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ORIGINAL RESEARCH

The Association Between Neonatal Respiratory Distress Syndrome and Plasma IgG N-Glycosylation: A Case-Control Study

Yingjie Wang[1,2,*], Qingqing Shen[3,*], Ruxu Yan[1,2], Meng Wang[2,4], Min Xu[5], Hanxiang Chen[1], Dong Li[2,6]

[1]Department of Clinical Laboratory Medicine, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Shandong Medicine and Health Key Laboratory of Laboratory Medicine, Jinan, Shandong, People's Republic of China; [2]School of Public Health and Health Management, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, Shandong, People's Republic of China; [3]Department of Neonatology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Shandong, People's Republic of China; [4]Jinshan District Center for Disease Control and Prevention, Shanghai, People's Republic of China; [5]Department of neonatology, Tai'an Maternal and Child Health Hospital, Tai'an, Shandong, People's Republic of China; [6]Jining Medical University, Jining, Shandong, People's Republic of China

*These authors contributed equally to this work

Correspondence: Hanxiang Chen; Dong Li, Email hxchen@sdfmu.edu.cn; jnmcdongli@163.com

Background: Neonatal respiratory distress syndrome (NRDS) is the leading cause of neonatal death. Changes in plasma immunoglobulin G (IgG) N-glycosylation have been demonstrated in a variety of diseases. However, its implications and clinical significance in NRDS remain to be clarified.

Methods: To determine the effect of IgG N-glycosylation on NRDS, we recruited 88 NRDS participants and 120 control participants from December 2021 to September 2022. Plasma was collected, IgG was isolated and purified, and the glycogram was analyzed by ultra performance liquid chromatography (UPLC) with fluorescence detector.

Results: The occurrence of premature rupture of membranes (PROM) [OR=9.043(1.036–78.966), P=0.046] and the elevation of γ-glutamyltransferase (GGT) [OR=1.015(1.001–1.029), P=0.032] were independent risk factors for the occurrence of NRDS. Furthermore, the area percentages of GP1, GP3, GP4, GP11, GP13, and GP24 were significantly higher in NRDS patients compared with control group. Conversely, GP14 was observed to be significantly lower. Furthermore, an increase in plasma IgG sialylation and core fucosylation was observed in NRDS, whereas the modification with galactosylation was decreased. The model constructed using GP1, GP13, GP14, PROM, and GGT as composite indices demonstrated robust predictive performance (AUC=0.902, 95% CI: 0.851–0.953).

Conclusion: Patients with NRDS frequently exhibit alterations in the glycosylation of plasma IgG. These findings provide new insights into the diagnosis of NRDS and clinical treatment.

Keywords: neonatal respiratory distress syndrome, IgG N-glycosylation, ultra performance liquid chromatography, premature rupture of membranes

1.Introduction

Neonatal respiratory distress syndrome (NRDS) is a clinical condition that generally presents within 4 to 12 hours after birth, characterized by progressive dyspnea, grunting respirations, and cyanosis, and is predominantly observed in preterm infants.[1] Despite advancements in medical technology reducing the incidence of NRDS, it remains a significant contributor to neonatal mortality and morbidity.[2,3] Several studies have developed models to predict the incidence of NRDS;[4] however, these studies predominantly focus on alterations in complete blood count indicators in pregnant women prior to delivery, while insufficiently addressing the variations in indicators among patients diagnosed with NRDS. A comprehensive exploration of NRDS pathogenesis is imperative to identify effective preventive and therapeutic strategies.

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Protein glycosylation denotes the process of covalently attaching carbohydrates to protein functional groups, constituting a prevalent yet intricate post-translational modification. This process not only diversifies the proteome of organisms but also influences protein function and stability significantly.[5,6] Immunoglobulin G (IgG) represents the predominant antibody subclass in the human circulatory system, pivotal in adaptive immune responses. IgG exhibits the highest N-glycosylation level among human serum proteins.[7] Structurally, IgG comprises two identical light chains and two identical heavy chains interconnected by disulfide bonds, with Fab fragments facilitating antigen recognition and neutralization, and Fc fragments triggering phagocytosis, complement activation, and inflammatory responses. Alterations in Fc core fucose, galactose, N-acetylglucosamine, and sialic acid impact IgG's affinity for diverse complement proteins and receptors, potentially eliciting the secretion of pro-inflammatory and anti-inflammatory cytokines.[8] Consequently, modifications in IgG Fc N-glycosylation can disrupt immune homeostasis, predisposing individuals to various diseases.

Recent research has increasingly demonstrated the significant association between IgG Fc N-glycosylation modification and the pathogenesis of various diseases. Studies by Ivan Gudelj et al have highlighted alterations in serum IgG glycosylation profiles in patients with different diseases, particularly those with underlying inflammatory components, showing an increase in agalactosylated IgG abundance.[9] This change has been linked to variations in inflammation and the expression of cardiometabolic markers, indicating a crucial role of IgG N-glycosylation in disease development. Additionally, Barbara Radovani et al observed distinct IgG N-glycosyl composition in patients with coronary artery disease (CAD), with a more pronounced difference noted in women, suggesting a potential influence of sex on IgG N-glycosyl composition. Furthermore, the presence of salivary N-glycan structure was found to be inversely correlated with CAD.[10] Anna Birukov et al have also reported associations between changes in IgG N-glycosylation and cardiometabolic risk.[11]

While these findings underscore the intricate and diverse roles of IgG N-glycosylation in various diseases, the impact of IgG N-glycosylation profiles on NRDS remains unclear. Furthermore, current diagnostic approaches for NRDS are hindered by delayed diagnosis, absence of early biomarkers, and the potential risk of radiation exposure.[12] Considering the emerging correlation between IgG N-glycosylation modifications and disease, we propose the hypothesis that IgG N-glycosylation modifications may undergo alterations in response to NRDS. This is based on the premise that the glycosylation profile has the potential to reflect disease subtypes and inform targeted therapeutic interventions. Accordingly, we conducted a comprehensive analysis of the alterations in clinical indicators among NRDS patients and investigated the modifications of plasma IgG N-glycosylation using Ultra Performance Liquid Chromatography (UPLC) technology. This investigation aims to establish a theoretical foundation for enhancing the understanding of the pathogenesis and clinical management of NRDS.

