

Serum eosinophilic cationic protein level and hematological parameters in infants with cow's milk protein allergy

Erkan Dogan, Eylem Sevinc

Abstract

Background Various biomarkers have been investigated in the diagnosis of cow's milk protein allergy (CMPA) in infants. To our knowledge, no prior studies have evaluated serum eosinophil cationic protein (sECP), neutrophil-lymphocyte ratio (NLR), and mean platelet volume (MPV) concurrently in infants with CMPA.

Objective To compare sECP levels, MPV, and NLR in infants with and without CMPA, as well as to investigate the suitability of these parameters as biomarkers in the diagnosis of CMPA.

Methods Fifty-six children with CMPA were compared to 40 healthy, similar to distribution of age and sex normal infants as controls. The serum ECP levels were detected by a chemiluminescence assay. The MPV values were calculated by devices in hemogram parameters. The NLR values were obtained by dividing the neutrophil count by the lymphocyte count.

Results The median sECP level in the CMPA group was significantly higher than in the control group (23.5 and 9.27 ng/mL, respectively; $P=0.001$). However, there were no significant differences between groups with regards to median MPV (8.5 and 8.6 fL, respectively; $P=0.149$) and median NLR (0.35 and 0.37 respectively; $P=0.637$). Correlation analysis of sECP level with MPV and NLR in the CMPA group revealed no significant relationships ($P>0.05$ for both). In the Receiver-operating characteristic (ROC) curve analysis, the optimal cut-off levels to identify CMPA for sECP, MPV, and NLR were 18.4 ng/mL (60.7% sensitivity, 97.5% specificity, and AUC: 0.831), 10.05 fL (54% sensitivity, 77.5% specificity, and AUC: 0.413) and 0.97 (14.3% sensitivity, 50% specificity, and AUC: 0.528), respectively.

Conclusions The sECP level and blood eosinophil count are significantly higher in infants with CMPA, but MPV and NLR do not differ between infants with and without CMPA. There are also no significant correlations in the CMPA group between

sECP and MPV, as well as sECP and NLR. Serum ECP might be useful as a potential biomarker for diagnosing CMPA. [Paediatr Indones. 2019;59:119-24; doi: <http://dx.doi.org/10.14238/pi59.3.2019.119-24>].

Keywords: neutrophil/lymphocyte ratio; mean platelet volume; eosinophilic cationic protein; biomarker; cow's milk protein allergy

Cow's milk protein allergy (CMPA) is the most common cause of food allergies in infancy and is characterized by an inflammatory reaction to milk proteins.¹ Although the incidence of CMPA has increased worldwide, CMPA pathogenesis is not entirely clear. T regulatory cells, antigen-specific T cells, and some mediators secreted by T and B lymphocytes, play roles in CMPA pathogenesis.² Although detailed medical

From the Department of Pediatrics, University of Karabuk, Karabuk, Turkey.

Corresponding author: Eylem Sevinc, Karabuk University Faculty of Medicine, Balıkklar Kayası Mevkii, Demir Çelik Campus Karabuk, Turkey. Phone: +905392958610; Email: dr.eylemsevinc@gmail.com.

Submitted February 26, 2018. Accepted April 29, 2019.

history, careful physical examination, diagnostic elimination diets, skin prick tests, and specific IgE measurements are helpful for evaluating CMPA, accurate diagnosis remains difficult. This has led to the development of new diagnostic tests.³

Eosinophil cationic protein (ECP) is a cytotoxic protein released from eosinophils upon activation. It is often elevated in some allergic diseases, such as asthma, atopic eczema, food protein-induced allergic proctocolitis (FPIAP), and CMPA. As ECP exists in various body fluids, such as serum, saliva, and feces, the measurement of ECP levels can be used as a non-invasive indicator to detect active inflammation in the body.^{4,5} In addition to ECP, neutrophil-lymphocyte ratio (NLR) and mean platelet volume (MPV), which can be easily measured in routine tests, have been reported to be potential diagnostic biomarkers for some inflammatory disorders.⁶

The aim of our study was to investigate the sECP levels, MPV, and NLR in infants with and without CMPA and to determine the suitability of these parameters as biomarkers to diagnose CMPA. To our knowledge, to date, these parameters have not been simultaneously evaluated in infants with CMPA.

