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The relationship between hematological parameters and infectious mononucleosis in children

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Abstract

Objective: To evaluate the association between hematological parameters and (EBV) in infants and to help with early warning, identification, and intervention in the development of infection.

Material and methods: This is a prospective and observational study of all infants admitted to the pediatric emergency department with cervical lymphadenopathy and fever from January 2021 to December 2022. Multivariate analysis was conducted to evaluate the independence of the association between the hematological parameters and the risk of EBV infection.

Results: A total of 89 children with fever and lymphadenopathy were enrolled during the study period. While EBV-VCA IgG value was negative in 52 children, EBV-VCA IgM was negative in 47 children. The optimal cut-off value for NLR was ≤ 0.9, with 54.76% sensitivity and 76.6% specificity. The area under the ROC curve for NLR was 0.66. The optimal cut-off value for ALT was 30 mg/dL, with 47.6% sensitivity and 89.3% specificity. The area under the ROC curve for ALT was 0.54. The optimal cut-off value for AST was greater than 31 mg/dL, with 57.1% sensitivity and 68.0% specificity. The area under the ROC curve for AST was 0.64. The optimal cut-off value for lymphocyte was >5.16, with 50% sensitivity and 87.2% specificity. The area under the ROC curve for lymphocyte was 0.67.

Conclusion: New inflammatory markers, NLR, MPV, and PDW, have the advantages of being simple, economical, and rapid. They also have a certain sensitivity and specificity for predicting the occurrence of EBV, and have important clinical application value.

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Introduction

Cervical lymphadenopathy is a remarkably common condition in children, with prevalence rates varying by age group (1). Studies estimate its occurrence ranges from 62% in infants aged 3 weeks to 6 months, 41% in children aged 2 to 5 years, and up to 90% in those aged 4 to 8 years (1-3). Viral infections are the leading cause of cervical lymphadenopathy in the pediatric population, with lymph node enlargement typically resolving as the underlying infection subsides. Common viruses such as rhinovirus, adenovirus, influenza, parainfluenza, and respiratory syncytial virus often result in self-limiting lymphadenopathy that requires minimal intervention (4-6). However, certain viral infections, including Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human immunodeficiency virus (HIV), may present with lymphadenopathy in acute (within 3 weeks), subacute (3–6 weeks), or chronic (beyond 6 weeks) phases. Among these, EBV, the causative agent of infectious mononucleosis, warrants special attention in children presenting with cervical lymphadenopathy and fever. Accurate differential diagnosis is crucial to guide appropriate management (6-8).

This study aims to explore the predictive value of hematological parameters in diagnosing EBV infection in children presenting with cervical lymphadenopathy and fever.

Materials and methods

Research subjects

This prospective and observational study was conducted at the Memorial Hospital Department of Pediatrics between January 2021 to December 2022. Children aged 9-12 years with fever and cervical lymphadenopathy were included in the study. On the basis of an ultrasonographic examination (US) incorporating Power Doppler utilizing a 7.5 MHz transducer on a Siemens Sonoline Elegra device, all patients were radiologically identified. Node morphology assessment included evaluation of nodal form by assessing the long to short axis ratio (L/S), US hilus, homogeneity of echo-texture, transparency of borders, and intranodal vascularity. Final diagnoses were made based on clinical or histological findings, laboratory tests, and US analysis.

The exclusion criteria were: (i) 3 years of age or older upon admission; (ii) congenital anomalies or genetic metabolic abnormalities; (iii) congenital immune disorders, (iv) bacterial infections, (v) cancer disorders and (vi) allergic problems.

Definition of diagnosis

A diagnosis of infectious mononucleosis is made with a compatible clinical picture, typical blood picture and positive heterophile antibody test or a positivity of specific antibody test against EBV antigens.

Data collection

Child's age, sex, physical examination findings, rash after medication and antibiotic use were all obtained from the hospital's electronic medical records system. The Neutrophil-Lymphocyte Ratio (NLR) is simply the number of neutrophils divided by the number of lymphocytes. Blood tests were conducted using routine samples taken from the children's veins within 24 hours of hospital admission with cervical lymphadenopathy and fever. Whole blood testing was collected within the first 24 hours of hospital admission. AST, ALT values and hemogram parameters (White Blood Cell, Lymphocyte count, Red Cell Distribution Width (RDW), Mean Platelet Value (MPV), Platelet Distribution Width (PDW), Neutrophil count, Lymphocyte count, and PLT count) were also noted.