Materials And Methods

Subjects and Sample Collection

Based on our recently published research findings, the mean albumin-globulin ratio in the general population is 1.98 ±0.336. In contrast, neonatal patients diagnosed with hypoxic-ischemic encephalopathy exhibit an average albumin-globulin ratio of 2.19±0.425.[13] Statistical analysis was performed with a significance level (α) of 0.05 and a statistical power (1-β) of 0.90. For the case-control study, the sample size for each group was calculated to compare the means of the two samples, resulting in a requirement of 59 participants per group.[14] To mitigate potential issues such as information bias, data loss, and damage to blood samples, we increased the sample size by 10%. Consequently, the total number of participants across both groups was adjusted to 130.

$$n_1 = n_2 = 2\left[\frac{\left(Z\alpha_{/2} + Z_\beta\right)}{\delta}\right]^2 = 58.4452 \approx 59$$

In alignment with the case-control design of this retrospective study, a total of 88 patients diagnosed with NRDS and 120 control patients were recruited between December 2021 and September 2022 from Tai'an Maternal and Child Health

Hospital and Shandong Provincial Hospital Affiliated to Shandong First Medical University. Of these, 21 NRDS patients and 53 concurrent controls were designated as the validation group, while the remaining participants constituted the training group. In order to be eligible for inclusion in the study, participants were required to meet the following criteria: 1. The diagnosis of NRDS was made by clinicians based on the patient's clinical presentation (eg, dyspnea, moaning, tri specular signs within 6 hours after birth), arterial blood gas analysis and chest X-ray (eg, ground-glass changes, air bronchogram signs). The diagnosis also met the criteria set out in the 6th edition of the European Guidelines for the Management of NRDS.[12] 2.Newborns with informed consent of guardians. The exclusion criteria were as follows: 1. Severe congenital heart disease, pulmonary heart disease, vascular disease, malignancy and other diseases; 2. Maternal use of drugs that may affect glycosylation during pregnancy (such as immunosuppressants); 3. Incomplete outcome data or irrelevant clinical data. All subjects met the inclusion criteria, and their guardians provided informed consent. The study was approved by the Ethics Committee of Shandong First Medical University (R202410280375), and was conducted according to the Declaration of Helsinki principles.

Immunoglobulin G N-Glycans Analysis

The plasma samples underwent centrifugation and were subsequently combined with an extraction plate embedded with IgG-specific binding protein G. Following this, the samples were subjected to a series of washes, elution, and denaturation using 1.33% SDS and 4% gepal. Finally, the samples were digested and purified using PNGase. The resultant mixture was subsequently incubated in a water bath maintained at 37°C for a duration of 18 to 20 hours. N-glycans were labeled with fluorescence employing 2-aminobenzamide (2-AB). Quantitative and qualitative analysis was conducted utilizing an UPLC system equipped with a fluorescence detector.[15] Each sample was injected with a volume of 10 microliters, and the chromatographic run time was set to 30 minutes per sample. Upon acquiring the chromatogram for each sample, the chromatogram was segmented into 24 initial Glycan peaks based on the reference Glycan peak map. The area of each peak was then normalized by dividing it by the total peak area, thereby yielding the 24 Glycan peak (GP) values for all samples. The composition and glycan profile of the IgG N-glycome were analyzed.

Statistical Analysis

For the statistical analysis, SPSS 26.0 and R 4.3.3 software were employed for data processing, and GraphPad Prism 10 was used for chart creation. The normality of the data distribution was evaluated using the Kolmogorov–Smirnov test. Continuous variables exhibiting a normal or approximately normal distribution were reported as mean ± standard deviation, whereas those with a skewed distribution were presented as median (interquartile range). Categorical variables were reported as n (%). Differences between two groups in continuous variables that followed a normal (or approximate normal) distribution were analyzed using an independent sample *t*-test. Non-normal distributed continuous variables' differences between two groups were evaluated using Wilcoxon rank-sum test. Chi-square test was employed for categorical variables analysis. Variables that exhibited inter-group differences in the aforementioned analysis were incorporated into a multivariate logistic regression analysis to further identify independent risk factors. Based on the inclusion of independent risk factors as covariates, logistic regression analysis identified 24 initial glycans and 17 derivative indicators associated with NRDS. Hosmer-Lemeshow test (H-L test) was used for the fit degree of the model, and $P > 0.05$ was considered to be a good calibration degree. Additionally, the model underwent evaluation through K-fold cross-validation, with k set to 10.[16] The significance level was set as $\alpha=0.05$. All P-values<0.05 were considered significant.

Results

Baseline Information of Participants

We statistically analyzed the basic information of 134 participants (Figure 1). The study cohort consisted of 134 newborns, including 73 males and 61 females. None of the newborns had a gestational age of 42 weeks or more; therefore, gestational age was used as a binary variable to classify the newborns as preterm or term. Birth weight was also used as a binary variable to distinguish between low birth weight and normal birth weight. Preterm rupture of

