Clin. Lab. 2022;68:XXX-XXX

ORIGINAL ARTICLE

Association between Serum Erythrocyte Immune Function Indexes and Blue Light Treatment or the Severity of PJON

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SUMMARY

Background: **The association between serum erythrocyte immune function indexes and blue light treatment effect and severity in child patients with pathological jaundice was testified.**
Methods: **One hundred and seven children with pathological jaundice and 69 children with physiological jaundice were enrolled to analyze the association between erythrocyte immune function indexes and blue light treatment or disease progression.**
Results: **The area under the ROC curve (AUC) of red blood cell immune complex rosettes (RBC-ICR) and red blood cell C3b receptor rosette (RBC-C3bR) in diagnosing pathological jaundice and assessing the efficacy of blue light therapy overweighed 0.8. Meanwhile, the RBC-ICR values of the child patients were positively correlated with the severity of the disease, and the RBC-C3bR and red blood cell immune affinity receptor (FEER) values were negatively correlated with them ($p < 0.05$).**
Conclusions: **The erythrocyte immune function indexes of child patients with pathological jaundice were relevant to the disease severity, and was provided with diagnostic value for pathological jaundice or assessed value for the efficacy of blue light therapy.**
(Clin. Lab. 2022;68:xx-xx. DOI: 10.7754/Clin.Lab.2021.210941)

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Manuscript accepted October 25, 2021

KEYWORDS

pathological jaundice of newborn, erythrocyte immune function index, blue light therapy efficacy, severity of the disease

INTRODUCTION

Pathological jaundice of newborn (PJON) is a prevalent neo-natal disease, which is caused by the accumulation of bilirubin in the body, resulting in elevated blood levels and then yellowing of the skin, eye sclera, and mucous membranes, etc [1]. Although most child patients are in fairly good condition after treatment, the poison of unconjugated bilirubin is available to cause permanent damage to the nervous system [2], so early evaluation of the severity of the patient's condition and timely intervention are crucial to suppress the occurrence of irreversible brain tissue damage [3]. Blue light therapy is one of the main treatment methods for the disease, because bilirubin is more efficient at absorbing light at

wavelengths between 450 and 460 nm, and the wavelength of blue light ranges from 425 to 475 nm [4]. After blue light treatment, bilirubin in child patients is available to undergo a series of chemical reactions to generate structural isomers, which are then eliminated from the body in the urine [5]. It is now tested that erythrocyte immune function is closely relevant to the occurrence and progression of neonatal jaundice [6]. Erythrocyte is a type of blood cell, which is available to aggravate the activity of natural killer cell and eliminate circulating immune complexes, as well as augmenting phagocyte function and immune function of T lymphocytes [7,8]. Immune abnormalities are available to aggravate PJON severity and delay recovery from the disease [9]. However, the association between the erythrocyte immune function indexes in child patients and the progression of the disease and clinical treatment outcome was not clearly documented. Accordingly, the association between serum erythrocyte immune function indexes of PJON and blue light therapy or the disease severity was tested, aiming to provide a reference basis for clinical treatment and evaluation of this disease.

MATERIALS AND METHODS

Clinical data

From May 2019 to May 2021, a total of 107 children with pathological jaundice were selected as the study group, and 69 children with physiological jaundice in the same period were the control group. There was no distinct difference in general data between the two groups ($p > 0.05$), as shown in Table 1.

Inclusion criteria: ① Patients in study group met the diagnostic criteria of pathological jaundice in *Expert Consensus on the Principles of Neonatal Jaundice Treatment*; ② Families of the children were informed of the purpose of the study and the enrollment consent forms were signed; ③ Single pregnancies.

Exclusion criteria: ① Congenital deformed children; ② Neonatal hemolysis; ③ Child patients with congenital genetic metabolic diseases; ④ Child patients with ischemic-hypoxic encephalopathy; ⑤ Child patients with central nervous system infection; ⑥ Child patients with hypoglycemia; ⑦ Child patients had other abnormal immune function diseases; ⑧ Mothers of the child patient had severe immune system diseases.

