

CORRELATION BETWEEN EBV-DNA LOAD AND IL-6/ESR LEVELS AND CONSTRUCTION OF A PREDICTIVE MODEL FOR DISEASE ASSESSMENT IN CHILDREN WITH INFECTIOUS MONONUCLEOSIS

KORELACIJA IZMEĐU EBV-DNK OPTEREĆENJA I NIVOA IL-6/SE I IZRADA PREDIKTIVNOG MODELA ZA PROCENU BOLESTI KOD DECE SA INFEKTIVNOM MONONUKLEOZOM

Qiong Xue, Tao Ning, HanJun Yin, JingJing Yan, TianTian Lu, JiBing Qiao, SuYue Zhu

Department of Pediatrics, The Affiliated Suqian Hospital of Xuzhou Medical University
Suqian City, Jiangsu Province, 223800, China

Summary

Background: To investigate the correlation between Epstein-Barr virus deoxyribonucleic acid (EBV-DNA) load and interleukin-6 (IL-6) and erythrocyte sedimentation rate (ESR) levels in children with infectious mononucleosis (IM), and to construct a predictive model for disease assessment.

Methods: A total of 100 children with IM were enrolled and divided into a low-load group ($<40 \times 10^3$ copies/mL, $n=29$), a medium-load group ($40-60 \times 10^3$ copies/mL, $n=48$), and a high-load group ($>60 \times 10^3$ copies/mL, $n=23$) based on admission EBV-DNA levels. They were also categorised into a mild group ($n=58$) and a severe group ($n=42$) according to systemic involvement. General information, clinical symptoms and signs, and laboratory indicators – including EBV-DNA load, IL-6, ESR, C-reactive protein (CRP), and lactate dehydrogenase (LDH) – were collected. Spearman's correlation analysis was used to assess the relationship between EBV-DNA load and IL-6 and ESR. Restricted cubic spline (RCS) analysis was applied to evaluate the dose-response relationship between these parameters and the risk of severe disease. Variables with $P < 0.05$ in the univariate analysis were included in a multivariate logistic regression model to identify independent predictors of severe IM. A nomogram prediction model was developed and validated using receiver operating characteristic curves, the Hosmer-Lemeshow (H-L) test, and decision curve analysis (DCA).

Results: The incidence of severe disease significantly differed among the EBV-DNA load groups: 24.14 (7/29) in the low-load group, 33.33 (16/48) in the medium-load group, and 82.61 (19/23) in the high-load group, show-

Kratak sadržaj

Uvod: Cilj je bio da se ispita korelacija između opterećenja Epstein-Barrovim virusom deoksiribonukleinske kiseline (EBV-DNK) i nivoa interleukina-6 (IL-6) i sedimentacije eritrocita (SE) kod dece sa infektivnom mononukleozom (IM), i konstruisanje prediktivnog modela za procenu težine bolesti.

Metode: U istraživanje je uključeno ukupno 100 dece sa IM i podeljeno u grupu sa niskim opterećenjem ($<40 \times 10^3$ kopija/mL, $n=29$), srednjim opterećenjem ($40-60 \times 10^3$ kopija/mL, $n=48$) i visokim opterećenjem ($>60 \times 10^3$ kopija/mL, $n=23$) na osnovu početnog nivoa EBV-DNK. Takođe su kategorizovani na grupu sa blagom ($n=58$) i teškom kliničkom slikom ($n=42$) u zavisnosti od sistemskog zahvatanja. Prikupljeni su opšti podaci, klinički simptomi i znaci, kao i laboratorijski parametri – uključujući opterećenje EBV-DNK, IL-6, SE, C-reaktivni protein (CRP) i laktat dehidrogenazu (LDH). Za procenu odnosa između opterećenja EBV-DNK i IL-6 i SE korišćena je Spirmanova korelaciona analiza. Za procenu odnosa doza-odgovor između ovih parametara i rizika od razvoja teške bolesti primenjena je analiza pomoću restriktivnog kubnog modela (RCS). Varijable sa $P < 0,05$ u univarijantnoj analizi uključene su u multivarijantnu logističku regresiju kako bi se identifikovali nezavisni prediktori teškog oblika IM. Razvijen je nomogram prediktivni model i validiran pomoću ROC krivih, Hosmer-Lemeshow (H-L) testa i analize krive odlučivanja (DCA).

Rezultati: Učestalost teške bolesti značajno se razlikovala između grupa prema nivou EBV-DNK: 24,14 (7/29) u grupi sa niskim opterećenjem, 33,33 (16/48) u grupi sa srednjim opterećenjem i 82,61 (19/23) u grupi sa visokim

Address for correspondence:

SuYue Zhu
Department of Pediatrics, The Affiliated Suqian
Hospital of Xuzhou Medical University
No. 138 Huanghe South Road, Suqian City,
Jiangsu Province, 223800, China
e-mail: zsyzy7878@163.com

ing an increasing risk with higher viral load. The severe group had significantly higher rates of hepatomegaly, splenomegaly, and fever duration >7 days ($P=0.015$, $P=0.044$, and $P=0.009$, respectively), as well as significantly elevated levels of CRP, LDH, atypical lymphocyte ratio, IL-6, ESR, and EBV-DNA load (all $P<0.05$). Significant positive correlations were observed between EBV-DNA load and both IL-6 ($r=0.619$, $P<0.05$) and ESR ($r=0.524$, $P<0.05$). RCS analysis demonstrated significant positive dose-response relationships between EBV-DNA load, IL-6, and ESR levels and the risk of severe IM (all $P<0.05$). Multivariate logistic regression identified fever duration >7 days, EBV-DNA load, IL-6, and LDH as independent predictors of severe IM ($P<0.05$), while ESR and atypical lymphocyte ratio were marginally significant ($P=0.06$ and $P=0.100$, respectively). The nomogram prediction model exhibited an area under the curve (AUC) of 0.93 (95% confidence interval [CI]: 0.87–0.98) in the training set and 0.82 (95% CI: 0.66–0.98) in the validation set. The H-L test indicated good calibration in both sets ($P=0.870$ and $P=0.594$, respectively), and DCA demonstrated favourable clinical utility.

