ASTHMA ALLERGY IMMUNOLOGY
ASTIM ALLERJİ İMMÜNOLOJİ

doi: 10.21911/aai.2024.390
Asthma Allergy Immunol 2024;22:I-XI

RESEARCH ARTICLE

Received: 19.08.2022 • Accepted: 08.12.2023
Online Published: 05.02.2024

Diagnostic Value of Systemic Inflammatory Markers in Predicting Severe Anaphylaxis in Children

Zeynep GULEC KOKSAL, Duygu ERGE, Pinar UYSAL

Department of Pediatric Allergy and Immunology, Aydin Adnan Menderes University Faculty of Medicine, Aydin, Turkey

Corresponding Author: Zeynep Gulec Koksal ✉ zynp.glc@hotmail.com

ABSTRACT

Objective: The neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR) are blood markers of systemic inflammatory response (SIR). The purpose of this study was to investigate the clinical utility of SIR markers for severe anaphylaxis in children.

Materials and Methods: The medical records of 103 children aged 0-18 years experiencing a total of 204 anaphylactic episodes between 2013 and 2020 were analyzed. The children were categorized into mild, moderate, and severe anaphylaxis groups. The SIR markers were measured during anaphylaxis.

Results: Anaphylaxis was mild in 47 (45.6%), moderate in 28 (27.2%), and severe in 28 (27.2%) children. NLR was higher in children with severe anaphylaxis (p=0.001). The ROC curve demonstrated by the increase in NLR yielded an area under the curve (AUC) of 0.666 (CI: 0.5-0.79), with a cut-off value of ≥2.1 in discriminating severe anaphylaxis from mild and moderate anaphylaxis, with sensitivity of 73.6% and specificity of 76.9%. The risk of severe anaphylaxis increased in drug- and venom-induced reactions (OR: 11.83, 95%CI: 2.89-48.38, p=0.001, and OR: 13.03, 95%CI: 2.82-60.22, p=0.001, respectively), and in case of NLR ≥ 2.1 (OR: 3.92, 95%CI: 1.53-10.04, p=0.004), particularly in children aged ≤6 years (OR: 9.33, 95%CI: 1.27- 68.59, p=0.028).

Conclusion: NLR was higher in severe anaphylaxis than mild and moderate, whereas MLR and PLR were not. NLR could be used as a quick and easily accessible marker to predict the severity of anaphylaxis in children.

Keywords: Anaphylaxis, neutrophil-to-lymphocyte ratio, pediatrics, severe anaphylaxis, systemic inflammatory marker

INTRODUCTION

Anaphylaxis is a rapidly progressing and severe multisystemic hypersensitivity reaction in children (1). The estimated incidence of anaphylaxis is around 10-20 / 100,000 population per year and lifetime prevalence of anaphylaxis is between 0.5% and 2% in the general population (2,3). The severity ranges from mild to life-threatening when susceptible individuals are exposed to a potential causative agent (3,4).

Anaphylaxis is diagnosed on the basis of a compatible medical history, recognition of characteristic signs and symptoms that occur within minutes to several hours after exposure to a triggering agent, and detailed physical examination (5).

Although a number of guidelines have been developed on the diagnosis and treatment of anaphylaxis, practical challenges remain (6). In some conditions, it is not always possible to (i) distinguish severe anaphylaxis from other suddenly developed respiratory and cardiac disorders, (ii) determine the trigger(s), (iii) predict biphasic reactions and the prognosis. Although serum tryptase is the main laboratory tool in the confirmation of anaphylaxis, this is not specific or sufficiently reliable for diagnosis and assessment of severity (7). Considering all these situations, meeting the diagnostic criteria for anaphylaxis recommended

ORCID Zeynep Gulec Koksal / 0000-0001-9464-4605, Duygu Erge / 0000-0003-1477-4739, Pinar Uysal / 0000-0003-0288-608X

in the guidelines is not a prerequisite for the administration of epinephrine in the treatment of patients undergoing an acute systemic reaction (8).

Anaphylaxis occurs through immunological or non-immunological mechanisms. Immunologic anaphylaxis is used to define IgE-mediated, possibly IgG-mediated and immune complex and/or complement-mediated reactions (9). While the main cells involved in anaphylaxis are mast cells and basophils, various cell subpopulations including neutrophils, platelets, monocytes, and macrophages also appear to play a role in its development (8,10). Neutrophils have been reported to play an important role in anaphylaxis (11). These rapidly increase in number, are activated very rapidly and systemically, and can be easily detected at the beginning of the anaphylactic reaction in both IgE- and IgG-mediated anaphylactic reactions (12). Neutrophils play a role in IgG-mediated anaphylaxis by secreting platelet-activating factor (PAF), and in IgE-mediated anaphylaxis by secreting histamine (13,14). Additionally, tryptase, PAF, and chemokine (C-C motif) ligand 3 (CCL3) are chemotactic factors for neutrophils and modulate degranulation after mast cell activation (15,16). Neutrophil activation during anaphylactic shock is more pronounced in severe cases than in mild cases (17). Moreover, the severity of the symptoms correlates directly with the number of infiltrating neutrophils (18).

There is currently no evidence for a marker capable of use at the onset of anaphylaxis and of predictive value in estimating its severity. A sensitive, low-cost, accurate, and feasible marker is therefore needed to confirm the diagnosis of anaphylaxis, particularly in cases in which (i) medical history cannot be obtained, (ii) symptoms are atypical, and (iii) prompt differential diagnosis, management and follow-up decisions are needed on an emergency basis. A novel biological marker may be capable of confirming the diagnosis of severe anaphylaxis and estimating biphasic reactions, particularly in children (3). The neutrophil-to-lymphocyte ratio (NLR), the platelet-to-lymphocyte ratio (PLR), and the monocyte-to-lymphocyte ratio (MLR) are markers of systemic inflammation. Such markers are readily available, fast, cheap, and can be easily calculated from whole blood sample.

The purpose of the present study was therefore to assess the association between markers of systemic inflammation and the severity of anaphylaxis in children.