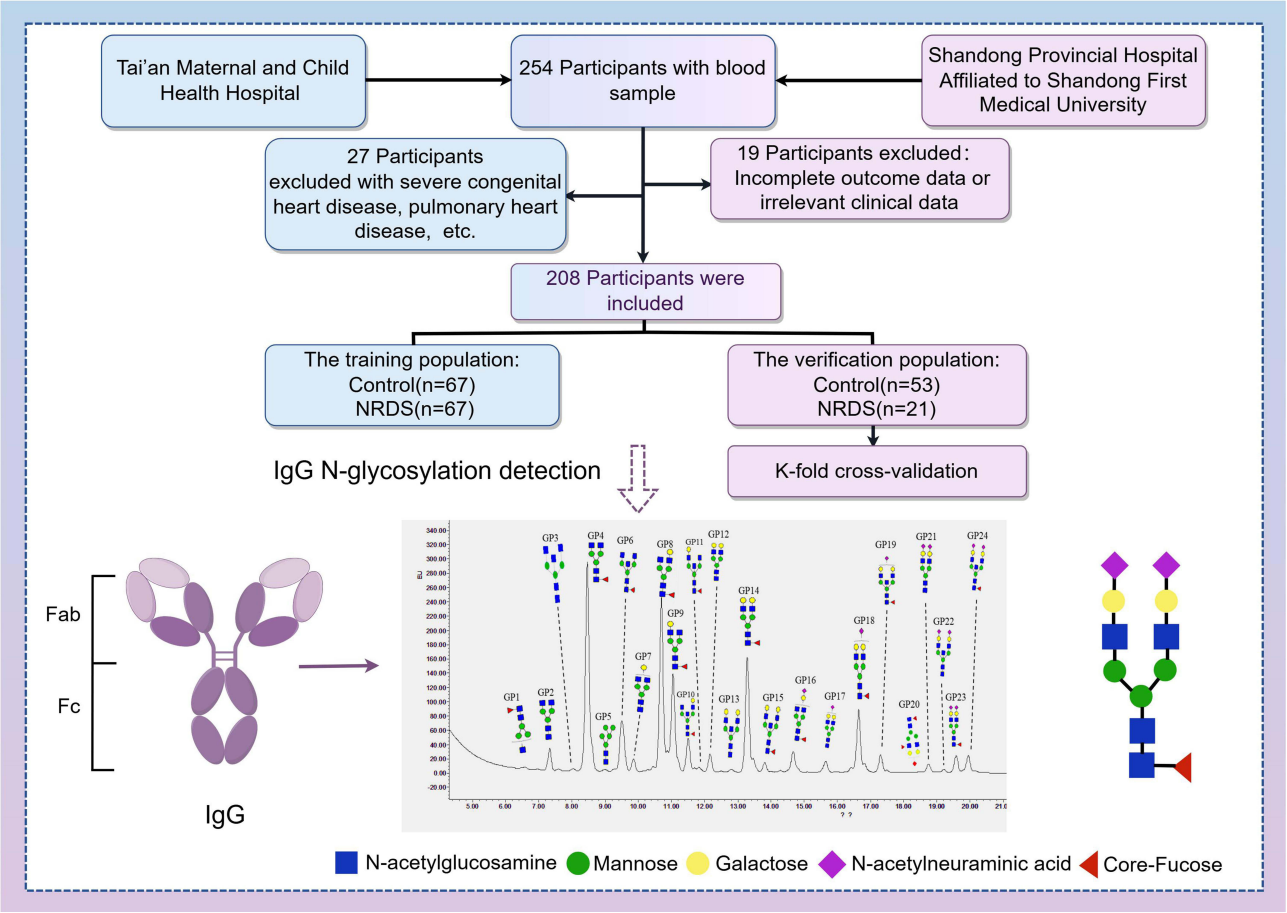


Figure 1 A schematic diagram of the study participants.

membranes (PROM) was defined as spontaneous rupture of membranes before delivery.[17] The results of the chi-squared test are shown in Table 1. Statistically significant differences were found between the NRDS group and the control group in terms of gestational age in weeks, birth weight in grams, history of miscarriage and PROM. The findings indicate that gestational age, birth weight, history of miscarriage, and PROM may contribute to the pathogenesis of NRDS, corroborating the results of previous research.[18,19] Furthermore, newborns with lower birth weight and earlier gestational age are at an increased risk, potentially due to compromised lung function, which may contribute to the development of NRDS.[20]

The Research Object of Clinical Indicators and Independent Risk Factors of NRDS

From the baseline data, we collected and analyzed the differences in clinical indicators between the two groups of neonates. The results of independent t-tests of the two samples showed that absolute neutrophil count (ANC), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), procalcitonin (PCT) and lactate dehydrogenase (LDH) levels were elevated in the NRDS group compared with the control group. In addition, the levels of α-hydroxybutyrate dehydrogenase (α-HBDH), alanine aminotransferase (ALT), total bilirubin (TBIL), serum albumin (ALB), prealbumin (PAB), total serum bile acids (STBA), triglyceride bile acids (CG) and cholinesterase (ChE) were also decreased (Table 2).

Incorporating the aforementioned baseline data and clinical indicators, along with between-group differences, into the logistic regression analysis revealed that both PROM and GGT are independent risk factors for NRDS. These factors remained statistically significant even after adjusting for the influence of other variables. (Table 3 and Figure S1). PROM is a frequent complication occurring in the mid to late stages of gestation, directly impacting maternal, fetal, and neonatal

Table 1 Comparison of Basic Characteristics of 134 Neonatal NRDS Group and Control Group [n (%)]