Methods

This cross-sectional study was carried out at the Department of Pediatric Gastroenterology of Karabuk University Medical Faculty in Karabuk, Turkey, from December 2017 to December 2018. Fifty-six infants with CMPA aged 1.5-11 months and forty healthy infants (similar to distribution of age and sex) were included in the study. The CMPA diagnosis was done according to the *European Society for Paediatric Gastroenterology Hepatology and Nutrition* (ESPGHAN) Guideline: Diagnosis and Management of CMPA.⁷ Children with genetic, metabolic, hematological, or infectious diseases were excluded from the study. The study was approved by the Ethics Committee for Non-invasive Clinical Research of Karabuk Training and Education Hospital. Subjects' parents provided written informed consent.

For the whole blood count, blood was collected in 2 mL, ethylenediaminetetraacetic acid (EDTA) tubes and analyzed within 2 hours using a Beckman

Coulter LH 780 analyzer. To measure ECP levels, blood was collected in 3 mL glass tubes and tested by a chemiluminescence method using an *Immulinite 2000 XPi analyzer Immunoassay System* (Germany).

The data were analyzed with *SPSS version 16.0* software for *Windows*. Results are expressed as mean (SD) or median (range). Shapiro-Wilk test was carried out to determine the normality of data distribution. Values of sECP, white blood cell count, absolute neutrophil count, absolute lymphocyte count, NLR, and MPV had abnormal data distribution, by Shapiro-Wilk test ($P < 0.05$), therefore, median values (interquartile range) between groups were determined and compared using Mann-Whitney U test. For age, absolute platelet counts were determined and compared using independent T-test, because of normal data distribution between groups ($P > 0.05$). The ROC curve were constructed for the sECP, MPV and NLR. The areas under the ROC curves with 95 % CIs were calculated and compared with each other. Optimal cutoff values for sECP, MPV and NLR, used to discriminate between infants with and without CMPA, were calculated by ROC curves. Sensitivity and specificity of the cutoff values were analyzed. Correlation analyses were evaluated with Spearman's correlation test. A P value of less than 0.05 was considered to be statistically significant.

Results

The mean age of 56 infants with CMPA (32 males, 57%) and 40 controls (23 males, 55%) were 5.30 (SD 1.67) and 5.15 (SD 1.99) months, respectively. There were no statistically significance differences between the two groups with respect to age or gender (**Table 1**).

The median sECP level in the CMPA group was significantly higher than that in the control group (23.5 and 9.27 ng/mL, respectively; $P = 0.001$) (**Figure 1**). However, the median MPV levels (8.5 and 8.6 fl, respectively; $P = 0.149$) were not significantly different between groups, nor were the median NLRs (0.35 and 0.37, respectively; $P = 0.637$) (**Figure 2**). We also noted that the median eosinophil count (320 and 18 mm³, respectively; $P = 0.001$) was significantly higher in the CMPA group than in the

Table 1. Comparison of socio-demographic and laboratory characteristics of the CMPA and control groups

