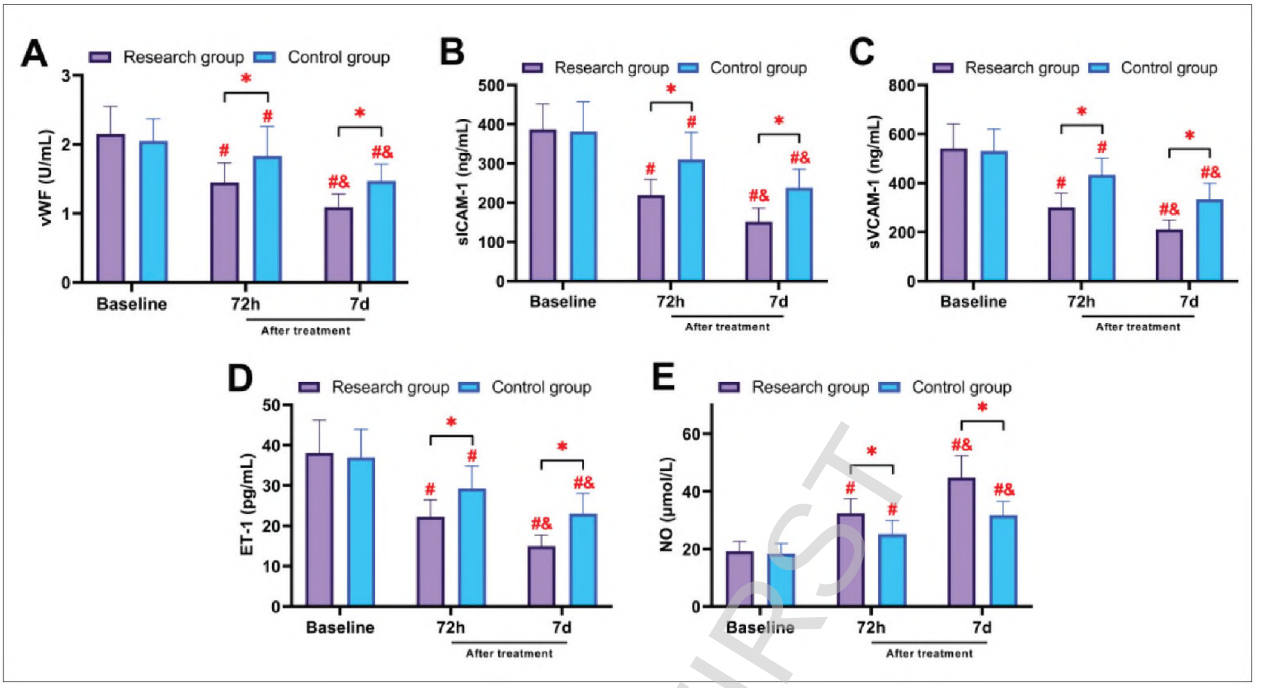

Figure 9 Changes in biochemical markers related to vascular endothelial injury and permeability.

(A) Serum vWF; (B) serum NO; (C) serum sICAM-1; (D) serum sVCAM-1; (E) serum ET-1. # indicates P<0.05 versus baseline within the same group; & indicates P<0.05 versus 72 h after treatment within the same group; * indicates P<0.05 for the research group compared with the control group at the same time point.

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indicators showed corresponding changes, with greater changes in the research group than in the control group (P<0.05, *Figure 8*).

Changes in biochemical markers related to vascular endothelial injury and permeability

At baseline, vascular endothelial injury was evident in both groups. At 72 h after PS treatment, vWF decreased in the research group, while NO increased to (44.73±7.60) μmol/L (P<0.05). At 7 days after treatment, vWF in the research group had returned to the reference range, whereas it remained elevated in the control group (P<0.05). Endothelial injury markers, such as sICAM-1, sVCAM-1, and ET-1, also decreased, and the degree of improvement was greater in the research group than in the control group (P<0.05; *Figure 9*).

Discussion

Using a standardised clinical biochemistry laboratory platform, this study examined dynamic changes in nine core biochemical pathways and more than 50 markers after exogenous pulmonary surfactant (PS) replacement therapy in preterm infants with neonatal respiratory distress syndrome. The results showed that changes observed in infants receiving PS treatment were not limited to pulmonary surfactant-related biochemical components in the lung. Rather, the observed changes involved several interconnected biochemical and metabolic axes, including oxidative stress balance, inflammatory cytokine networks, coagulation-fibrinolytic rhythm, vascular endothelial activity, mitochondrial energy metabolism, and hepatic synthetic function. Together, the coordinated changes in these multidimensional biochemical indicators are consistent with systemic biochemical changes after PS treatment and offer a laboratory perspective for understanding its possible multiorgan associations.