Characteristics	Total (n=134)	NRDS (n=67)	Control (n=67)	χ^2	*P*
Gender				<0.030	0.862
Male	73 (54.48)	37 (55.22)	36 (53.73)		
Female	61 (45.52)	30 (44.78)	31 (46.27)		
Gestational Age (weeks)				50.585	**<0.001*** **
<37	61 (45.52)	51 (76.12)	10 (14.93)		
≥37	73 (54.48)	16 (23.88)	57 (85.07)		
Birth Weight (g)				40.659	**<0.001*** **
<2500	37 (27.61)	35 (52.24)	2 (2.99)		
≥2500	97 (72.39)	32 (47.76)	65 (97.01)		
Mode of Delivery				0.132	0.716
Natural Delivery	46 (34.33)	22 (32.84)	24 (35.82)		
Cesarean Delivery	88 (65.67)	45 (67.16)	43 (64.18)		
Number of Pregnancies				1.639	0.200
<2	45 (33.58)	19 (28.36)	26 (38.81)		
≥2	89 (66.42)	48 (71.64)	41 (61.19)		
History of Abortion				11.268	**0.001*** **
Yes	53 (39.55)	36 (53.73)	17 (25.37)		
No	81 (60.45)	31 (46.27)	50 (74.63)		
PROM				15.462	**<0.001*** **
Yes	26 (19.40)	22 (32.84)	4 (5.97)		
No	108 (80.60)	45 (67.16)	63 (94.03)		
Fetal Distress				0.119	0.730
Yes	9 (6.72)	5 (7.46)	4 (5.97)		
No	125 (93.28)	62 (92.54)	63 (94.03)		
Gestational Diabetes Mellitus				<0.001	1.000
Yes	8 (5.97)	4 (5.97)	4 (5.97)		
No	126 (94.03)	63 (94.03)	63 (94.03)		
Gestational Hypertension				0.075	0.784
Yes	15 (11.19)	8 (11.94)	7 (10.45)		
No	119 (88.81)	59 (88.06)	60 (89.55)		
Anemia during Pregnancy				0.052	0.819
Yes	23 (17.16)	12 (17.91)	11 (16.42)		
No	111 (82.84)	55 (82.09)	56 (83.58)		

Notes: Bold and *** indicate a p-value of less than 0.001, all statistical symbols are presented in italics.

health, and is linked to the onset of NRDS[21] Serum GGT, primarily originating from the hepatobiliary system, is recognized as a biomarker for liver damage, cancer, and low-grade chronic inflammation.[22] This association may represent one of the potential mechanisms underlying its involvement in NRDS.

IgG N-Glycosylation Levels in Plasma of Populations

Subsequently, we conducted an extensive analysis to investigate the association between IgG N-glycosylation and the incidence of NRDS utilizing UPLC technology. The results of logistic regression analysis of primary glycans and their derived metrics with NRDS were shown in Figure 2 (see Table S1 for detailed data), and the levels of GP1, GP3, GP13, GP14, GP21, GP24, and their derived features, GPS, S1, S2, G0, G1, G2, F, and FG2, were different in the case group compared with the control group. Subsequent analysis of baseline sugar levels and their derived indices, using PROM and GGT as covariates, showed that the levels of GP1, GP3, GP4, GP11, GP13, GP14, and GP24 were significantly different in the NRDS compared to the control group. In contrast, the levels of GP21 were not statistically significantly different. In addition, derived indices such as GPS, S1, G0, G1, G2, F, BS, FG0, FG2 and Gal ratio were also statistically

Table 2 Comparison of Laboratory Examination Indexes Between NRDS Group and Control Group

Clinical Index	NRDS (n=67)	Control (n=67)	t/t'	P
ANC	12.46±8.27	6.24±4.72	−5.348	**<0.001*** **
ALT	8.00±4.89	12.37±6.99	4.195	**<0.001*** **
AST	47.70±22.32	35.73±13.72	−3.740	**<0.001*** **
GGT	190.43±124.01	106.73±62.63	−4.932	**<0.001*** **
Adenosine Deaminase	7.37±4.15	7.62±3.59	0.361	0.719
Alkaline Phosphatase	174.72±49.57	170.52±52.89	−0.474	0.637
TBIL	84.97±43.15	146.73±68.58	6.239	**<0.001*** **
PCT	10.10±15.10	1.51±3.46	−4.540	**<0.001*** **
ALB	30.25±3.74	34.29±2.99	6.897	**<0.001*** **
Globulin	15.43±3.78	16.34±2.49	1.644	0.103
PAB	90.94±25.66	113.64±22.14	5.483	**<0.001*** **
STBA	8.49±5.40	12.50±9.43	3.021	**0.003****
CG	2.44±1.44	4.49±3.67	4.246	**<0.001*** **
ChE	4934.79±1147.90	5602.66±1637.13	2.734	**0.007****
Creatine Kinase	356.99±397.08	249.10±257.89	−1.865	0.064
LDH	581.90±186.26	408.25±113.58	−6.515	**<0.001*** **
α-hbdh	421.43±148.78	297.51±83.33	−5.949	**<0.001*** **

Notes: Bold and ** indicate a p-value of less than 0.01, and bold and *** represent a p-value of less than 0.001. All statistical symbols are presented in italics.

Table 3 Analysis of Influencing Factors of Neonatal Respiratory Distress Syndrome Based on Multivariate Logistic Regression

Influence Factors (Reference)	*β*	*SE*	Waldχ2	*OR*	95% *CI* of *OR*		*P*
					LCI	UCI	
Gestational Age (≥37 weeks)	1.460	1.130	1.669	4.308	0.470	39.485	0.196
Birth Weight (≥2500g)	1.271	1.150	1.221	3.564	0.374	33.950	0.269
History of Abortion (No)	−0.591	0.869	0.462	0.554	0.101	3.042	0.497
PROM (No)	2.202	1.106	3.966	9.043	1.036	78.966	**0.046***
ALT	0.069	0.086	0.654	1.072	0.906	1.268	0.419
ANC	0.137	0.098	1.966	1.147	0.947	1.390	0.161
AST	−0.049	0.036	1.841	0.952	0.888	1.022	0.175
GGT	0.015	0.007	4.608	1.015	1.001	1.029	**0.032***
TBIL	−0.015	0.011	1.868	0.985	0.964	1.007	0.172
PCT	0.020	0.063	0.097	1.020	0.901	1.154	0.756
ALB	−0.408	0.219	3.465	0.665	0.433	1.022	0.063
PAB	−0.007	0.014	0.222	0.993	0.966	1.022	0.637
TBA	0.318	0.200	2.537	1.375	0.929	2.035	0.111
CG	−1.206	0.740	2.655	0.300	0.070	1.277	0.103
ChE	0.001	0.000	1.623	1.001	1.000	1.001	0.203
LDH	0.004	0.010	0.170	1.004	0.985	1.024	0.680
α-hbdh	0.012	0.015	0.625	1.012	0.983	1.041	0.429