	CMPA group (n= 56)	Control group (n=40)	P value
Mean age (SD), months	5.30 (1.67)	5.15 (1.99)	>0.05 ^a
Males, n (%)	32 (57)	23 (55)	>0.05
Median sECP (P25-P75), ng/mL	23.5 (4.74-155)	9.27 (3-19)	0.001 ^b
Median white blood cell count (P25-P75), ×103/μL	9.01 (6.15-16.65)	9.88 (3.66-14.23)	0.233 ^b
Median absolute neutrophil count (P25-P75), ×103/μL	2.17 (1.05-11.49)	2.42 (1.06-5.38)	0.879 ^b
Median absolute lymphocyte count (P25-P75), ×103/μL	5.96 (1.1-10.39)	6.14 (2.19-10.71)	0.146 ^b
Median eosinophil count (P25-P75), /mm ³	320 (3-1770)	18 (12-46)	0.001 ^b
Median NLR (P25-P75)	0.35 (0.16-9.5)	0.37 (0.19-1.5)	0.637 ^b
Platelet count (SD), ×103/μL	289 (49)	293 (57)	0.712 ^a
Median MPV (P25-P75), fl	8.5 (7-10.1)	8.6 (7.6-11.1)	0.149 ^b

^aIndependent sample T-test; ^bMann-Whitney U test

control group. There were no statistically significant differences between the two groups with respect to white blood cell counts, neutrophils, lymphocytes, and platelet counts (Table 1). In the CMPA group, correlation analysis of sECP levels with MPV and NLR revealed no significant relationships ($P > 0.05$), but the sECP level was positively correlated to eosinophil count ($r = 0.666$; $P = 0.001$) in the CMPA group (Table 2).

The ROC curve analysis was carried out to determine the diagnostic value of sECP and other markers in differentiating between infants with and without CMPA. The optimal cut-off levels for sECP, MPV, and NLR were 18.4 ng/mL (sensitivity 60.7%, specificity 97.5%, and AUC: 0.831), 10.05 fL (sensitivity 54%, specificity 77.5%, and AUC: 0.413),

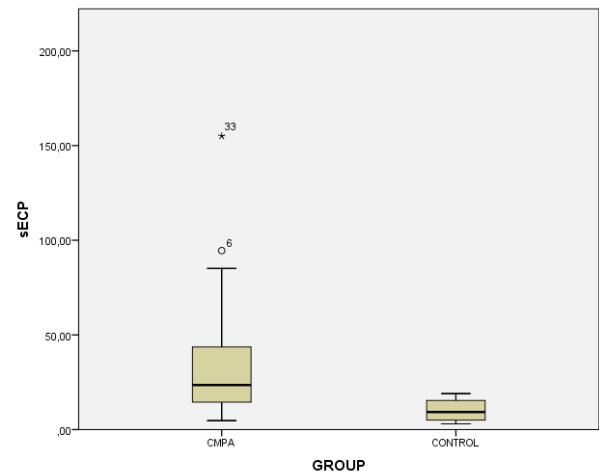


Figure 1. Comparison of median sECP levels in the CMPA and control groups

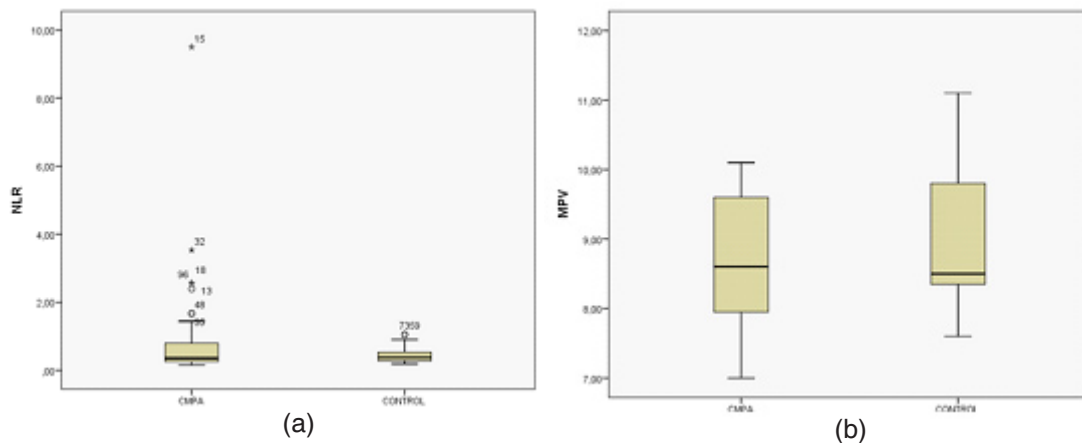


Figure 2. Comparison of median (a) NLR and (b) MPV values in the CMPA and control groups