MATERIALS and METHODS

Study Design and Population

This single-center retrospective cohort study was conducted at the tertiary referral hospital pediatric allergy and immunology clinic. A total of 103 patients with 204 anaphylactic reactions who were followed up in the pediatric wards, emergency department, and intensive care unit for anaphylaxis and consulted with pediatric allergy immunology specialists between 2013 and 2020, were included.

Participants were identified from the medical records of anaphylactic reactions coded as anaphylactic shock caused by adverse food reaction (T78.0), other adverse food reactions (T78.1), undefined anaphylactic shock (T78.2), and contact with wasps and bees (X23) according to the International Classification of Disease (ICD-10).

Inclusion criteria were as follows: patients both of genders, ranging in age from 0-18 years, diagnosis of anaphylaxis based on history and physical examination according to widely recognized clinical criteria were included in the study (19).

Exclusion criteria were as follows: chronic infections, chronic heart and/or lung disease, malignancy, immune deficiency, metabolic disease, connective tissue diseases, use of oral corticosteroids, and hospitalization in the previous week.

Definitions

The diagnosis of anaphylaxis was based on the guidelines from the European Academy of Allergy and Clinical Immunology (EAACI) (19). Anaphylaxis was classified as mild, moderate, or severe. The severity of anaphylaxis episode was based on the position paper of the European Academy of Allergy and Clinical Immunology (Table I) (20).

Data Sources and Handling

Patients' demographic characteristics of the medical histories, physical examination findings, and laboratory parameters were recorded using a standard questionnaire investigating age, gender, comorbidities, and parental atopy history. The demographic characteristics of the reactions were triggers (foods, drugs, and insects), location

Table I: Grading the severity of anaphylactic reactions

Grade	Skin	Gastrointestinal tract	Respiratory	Cardiovascular	Neurological
Mild	Sudden itching of eyes and nose, generalized pruritus, flushing, urticaria, angioedema,	Oral pruritus, oral 'tingling', mild lip swelling, nausea or emesis, mild abdominal pain	Nasal congestion and/ or sneezing, rhinorrhoea, throat pruritus, throat tightness, mild wheezing	Tachycardia (increase >15 beats/min)	Change in activity level plus anxiety
Moderate	Any of the above	Any of the above, crampy abdominal pain, diarrhoea, recurrent vomiting	Any of above, **hoarseness, 'barky' cough, difficulty swallowing, stridor, dyspnoea, moderate wheezing**	As above	'Light headedness' feeling of 'pending doom'
Severe	Any of the above	Any of the above loss of bowel control	Any of the above, **cyanosis** or saturation <92%, **respiratory arrest**	**Hypotension[1] and/or collapse, dysrhythmia, severe bradycardia and/or cardiac arrest**	Confusion, loss of consciousness

The severity score should be based on the organ system most affected.

Boldface symptoms and signs are mandatory indications for the use of epinephrine.

[1] Hypotension defined as systolic blood pressure: 1 month to 1 year <70 mmHg; 1-10 years< [70 mmHg + (2 x age)]; 11-17 years <90 mmHg.

of the reaction, cofactors, history of a previous anaphylactic reaction, time to symptom onset after the triggers, number of allergens, symptoms, biphasic reaction, admission, epinephrine administration rates and dosages, time to epinephrine administration after the onset of anaphylaxis, and self-injectable epinephrine prescription and administration rates. Demographic characteristics were compared among different anaphylaxis severity groups - mild, moderate, and severe.

Laboratory Parameters

Blood samples were collected while intravenous access was provided during the anaphylaxis. They were analyzed in terms of neutrophil, lymphocyte, eosinophil, basophil, and platelet counts, mean platelet volume (MPV), NLR, PLR, and MLR. Blood counts were studied using an automated hematology analyzer (MINDRAY BC-6800 Hematology Analyzer; Shenzhen, P.R. China). NLR was calculated as the ratio of absolute neutrophil to lymphocyte count.

Serum total immunoglobulin E (IgE), allergen (food, drug, and venom) specific IgE (sIgE) and tryptase levels were recorded, if available. Serum total IgE, sIgE and tryptase levels were measured using a Pharmacia CAP system (ImmunoCAP; Pharmacia &Upjohn, Uppsala, Sweden). Levels ≥ 0.35 kUA/ L^{-1} and ≥11.4 ng/ml were considered positive for serum sIgE and serum tryptase levels, respectively.

Laboratory parameters were compared among different anaphylaxis severity groups - mild, moderate, and severe.

The anaphylaxis treatment approach was routinely performed in accordance with the EAACI guidelines. Immediately after the administration of intramuscular epinephrine in all anaphylaxis cases, the vascular access was established and blood samples were taken. Subsequently, other intravenous treatments were administered as indicated (fluids, antihistamine, and corticosteroid) (19).

Ethics

The study was approved by the local research ethics committee (Project no. 2020/85). Informed consent was obtained from all participants.

Statistical Analysis

Statistical analysis was performed on the SPSS Statistics for Windows v.21.0 package program (IBM Corp., Armonk, NY, USA). Data were expressed as mean ± standard deviation (SD) and median (IQR, interquartile range). Descriptive statistics were used to estimate the percentage of children diagnosed with anaphylaxis, reactions, and treatment. Continuous variables were compared among the different anaphylaxis severity groups using one-way analysis of variance (ANOVA) and the Kruskal-Wallis test. Bonferroni *post hoc* tests were applied to compare data

within the subgroups. Pearson and Spearman's correlation analyses were applied for continuous variables. Receiver operating characteristic (ROC) curves were produced for sensitivity and specificity analysis for NLR in predicting severe anaphylaxis. Univariate or multivariate regression analyses were performed to evaluate associations between clinical and demographic parameters and the severity of anaphylaxis. Factors identified as significant at univariate analysis were included into multivariate regression analysis as covariates. Regression analysis results were expressed as odds ratio (OR) and 95% confidence interval (CI). Since lymphocyte and neutrophil counts vary according to age, the analyses were adjusted for age. p values <0.05 were considered statistically significant.