Notes: Bold and * indicate a p-value of less than 0.05, all statistical symbols are presented in italics.

significantly different between the two groups (Figure 3 and Table S2). This suggests a correlation between the occurrence of NRDS and various modifications of plasma IgG, including sialylation, monosialylation, agalactosylation, monogalactosylation, bisgalactosylation, core fucosylation, agalactosylated core fucosylation, bisgalactosylated core fucosylation, and galactosylation. The sialylation of IgG plays a role in modulating inflammation, while an increase in core fucosylation of IgG can augment antibody-dependent cell-mediated cytotoxicity (ADCC). Additionally, the absence

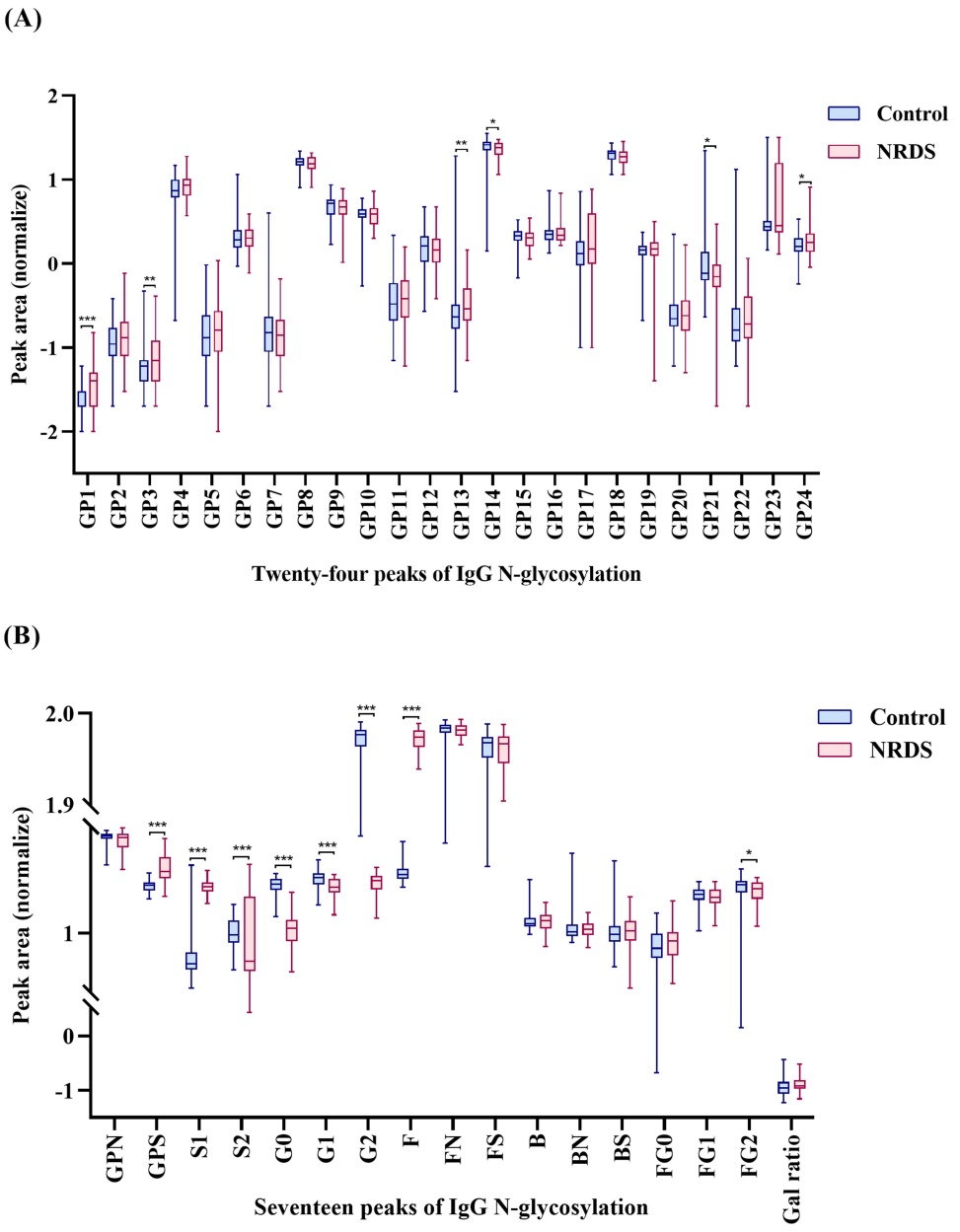


Figure 2 Box plots of 24 glycan peaks and derived indices of IgG N-glycosylation. **(A)** The difference between the NRDS group and the control group in the proportion of the 24 glycosyl peak areas in the total IgG glycosylated area normalized by log10; **(B)** The difference between the NRDS group and the control group after log10 normalization of 17 IgG N-glycosylation derived indicators. Each box represents the 25th to 75th percentile. The lines inside the box indicate the median. The lines outside the box indicate the 0th and 100th percentiles. *P < 0.05; **P < 0.01; ***P < 0.001. P values were calculated with the Wilcoxon rank-sum test. GP, glycan peak; IgG, immunoglobulin G; NRDS, neonatal respiratory distress syndrome; B, bisecting N-acetylglucosamine (GlcNAc); F, fucose; G, galactose; S, sialic acid; N, neutral glycosylation.

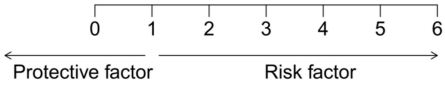
of galactosylation may influence IgG functionality.[23] These findings suggest a potential mechanistic link between IgG N-glycosylation modifications and the development of NRDS.