Table 2. Correlation of sECP with NLR, MPV, and eosinophil count

Parameters	r Value ^a	P value
NLR	0.218	0.17
MPV, fl	0.638	0.64
Eosinophil, mm ³	0.666	0.001

^aSpearman's rank correlation

and 0.97 (sensitivity 14.3%, specificity 50%, and AUC: 0.528) respectively (Table 3). The area under the ROC curve of sECP was statistically significantly higher than the MPV and NLR variables (P=0.001, P=0.637, P=0.149, respectively) (Figure 3).

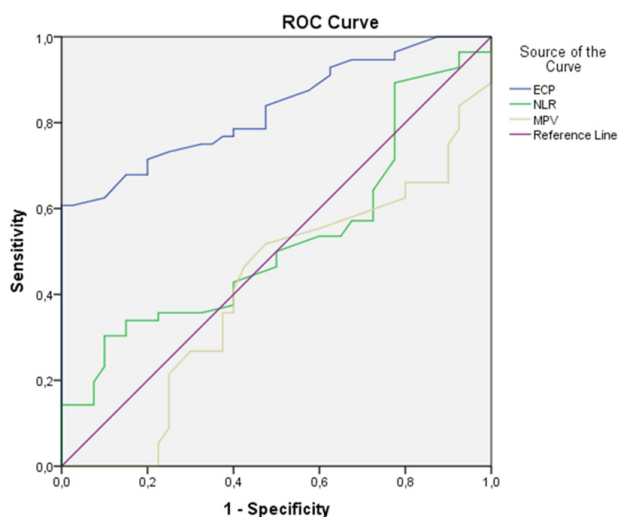


Figure 3. Comparison of ROC curves of ECP, MPV, and NLR.

Discussion

In our study, infants with CMPA had higher sECP levels than controls. On the other hand, there were no significant differences in MPV or NLR values between groups. Moreover, no correlations were observed between sECP level and MPV or NLR values

in infants with CMPA.

Eosinophils have large cytoplasmic granules containing protein, such as eosinophil protein X, major basic protein, eosinophil-derived neurotoxin, and ECP.⁸ The ECP level and numbers of circulating eosinophils can be increased in several allergic disorders like asthma, atopic dermatitis, and inflammatory diseases. Hence, measurement of ECP is used extensively as an indicator in allergic and inflammatory diseases.⁹

Suomalainen et al. reported that in 5.8 to 43.0-month-old children with CMPA, sECP levels were significantly higher after an oral cow's milk challenge.¹⁰ Consistent with these results, Hidvégi et al. reported that the basic sECP level in children with CMPA (12.4 µg/L) was statistically higher than in the controls (7 µmol/L).¹¹ Saarinen et al. conducted a study in 239 infants with CMPA and reported significantly elevated sECP levels (≥20µg/L) in 35 (15%) infants.¹² Consistent with previous studies, we found significantly higher sECP levels in infants with cows' milk protein induced proctocolitis (CMPPI) compared to controls (23.5 and 9.27 ng/mL, respectively; P=0.001).