RESULTS

Demographics

Participant Characteristics

The characteristics of the 103 patients are shown in Table II. The patients' median (IQR) age was 10 (4 - 14) years, and the median age at first anaphylaxis episode was 7 (2 - 12) years. The median age for the onset of initial reactions to food-induced anaphylaxis was 3 (1-9) years. In the case of drug-induced anaphylaxis, the median age was 12 (7-14) years, and for anaphylaxis caused by insect stings, the median age was 8 (5-13) years. Fifty-six (54.4%) patients were boys. The age of the participants, age at first anaphylaxis episode, and NLR were higher in females than males (p=0.005, p=0.041, and p<0.001, respectively) (data not shown). Among the 103 patients who experienced mild, moderate, and severe anaphylaxis, 41 (39.8%) underwent tryptase level assessments, and the median tryptase value was <11.4 ng/mL in all groups.

The characteristics of the 204 anaphylactic reactions are presented in Table II. The intramuscular epinephrine administration rate was 60.8% (n=124), with epinephrine being administered once per after anaphylaxis episode in 94 (75.8%) patients, and ≥2 times in 30 (24.2%). The median (IQR) time to administration of epinephrine after symptoms onset of anaphylaxis was 17.3 (8.9 - 31.3) minutes (data not shown). The self-injectable epinephrine prescription rate was 79.6%, (n=82). It was administered in 3.9% (n=4) of patients by their parents or themselves.

Severity of Anaphylaxis

Anaphylaxis was mild in 47 (45.6%) children, moderate in 28 (27.2%), and severe in 28 (27.2%). The most severe reaction was combination of hypotension, tachycardia, hypoxia, and loss of consciousness during the oral food challenge test with egg yolk in a six-month-old boy with severe atopic dermatitis and a negative skin prick test. Cardiovascular symptoms were observed in 67.9% of the children with severe anaphylaxis, and the rate of cardiovascular system findings in patients with drug-induced anaphylaxis was 2.84 times (CI:1.15-7.05) higher than in patients with food-induced anaphylaxis. No cardiac arrest or mortality occurred in our study population.

The severity of anaphylaxis was significantly greater in children experiencing anaphylaxis between 12 and 18 years of age than in children aged 0-6 or 7-12 years (p=0.005).

Intramuscular epinephrine was administered in 24/28 children (85.7%) with severe anaphylaxis.

Comparison of Anaphylaxis Parameters Among the Anaphylaxis Severity Groups

A comparison of demographics among the mild, moderate, and severe anaphylaxis groups are presented in Table III. Age at diagnosis and at initial reaction, cardiovascular symptoms, admissions to the intensive care unit, epinephrine administration rates, and epinephrine administrations ≥2 were significantly higher in children with severe anaphylaxis compared to those with mild and moderate anaphylaxis (p<0.01). However, food was a more common allergen in the mild anaphylaxis group compared to the moderate and severe anaphylaxis groups (<0.001) (Table III).

A comparison of laboratory data among the mild, moderate, and severe anaphylaxis is presented in Table IV and shown in Figure 1. The neutrophil count and NLR were higher in the severe anaphylaxis group than in the other two groups (p=0.001 and p=0.002, respectively), when the age and gender were adjusted.

Viral upper airway infection was found to be a cofactor in 28 anaphylactic reactions (Table II). When these patients were excluded from the analysis, a significant difference was found among mild, moderate, and severe anaphylaxis in terms of NLR and neutrophil count. In addition, when subgroup analysis was performed, NLR and neutrophil count were significantly higher in severe anaphylaxis than the others (p<0.01). When children with viral infection were also analyzed, neutrophil count and NLR were not significantly different among these three groups (p>0.05) (Data not shown).

Table II: Characteristics of children with anaphylaxis and anaphylactic reactions

Characteristics	Frequency (%)
Participants (n=103)	
Gender	
Male	56 (54.4)
Female	47 (45.4)
Age (median, IQR)	
Age at diagnosis	10 (4-14)
Age of first anaphylaxis episode	7 (2-12)
Co-morbidities (recent personal medical history) (n= 79, 76.7%)	
Asthma	24 (23.3)
Allergic rhinitis	13 (12.6)
Atopic dermatitis	10 (9.7)
Urticaria	3 (2.9)
Food allergy (IgE-mediated)	29 (28.2)
Parental history of atopy	
Atopic	32 (31.1)
Non-atopic	71 (68.9)
Reactions (n=204)	
Triggers	
Food (n=115, 56.4%)	
Cow's milk	24 (11.77)
Hen's egg	16 (7.85)
Fishes	12 (5.85)
Hazelnut	8 (3.92)
Chicken	8 (3.92)
Walnut	7 (3.44)
Sesame	7 (3.44)
Spices - food additives	7 (3.44)
Legumes	6 (2.94)
Fruits	5 (2.45)
Peanut	4 (1.96)
Soy	4 (1.96)
Lentil	4 (1.96)
Wheat	3 (1.47)
Drugs (n=55, 26.9%)	
Antibiotics	27 (13.24)
NSAID	14 (6.86)
Immunotherapy	8 (3.92)
Other medications	6 (2.94)
Insects (n=34, 16.7%)	
Venom - Apis mellifera sp.	16 (7.85)
Venom - Vespula sp.	10 (4.90)
Other insects	8 (3.92)
Location of the reaction	
Child's own home	91 (44.7)
Kindergarten - School	12 (5.8)
Restaurant	16 (7.8)
Outdoor	40 (19.4)
Hospital	45 (22.3)