Laboratory analysis

EBV-VCA IgM (Epstein-Barr Virus Viral Capsid Antigen Immunoglobulin M) is a type of antibody produced by the immune system in response to the Epstein-Barr virus (EBV). This antibody is typically detected in the early stage of an EBV infection and usually disappears after a few months (9). The presence of EBV-VCA IgM antibodies in the blood indicates an acute or recent infection with EBV. While the presence of EBV-VCA IgM antibodies suggests an active infection, it is not a definitive diagnosis. Further testing, including a blood test for EBV-VCA IgG antibodies and a monospot test, may be needed to confirm diagnosis. EBV-VCA IgM <0.80 mg/dL was accepted as negative.

EBV-VCA IgG (Epstein-Barr Virus Viral Capsid Antigen Immunoglobulin G) is an antibody that is produced by the immune system in response to the EBV. The presence of EBV-VCA IgG antibodies indicates a past or current infection with the EBV, which is considered the causative agent of IM (9,10). However, it is important to note that the presence of IgG antibodies alone is not sufficient for the diagnosis of IM, as they

may persist for years after the initial infection. EBV-VCA IgG <16 mg/dL was accepted as negative.

EBV-EBNA IgG is a blood test that measures the number of antibodies in the blood against the EBV nuclear antigen. After infection with EBV, the immune system develops antibodies against the virus. These antibodies can remain in the blood for the person's lifetime, indicating a past infection with EBV. The EBV-EBNA IgG test is commonly used to diagnose and monitor EBV infections, as well as to distinguish between an acute or past infection. EBV-EBNA IgG <0.1 mg/dL was accepted as negative.

Statistical analysis

SPSS 26.0 statistical software was applied for data analysis. Statistical data were expressed as cases (%), and the $\chi 2$ test or Fisher exact test was used for comparison between groups; normally distributed measures were expressed as mean and standard deviation, and the t-test for two independent samples was used for comparison between groups; non-normally distributed measures were expressed as median (Q1, Q3), and the Kruskal-Wallis rank sum test was used for comparison between groups. In the multi-factor logistic regression model, the risk factors with important significance in the univariate analysis were selected as covariates. The receiver operator characteristic curve (ROC) was used to analyze the predictive value of laboratory markers. Differences were considered statistically significant at $p<0.05$.

Results

A total of 89 children with fever and lymphadenopathy enrolled during the study period. 66.3% of the children included in the study were male. The mean

Table 1: General characteristics of the children

	n (%)
Gender	
Female	30 (33.7)
Male	59 (66.3)
EBV-VCA IGG	
Normal	52 (58.4)
Abnormal	37 (41.6)
EBV-VCA IGM	
Normal	47 (52.8)
Abnormal	42 (47.2)
EBV-EBNA IGG	
Normal	62 (69.7)
Abnormal	27 (30.3)
Hepatomegaly	
Negative	23 (54.8)
Positive	19 (45.2)
Splenomegaly	
Negative	27 (64.3)
Positive	15 (35.7)
Rash	
Negative	40 (95.2)
Positive	2 (4.8)
Usage of Antibiotic	
Negative	10 (23.8)
Positive	32 (76.2)
Lymphadenopathy	
Negative	0 (0)
Positive	42 (100)

n: number, %: percent

age of the study group was determined as 9.04 years. While hepatomegaly was present in 45% of cases, splenomegaly was present in 35.7%. The rate of antibiotic use was 76.2%. The basic clinical characteristics