Conclusions: EBV-DNA load is positively correlated with IL-6 and ESR levels, and all three are associated with the risk of severe IM. The predictive model based on fever duration, EBV-DNA load, IL-6, ESR, LDH, and atypical lymphocyte ratio effectively assesses the risk of severe disease in children with IM. This approach not only confirms the diagnostic value of biochemical and laboratory indicators but also provides an objective, quantitative basis for precise clinical diagnosis and treatment.

Keywords: pediatric infectious mononucleosis, EBV-DNA load, interleukin-6, erythrocyte sedimentation rate, disease assessment

Introduction

Infectious mononucleosis (IM) is an abnormal lymphoproliferative disease primarily caused by Epstein-Barr virus (EBV) infection (1). It predominantly affects children aged 4–6 years and is clinically characterised by fever, pharyngitis, lymphadenopathy, hepatosplenomegaly, and an increased number of atypical lymphocytes in peripheral blood (2, 3). Due to its atypical and non-specific manifestations, IM is often misdiagnosed as a respiratory infection. The clinical course can be complex and variable, with severe cases potentially life-threatening (4). Although the disease is generally self-limiting with a favourable prognosis, its diverse clinical presentations and multi-system involvement make early diagnosis challenging. Missed or delayed diagnosis may lead to secondary malignant diseases or serious complications (5). Accurate assessment of disease severity is essential for developing appropriate treatment strategies, improving prognosis, and enhancing the quality of life in affected children. However, there is currently a lack of simple, effective, and highly specific methods for evaluating IM severity, which hinders precise treatment and effective management. Therefore, identifying biochemical and laboratory indicators that objectively reflect the patholog-

opterećenjem, pokazujući porast rizika sa povećanjem virusnog opterećenja. Grupa sa teškom kliničkom slikom imala je značajno višu učestalost hepatomegalije, splenomegalije i trajanja febriliteta >7 dana ($P=0.015$, $P=0.044$ i $P=0.009$, respektivno), kao i značajno povećane vrednosti CRP-a, LDH, procenta atipičnih limfocita, IL-6, SE i opterećenja EBV-DNK (sve $P<0.05$). Utvrđene su značajne pozitivne korelacije između opterećenja EBV-DNK i IL-6 ($r=0.619$, $P<0.05$) i SE ($r=0.524$, $P<0.05$). RCS analiza je pokazala značajne pozitivne odnose doze i odgovora između opterećenja EBV-DNK, nivoa IL-6 i SE i rizika od teškog oblika IM (sve $P<0.05$). Multivarijantna logistička regresija identifikovala je trajanje febriliteta >7 dana, opterećenje EBV-DNK, IL-6 i LDH kao nezavisne prediktore teške IM ($P<0.05$), dok su SE i procenat atipičnih limfocita bili granično značajni ($P=0.06$ i $P=0.100$, respektivno). Nomogram model predviđanja pokazao je AUC od 0,93 (95% CI: 0,87–0,98) u trening skupu i 0,82 (95% CI: 0,66–0,98) u validacionom skupu. H-L test je ukazao na dobro slaganje modela u oba skupa ($P=0.870$ i $P=0.594$, respektivno), dok je DCA potvrdila povoljnu kliničku prikladnost.

Zaključak: Opterećenje EBV-DNK pozitivno korelira sa nivoima IL-6 i SE, a sva tri parametra su povezana sa rizikom od teške infektivne mononukleoze. Prediktivni model zasnovan na trajanju febriliteta, opterećenju EBV-DNK, IL-6, SE, LDH i procentu atipičnih limfocita efikasno procenjuje rizik od razvoja teške bolesti kod dece sa IM. Ovaj pristup ne samo da potvrđuje dijagnostičku vrednost bihemijskih i laboratorijskih parametara već pruža objektivnu, kvantitativnu osnovu za preciznu kliničku dijagnostiku i terapiju.

Ključne reči: infektivna mononukleoza u pedijatriji, opterećenje EBV-DNK, interleukin-6, sedimentacija eritrocita, procena težine bolesti

ical process of IM and constructing a scientific disease prediction system have become research hotspots in pediatric infectious diseases.

In the pathogenesis of IM, EBV infection triggers a series of complex immune responses. Epstein-Barr virus deoxyribonucleic acid (EBV-DNA) load, a key indicator of viral activity, is closely associated with disease progression and severity (4, 6). Elevated EBV-DNA levels reflect active viral replication, which may lead to excessive immune activation, inflammatory responses, and tissue damage. Although some theoretical and empirical evidence support a relationship between EBV infection and clinical features, several studies have reported that the correlation between blood EBV-DNA load and clinical manifestations in children with IM remains uncertain (7, 8). Meanwhile, interleukin-6 (IL-6) plays a critical role in infection-induced cytokine storms (9). As an important inflammatory factor, IL-6 is involved in the development and progression of various inflammatory diseases. EBV infection activates immune responses and induces IL-6 release, contributing to related clinical symptoms (10). The erythrocyte sedimentation rate (ESR), a classical inflammatory marker, reflects the overall activity of systemic inflammation and is widely used in

evaluating infectious diseases. In healthy individuals, ESR remains within a narrow range, but it increases rapidly during infections (11). Nevertheless, studies on the correlation between EBV-DNA load and IL-6 or ESR levels in children with IM remain limited. The dose-response relationship between these markers and the risk of severe IM has not been fully elucidated, and there is a lack of systematic research on predictive models based on these and other key indicators.