Treatment method: Through the adoption of Light Emitting Diode (LED) cold light source double-sided blue light therapy machine (Ningbo David Medical Device Co., Ltd.), the naked children were placed in incubators with a small diaper to protect the perineum and a black opaque eye mask to cover both eyes. The blue light was irradiated for 12 hours within 24 hours. After 12 hours of continuous irradiation and 12 hours of no irradiation, the children were treated with bacillus subtilis bigeminy viable bacteria particles (Hanmi Pharm. Co., Ltd., Beijing, China, Batch No. S20020037).
Assessment criteria for the efficacy of blue light therapy [10]: ineffective = total serum bilirubin increased by 86 μmol/L/hour after 4 - 6 hours of phototherapy.

Classification of disease severity: Total bilirubin over 307.8 μmol/L on the 3rd day after birth was defined as the severe group, 256.5 - 307.8 μmol/L was as the moderate group, and 205.2 - 256.5 μmol/L was as the mild group in line with the relevant criteria in *Practical Neonatology*.

Erythrocyte immune function index detection:
Fasting venous blood was collected from the children prior to treatment, and centrifuged at 3,000 r/minute. Red blood cell immune complex rosettes (RBC-ICR), red blood cell C3b receptor rosette (RBC-C3bR), and red blood cell immune affinity receptor (FEER) were examined using an erythrocyte immune adhesion test and matching kit.

Observation indexes: (1) The comparison of erythrocyte immune function indexes of the study and the control groups and analysis of their diagnostic value for pathological jaundice were employed. (2) The comparison of erythrocyte immune function indexes of the effective and the ineffective groups was to analyze the predictive value of blue light treatment for PJON and its association with clinical efficacy. (3) The comparison of cellular immune function indexes of patients with different severity and analysis of the correlation between the indexes and the disease severity were adopted.

Statistical treatment: SPSS 22 software was applied to process the data, the count data were expressed as percent, and χ^2 test was used for the comparison of the differences between groups. The measurement data were expressed as ($\bar{x} \pm s$) after normal test. The *t*-test was used for the comparison of the differences between two groups, with that of the differences between multiple groups using one-way ANOVA. Multiple logistic regression was employed to analyze the association between erythrocyte immune function indexes and blue light treatment of PJON. $p < 0.05$ was accepted as indicative of distinct differences.

RESULTS

Comparison of erythrocyte immune function indexes between the study and the control groups

The RBC-ICR of the study outweighed that of the control, while the RBC-C3bR and FEER were inferior to those of the control ($p < 0.05$), as manifested in Table 2.

Table 1. Comparison of general data between study and control groups.

Classification		Study (n = 107)	Control (n = 69)	χ^2/t	p
Gestational age (weeks)		38.06 ± 1.89	38.24 ± 1.53	0.663	0.508
Gender (male or female)		65/42	41/28	0.031	0.861
Age (d)		9.68 ± 1.37	9.50 ± 1.42	0.839	0.403
Body weight (kg)		2.96 ± 0.23	2.91 ± 0.27	1.314	0.191
Serum total bilirubin (μmol/L)		253.69 ± 22.59	153.12 ± 20.31	29.979	< 0.001
Type of delivery (cases)	vaginal delivery	81	51	0.072	0.789
	cesarean delivery	26	18		
Premature babies	yes	9	3	1.090	0.296
	no	98	66		

Table 2. Comparison of erythrocyte immune function indexes between the study and the control groups.

Groups	n	RBC-ICR (%)	RBC-C3bR (%)	FEER (%)
Study	107	10.32 ± 2.11	7.97 ± 1.74	27.15 ± 3.95
Control	69	7.16 ± 1.59	12.15 ± 2.06	33.64 ± 4.17
t		10.640	14.465	10.411
p		< 0.001	< 0.001	< 0.001

Table 3. Analysis of the diagnostic value of erythrocyte immune function indexes for pathological jaundice.

Indexes	Cutoff values	AUC	SE	95% CI	Sensitivity (%)	Specificity (%)
RBC-ICR	8.90%	0.885	0.025	0.837 - 0.933	81.31	79.71
RBC-C3bR	9.71%	0.928	0.02	0.888 - 0.967	88.79	85.51
FEER	31.57%	0.817	0.032	0.754 - 0.879	79.44	71.01

Table 4. Comparison of erythrocyte immune function indexes between the effective and the ineffective groups prior to treatment.

Groups	n	RBC-ICR (%)	RBC-C3bR (%)	FEER (%)
Effective	79	9.53 ± 1.71	8.36 ± 1.94	27.69 ± 3.96
Ineffective	28	12.55 ± 1.85	6.87 ± 1.82	25.63 ± 4.28
t		7.860	3.547	2.316
p		< 0.001	< 0.001	0.023

Table 5. Analysis of the predictive value of erythrocyte immune function indexes on the efficacy of blue light therapy for PJON.