Characteristics	Frequency (%)
Participants (n=103)	
Cofactors (n=41)	
Viral infection	28 (13.7)
Physical exercise	7 (3.4)
Unknown	6 (2.9)
History of previous anaphylactic reaction	
Food	132 (64.5)
Drug	52 (25.8)
Venom	20 (9.7)
The time to symptom onset after the triggers of the anaphylaxis (min) (%)	
0-15	125 (61.2)
15-60	57 (28.1)
≥ 60	22 (10.7)
Number of allergens (%)	
Single	169 (82.84)
Multiple	35 (17.16)
Symptoms (%)	
Cutaneous*	91 (44.60)
Respiratory	89 (43.62)
Gastrointestinal	44 (21.56)
Cardiovascular	41 (20.09)
Neurological	5 (2.45)
Cutaneous + respiratory	80 (39.21)
Cutaneous + gastrointestinal	36 (17.64)
Other combinations of two	11 (5.39)
Other combinations of three	3 (1.47)
Biphasic reaction (%)	8 (3.9)
Admission (n=204) (%)	
Emergency department	101 (49.50)
Inpatient ward	50 (24.50)
Intensive care unit	53 (26.0)
Epinephrine administration rate (%)	124 (60.78)
Time to administration of epinephrine after symptom onset of anaphylaxis (n=124, 60.8%)	
15 min	60 (48.39)
16-60 min	44 (35.48)
≥61 min	20 (16.13)
Dosage of epinephrine (n=124) (%)	
1	94 (75.80)
≥2	30 (24.20)
Self-injectable epinephrine prescription (n=103) (%)	82 (79.61)
Self-injectable epinephrine administration (n=103) (%)	4 (3.9)

NSAID: Non-steroid anti-inflammatory drug, **n:** Number, **yr:** Year, **%:** percent.

Table III: Comparison of demographics among mild, moderate, and severe anaphylaxis

Variables	Severity of anaphylaxis			*p* value
Participants (n=103)	**Mild (n=47)**	**Moderate (n=28)**	**Severe (n=28)**	
Gender (%)				
Sex (male)	27 (48.21)	14 (25)	15 (26.79)	0.818
Age (yr) (median, IQR)				
At diagnosis	6 (3 - 12)	10.5 (5.5 - 14.7)	13** (6.2 - 15.7)	**0.007**
At initial reaction	4 (1 - 11)	7 (2.3 - 11.5)	12** (6.2 - 13.7)	**0.008**
Reactions (n=204)	**Mild (n=90)**	**Moderate (n=54)**	**Severe (n=60)**	
Trigger of anaphylactic reaction (%)				
Food	74 (56.52)**	52 (39.13)	6 (4.35)	
Drug	22 (42.31)	20 (38.46)	10 (19.23)	**<0.001**
Venom	8 (40)	4 (20)	8 (40)	
The time to symptom onset after the triggers of the anaphylaxis (min) (%)				
0-15	48 (38.40)	36 (28.80)	41 (32.80)	
15-60	33 (57.90)	12 (21.05)	12 (21.05)	0.204
≥ 60	14 (63.64)	4 (18.18)	4 (18.18)	
Number of allergens (%)				
Single	77 (45.56)	44 (26.04)	48 (28.40)	0.778
Multiple	16 (45.71)	12 (34.29)	7 (20)	
Symptoms (%)				
Cutaneous*	44 (48.35)	26 (28.57)	21 (23.08)	0.052
Respiratory	41 (46.06)	24 (26.97)	24 (26.97)	0.975
Gastrointestinal	14 (31.82)	15 (34.09)	15 (34.09)	0.052
Cardiovascular	12 (29.26)	10 (24.40)	19 (46.34)**	**0.001**
Neurological	N/A	1 (20)	4 (80)	N/A
Cutaneous + respiratory	39 (48.75)	22 (27.50)	19 (23.75)	0.312
Cutaneous + gastrointestinal	12 (33.33)	13 (36.11)	11 (30.56)	0.158
Other combinations of two	2 (18.19)	3 (27.27)	6 (54.54)	0.071
Other combinations of three	1 (33.33)	1 (33.33)	1 (33.33)	N/A
Biphasic reaction (%)	2 (25.0)	2 (25.0)	4 (50.0)	N/A
Admission (%)				
Emergency department	73 (72.3)	22 (21.78)	6 (5.92)	
Ward	22 (44.0)	18 (36.0)	10 (20.0)	**<0.001**
Intensive care unit	6 (11.32)	9 (16.98)	38 (71.70)**	
Epinephrine administration rate (n=124) (%)	20 (16.13)	36 (29.03)	68 (54.84)**	**<0.001**
Time to administration of epinephrine after symptom onset of anaphylaxis (n=124) (%)				
15 min	35 (36.2)	16 (28.6)	29 (50)	
16-60 min	16 (36.36)	12 (27.28)	16 (36.36)	0.544
≥61 min	8 (8.5)	8 (14.3)	4 (7.1)	
Dosage of epinephrine (n=124) (%)				
1	34 (36.17)	34 (36.17)	26 (27.66)	**0.002**
≥2	4 (13.33)	4 (13.33)	22 (73.34)**	
Self-injectable epinephrine prescription (n=103) (%)	36 (43.90)	23 (28.05)	23 (28.05)	0.785
Self-injectable epinephrine administration at one-year follow-up (n=103) (%)	N/A	N/A	4 (3.9)	N/A

IQR: Interquartile range, **min:** Minute; **n:** Number, **yr:** Year, **%:** percent, *The isolate mucocutaneous reaction was not accepted as a clinical criteria;**p<0.016 after Bonferroni correction.

Table IV: Comparison of laboratory parameters among mild, moderate, and severe anaphylaxis

	Severity of anaphylaxis			
Variables	**Mild (n=47)**	**Moderate (n=28)**	**Severe (n=28)**	***p* value***
Lymphocyte (x10³/μL)	3430 (2515 - 4985)	4110 (1230 - 9170)	3710 (1503 - 9405)	0.883
Neutrophil (x10³/μL)	3960 (2980 - 5895)	6540 (1480 - 15830)	8375** (2155- 17250)	**<0.001**
Monocyte (x10³/μL)	655 (432 - 759)	620 (270 - 980)	670 (210 - 1230)	0.658
Platelet (x10³/μL)	330 (280 - 400)	380 (200 - 660)	360 (210 - 580)	0.223
NLR	1.94 (1.64 - 7.62)	1.84 (0.48 - 5.07)	8.936** (7.57 - 10.2)	**<0.001**
MLR	0.14 (0.10 - 2.75)	0.19 (0.05 - 0.47)	0.27 (0.08 - 0.73)	0.781
PLR	92.48 (79.45 - 104.30)	111.41 (39.54 - 256.33)	117.05 (49.89 - 306.17)	0.351
MPV	9.40 (8.10 - 10.10)	8.89 (6.77 - 10.82)	8.67 (7.21 - 10.62)	0.266
Eosinophil	190 (90 - 400)	165 (77.5 - 585)	155 (92.5 - 225)	0.434
Basophil	40 (20 - 50)	30 (17.5 - 50)	30 (20 - 50)	0.302
Total IgE (IU/mL)	49.95 (17.2 - 197)	159(48.5- 360.5)	167 (15.5 - 972.2)	0.183
Tryptase (ng/mL) (n=41)	8.75 (6.78 - 14.12)	9.12 (8.17 - 16.21)	10.13 (9.34 - 17.23)	0.343