The MPV level has been associated with the intensity of inflammation and acts as an acute phase reactant. The severity and duration of inflammation may induce rapid (minutes-hours) shifts in MPV levels.¹³ Low MPV levels are seen to represent enhanced consumption of large platelets in inflammatory states like rheumatoid arthritis and familial Mediterranean fever. High MPV levels are associated with various cardiovascular diseases like coronary artery disease, hypertension, and stroke.¹⁴ Recently, conflicting results have been reported on the reliability of MPV as an inflammatory marker for allergic diseases.^{15,16} Akelma et al. conducted a study in 40 children with chronic urticaria (CU). They reported that the MPV levels of children with CU [7.42 (SD 0.77) fL] were significantly lower than that of controls [7.89 (SD 0.65) fL].¹⁷ Consistently, Topal et al. noted that in 6 to 18-year-old children

Table 3. Accuracy and ROC analyses of sECP and other biomarkers to differentiate between infants with and without CMPA

Parameters	Cut-off	AUC	Sensitivity (%)	Specificity (%)	95%CI	P value
sECP	18.4	0.831	60.7	97.5	0.751 to 0.910	0.001
MPV	10.05	0.413	54.0	77.5	0.297 to 0.530	0.637
NLR	0.97	0.528	14.3	50.0	0.411 to 0.645	0.149

with allergic rhinitis (AR), MPV levels were lower than that of controls (7 and 7.6 fL, respectively; $P < 0.001$).¹⁸ On the other hand, Nacaroglu et al. investigated MPV levels in asthmatic children and found no significant differences between groups [8.1 (SD 0.8) fL and 8.2 (SD 0.9) fL, respectively].¹⁹ Likewise, Nacaroglu et al. reported that MPV levels were significantly higher in children with food protein-induced allergic proctocolitis (FPIAP) than in controls [6.87 (SD 1.3) fL and 8.29 (SD 1) fL, respectively].²⁰ In contrast, we found no significant difference in MPV levels of infants with and without CMPA (8.5 and 8.6 fL, respectively; $P = 0.149$). All the above studies including ours suggest that alterations of MPV levels may be easily affected by the type and severity of inflammation.

Neutrophil-to-lymphocyte ratio (NLR) is another parameter used to evaluate inflammatory status.⁷ Despite numerous studies conducted in adults, few studies have evaluated NLR in childhood.^{21,22} Moreover, most pediatric studies have focused on NLR in asthma.^{23,24} Like studies measuring MPV levels, different results have been reported on NLR in asthma. Zhang et al. observed that NLR was not altered in eosinophilic asthma but increased in neutrophilic asthma.²⁵ In our study, there was no significant difference in NLR between infants with CMPA and controls (0.35 and 0.37, respectively; $P = 0.637$). Consistent with our findings, Nacaroglu et al. reported no significant differences in NLR values of children with and without FPIAP [0.61 (SD 0.78) and 0.63 (SD 0.87), respectively; $P = 0.883$].²⁰

As mentioned above, various markers have been investigated in the diagnosis of inflammation-related disease. In our study, the area under the ROC curve of sECP performed significantly better than did NLR or MPV (AUC: 0.831, $P = 0.001$; AUC: 0.528, $P = 0.149$; and AUC: 0.413, $P = 0.637$, respectively). Moreover, ECP showed moderate sensitivity and high specificity for diagnosing CMPA (60.7% sensitivity, 97.5% specificity). On the other hand, NLR and MPV showed lower sensitivity and specificity for the diagnosing CMPA (14.3% sensitivity, 50% specificity and 54% sensitivity, 77.5% specificity, respectively).

There were some limitations in this study. As there have been relatively few studies evaluating NLR, MPV and sECP levels in CMPA, we compared our findings to only a small number of studies. Also,

other inflammatory markers such as fecal ECP and calprotectin were not evaluated due to funding issues.

In conclusion, infants with CMPA have significantly higher sECP levels than control infants, but NLR and MPV levels do not differ between the two groups. Also, the diagnostic performance of ECP is found to be higher than that of MPV and NLR. Even though NLR and MPV have recently been the source of inspiration as biomarkers for some clinical trials, the ECP might be considered as a potential biomarker for diagnosing CMPA.

Conflict of Interest

None declared.

Funding Acknowledgment

The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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