Indexes	Cutoff values	AUC	SE	95% CI	Sensitivity (%)	Specificity (%)
RBC-ICR	11.97%	0.941	0.024	0.894 - 0.988	82.14	96.20
RBC-C3bR	7.38%	0.806	0.043	0.721 - 0.891	75.00	73.42
FEER	26.80%	0.514	0.072	0.374 - 0.654	64.29	17.72

Table 6. Univariate analysis of erythrocyte immune function indexes and the efficacy of blue light therapy for PJON.

Indexes		Effective (n = 79)	Ineffective (n = 28)	χ^2	p
RBC-ICR	≥ 11.97%	25	20	13.426	< 0.001
	< 11.97%	54	8		
RBC-C3bR	≥ 7.38%	57	9	14.001	< 0.001
	< 7.38%	22	19		
FEER	≥ 26.80%	58	7	20.324	< 0.001
	< 26.80%	21	21		

Table 7. Multifactorial analysis of erythrocyte immune function indexes and the efficacy of blue light therapy for PJON.

Indexes	β	SE	wald χ^2	OR	95% CI	p
RBC-ICR	1.105	0.144	58.884	3.019	2.277 - 4.004	< 0.001
RBC-C3bR	-0.549	0.165	11.071	0.578	0.418 - 0.798	< 0.001
FEER	-0.286	0.751	0.145	0.751	0.172 - 3.274	0.704

Table 8. Comparison of erythrocyte immune function indexes in child patients with different severity.

Groups	n	RBC-ICR (%)	RBC-C3bR (%)	FEER (%)
Mild	52	9.04 ± 1.79	8.79 ± 1.58	28.69 ± 3.28
Moderate	36	10.95 ± 2.03	7.65 ± 1.43	26.83 ± 2.96
Severe	19	12.63 ± 2.36	6.33 ± 1.27	23.54 ± 3.91
F		25.608	20.477	17.231
p		< 0.001	< 0.001	< 0.001

Analysis of the diagnostic value of erythrocyte immune function indexes for pathological jaundice

The AUC of RBC-ICR, RBC-C3bR, and FEER in the diagnosis of pathological jaundice all outweighed 0.8, as shown in Table 3 and Figure 1.

Comparison of erythrocyte immune function indexes between the effective and ineffective groups prior to treatment

The RBC-ICR of the effective was inferior to that of the ineffective, and the RBC-C3bR and FEER outweighed those ($p < 0.05$), as shown in Table 4.

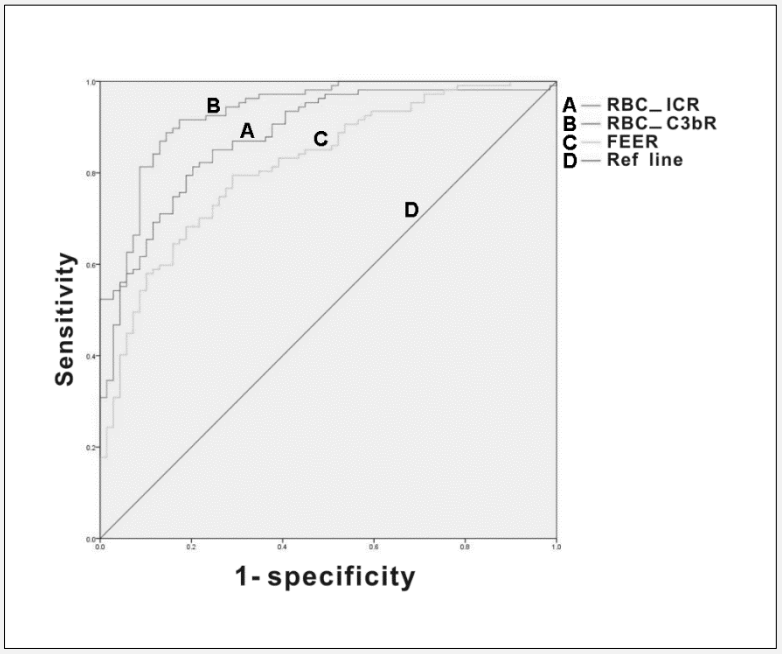

Figure 1. ROC curve analysis of erythrocyte immune function indexes in the diagnosis of pathological jaundice.