In light of this, this study aims to thoroughly investigate the correlation between EBV-DNA load and IL-6 and ESR levels in children with IM. Through comprehensive analysis of these indicators, independent factors closely associated with disease severity will be identified, and a nomogram-based prediction model using core biochemical and laboratory indicators will be constructed. The study aims to assess the diagnostic value of biochemical indicators in the pathologic process of IM, providing an objective, quantitative biochemical basis for accurate clinical assessment of disease severity in affected children.

Materials and Methods

Study population

This prospective single-centre study was conducted after approval from the Medical Ethics Committee (Approval No. 2023050). A standardised enrollment screening form was developed, and healthcare personnel were trained to ensure standard implementation. A total of 100 children with IM admitted to the hospital between January 2024 and June 2024 were enrolled as study subjects. The diagnostic criteria were based on the Expert Consensus on the Diagnosis and Treatment Principles of EBV Infection-Related Diseases in Children (2021). Clinical criteria included: (1) presence of fever; (2) cervical lymphadenopathy; (3) pharyngotonsillitis; (4) hepatomegaly; (5) eyelid oedema; (6) splenomegaly. Laboratory criteria included: (1) positive anti-EBV viral capsid antigen (VCA)-IgM and anti-EBV-VCA-IgG antibodies with negative anti-EBV nuclear antigen (EBNA)-IgG; (2) a fourfold or greater increase in anti-EBV-VCA-IgG antibody titers in paired serum samples; (3) positive anti-EBV-VCA-IgG antibodies with low affinity and negative anti-EBV-VCA-IgM; (4) a proportion of atypical lymphocytes in peripheral blood $>10\%$; or a lymphocyte proportion $>50\%$ or an absolute lymphocyte count $>5.0 \times 10^9/L$ in children over 6 years old. A diagnosis of IM was established if the patient met any three of the six clinical criteria and one of the laboratory criteria.

Inclusion criteria: (1) Children who met the above diagnostic criteria for IM. (2) Children aged under 18 years. (3) Children experiencing their first episode and who had not received systematic treatment. Exclusion criteria: (1) Children who did not

undergo quantitative blood EBV-DNA PCR testing or whose test results were negative. (2) Children co-infected with other viral pathogens, such as *Mycoplasma pneumoniae* or cytomegalovirus. (3) Children with organ dysfunction, abnormal growth and development, or congenital malformations. (4) Children with a history of immunomodulator or corticosteroid use within the past 6 months. (5) Children with autoimmune deficiencies or haematological diseases. (6) Children with malignant tumours. (7) Children with unclear examination results or missing clinical data. Additionally, 50 children undergoing health examinations during the same period were selected as the control group. The Ethics Committee of the Affiliated Suqian Hospital of Xuzhou Medical University approved this study. All subjects and their guardians provided informed consent and voluntarily participated in the study.

Methods

Baseline demographic characteristics (age, gender) and clinical data, including symptoms and signs (such as pharyngitis, cervical lymphadenopathy, peak fever temperature, hepatomegaly, splenomegaly, and duration of fever), were collected for all participants. Fasting venous blood samples (3 mL each) were obtained from every child on the second morning following admission. Of these, 3 mL was collected in an EDTA tube for ESR and EBV-DNA testing. Another 3 mL was collected in a red-capped vacuum tube and centrifuged at 3000 r/min for 10 minutes to separate the serum for IL-6 measurement. ESR was measured using a fully automated ESR analyser (model LBV-XC40; automated Westergren method; Beijing Precil Instrument Co., Ltd.). IL-6 levels were measured utilising enzyme-linked immunosorbent assay kits (kt34251, Merck Biological Co., Ltd.) in strict accordance with the manufacturer's instructions. Absorbance values were measured with a fully automated microplate reader (Thermo Fisher Scientific, USA). Quantitative real-time polymerase chain reaction (Applied Biosystems 7500, USA) was used to detect EBV-DNA load in peripheral whole blood. EBV-DNA loads were categorised as follows: low ($<40 \times 10^3$ copies/mL), moderate ($40\text{--}60 \times 10^3$ copies/mL), or high ($>60 \times 10^3$ copies/mL). Additionally, levels of Immunoglobulin A (IgA), Immunoglobulin G (IgG), and Immunoglobulin M (IgM) were measured using a fully automated protein analyser (Beckman IMMAGE 800). An automated biochemical analyser (Hitachi 7600) was used to assess Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and lactate dehydrogenase levels. For assessment of atypical lymphocyte ratio, blood smears were prepared and stained with Wright-Giemsa. A laboratory technician counted 100 white blood cells under an oil immersion microscope to determine the percentage of atypical lymphocytes.

There is currently no universally accepted definition or diagnostic criteria for severe IM. Severity is generally assessed based on the extent of clinical manifestations and the presence of complications. In this study, patients were classified into mild and severe groups based on the number of organs or systems involved. Patients in the mild group had IM with involvement of at most one organ/system, while those in the severe group had IM with involvement of two or more organs/systems (12).