The time-course changes in bronchial development-related markers indicate that infants receiving PS treatment showed biochemical changes consistent with less airway epithelial injury and altered tissue remodelling. The gradual decline in epithelial injury markers such as CC16 and SP-A after treatment may reflect, at least in part, the stabilisation of type II alveolar epithelial cell membranes by PS phospholipids, with subsequent reductions in cell necrosis and shedding. At the same time, PS treatment was associated with a more favourable MMP-9/TIMP-1 profile, which may reflect attenuation of extracellular matrix degradation and airway remodelling. Previous biochemical studies have confirmed that exogenous PS can downregulate serum MMP-9 levels in children with NRDS. On this basis, the present study further describes the overall

evolution of markers of bronchial development, rather than relying on isolated changes in a single indicator. This provides a more comprehensive laboratory basis for evaluating airway repair status.

The detection results for biochemical markers of intracranial injury showed that PS intervention reduced S100β and NSE expression levels in both CSF and serum, with highly consistent trends across the two specimen types. From a biochemical and metabolic perspective, this phenomenon may be associated with improved pulmonary gas exchange after PS treatment, which increases cerebral oxygen supply and thereby alleviates hypoxia-mediated neuronal metabolic injury (12, 13). Although CSF and serum S100β levels decreased in parallel in infants receiving PS treatment, the absence of a significant change in the CSF/serum S100β ratio suggests that the decline in CSF S100β is unlikely to be explained mainly by altered blood-brain barrier permeability. A more cautious interpretation is that infants receiving PS treatment had lower production or release of S100β within the central nervous system, possibly secondary to improved oxygenation and reduced hypoxia-related glial or neuronal injury. This interpretation remains indirect and should be validated in studies that include both biochemical markers and clinical neuroimaging outcomes. In addition, lower systemic inflammatory activity after PS treatment may theoretically be associated with less inflammatory stress on the blood-brain barrier (14, 15). However, the unchanged CSF/serum S100β ratio in the present study does not directly support a measurable reduction in blood-brain barrier permeability. Therefore, the decrease in S100β should be interpreted more cautiously as a biochemical change possibly related to reduced central release or production of S100β, rather than as direct evidence of blood-brain barrier protection. Some studies have also suggested that the risk of severe intracranial haemorrhage in preterm infants may be related to early postnatal resuscitation, tracheal intubation, respiratory management strategies, and perinatal hemodynamic fluctuations (16, 17). Therefore, changes in IVH risk cannot simply be attributed to the PS drug itself. These discrepancies may partly reflect differences in the baseline biochemical status of enrolled populations, specimen processing and storage procedures, reagent batches, and internal quality control standards. Differences in the timing of observation across studies may also contribute to inconsistent findings (18). In the present study, all specimens were processed and stored according to a unified protocol, and inter-batch variation was strictly controlled, thereby reducing measurement-related variability.

Oxidative stress and inflammation are central to the pathophysiology of NRDS, and these two processes can amplify each other through multiple signalling pathways (19–21). In this study, infants receiving PS treatment showed lower oxidative injury markers and higher endogenous antioxidant activity, with changes in the GSH/GSSG ratio being particularly evident. The potential biochemical mechanism may involve the regulatory effects of PS and its phospholipid components on the pulmonary oxidative-inflammatory microenvironment. Recent studies have suggested that exogenous PS or its phospholipid-related components can alleviate inflammation and redox imbalance, and may influence NF- B- or inflammasome-related pathways, thereby reducing lipid peroxidation and inflammatory amplification (22–25). Previous studies have often examined the antioxidant or anti-inflammatory effects of PS in isolation (26).

In contrast, the present study approaches this issue at the network level. It shows that PS treatment was associated with coordinated changes in the oxidative-inflammatory interaction axis. These results add a more integrated laboratory basis for interpreting the systemic protective effects of PS.