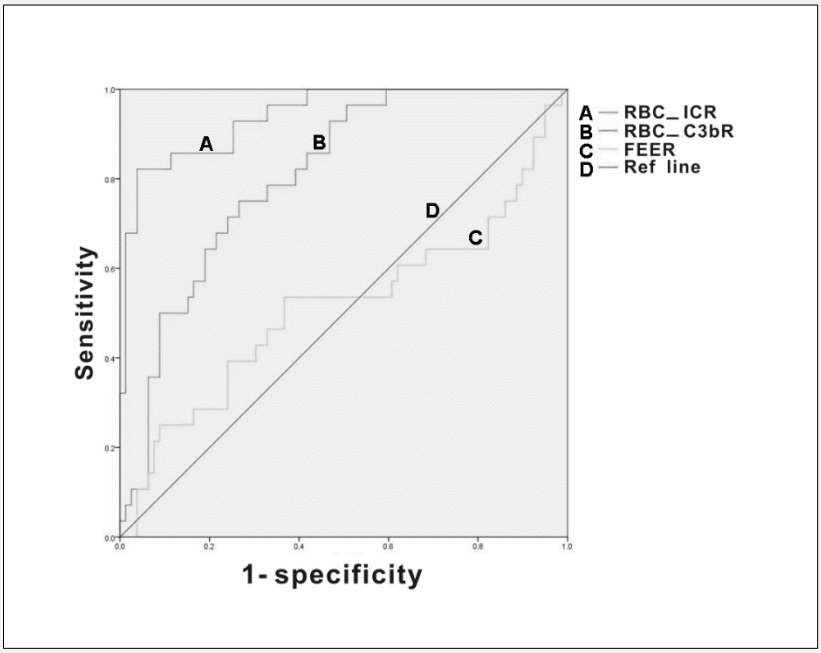

Figure 2. ROC curve analysis of erythrocyte immune function indexes is conducted to predict the efficacy of blue light treatment for PJON.

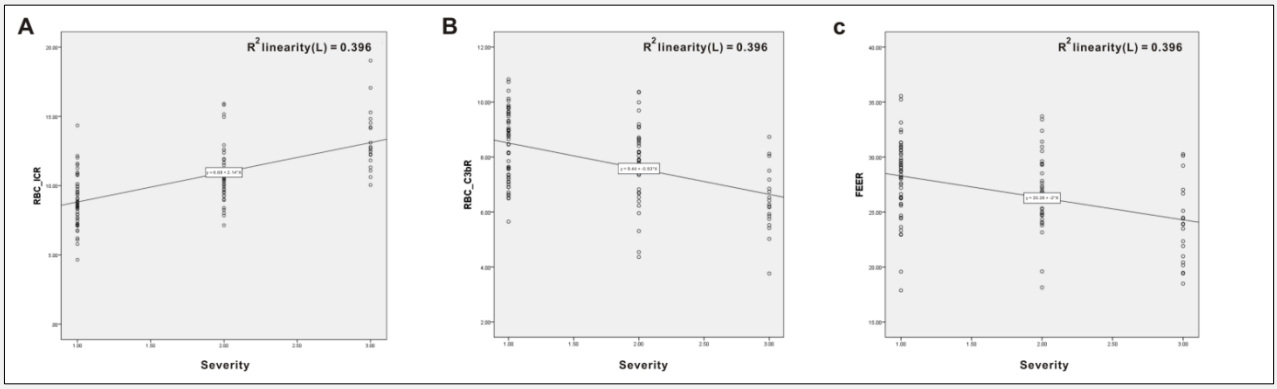

Figure 3. Fitting line analysis of the correlation between erythrocyte immune function indexes and disease severity.

A - C, fit lines of the correlation between RBC-ICR, RBC-C3bR and FEER and severity of disease separately.

Analysis of the predictive value of erythrocyte immune function indexes on the efficacy of blue light therapy for PJON

The AUC of RBC-ICR and RBC-C3bR in predicting the efficacy of blue light treatment for PJON outweighed 0.8, as presented in Table 5 and Figure 2.

Analysis of the association between erythrocyte immune function indexes and the efficacy of blue light therapy for PJON

RBC-ICR with 11.97% or more and RBC-C3bR with less than 7.38% were the risk factors affecting the efficacy of blue light therapy for PJON ($p < 0.05$), as presented in Tables 6 and 7.