Statistical analysis

Statistical analysis was performed using SPSS software (version 25.0). Normality tests were conducted for all measurement data. Data conforming to a normal distribution are summarised as mean $\pm$ standard deviation, with group comparisons performed using the independent-samples *t*-test. Non-normally distributed data are summarised as median (interquartile range: P25, P75) and analysed using the Mann-Whitney U test. Categorical variables are reported as counts and percentages [*n* (%)], and group differences were analysed using the chi-square test. For correlation analysis, normality tests were performed on the data. Pearson correlation analysis was used for normally distributed continuous data, while Spearman's rank correlation was applied for non-normally distributed continuous data. The relationship between EBV-DNA load, IL-6, ESR levels, and the risk of severe IM was evaluated using restricted cubic splines (RCS) in R (version 4.0.5). The dataset was randomly split into a training set (70 %) and a validation set (30 %). In the training set, the outcome variable was IM disease severity (mild or severe). Factors with a significance level of $P < 0.05$ in univariate analysis were included as independent variables. A backwards-stepwise multivariate logistic regression was used to identify influencing factors and to construct a predictive model. A nomogram was developed in R. Predictive accuracy was evaluated by calculating the area under the receiver operating characteristic curve (AUC) in both training and validation sets. Model calibration was assessed using the Hosmer–Lemeshow test (H-L test), implemented on the online statistical platform www.medsta.cn/software. A decision curve analysis (DCA) was conducted to assess the model's clinical utility. A P -value < 0.05 was considered statistically significant.

Results

Disease status and clinical characteristics in children

This study enrolled 100 pediatric patients who met the inclusion criteria. EBV-DNA levels were measured in all subjects at admission. Among them, 29 cases (29.00 %) had a low EBV-DNA load ($< 40 \times 10^3$

copies/mL), 48 cases (48.00 %) had a moderate load ($40\text{--}60 \times 10^3$ copies/mL), and 23 cases (23.00 %) had a high load ($> 60 \times 10^3$ copies/mL). In the low EBV-DNA load group, 7 cases were severe; in the moderate load group, 16 cases were severe; and in the high load group, 19 cases were severe. According to the extent of systemic involvement, the children were categorised into a mild group ($n=58$) and a severe group ($n=42$). As shown in Table 1, no statistically significant differences were observed between the two groups regarding age or gender distribution ($P=0.303$ and $P=0.538$, respectively), indicating balanced baseline demographic characteristics. Regarding clinical symptoms and signs, the incidence of pharyngitis, cervical lymphadenopathy, and peak fever temperature did not differ significantly (all $P > 0.05$). However, the severe group demonstrated a significantly higher incidence of hepatomegaly (61.90 vs. 36.21 %) and splenomegaly (57.14 vs. 36.21 %), and a higher proportion of children with a duration of fever exceeding 7 days (59.52 vs. 32.76 %) than the mild group. These differences were statistically significant ($P=0.015$, $P=0.044$, and $P=0.009$, respectively). In terms of laboratory indicators, there were no significant differences in immunoglobulins (IgA, IgG, IgM), liver function markers (AST, ALT, Total Bilirubin), or white blood cell count between the two groups (all $P > 0.05$). In contrast, the severe group showed significantly higher levels of C-reactive protein (CRP), lactate dehydrogenase (LDH), and atypical lymphocyte ratio compared to the mild group ($P=0.003$, $P=0.004$, and $P=0.004$, respectively). Notably, the levels of IL-6, ESR, and EBV-DNA load – which were the core focus of this study – were all significantly higher in the severe group than in the mild group, with all differences being statistically significant (all $P < 0.001$). These findings suggest that IL-6, ESR, and EBV-DNA load are closely associated with the severity of IM in children.

Levels of IL-6 and ESR and their correlation with EBV-DNA load in children with IM

Spearman analysis revealed that peripheral blood levels of IL-6 ($r_s=0.619$, $P < 0.05$) and ESR ($r_s=0.524$, $P < 0.05$) were positively correlated with EBV-DNA load in children with IM (Figure 1). To investigate the dose-response relationship between the continuous exposure variables (IL-6 and ESR) and severe IM, RCS analysis was performed. After adjusting for age and gender as confounding factors, the analysis demonstrated a positive dose-response relationship between EBV-DNA load, IL-6, and ESR levels and the risk of severe IM (all $P < 0.05$), as shown in Figure 2. When EBV-DNA load exceeded 47.085×10^3 copies/mL, IL-6 exceeded 10.01 pg/mL, and ESR exceeded 29.185 mm/h, the risk of severe IM progressively increased with rising levels. However, no significant nonlinear association was observed (P for nonlinearity > 0.05), suggesting a potential linear

Table I Comparison of general characteristics of children with IM.

Characteristics	Severe Group (n=42)	Mild Group (n=58)	P-value
Age (years)	5 (4, 7)	6 (4, 7)	0.303
Gender			0.538
Male	23 (54.76)	36 (62.07)	
Female	19 (45.24)	22 (37.93)	
Clinical Symptoms			
Herpangina	31 (73.81)	39 (67.24)	0.515
Cervical Lymphadenopathy	32 (76.19)	41 (70.69)	0.650
Hepatomegaly	26 (61.90)	21 (36.21)	0.015
Splenomegaly	24 (57.14)	21 (36.21)	0.044
Duration of Fever			0.009
≤7 days	17 (40.48)	39 (67.24)	
>7 days	25 (59.52)	19 (32.76)	
Peak Body Temperature (°C)	39.14±0.61	39.05±0.68	0.499
Laboratory Parameters			
IgA (g/L)	2.47 (1.58, 2.72)	2.09 (1.35, 2.48)	0.086
IgG (g/L)	12.80±3.09	12.13±2.32	0.222
IgM (g/L)	1.95±0.90	1.74±0.80	0.201
AST (U/L)	57.43±21.34	52.76±20.47	0.271
ALT (U/L)	71.18±29.21	63.25±24.76	0.146
TBIL (μmol/L)	7.68±0.85	7.42±0.81	0.124
WBC (×10 ³ /L)	12.65 (11.47, 14.34)	11.94 (9.93, 14.43)	0.178
CRP (mg/L)	12.69±2.21	11.48±1.67	0.003
LDH (U/L)	483.69 (398.48, 572.79)	440.71 (341.93, 516.88)	0.004
IL-6 (pg/mL)	11.77±3.13	9.05±3.15	<0.001
ESR (mm/h)	33.03 (27.85, 38.64)	25.43 (19.32, 31.39)	<0.001
EBV-DNA Load (×10 ³ copies/mL)	53.94 (43.13, 74.29)	44.29 (32.07, 50.35)	<0.001
Atypical Lymphocyte Ratio ()	23.53±3.12	21.67±2.88	0.004

relationship between EBV-DNA load, IL-6, and ESR levels and the risk of severe IM.