Construction of NRDS Identification Model

In order to assess the clinical predictive value of alterations in these indicators for NRDS, a diagnostic model was constructed. Concurrently, to address the issue of multicollinearity among the independent variables (Figure S2), stepwise logistic regression was employed for variable selection (Table S3). We developed Model A and Model B based on clinical and glycosylation indicators, respectively. Model C was subsequently constructed by integrating the variables from both models to identify the predictive model with the optimal classification performance. The AUC for

(A)

Initial glycans and their derived indices	OR (95%CI)	P
GP1	2.858 (1.845−4.425)	<0.001
GP2	1.310 (0.911−1.883)	0.146
GP3	1.608 (1.124−2.301)	0.009
GP4	1.495 (1.033−2.164)	0.033
GP5	1.397 (0.992−1.969)	0.056
GP6	1.165 (0.822−1.651)	0.392
GP7	0.882 (0.617−1.259)	0.488
GP8	0.812 (0.570−1.156)	0.247
GP9	0.949 (0.665−1.353)	0.771
GP10	0.918 (0.645−1.305)	0.632
GP11	1.477 (1.020−2.140)	0.039
GP12	0.804 (0.568−1.137)	0.218
GP13	1.894 (1.293−2.772)	0.001
GP14	0.568 (0.388−0.831)	0.004
GP15	0.727 (0.506−1.045)	0.085
GP16	1.196 (0.843−1.697)	0.315
GP17	1.208 (0.855−1.707)	0.284
GP18	0.721 (0.504−1.033)	0.075
GP19	1.320 (0.918−1.898)	0.134
GP20	1.009 (0.711−1.433)	0.960
GP21	0.746 (0.524−1.061)	0.103
GP22	1.217 (0.869−1.705)	0.253
GP23	1.220 (0.862−1.727)	0.262
GP24	1.647 (1.128−2.405)	0.010



(B)

Initial glycans and their derived indices	OR (95%CI)	P
GPN	0.753 (0.525−1.079)	0.122
GPS	10.043 (4.466−22.587)	<0.001
S1	5.135 (2.929−9.003)	<0.001
S2	0.711 (0.489−1.034)	0.075
G0	0.008 (0.001−0.063)	<0.001
G1	0.489 (0.330−0.724)	<0.001
G2	0.115 (0.053−0.252)	<0.001
F	14.008 (5.200−37.736)	<0.001
FN	0.737 (0.519−1.047)	0.089
FS	0.895 (0.630−1.271)	0.535
B	1.366 (0.951−1.963)	0.092
BN	1.332 (0.935−1.897)	0.112
BS	1.485 (1.023−2.155)	0.037
FG0	1.495 (1.033−2.164)	0.033
FG1	0.877 (0.618−1.245)	0.462
FG2	0.568 (0.388−0.831)	0.004
Gal ratio	1.750 (1.183−2.590)	0.005

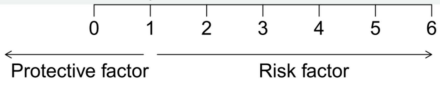


Figure 3 Correlation analysis of IgG N-glycosylation related indicators and NRDS. **(A)** Univariate logistic analysis of initial glycans with covariates of PROM and GGT; **(B)** Univariate logistic analysis of IgG N glycosylation derived index with covariates of PROM and GGT. Orange confidence intervals indicate that the variable is a statistically significant risk factor and blue is a protective factor. OR, odds ratio; CI, confidence interval. GP, glycan peak; IgG, immunoglobulin G; NRDS, neonatal respiratory distress syndrome; B, bisecting N-acetylglucosamine (GlcNAc); F, fucose; G, galactose; S, sialic acid; N, neutral glycosylation.

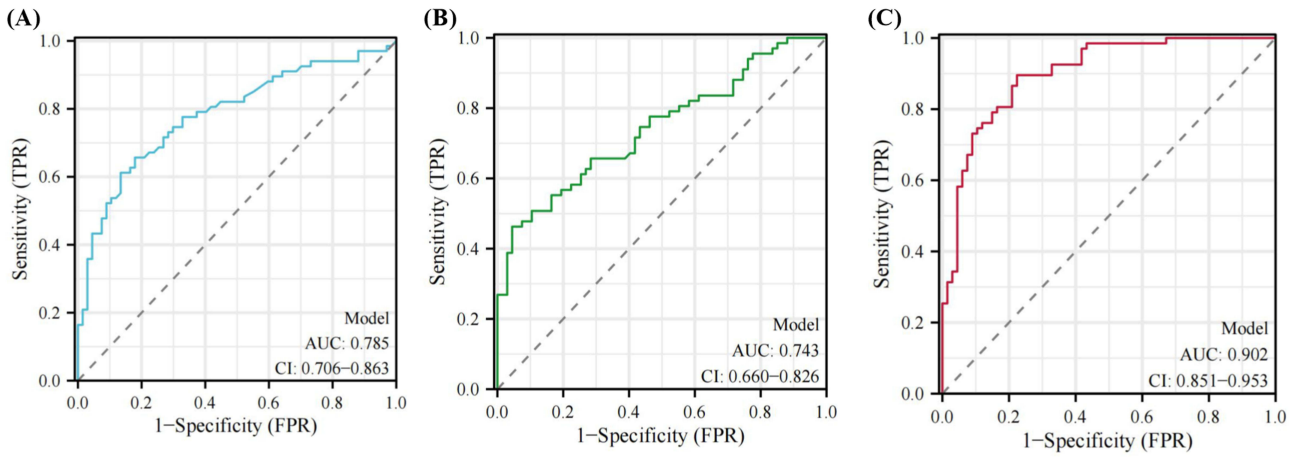


Figure 4 ROC curve characteristics of three diagnostic models constructed by binary logistic regression. **(A)** ROC curve constructed with PROM and GGT as variables; **(B)** ROC curve constructed with GP1 and GP14 as variables; **(C)** ROC curve constructed with PROM, GGT, GP1, GP13, and GP14 as combined indicators.