Comparison of erythrocyte immune function indexes in children with different severity

The values of RBC-ICR in children with pathological jaundice tended to ascend with the severity of the disease, while the values of RBC-C3bR and FEER tended to drop ($p < 0.05$), as presented in Table 8.

Correlation analysis between erythrocyte immune function indexes and severity of the disease

The values of RBC-ICR in children with pathological jaundice were positively correlated with the disease severity, while the RBC-C3bR and FEER values were negatively correlated with them ($p < 0.05$), as presented in Figure 3.

DISCUSSION

Pathological jaundice leads to elevated bilirubin causing yellow staining of the sclera and skin. Related studies have testified that augmented erythrocytes and erythrocyte destruction are risk factors affecting the occurrence of pathological jaundice [11,12]. In contrast, it is currently documented that augmented erythrocyte destruction is available to affect erythrocyte immune function, so erythrocyte immune function is supposed to be somehow associated with the occurrence of pathological jaundice [13]. Erythrocyte immune function exerts a critical impact on the regulation of T lymphocyte and NK cell activity, anti-infection, etc., and secondly it is realized through the C3b receptors on the surface of erythrocyte membrane, which plays a critical part in the conveyance and clearance of circulating immune complexes [14].

Moreover, excessive proliferation of bone marrow erythrocytes in child patients with pathological jaundice allows the release of incompletely formed erythrocytes from the RBC-C3bR site into the blood and the augmentation in hematoma circulating complexes competitively occupies the C3b site, thus affecting erythrocyte function [15]. In this study, the RBC-ICR in the study group outweighed that in the control group, while the RBC-C3bR and FEER were inferior, further confirming the abnormal erythrocyte immune function in the pathological children, which was mainly relevant to the erythrocyte senescence caused by elevated serum bilirubin levels. Additionally, RBC-ICR, RBC-C3bR, and FEER showed diagnostic value for pathological jaundice, implying that the indexes were supposed to apply to the clinical diagnosis of pathological jaundice.

Declined erythrocyte immune adhesion in child patients with PJON may repress the removal of circulating immune complexes. Further formation of lipid peroxidation lipids via unsaturated fatty acid radicals is unavailable to be suppressed, which triggers a chain reaction leading to cell destruction. Erythrocyte damage is associated with the occurrence of pathological jaundice, so the interaction of the two mechanisms is available to create a vicious circle, further aggravating the disease severity [16]. The above studies confirmed that the abnormal erythrocyte immune function was in pathologic child patients, suggesting that the relevant indexes were also supposed to be somehow relevant to disease progression. The study documented that the RBC-ICR values of child patients with pathological jaundice were positively correlated with the disease severity, while the RBC-C3bR and FEER values were negatively correlated with it, manifesting the correlation of the erythrocyte immune function and the disease severity in the child patients, which is mainly relevant to the excess bilirubin in the blood and its metabolites that are available to repress the activity of the RBC-C3bR locus and accelerate erythrocyte senescence [17].

Blue light therapy is the main therapeutic method for PJON [18], in which bilirubin in the newborn bodies constantly rises to the skin surface and decomposes. Unconjugated bilirubin breaks down into water-soluble isomers and ultimately is excreted from the body [19]. Presently, the efficacy of treatment is mostly determined by testing variations in total serum bilirubin in child patients, but the sensitivity of a single index test is on the low side [20]. Therefore, the association between erythrocyte immune function indexes and clinical efficacy was analyzed by the authors, detecting that RBC-ICR with 11.97% or more and RBC-C3bR with less than 7.38% were risk factors affecting the efficacy of blue light therapy for PJON. It showed that abnormal erythrocyte immune function was available to delay disease recovery, but the specific mechanism is yet unknown. The authors consider that this may be relevant to immune dysfunction leading to abnormal secretion of inflammatory cytokines [21]. Additionally, the AUC of RBC-ICR and RBC-C3bR predicting the efficacy of blue light therapy for PJON outweighed 0.8, implying that the efficacy of blue light therapy in child patients was available to be assessed by testing the two.

In short, abnormalities of erythrocyte immune function were seen in child patients with pathological jaundice, and its relevant indexes correlate with pathological jaundice severity. These may support the diagnosis and prediction for pathological jaundice and the efficacy of blue light therapy.

Acknowledgment:
Not applicable.

Source of Funds:
District-level scientific research project of medical and health institutions in Longhua district of Shenzhen (Item number: 2020178).

Declaration of Interest:
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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