Table 2: Evaluation of characteristics according to EBV-EBNA IgG status

	EBV-EBNA IGG		p
	Normal (n = 62)	Abnormal (n = 27)	
Gender (F)*	22 (35.5)	8 (29.6)	0.635 [c]
Age (month)	105.34 (51.65)	117.15 (35.99)	0.229 [t]
Age (year)	8.76 (4.31)	9.7 (2.97)	0.241 [t]
AST (mg/dL)	31.5 (17 / 276)	27 (12 / 127)	0.262 [u]
ALT (mg/dL)	16 (7 / 458)	19 (7 / 149)	0.286 [u]
CRP (mg/dL)	15.18 (0.2 / 163)	9.3 (0.23 / 104.55)	0.406 [u]
WBC	10.94 (3.54 / 27.98)	10.67 (4.93 / 21.39)	0.608 [u]
RDW	14.05 (11.9 / 18.9)	14.7 (12.4 / 18.2)	0.336 [u]
MPV	8.8 (7.4 / 11.9)	8.9 (7.9 / 10.9)	0.931 [u]
PDW	9.9 (8 / 16.7)	10 (8.5 / 13.5)	0.942 [u]
Neutrophile count	5.07 (0.76 / 21.38)	3.75 (0.1 / 11.4)	0.120 [u]
Lymphocyte count	3.52 (0.62 / 14.15)	4.6 (1.07 / 14.38)	0.198 [u]
N/L Ratio	1.39 (0.11 / 22.9)	0.88 (0.02 / 6.57)	0.070 [u]
Sedimentation	11 (3 / 94)	12 (4 / 62)	0.803 [u]
Hepatomegaly*	11 (36.7)	8 (66.7)	0.098 [c]
Splenomegaly*	9 (30)	6 (50)	0.292 [f]
Rash*	1 (3.3)	1 (8.3)	0.495 [f]
Usage of Antibiotic*	22 (73.3)	10 (83.3)	0.696 [f]
Lymphadenopathy*	30 (100)	12 (100)	-

[c] Pearson Chi Square Test (Monte Carlo), [t] Independent Samples T Test (Boostrap), [u] Mann Whithey U Test (Monte Carlo), [f] Fisher Exact Test (Monte Carlo), AST: Aspartate Aminotransferase, ALT: Alanin Aminotransferase, CRP: C-Reactive protein, WBC: White blood cell, RDW: Red cell distribution width, MPV: Mean platelet volüme, N: neutrophile, L: Lymphocyte, * variables were given as n(%). Other variables were given as mean (SD) or median (min-max).

of the children in the study group are summarized in Table 1. While the EBV-VCA IgG value was negative in 52 children, EBV-VCA IgM was negative in 47 children. MPV, PDW values were found to be significantly higher in cases where EBV-VCA IgG was positive (p=0.031 and p=0.026, respectively). In cases where EBV-VCA IgM was positive, AST, ALT, neutrophil count and the NLR were found to be significantly higher. The evaluation according to EBV-EBNA IgG is given in Table 2, and no difference was observed between the groups. The analysis is given in Table 2. In the evaluation of physical examination findings and laboratory values, it was observed that hepatomegaly and splenomegaly were more common in cases where EBV-VCA-IgM was positive (Table 3).

The optimal cut-off value for NLR was ≤ 0.9, with 54.76% sensitivity and 76.6% specificity and the area under the ROC curve for NLR was 0.66. The optimal cut-off value for ALT was 30 mg/dL, with 47.6% sensitivity and 89.3% specificity and the area under the ROC curve for ALT was 0.54. The optimal cut-off value for AST was >31 mg/dL, with 57.1% sensitivity and 68.0% specificity and the area under the ROC curve for PMI was 0.64. The optimal cut-off value for lymphocyte was >5.16, with 50% sensitivity and 87.2% specificity and the area under the ROC curve for lymphocyte was 0.67. Detailed analysis results are shown in Table 4. with sensitivity and specificity curve data in Figure 1.