Model A, which was developed using PROM and GGT as combined biomarkers, was 0.785 (95% CI: 0.706–0.863). The H-L test indicated a χ^2 value of 1.840 with a *P*-value of 0.986. In contrast, Model B, utilizing GP1 and GP14 as predictors, demonstrated a lower predictive capability with an AUC of 0.743 (95% CI: 0.660–0.826), and the H-L test yielded a χ^2 value of 6.950 with a *P*-value of 0.542.The composite model C using GP1, GP13, GP14, PROM and GGT as composite indicators had an AUC of 0.902 (95% CI: 0.851–0.953), H-L test showed that χ^2 value was 9.461, *P*= 0.305, which greatly exceeded the first two models (Figure 4). The calibration curves for each model within both the training and validation cohorts were generated (Figure 5). The proximity of the model's calibration curve to the diagonal line is indicative of the model's stability and the concordance between predicted and actual results. Among the models evaluated, only Model C demonstrated superior stability and fit across both the training and validation cohorts. To evaluate the internal stability of the models, a 10-fold cross-validation method was employed. The results of this cross-validation for the validation cohort revealed that Model C achieved an R-squared value of 0.417, whereas Models A and B attained R-squared values of 0.383 and 0.206, respectively (Table S4). Among the three models evaluated, only Model C exhibited superior fit in the validation cohort.

Discussion

Globally, approximately 15 million premature infants are born annually.[24] NRDS remains the leading cause of mortality in neonates, particularly in preterm infants, and the leading cause of respiratory failure in this population.[25] It is noteworthy that numerous studies have demonstrated a correlation between alterations in IgG N-glycosylation and the onset of various pathological conditions, but its role in NRDS has not been clarified.

The underlying cause of NRDS is the deficiency of pulmonary surfactant (PS) and the presence of immature lung tissue structure.[26] The synthesis of PS is markedly affected by the presence of inflammatory mediators. Additionally, inflammatory markers hold substantial prognostic and predictive value in the context of NRDS. For instance, plateletcrit has been identified as a potential predictor for the occurrence of NRDS.[27] Elevated PCT levels are correlated with an increased risk of developing NRDS.[28] Furthermore, there is an elevated likelihood of lung infections in newborns with NRDS, especially subsequent to the administration of invasive respiratory support. IgG Glycosylation represents a pivotal post-translational modification, influencing both adaptive and innate FC-γR signaling pathways. This is achieved by modulating the structure of Fc segments, thereby activating a range of immune effects.[29] Conversely, modifications in the glycosylation of the IgG Fc segment are dependent on the variations in inflammatory processes that transpire throughout the disease's progression. Consequently, IgG glycosylation may serve both predictive and regulatory functions in the development of NRDS.

The current study demonstrated a significantly elevated level of IgG sialylation and fucosylation in the NRDS cohort compared to the control group. IgG sialylation may function as an anti-inflammatory modulator throughout all stages of

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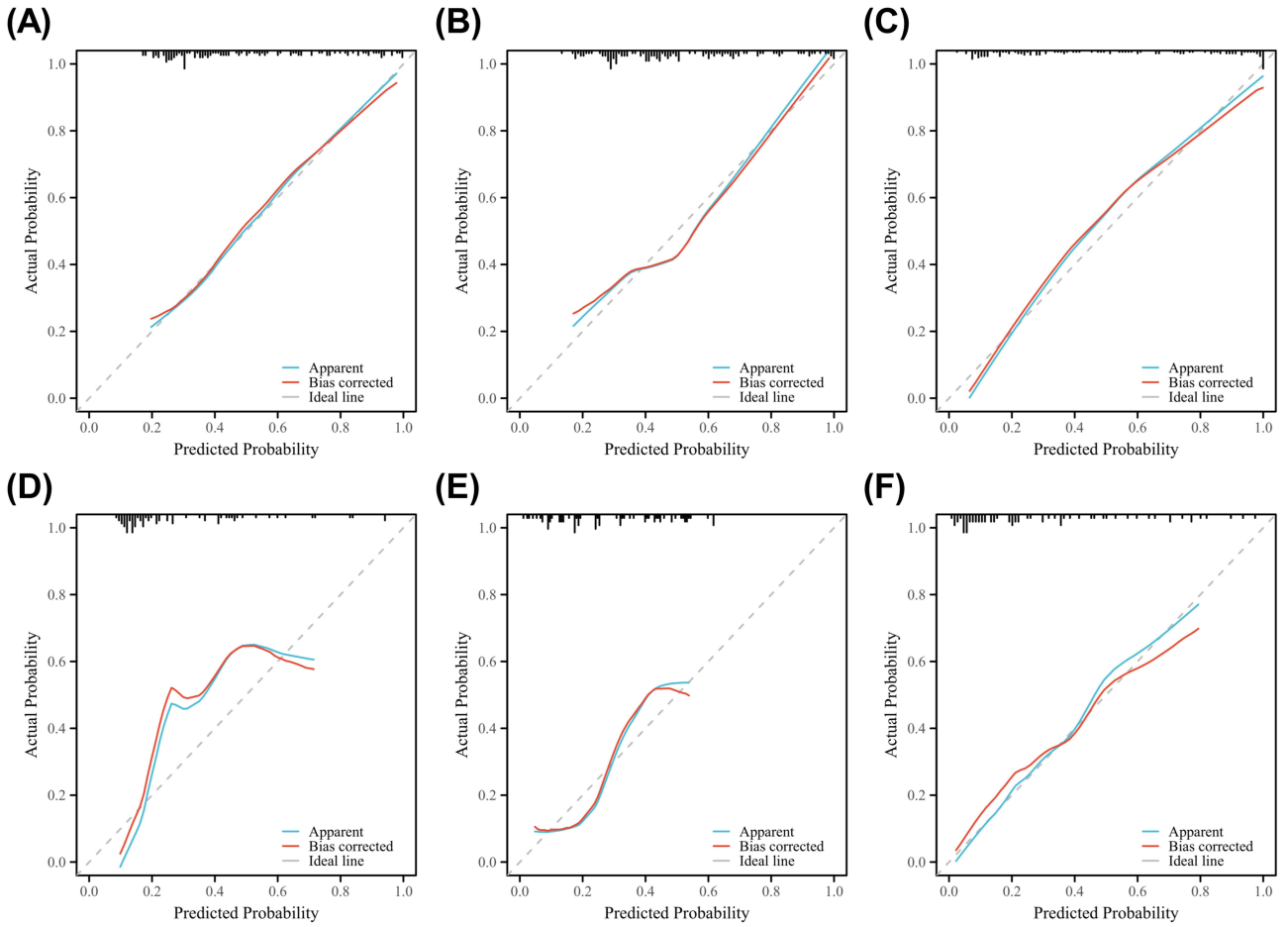