Construction of a clinical prediction model for severe IM

To identify independent factors associated with severe IM, variables that were statistically significant in the baseline data were further analysed using logistic regression. Variables with $P<0.05$ in univariate analysis were included in a backward stepwise multi-

variate logistic regression model, with IM severity as the dependent variable (mild=0, severe=1). Age and gender were included as adjustment variables, while seven independent variables – duration of fever, EBV-DNA load, IL-6, ESR, CRP, LDH, and atypical lymphocyte ratio – were analysed as continuous variables. All variables passed collinearity diagnostics, confirming the absence of multicollinearity.

As shown in *Table II*, fever duration >7 days, EBV-DNA load, IL-6, and LDH were identified as independent risk factors for severe IM ($P<0.05$).

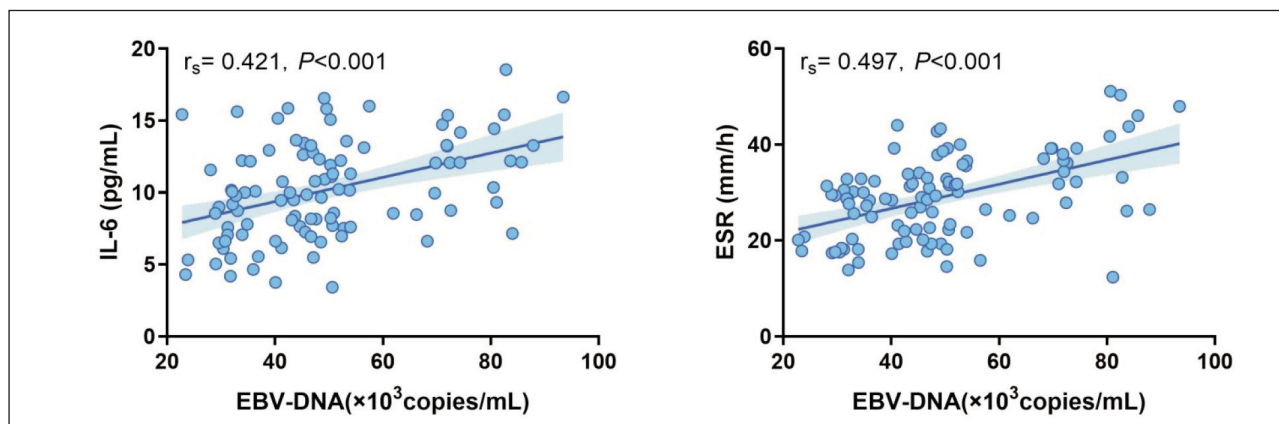


Figure 1 Spearman correlation analysis of IL-6 and ESR levels with EBV-DNA load in children with IM.