IU: International unit, **mL:** Milliliter, **μL:** Microliter, **MPV:** Mean platelet volume, **n:** Number, **ng:** Nanogram, **NLR:** Neutrophil lymphocyte ratio, **MLR:** Monocyte lymphocyte ratio, **PLR:** Platelet lymphocyte ratio, *adjusted for age and gender; **p<0.016 after Bonferroni correction.

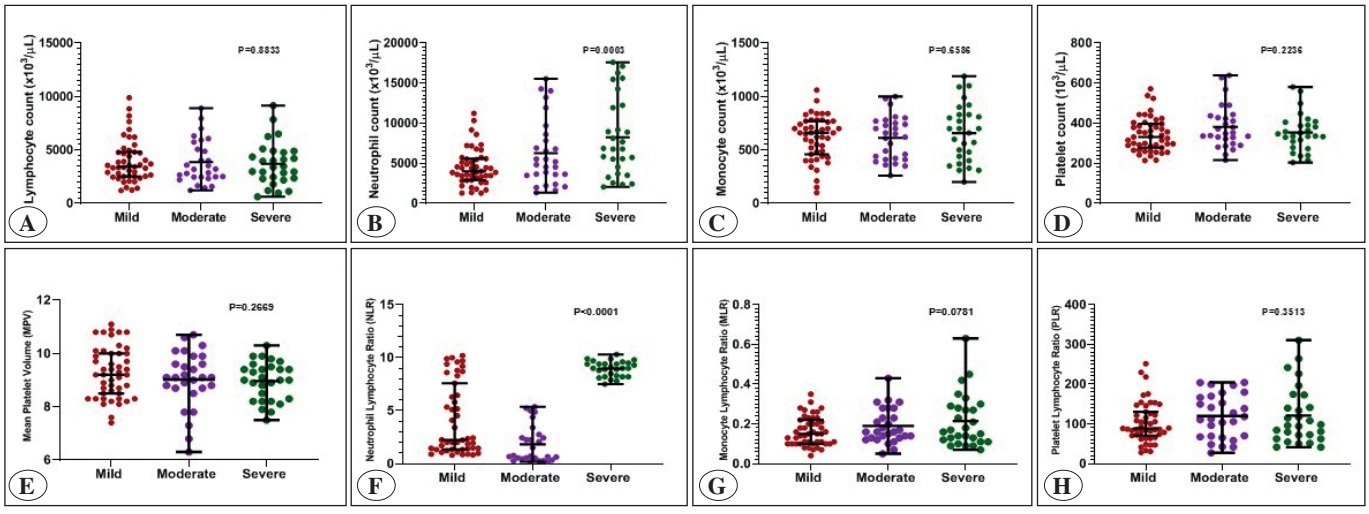

Figure 1. Comparison of laboratory data among the mild, moderate, and severe anaphylaxis patients

Correlation Analysis

The mean age of the anaphylaxis patients (r= 0.374, p<0.001) and age at initial anaphylactic reaction (r= 0.419, p<0.001) were both correlated with NLR. However, the frequency of anaphylaxis was negatively correlated with the patient's age (r= -0.532, p<0.001) and basophil count (r= -0.309, p=0.026).

ROC Analysis

The ROC curve demonstrated by the increase in NLR yielded an area under the curve (AUC) of 0.666 (CI: 0.54 - 0.79), with a cut-off value of ≥2.1 in discriminating severe from mild and moderate anaphylaxis, with sensitivity of 73.6% and specificity of 76.9% as shown in Figure 2.

Table V: Logistic regression analysis for prediction of severe anaphylaxis

Variable	Exp (B)	95% Confidence Interval		p value
		Lower Bound	Upper Bound	
Constant	0.044			<0.001
NLR	1.23 2.54	1.03 1.57	1.47 10.37	0.03 0.012*
Drug-induced reaction	11.83	2.89	48.38	0.001
Venom-induced reaction	13.03	2.82	60.22	0.001
R^2=0.26 (Cox&Snell), 0.36 (Nagelkerke), X2(8)=0.823 (Hosmer&Lemeshow)				

NLR: Neutrophil-to-lymphocyte ratio; **%:** Percentage; * in children ≤6 years of age.

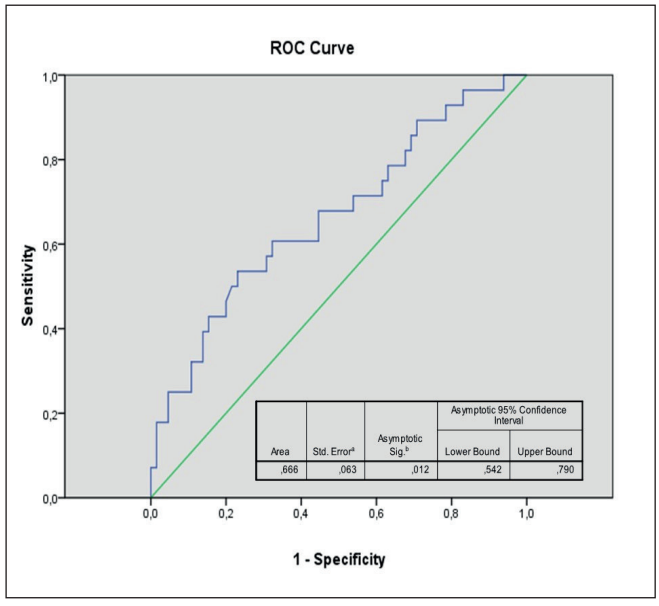

Figure 2. Comparison of laboratory data among the mild, moderate, and severe anaphylaxis patients

Multivariate Regression Analysis

When the statistically significant variables of NLR, age, and the triggers of venom and drug obtained in the univariate analysis were included in the binary logistic regression analysis, the significant effect of age on the severe anaphylaxis was no longer apparent.