Discussion

Lymphadenopathy is a common condition in childhood where the lymph nodes are enlarged. Lymph nodes are small bean-shaped structures that are part of the lymphatic system. Their function is to filter lymphatic fluid, which contains immune cells, pathogens, and other waste products. Lymphadenopathy is very

common in childhood, accounting for about 40% even among healthy children in the pediatric population (11). Considering the fact that all pediatricians and even family physicians encounter lymphadenopathy in their daily practice, the importance of the clinical approach and differential diagnosis presents. Since lymphadenopathy is a common abnormal finding that preoccupies a great deal of physicians in our daily practice, patients and physicians alike have varying degrees of anxiety about the subject. Therefore, rapid diagnosis is very important, especially in cases with fever. Viral infections in particular play a major role in the differential diagnosis (12). Typical manifestations of EBV are fever, pharyngitis, lymphadenopathy, fatigue, and atypical lymphocytosis. Splenomegaly occurs in 50-60% of cases with IM, although spleen rupture is rare (13). Hepatomegaly is observed in 10-35% in EM and more than 65% of cases have elevated transaminases (14). Although it is mostly benign, complications can be seen. As a matter of fact, our study aimed to investigate EBV infection with simple laboratory methods in those cases, who applied with fever and cervical lymphadenopathy. In our study, hepatomegaly was observed in 45% and splenomegaly in 35.7% of cases. In cases where EBV-VCA-IgM was positive, the incidence of hepatomegaly and splenomegaly was also high. Similarly, elevated transaminase levels were also detected. Since the most common finding was lymphocytosis, laboratory parameters were examined and a significant increase in NLR and neutrophil counts was observed in cases where EBV-VCA-IgM was positive. Niedzielska et al (15) conducted a study in which 87 children with cervical LAP were evaluated. Mononucleosis was detected in only 2 children. However, in our study, EBV-VCA-IgM was positive in 45 children. EBV-VCA-IgG was positive in 37 children. In another trial from Türkiye, researchers sought to determine the importance of demographic, clinical, and laboratory variables in the prediction of malignancy as well as the origin of cervical lymphadenopathies in children (16). They detected only EBV infection in 6.9% of them and children's cervical lymphadenopathies were often benign, but early biopsy should be explored for individuals with persistent cervical lymphadenopathy, for those who are in their teen years, and those with associated systemic symptoms, and abnormal laboratory results.

Table 3: Evaluation of characteristics according to EBV-VCA IgM status

	EBV-VCA IGM	p
	Median (min / max)	
Hepatomegaly		**0.001**
Negative	1.85 (0.83 / 90)	
Positive	3.94 (1.16 / 181)	
Splenomegaly		**0.004**
Negative	1.90 (0.83 / 90.00)	
Positive	3.67 (1.40 / 181.00)	
Rash		0.340
Negative	2.67 (0.83 / 181)	
Positive	5.77 (3.14 / 8.4)	
Usage of Antibiotic		0.370
Negative	2.29 (0.9 / 7.95)	
Positive	3.28 (0.83 / 181)	
Lymphadenopathy		-
Negative	-	
Positive	2.75 (0.83 / 181)	

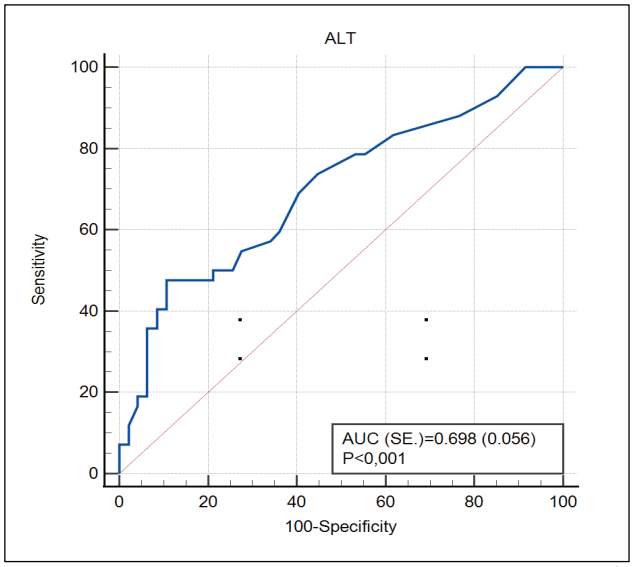
Young infants and toddlers typically have primary EBV infections, which are frequently asymptomatic. Several manifestations, including otitis media, diarrhea, stomach problems, upper respiratory infection, and IM, have been seen when symptoms do arise (17,18). From a population of 200 children, 32 children under the age of four were diagnosed with IM in one series after blood smear analysis (more than 50% of cells mononuclear and more than 10% atypical lymphocytes) (18). Significant cervical adenopathy and tonsillar pharyngitis were among the clinical symptoms present in the majority of these kids, and respiratory problems were commonly present, especially in newborn babies. Only 27% of children aged 10 to 24 in this group tested positive for heterophile antibodies, whereas 60% of babies tested positive for the antiviral capsid antigen (VCA) IgM (18). EBV-specific serologic testing is often needed to confirm the diagnosis, since heterophile antibody tests are more likely to be negative in newborns and