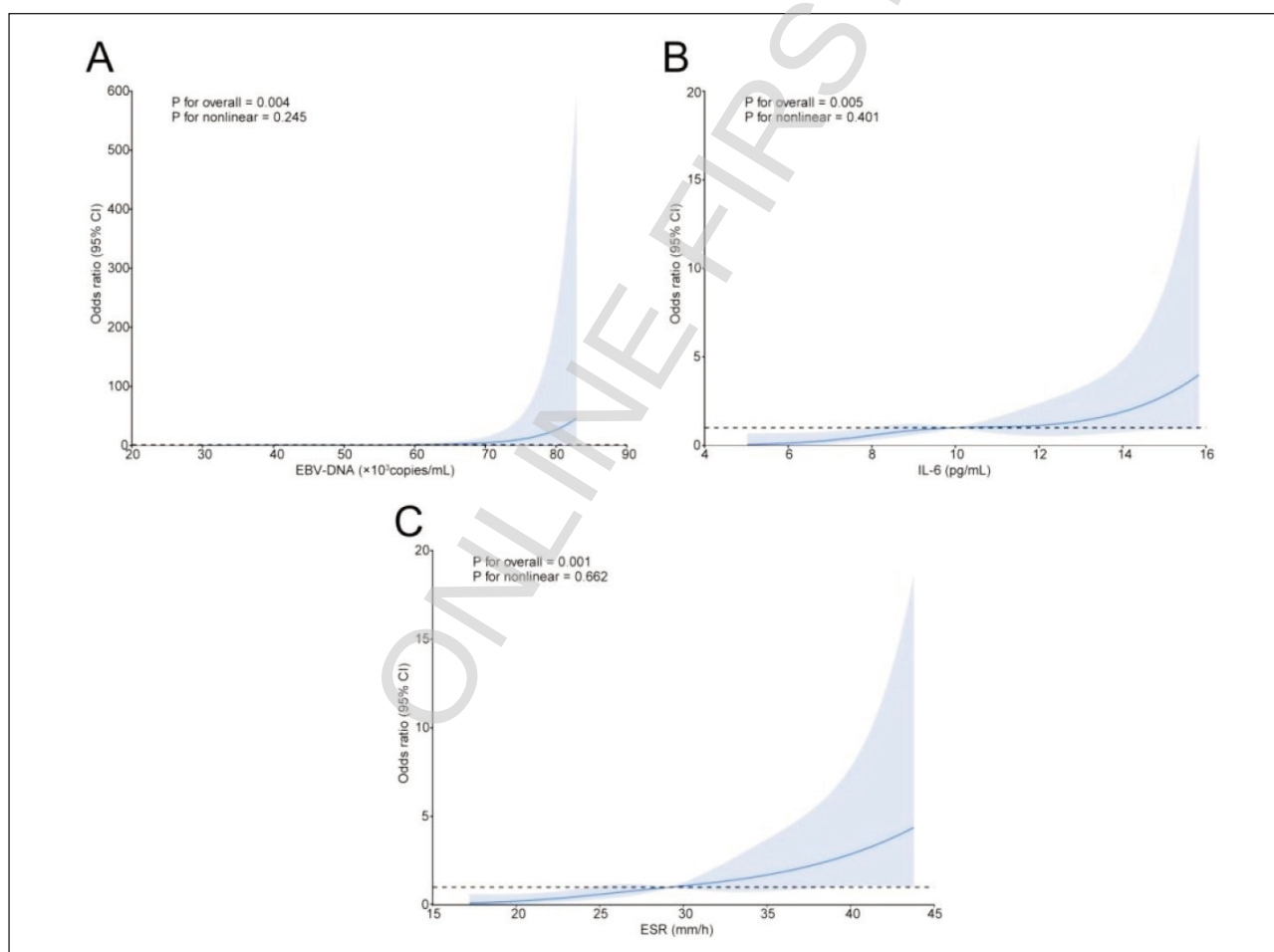


Figure 2 RCS plots of the relationship between EBV-DNA load, IL-6, ESR levels and severe IM after adjusting for age and gender.

(A) EBV-DNA load; (B) IL-6; (C) ESR. P for overall, overall association; P for nonlinear, nonlinear association. P for overall <0.05 and P for nonlinear >0.05 indicate a linear exposure-response relationship. The shaded areas represent the 95% confidence intervals.

Although ESR did not show a statistically significant association ($P=0.06$), an odds ratio (OR) of 1.11 suggested a trend toward increased risk with marginal significance. Atypical lymphocyte ratio was identified

as a potential influencing factor (OR=1.24, 95% confidence interval [CI]: 0.96–1.61, $P=0.100$).

Based on the backward stepwise logistic regression results, duration of fever, EBV-DNA load, IL-6,

Table II Factors associated with severe IM in children identified by backward stepwise logistic regression analysis.

Variables	Univariate Analysis					Multivariate Analysis				
	β	S.E	Z	P	OR (95 CI)	β	S.E	Z	P	OR (95 CI)
Age	-0.19	0.13	-1.49	0.136	0.82 (0.64~1.06)					
Gender										
0					1.00 (Reference)					
1	0.05	0.50	0.10	0.921	1.05 (0.40~2.79)					
Hepatomegaly										
0					1.00 (Reference)					
1	0.88	0.50	1.74	0.082	2.40 (0.90~6.43)					
Splenomegaly										
0					1.00 (Reference)					
1	0.77	0.50	1.56	0.120	2.17 (0.82~5.74)					
Fever Duration (days)										
0					1.00 (Reference)					1.00 (Reference)
1	1.39	0.52	2.69	0.007	4.02 (1.46~1.05)	2.42	0.94	2.58	0.010	11.22 (1.78~70.55)
EBV-DNA	0.07	0.02	3.52	<.001	1.07 (1.03~1.12)	0.07	0.03	2.08	0.038	1.08 (1.01~1.15)
IL6	0.28	0.09	3.23	0.001	1.32 (1.12~1.57)	0.36	0.16	2.26	0.024	1.43 (1.05~1.95)
ESR	0.15	0.04	3.76	<.001	1.16 (1.08~1.26)	0.10	0.05	1.88	0.060	1.11 (1.00~1.23)
CRP	0.30	0.14	2.23	0.026	1.35 (1.04~1.77)					
LDH	0.01	0.00	2.64	0.008	1.01 (1.01~1.01)	0.01	0.00	2.07	0.038	1.01 (1.01~1.02)
ALY	0.26	0.09	2.87	0.004	1.30 (1.09~1.56)	0.22	0.13	1.64	0.100	1.24 (0.96~1.61)

Note: OR, odds ratio; CI, confidence interval; Ref, reference; S.E., standard error; EBV-DNA, Epstein-Barr virus DNA load; IL-6, interleukin-6; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; LDH, lactate dehydrogenase; ALY, atypical lymphocyte percentage.

ESR, LDH, and atypical lymphocyte ratio were incorporated into the prediction model, and a nomogram was constructed (Figure 3A). In the nomogram, the length of each variable's line segment reflects its relative contribution to predicting severe IM progression. The scale on the horizontal axis for each variable aligns with the »Points« scale at the top, indicating the score for specific values of that variable. For a given patient, the scores for each of the six risk factors are determined based on admission data, summed to yield »Total Points,« and the corresponding »Risk« value indicates the predicted probability of progression to severe IM.

The predictive model was further validated and evaluated using ROC curve analysis, the H-L test, and DCA. ROC curve analysis showed an AUC of 0.93 (95 CI: 0.87–0.98) for the training set and 0.82 (95 CI: 0.66–0.98) for the validation set, indicating good discriminatory ability in both cohorts. Calibration performance, assessed using the H-L test, produced $\chi^2=3.853$ ($P=0.870$) for the training set and $\chi^2=6.474$ ($P=0.594$) for the validation set, suggesting good calibration in both sets.