Figure 5 Calibration diagrams of prediction models. **(A-C)** Calibration curves for models A, B, and C within the training population, respectively; **(D-F)** Calibration curves for models A, B, and C within the validation population, respectively.

the inflammatory response.[23,30] Additionally, the observed increase in IgG fucosylation has the potential to markedly enhance the binding affinity between the monoclonal antibody Fc region and FcγRIIIα, thereby significantly augmenting ADCC.[23,31] Consistent with our findings, in cases of fetal or neonatal alloimmune thrombocytopenia (FNAIT), there is a tendency for IgG Fc glycosylation against platelet and red cell antigens to decrease, while modifications in galactosylation and sialylation are increased.[32] Furthermore, elevated IgG sialylation has also been observed in tumors such as renal cell carcinoma (RCC).[33] Reduced sialylation has also been documented in other pathological conditions, including pediatric meningococcal septicemia and endometrial cancer (EC).[34,35] It is postulated that these variations are predominantly linked to immune responses involved in the pathogenesis of various diseases. Analogously, the expression of core fucosylation exhibits variability across different disease states.

In contrast, the galactosylation process was observed to be diminished in neonates diagnosed with NRDS. Specifically, there was a notable reduction in the levels of agalactosylated IgG, monogalactosylated IgG, and bisgalactosylated IgG. The deficiency in galactosylation can not only impact IgG functionality but also precipitate excessive inflammatory responses, such as those mediated by Immunoglobulin A (IgA), under conditions of inflammatory signal regulation.[23,36] Similarly, variations in IgG galactosylation exhibit distinct patterns across different disease states. Elevated levels of IgG galactosylation have been observed in acute Lyme disease (LD), early thyroid cancer (ETC), and Parkinson's disease.[37–39] Conversely, a deficiency in galactosylation has been reported in cases of urinary tract infections (UTIs), severe forms of coronavirus disease 2019 (COVID-19), and Guillain-Barré syndrome.[33,40,41] These

discrepancies in outcomes may be attributed to the specific characteristics of the participants, as well as the underlying mechanisms of the respective diseases.

Interestingly, in addition to illustrating the correlation between IgG N-glycosylation and NRDS, the model constructed with GP1, GP13, GP14, GGT, and PROM, following a stepwise logistic regression screening process, demonstrated considerable classification efficacy (AUC: 0.902, 95% CI: 0.851–0.953). This study further elucidates the potential clinical significance of IgG N-glycosylation in NRDS. Within this model, we examined the relevance of GGT and PROM in relation to demographic and clinical characteristics. GGT is an enzyme widely distributed throughout the human body, playing a significant role in both secretion and absorption processes. Its primary site of activity is the hepatobiliary system; nonetheless, it is also present in the lungs.[42] Beyond its potential involvement in PS formation, GGT expression is elevated in inflammatory conditions and is associated with oxidative stress.[22] The inflammatory response is crucial in the modification of IgG N-glycosylation, suggesting a potential link between GGT and NRDS. Furthermore, GGT levels have been shown to correlate with gestational age. Importantly, the gestational age of neonates in the NRDS group was significantly lower than that of the control group. It is plausible to suggest that the inclusion of GGT in the model was informed by these considerations. However, this does not eliminate the potential impact of sample size on the findings. Similarly, pregnant women with small pregnancy-age PROM are independent risk factors for NRDS, which supports our results.[43]

While this study has elucidated the relationship between IgG-N glycosylation modification and the onset of NRDS from an uncovered perspective, thereby potentially enhancing the understanding of NRDS pathogenesis, several limitations must be acknowledged: (1) the comprehensive evaluation and analysis of neonates in the postpartum period are relatively insufficient; (2) as a case-control study, the ability to establish a strong causal relationship is limited. Furthermore, additional investigation is required to bridge the gap between laboratory testing and clinical practice. This includes the standardization of operating procedures, the validation of larger sample sizes, and the reduction of associated costs, among other considerations. Future research should involve cohort studies with larger sample sizes, as well as cell or animal experiments, to further investigate the underlying mechanisms.

In summary, alterations in IgG N-glycosylation are linked to NRDS. Specifically, the levels of IgG core fucosylation and IgG sialylation are elevated in newborns with NRDS, contributing to anti-inflammatory processes. Conversely, the modifications with agalactosylation, single galactosylation, and double galactosylation are reduced in these neonates, potentially influenced by the underlying disease mechanisms, as well as genetic and environmental factors. The models of GGT, PROM, GP1, GP13, and GP14 developed on this basis exhibited satisfactory classification efficiency, indicating potential clinical value in the prediction and prognosis of NRDS.

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Disclosure

The authors report no conflicts of interest in this work.

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