While NLR ≥2.1 was determined as an independent risk factor for severe anaphylaxis (OR: 3.92, 95% CI: 1.53-10.04, p=0.004), the risk was particularly higher for children ≤6 years (OR: 9.33, 95% CI: 1.27- 68.59, p=0.028). Drug- and venom-induced reactions also significantly increased the risk of severe anaphylaxis (OR:11.83, 95% CI:2.89-48.38, p=0.001; OR:13.03, 95% CI:2.82-60.22, p=0.001, respectively) (Table V).

DISCUSSION

To the best of our knowledge, this is the first study of children with anaphylaxis to evaluate the relationship between reaction severity and systemic inflammatory markers. The most important finding of the present study is the association between high NLR levels and severe anaphylaxis in children. A cut-off value of NLR ≥ 2.1 may be used for the prediction of severe anaphylaxis, and this also emerged as a risk factor for severe anaphylaxis.

The potential functions of neutrophils in patients with anaphylaxis have recently been reviewed in detail (18). The neutrophils have receptors for both IgE and IgG, enabling them to directly respond to the allergen (21). It is possible that in individuals with more severe reactions, either their neutrophils are more sensitive to the allergen or they have a larger number of neutrophils, leading to a stronger inflammatory response (12). A microarray study on human anaphylaxis uncovered increased activity in the inflammatory pathways, notably the triggering receptor expressed on myeloid cells-1 (TREM-1) pathway. This indicates early involvement of the innate immune system and the activation of neutrophils (22). When neutrophils are activated, they release cytokines like IL-1, IL-6, IL-8, IL-12, tumor necrosis factor-α, and transforming growth factor-β. These cytokines can further intensify the anaphylactic response by activating more neutrophils and other immune cells (23). The major enzyme stored in neutrophils is myeloperoxidase (MPO). A recent report has shown that circulating MPO levels are higher in patients with anaphylaxis compared to healthy controls. It is likely that neutrophil activation contributes to the physiological changes in anaphylaxis, and increased MPO concentrations, among other neutrophil products, may lead to more severe symptoms (12). The activation of neutrophils likely plays a crucial role in rapidly and broadly activating the

immune system during anaphylaxis (12). Although the functions of neutrophils have not been specifically evaluated in our study, the hypothesis that an increased NLR may indicate the severity of anaphylaxis could be associated with an elevated neutrophil count.

No useful serologic marker is currently available for the rapid and reliable evaluation of severe anaphylaxis and accurate differential diagnosis in the emergency setting. Although serum tryptase levels are widely used for this purpose, these are less consistently elevated in children presenting with food-induced anaphylaxis, and also normal levels of tryptase also do not rule out anaphylaxis (24). Moreover, tryptase is not able to predict the severity of the reaction, as well as being a time-consuming, expensive, and not easily accessible marker in the emergency department setting (3,19). In our study, the tryptase level was within normal ranges in all patients who could be analyzed during anaphylaxis. Although few patients were studied, our study supported that tryptase is not a reliable sufficient biomarker for predicting severe anaphylaxis. We therefore suggest that the NLR may be used to predict the severe anaphylaxis at prompt evaluation due to its being practical, inexpensive, and easily accessible.

The most common triggering factors in the present study were foods (cow's milk, egg, and fish), drugs (antibiotics and NSAIDs), and venoms (Apis mellifera and Vespula *spp*.). While food products were associated with mild anaphylaxis, venom was found to be associated with severe anaphylaxis. The risk of severe anaphylaxis was found to be 11.8 times higher in the presence of drug allergy and 13 times higher in the presence of venom allergy. However, to the best of our knowledge, only limited studies have investigated the relationship between anaphylaxis severity and triggers in children. Jeon et al. have reported that the risk of drug anaphylaxis was 2.7 times higher in cases of severe anaphylaxis (25). However, whether one or more allergens were involved was not significant in terms of anaphylaxis severity.

Reported age at time of diagnosis ranges between 7 and 10 years in pediatric anaphylaxis cases (26,27). In the present study, children with severe anaphylaxis were older and had a higher frequency of venom allergy compared to those with mild and moderate anaphylaxis. A higher risk of serious or fatal anaphylaxis has been reported at older ages in a number of studies, although no reason for this observation has been suggested (28-34). Consistent with our findings, the risk of anaphylaxis due to insect venom was higher and the condition was more severe at older ages in these studies (31-37).

Consistent with previous studies involving children, the most common symptom in the present research was cutaneous involvement, followed by respiratory, cardiovascular and gastrointestinal symptoms (26,27,38-41). In this study, the rate of cardiovascular system findings in patients with drug-induced anaphylaxis was 2.84 times higher than in patients with food-induced anaphylaxis. Similarly, in Jeon et al.'s multicenter retrospective study, the frequency of cardiovascular symptoms in infantile cases were 2.5 times higher in drug-related anaphylaxis than in food-related anaphylaxis (25). We speculate that the higher frequency of cardiovascular symptoms in severe anaphylaxis in our study population may be due to the referral of severe cases to our hospital as a tertiary center, the accurate recording of physical examinations at the time of hospital admission, and the large number of cases consulted with allergy specialists in the emergency department.

The rate of epinephrine administration was significantly higher in severe anaphylaxis in the present study, at 85.7%. As the severity of anaphylaxis increased, both the administration rate and the repeated doses of epinephrine rose significantly. However, no significant relationship was found between time to epinephrine administration and the severity of anaphylaxis. Similarly, in Dubus et al.'s study, the rate of epinephrine administration in severe anaphylaxis was 84.8%, and was significantly higher in severe anaphylaxis compared to the moderate and mild anaphylaxis groups (27).