Changes in the coagulation-fibrinolytic system also paralleled those observed in vascular endothelial function. After PS treatment, infants in the PS treatment group showed lower levels of endothelial activation or injury markers, such as vWF and sICAM-1. These findings should not be interpreted as evidence of a direct pharmacological action of intratracheally administered PS on the systemic vascular endothelium. A more plausible explanation is indirect: improved alveolar stability and oxygenation after PS treatment may reduce hypoxia-reoxygenation stress and attenuate pulmonary and systemic inflammatory signalling, thereby leading to lower endothelial activation markers and a more favourable coagulation-fibrinolytic profile (27). At the same time, the gradual recovery of the t-PA/PAI-1 ratio indicates that PS treatment was associated with partial restoration of fibrinolytic balance and improvement of the hypercoagulable biochemical profile in the early stage of disease. Together, these biochemical changes may reduce microvascular thrombosis and improve the biochemical milieu associated with intracranial haemorrhage risk. The temporal changes in energy metabolism and mitochondrial function indicators also suggest that, after improvement in tissue oxygen supply, mitochondrial respiratory chain function gradually recovers, lactate and other metabolic wastes accumulate less, and the synthesis of high-energy phosphate compounds increases (28). The synchronised recovery of hepatic synthetic and metabolic indicators reflects the gradual reconstruction of systemic metabolic homeostasis at the biochemical level, addressing a gap in previous studies that focused mainly on local pulmonary

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biochemical changes while paying less attention to the systemic metabolic profile (29, 30).

However, as a single-centre retrospective analysis of laboratory markers, this study has several limitations. First, all tests were performed on a single laboratory platform. The lack of parallel data across multiple centres and laboratories makes it difficult to completely exclude the potential influence of inter-laboratory differences in instrument performance, reagent batches, and internal quality control standards on biochemical test results. In addition, although the same laboratory platform, reagent lots, calibrators, and internal quality control procedures were used to reduce analytical variation, potential residual batch effects could not be completely excluded because not all three time-point specimens were consistently confirmed to have been tested in a single analytical run. Second, only three observation time points were set: before treatment, 72 h after treatment, and 7 d after treatment. These time points can reflect only short-term changes in biochemical markers. They cannot capture long-term steady-state changes in metabolic pathways over months or years after treatment. Third, a large number of biochemical indicators were examined. Although planned pairwise comparisons were performed, no global multiple-testing correction across all biomarkers was applied; therefore, some statistically significant findings may represent false positives. Fourth, residual confounding cannot be excluded. Disease severity, duration of invasive or non-invasive ventilation, oxygen exposure, hemodynamic instability, transfusion, caffeine use, antibiotics, vasoactive medications, and other concomitant therapies were not fully adjusted for in the present analysis. In particular, respiratory support intensity and detailed disease severity indices were neither balanced by propensity score matching nor adjusted for in multivariable models, which may have influenced the observed biomarker trajectories. Fifth, CSF specimens were available only for infants who underwent lumbar puncture for clinical indications and had residual CSF samples at all three time points. This may have introduced selection bias. Sixth, this study measured biochemical markers only at the body-fluid level. It did not include further validation at the cellular, pathway-protein, or gene level. Therefore, the upstream molecular targets and specific signalling interactions underlying these biochemical changes remain unclear. Finally, because of the retrospective non-randomised design, the present findings should be interpreted as associations rather than evidence of causality.

Conclusion

This retrospective cohort study, using comprehensive biochemical marker analysis, showed that PS replacement therapy was associated with multi-domain biochemical changes in preterm infants with NRDS. The findings confirmed changes in indicators of the pulmonary surfactant system. Further, they showed coordinated associations with systemic oxidative stress, inflammatory response, coagulation-fibrinolytic balance, vascular endothelial markers, energy metabolism, and hepatic synthetic function. These temporal biochemical changes showed laboratory consistency and pathophysiological plausibility, but they should be interpreted as associative and hypothesis-generating. These findings may provide a laboratory basis for constructing multi-marker combinations to monitor treatment response and to support early risk assessment of long-term complications in children with NRDS. Future multicentre studies with larger sample sizes, adjustment for clinical confounders, and mechanistic validation are needed to confirm these findings and to clarify the molecular pathways underlying the observed biochemical changes.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Authors' contribution

Huifen Chen and Xingyu Bai contributed equally to this work. YXQ and HFC contributed to conceptualisation, data curation, formal analysis, and writing the original draft, as well as reviewing and editing the manuscript. XYB and MZ were responsible for validation, visualisation, and funding acquisition. DWW and SZ contributed to visualisation. MBZ and SZ were involved in the investigation, methodology, and project administration. HFC and XYB contributed resources, software, and supervision. All authors read and approved the final manuscript.

Acknowledgements. None.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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