Table 4: ROC analysis of laboratory markers

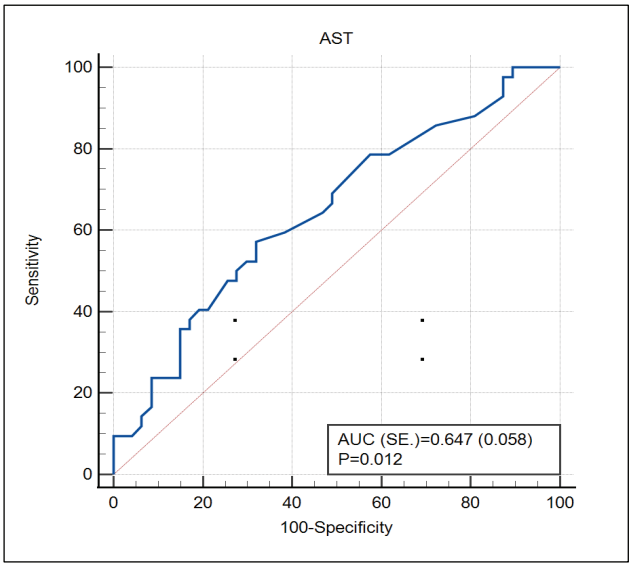
Reference	Cut off	Sensitivity	Specificity	+PV	-PV	AUC ± SE.	P
AST (mg/dL)	> 31	57%	68%	62%	64%	0.647 ± 0.058	**0.012**
ALT (mg/dL)	>30	47%	89%	80%	66%	0.549 ± 0.064	**<0.001**
Lymphocyte count	> 5.16	50%	87%	78%	66%	0.675 ± 0.058	**0.003**
N/L ratio	≤ 0.9	54%	76%	68%	66%	0.665 ± 0.059	**0.005**

ROC (Receiver Operating Curve) Analysis (Honley&Mc Nell - Youden index J), AUC: Area under the ROC curve, SE: Standard Error, +PV: Positive Predictive Value, -PV: Negative Predictive Value, N: neutrophile, L: Lymphocyte

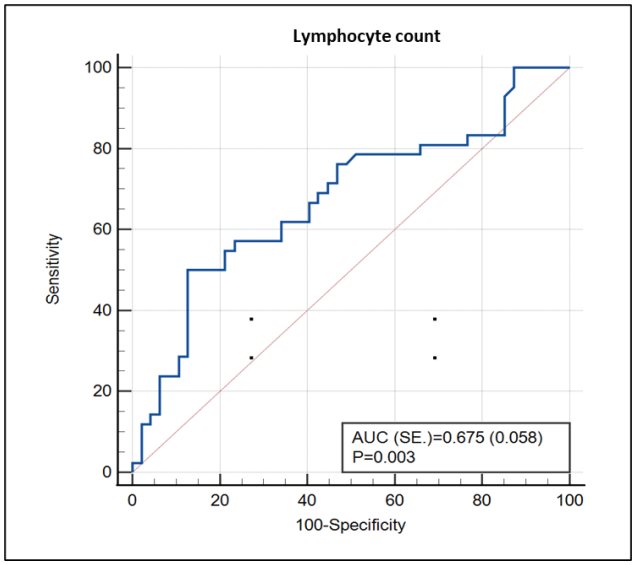
young children than in older children. Although our study group was older than these patients, it consisted of patients with cervical adenopathy and high fever. EBV-specific serologic testing was done and positively detected in 42 children. Prompt serological testing is important when EBV is suspected. AST, ALT, neutrophil count and NLR in particular were found to be significantly higher when EBV-VCA IgM was positive. In another trial conducted by Li et al (9), the researchers looked to investigate the usefulness of two laboratory tests in the diagnosis of infectious mononucleosis (EBV-IM) linked with the Epstein-Barr virus in children.