DCA was employed to evaluate the clinical utility of the prediction model. The threshold probability (P)

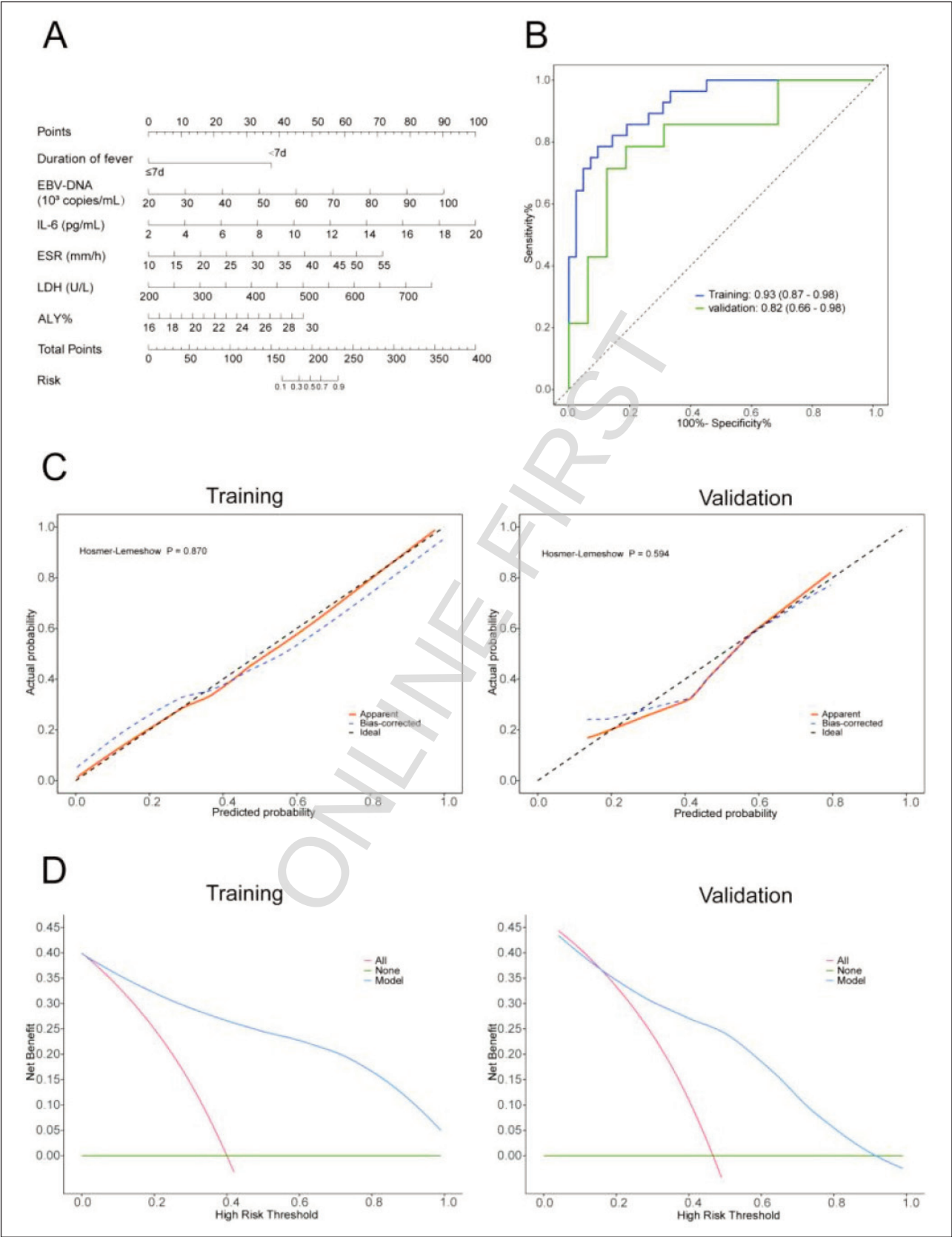


Figure 3 Clinical prediction model for severe IM.

(A) Nomogram for predicting disease severity in children with IM; (B) ROC curve of the prediction model for IM severity; (C) Calibration curve of the prediction model for IM severity; (D) Decision curve of the prediction model for IM severity.

ranges from 0 to 1, where $P=0$ indicates no treatment benefit for any patient and $P=1$ indicates benefit for all patients. The net benefit, represented on the y-axis, reflects the difference between the predicted and true probabilities. As shown in Figure 3, the blue curve represents the prediction model. In the training set, most of the curve lies above $y=0$, indicating a positive clinical net benefit. In the validation set, the model demonstrated a satisfactory net benefit was observed within the threshold probability range of approximately 0.2 to 0.9.

Discussion

IM, a common pediatric disease triggered by primary EBV infection, is characterised by significant clinical heterogeneity, which poses persistent challenges for early identification of severe cases in clinical practice (13). Studies indicate that delayed treatment in children with IM can lead to varying degrees of damage to the respiratory, digestive, neurological, and cardiac systems. More severe cases are associated with a higher incidence of complications, resulting in dysregulated immune responses, induction of specific antibodies, exacerbated organ damage, and, in critical cases, death (14). EBV, a member of the human gammaherpesvirus family, survives readily in alkaline environments and persists in the host for extended periods. During latent infection, EBV primarily resides in B lymphocytes without expressing nuclear proteins, thereby remaining non-pathogenic. However, during the differentiation of memory B lymphocytes into plasma cells, viral particles are released, which in turn activate cytotoxic T cells and induce substantial secretion of inflammatory mediators. This cascade disrupts the immune-inflammatory balance, facilitating the transition of EBV from latent to lytic replication and thereby exacerbating IM progression (15, 16).