We observed a higher rate of self-injectable epinephrine prescriptions (79.6%) after discharge. However, a lower rate (3.9%) of self-administration of epinephrine was reported by patients or their families in the 63 (61.2%) patients who had recurrent anaphylaxis. The self-injectable epinephrine administration rate in children with food-related anaphylaxis was 2.7% in a study from Turkey (42). A multi-center study from Europe reported a rate of self-injectable epinephrine administration in children of 16.7% (43). We speculate that the low rate of self-injectable epinephrine administration may be due to the easy access to our hospital. We suggest that further encouragement of self-injectable epinephrine administration is still needed.

The particular strengths of this study were (i) the high numbers of cases and reactions for a single-center study,

and (ii) the fact that diagnosis, evaluation of the severity, and management protocols of anaphylaxis were established by allergy specialists.

The limitations of the study were its retrospective design, lack of control group and that number of tryptase specimens obtained from the patients was insufficient to evaluate its potential association with NLR. The NLR may be affected due to the fact that the blood samples were taken after the administration of epinephrine.

CONCLUSION

Age ≥12 years, venom and drug allergy, and cardiovascular symptoms were associated with severe anaphylaxis in children. In addition, the rate of epinephrine administration and the administration of repeated doses increased with the severity of anaphylaxis. Early diagnosis of severe anaphylaxis and administration of epinephrine therapy is important for the prevention of life-threatening events. However, the serum tryptase level is not sufficiently helpful in this regard. We think that it will be beneficial to use new diagnostic tests that yield results during anaphylaxis to overcome the deficiency on this subject in the literature. NLR might be used as an easily accessible biomarker for confirmation of severe anaphylaxis. Further large-scale prospective studies are now needed to verify our findings.

Acknowledgement

The authors are indebted to specialist nurse Nazmiye Ozdemir for the childcare and collection of analysis data. Pediatric assistant Sercan Ozturk provided guidance at the data analysis stage.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

None.

Author Contributions

Concept: **Zeynep Gulec Koksal, Duygu Erge,** Design: **Zeynep Gulec Koksal, Pinar Uysal,** Data collection or processing: **Zeynep Gulec Koksal, Duygu Erge,** Analysis or Interpretation: **Zeynep Gulec Koksal, Pinar Uysal,** Literature search: **Zeynep Gulec Koksal, Pinar Uysal,** Writing: **Zeynep Gulec Koksal, Pinar Uysal,** Approval: **Zeynep Gulec Koksal, Pinar Uysal.**

REFERENCES

1. Turner PJ, Worm M, Ansotegui IJ, El-Gamal Y, Rivas MF, Fineman S, et al. Time to revisit the definition and clinical criteria for anaphylaxis? World Allergy Organ J 2019;12(10):100066.
2. Grabenhenrich LB, Dölle S, Ruëff F, Renaudin JM, Scherer K, Pföhler C, et al. Epinephrine in severe allergic reactions: the European anaphylaxis register. J Allergy Clin Immunol Pract 2018;6(6):1898-906.e1.
3. Sampson HA, Muñoz-Furlong A, Campbell RL, Adkinson NF, Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: Summary report - Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. J Allergy Clin Immunol 2006;117(2):391-7.
4. Simons FE. 9. Anaphylaxis. J Allergy Clin Immunol 2008;121(2 Suppl):S402-7; quiz S420.
5. Giavina-Bianchi P, Aun MV, Kalil J. Drug-induced anaphylaxis: is it an epidemic? Curr Opin Allergy Clin Immunol 2018;18(1):59-65.
6. Dinakar C. Anaphylaxis in children: current understanding and key issues in diagnosis and treatment. Curr Allergy Asthma Rep 2012;12(6):641-9.
7. Vadas P, Perelman B, Liss G. Platelet-activating factor, histamine, and tryptase levels in human anaphylaxis. J Allergy Clin Immunol 2013;131(1):144-9.
8. Shaker MS, Wallace DV, Golden DBK, Oppenheimer J, Bernstein JA, Campbell RL, et al. Anaphylaxis—a 2020 practice parameter update, systematic review, and Grading of Recommendations, Assessment, Development and Evaluation (GRADE) analysis. J Allergy Clin Immunol 2020;145(4):1082-123.
9. Johansson SGO, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol 2004;113(5):832-6.
10. Reber LL, Hernandez JD, Galli SJ. The pathophysiology of anaphylaxis. J Allergy Clin Immunol 2017;140(2):335-48.
11. Francis A, Bosio E, Stone SF, Fatovich DM, Arendts G, Macdonald SPJ, et al. Markers involved in innate immunity and neutrophil activation are elevated during acute human anaphylaxis: Validation of a microarray study. J Innate Immun 2018;11(1):63-73.
12. Francis A, Bosio E, Stone SF, Fatovich DM, Arendts G, Nagree Y, et al. Neutrophil activation during acute human anaphylaxis: analysis of MPO and sCD62L. Clin Exp Allergy 2017;47(3):361-70.
13. Jönsson F, De Chaisemartin L, Granger V, Gouel-Chéron A, Gillis CM, Zhu Q, et al. An IgG-induced neutrophil activation pathway contributes to human drug-induced anaphylaxis. Sci Transl Med 2019;11(500): eaat1479.
14. Kraft S, Kinet JP. New developments in FcεRI regulation, function and inhibition. Nat Rev Immunol 2007;7(5):365-78.
15. Walls AF, He S, Teran LM, Buckley MG, Jung KS, Holgate ST, et al. Granulocyte recruitment by human mast cell tryptase. Int Arch Allergy Immunol 1995;107(1-3):372-3.
16. da Silva EZ, Jamur MC, Oliver C. Mast cell function: a new vision of an old cell. J Histochem Cytochem 2014;62(10):698-738.