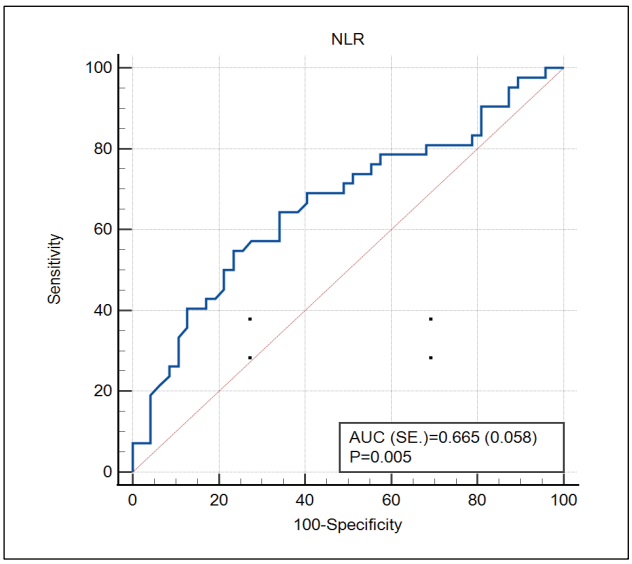
a. ALT levels



b. AST levels



c. Lymphocyte count



d. NLR ratio

Figure 1: ROC analysis of laboratory markers [a) ALT, b) AST, c) Lymphocyte, d) Neutrophile/Lymphocyte ratio]

In that study, 166 individuals with EBV-IM were enrolled from January 2018 to December 2020. Low-affinity EBV-CA IgG and EBV-CA IgM had the best diagnostic effectiveness when EBV antibodies were detected using the immunofluorescence approach, and their AUC values were 0.798 (0.751-0.840) and 0.663 (0.609-0.713), respectively (9).

Generally, inflammation is usually accompanied by relative changes in the absolute values of circulating peripheral blood leucocyte subpopulations such as neutrophils and lymphocytes. In recent years, leukocyte and their subpopulation counts have been used as markers of the degree of inflammation in several diseases such as acute appendicitis, allergic rhinitis, chronic obstructive pulmonary disease, and acute pulmonary embolism (19,20). Neutrophils are important cells in the immune defense system and regulate the functions of mast cells, epithelial cells, and macro cells (21). The NLR is a marker of inflammation and is used in conjunction with other inflammatory markers to determine the severity of the body's condition in some inflammatory diseases. NLR was significantly higher in patients with EBV-VCA-IgM positive cases. In our research, we found that the NLR in the EBV-VCA-IgM positive cases was higher than that in the negative cases. We speculated that the NLR had the capacity to predict EBV infection in the early period in cases with fever and lymphadenopathy.

Strengths and limitations

The study, however, has some limitations. This trial was a single-center observational study, it was challenging to ensure the consistency of the clinical data because patients were not all treated by the same physicians. Last but not least, this study only included Turkish people, which limits the data available to assess the impact of ethnic diversity. If the level of evidence for the relationship between the NLR, and IM is to be improved, future multi-center, large-sample studies will need to be conducted to further validate its association with IM.

Conclusions

In conclusion, as new inflammatory markers, NLR, MPV, and PDW, have the following advantages: they are simple, economical, and rapid; they exhibit a necessary degree of sensitivity and specificity for predicting the occurrence of EBV; and they have important clinical application value.

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Data availability: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Contributions

Research concept and design: HO, MAK

Data analysis and interpretation: HO, MAK

Collection and/or assembly of data: HO, MAK

Writing the article: HO, MAK

Critical revision of the article: HO, MAK

Final approval of the article: HO, MAK

All authors read and approved the final version of the manuscript.

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