EBV exhibits a strong replicative capacity, and EBV-DNA detection is one of the primary methods for diagnosing IM. It reflects the viral load in infected children and plays a crucial role in evaluating clinical efficacy and disease progression (4). Regarding the relationship between EBV-DNA load and disease severity, as the causative pathogen of IM, EBV's replication activity directly influences disease progression (17). Although this study does not directly demonstrate this outcome, based on relevant theories and previous research, it can be hypothesised that an elevated EBV-DNA load indicates active viral replication, which may trigger more intense immune responses and cellular damage. The virus can directly infect lymphocytes, leading to their proliferation and activation, as well as the release of various cytokines and inflammatory mediators. This exacerbates systemic inflammation and tissue injury, driving the disease toward greater severity. These findings align with previous studies reporting positive correlations between EBV-

DNA load and IM severity, further emphasising the importance of monitoring EBV-DNA load in assessing IM progression.

IL-6, a key pro-inflammatory cytokine, plays a critical role in the pathogenesis of IM. Previous studies suggest that inflammatory responses and immune system imbalance may be significant contributors to poor prognosis in IM (18). Under EBV stimulation, immune cells such as monocytes and macrophages are activated, leading to increased IL-6 secretion (19). IL-6 not only promotes the infiltration and activation of inflammatory cells, exacerbating local tissue inflammation, but also disrupts immune regulatory functions, resulting in immune imbalance (20). Consistent with the study by Zhang et al. (21), the present study found that severe cases showed elevated IL-6 levels compared to mild cases, and a positive correlation was observed between EBV-DNA load and IL-6 levels in children with IM ($P<0.001$) (22).

ESR, a traditional inflammatory marker, though relatively non-specific, remains helpful in assessing IM severity. During inflammatory processes, elevated levels of acute-phase proteins, such as fibrinogen and immunoglobulins, reduce repulsive forces between red blood cells, accelerating their sedimentation rate and elevating the ESR (23, 24). This study identified a positive correlation between EBV-DNA load and ESR levels in children with IM ($P<0.001$). In the study by Prtorić et al., ESR was identified as one of the most accurate predictors of hospitalisation duration (25). Although various factors can influence ESR elevation, its dynamic changes – when other confounding factors are excluded – can assist clinicians in evaluating disease progression and treatment response. For instance, persistently high or rising ESR during treatment may indicate inadequate disease control or the development of complications, necessitating further investigation and adjustment of therapeutic strategies.

Active EBV infection can cause severe damage to multiple organs, systems, and immune function. The diverse and complex symptoms in children often lead to high rates of missed or misdiagnosis, highlighting the need for an effective and convenient method to improve early diagnosis of IM, which is particularly important for primary care physicians. The nomogram prediction model constructed in this study, based on duration of fever, EBV-DNA load, IL-6, ESR, LDH, and atypical lymphocyte ratio, demonstrates good discriminatory ability (training set AUC=0.93, validation set AUC=0.82), calibration (H-L test $P>0.05$), and clinical net benefit, highlighting the advantages of multi-indicator joint prediction. The duration of fever, as an important indicator of disease persistence, is closely associated with disease severity (26, 27). Prolonged fever may lead to increased physical exhaustion, reduced immunity, and a higher risk of complications. EBV-DNA load, IL-6, and ESR reflect viral activity, inflammatory intensity,

and cellular damage from different perspectives. While existing studies are often limited to correlation analyses of single or a few indicators, this study developed a multi-dimensional integrated model that combines these indicators, overcoming the limitations of single-marker prediction. This approach allows for a more comprehensive and accurate assessment of disease severity in children with IM, providing a scientific basis for clinical decision-making.

However, several limitations should be considered. First, due to its single-centre design and relatively restricted sample size, the findings may be subject to intrinsic selection bias, and the external validity of the results warrants further confirmation. Future multi-centre, large-sample studies are needed to expand the scope and improve the reliability and representativeness of the findings. The limited sample size may also pose a potential risk of overfitting; meanwhile, the lack of multi-centre external validation means the model's generalizability across different healthcare settings and populations has not been confirmed, and its clinical application requires further verification. Second, this study focused solely on the relationship between EBV-DNA load, IL-6, ESR, and disease severity, without accounting for other potential influencing factors, such as genetic background, nutritional status, and environmental factors. Future research should incorporate multiple factors to refine the disease assessment system further. Additionally, the predictive model developed in this study requires further validation and optimisation in broader clinical practice to enhance its clinical applicability.

Conclusion

In summary, EBV-DNA load shows significant correlations with IL-6 and ESR levels in children with IM. The disease-assessment prediction model, developed using these clinical biochemical indicators, demonstrates promising potential for clinical application. These findings not only confirm the central role of biochemical indicators in the pathological process of IM but also highlight the value of biochemical techniques in the precise assessment of pediatric infectious diseases. Future studies should expand the sample size, incorporate multiple factors, and further refine the disease assessment system to provide more

accurate guidance for the clinical diagnosis and treatment of IM.

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Availability of data and materials

The datasets used and/or analysed during the present study are available from the corresponding author on reasonable request.

Ethics approval

The present study was approved by the Ethics Committee of The Affiliated Suqian Hospital of Xuzhou Medical University (No. 2023050), and written informed consent was obtained from all patients' parents or guardians before the study start. All procedures were performed in accordance with the ethical standards of the Institutional Review Board and the Declaration of Helsinki, and its later amendments or comparable ethical standards.

Author's contribution

Qiong Xue and Tao Ning designed the research study. Qiong Xue and Tao Ning performed the research. HanJun Yin, JingJing Yan and TianTian Lu provided help and advice. HanJun Yin, JiBing Qiao and SuYue Zhu analysed the data. Qiong Xue and Tao Ning wrote the manuscript. SuYue Zhu reviewed and edited the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Conflict of interest statement

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

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