17. Anaphylactic shock: IgG antibodies and neutrophils play an unexpected role | Newsroom | Inserm [Internet]. [cited 2020 Sep 4]. Available from: https://presse.inserm.fr/en/anaphylactic-shock-igg-antibodies-and-neutrophils-play-an-unexpected-role/35699/
18. Jönsson F, Mancardi DA, Albanesi M, Bruhns P. Neutrophils in local and systemic antibody-dependent inflammatory and anaphylactic reactions. J Leukoc Biol 2013;94(4):643-56.
19. Muraro A, Roberts G, Worm M, Bilò MB, Brockow K, Fernández Rivas M, et al. Anaphylaxis: Guidelines from the European Academy of Allergy and Clinical Immunology. Allergy 2014;69(8):1026-45.
20. Muraro A, Roberts G, Clark A, Eigenmann PA, Halken S, Lack G, et al. The management of anaphylaxis in childhood: Position paper of the European academy of allergology and clinical immunology. Allergy 2007;62(8):857-71.
21. Gounni AS, Lamkhioued B, Koussih L, Ra C, Renzi PM, Hamid Q. Human neutrophils express the high-affinity receptor for immunoglobulin E (Fc epsilon RI): role in asthma. FASEB J 2001;15(6):940-9.
22. Stone SF, Bosco A, Jones A, Cotterell CL, van Eeden PE, Arendts G, et al. Genomic responses during acute human anaphylaxis are characterized by upregulation of innate inflammatory gene networks. PLoS One 2014;9(7):e101409.
23. Wright HL, Moots RJ, Bucknall RC, Edwards SW. Neutrophil function in inflammation and inflammatory diseases. Rheumatology (Oxford) 2010;49(9):1618-31.
24. Lin RY, Schwartz LB, Curry A, Pesola GR, Knight RJ, Lee HS, et al. Histamine and tryptase levels in patients with acute allergic reactions: An emergency department-based study. J Allergy Clin Immunol 2000;106(1 Pt 1):65-71.
25. Jeon YH, Lee S, Ahn K, Lee SY, Kim KW, Kim HH, et al. Infantile anaphylaxis in Korea: A multicenter retrospective case study. J Korean Med Sci 2019;34(13):1-12.
26. Orhan F, Canitez Y, Bakirtas A, Yilmaz O, Boz AB, Can D, et al. Anaphylaxis in Turkish children: A multi-centre, retrospective, case study. Clin Exp Allergy 2011;41(12):1767-76.
27. Dubus JC, Lê MS, Vitte J, Minodier P, Boutin A, Carsin A, et al. Use of epinephrine in emergency department depends on anaphylaxis severity in children. Eur J Pediatr 2019;178(1):69-75.
28. Triggiani M, Patella V, Staiano RI, Granata F, Marone G. Allergy and the cardiovascular system. Clin Exp Immunol 2008;153(suppl 1):7-11.
29. Mueller UR. Cardiovascular disease and anaphylaxis. Curr Opin Allergy Clin Immunol 2007;7(4):337-41.
30. TenBrook JA, Wolf MP, Hoffman SN, Rosenwasser LJ, Konstam MA, Salem DN, et al. Should β-blockers be given to patients with heart disease and peanut-induced anaphylaxis? A decision analysis. J Allergy Clin Immunol 2004;113(5):977-82.
31. Ruëff F, Przybilla B, Biló MB, Müller U, Scheipl F, Aberer W, et al. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase-a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. J Allergy Clin Immunol 2009;124(5):1047-54.
32. Jerschow E, Lin RY, Scaperotti MM, McGinn AP. Fatal anaphylaxis in the United States, 1999-2010: Temporal patterns and demographic associations. J Allergy Clin Immunol 2014;134(6):1318-28.e7.
33. Simons FER, Frew AJ, Ansotegui IJ, Bochner BS, Golden DBK, Finkelman FD, et al. Risk assessment in anaphylaxis: Current and future approaches. J Allergy Clin Immunol 2007;120(1 Suppl):S2-24.
34. Clark S, Wei W, Rudders SA, Camargo CA. Risk factors for severe anaphylaxis in patients receiving anaphylaxis treatment in US emergency departments and hospitals. J Allergy Clin Immunol 2014;134(5):1125-30.
35. Simons FER, Ardusso LRF, Dimov V, Ebisawa M, El-Gamal YM, Lockey RF, et al. World allergy organization anaphylaxis guidelines: 2013 update of the evidence base. Int Arch Allergy Immunol 2013;162(3):193-204.
36. Harduar-Morano L, Simon MR, Watkins S, Blackmore C. A population-based epidemiologic study of emergency department visits for anaphylaxis in Florida. J Allergy Clin Immunol 2011;128(3):594-600.e1.
37. Chapsa M, Roensch H, Langner M, Beissert S, Bauer A. Predictors of severe anaphylaxis in Hymenoptera venom allergy: The importance of absence of urticaria and angioedema. Ann Allergy Asthma Immunol 2020;125(1):72-7.
38. Grabenhenrich LB, Dölle S, Moneret-Vautrin A, Köhli A, Lange L, Spindler T, et al. Anaphylaxis in children and adolescents: the European anaphylaxis registry. J Allergy Clin Immunol 2016;137(4):1128-37.e1.
39. Lee WS, An J, Jung YH, Jee HM, Chae KY, Park YA, et al. Characteristics and Treatment of Anaphylaxis in Children Visiting a Pediatric Emergency Department in Korea. Biomed Res Int 2020;2020:2014104.
40. Silva R, Gomes E, Cunha L, Falcão H. Anaphylaxis in children: A nine years retrospective study (2001-2009). Allergol Immunopathol (Madr) 2012;40(1):31-6.
41. de Silva IL, Mehr SS, Tey D, Tang ML. Paediatric anaphylaxis: a 5 year retrospective review. Allergy 2008;63(8):1071-6.
42. Kahveci M, Akarsu A, Koken G, Sahiner UM, Soyer O, Sekerel BE. Food-induced anaphylaxis in infants, as compared to toddlers and preschool children in Turkey. Pediatr Allergy Immunol 2020;31(8):954-61.
43. Noimark L, Wales J, Du Toit G, Pastacaldi C, Haddad D, Gardner J, et al. The use of adrenaline autoinjectors by children and teenagers. Clin Exp Allergy 2012;42(2):284-92.