

Original Article



CBC-Derived Inflammatory Indices in Pediatric Acute Gastroenteritis: Association with Stool Culture Status, Disease Severity, and Length of Hospital Stay

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Summary

Introduction: Acute gastroenteritis (AGE) is a leading cause of pediatric hospitalization worldwide. Early identification of pediatric patients at risk for prolonged hospitalization or severe disease remains an important challenge in clinical practice. CBC-derived inflammatory indices are simple, low-cost markers that may support routine clinical assessment and reduce reliance on advanced diagnostic tests.

Methods: This prospective observational cohort study included 385 children hospitalized with acute gastroenteritis. We classified patients as culture-positive or culture-negative based on stool culture results. Disease severity was assessed using the Vesikari scoring system. CBC-derived inflammatory indices, including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), eosinophil-to-lymphocyte ratio (ELR), eosinophil-to-neutrophil ratio (ENR), and mean platelet volume-to-platelet count ratio (MPV/PLT), were calculated. Associations with stool culture status and disease severity were evaluated using group-comparison analyses, whereas associations with hospitalization duration were assessed using correlation analysis and exploratory multivariable linear regression.

Results: Among 385 children, 262 (68.1%) were culture-negative, and 123 (31.9%) were culture-positive. The mean age was 2.79 ± 0.71 years, and the mean hospital stay was 3.11 ± 1.3 days. All inflammatory indices were significantly higher in the culture-negative group ($P < 0.01$). NLR and PLR showed significant inverse correlations with hospitalization duration, while ELR, ENR, and MPV/PLT were not. Most inflammatory indices tended to decrease with increasing disease severity, whereas ENR showed no significant association with severity. In the exploratory multivariable analysis, NLR was the only CBC-derived index that remained significantly associated with hospitalization duration ($\beta = -0.14$, $P = 0.01$).

Conclusion: CBC-derived inflammatory indices, including NLR, were associated with stool culture status and hospitalization duration in pediatric acute gastroenteritis. However, associations with disease severity were limited, and culture-negative status should not be interpreted as confirmed viral etiology in the absence of virologic testing. These findings are exploratory and hypothesis-generating; prospective multicenter studies with formal evaluation of discrimination, calibration, and clinical utility are required before clinical implementation.

Keywords: Acute gastroenteritis, CBC-derived inflammatory indices, Disease severity, Inflammatory biomarkers, Stool culture

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Introduction

Acute gastroenteritis is one of the most common causes of pediatric healthcare visits worldwide and remains a major public health concern during childhood. Recent evidence indicates that diarrheal diseases continue to contribute substantially to morbidity and mortality among children under five years of age, particularly in low- and middle-income countries.¹

A wide range of infectious pathogens, including bacteria, viruses, and parasites, may cause acute gastroenteritis in children.² However, considerable overlap in clinical manifestations often makes differentiation of underlying etiologies difficult based solely on symptoms and physical examination findings. Definitive microbiological diagnosis may require stool culture, antigen detection assays, or

molecular diagnostic techniques such as polymerase chain reaction (PCR), which may be costly, time-consuming, or unavailable in resource-limited settings.

Disease severity in pediatric acute gastroenteritis is commonly assessed using the Vesikari scoring system, a validated clinical tool evaluating diarrhea, vomiting, fever, dehydration, and treatment requirements.³ Nevertheless, simple and accessible biomarkers capable of predicting clinical outcomes and disease progression remain clinically desirable in routine pediatric practice.⁴

In recent years, inflammatory indices derived from complete blood count (CBC) parameters have emerged as inexpensive and readily available indicators of systemic inflammation. Parameters such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio



(PLR), eosinophil-based ratios, and mean platelet volume-to-platelet count ratio (MPV/PLT) may reflect interactions between innate and adaptive immune responses.^{5,6} Previous studies have suggested potential roles for these markers in infectious and inflammatory conditions; however, their diagnostic and prognostic utility in pediatric acute gastroenteritis remains controversial.^{7,8,9}

Given the limited evidence regarding the clinical significance of CBC-derived inflammatory indices in Iranian pediatric populations, the present study aimed to investigate the association between CBC-derived inflammatory indices, stool culture status, disease severity based on the Vesikari score, and hospitalization duration in children with acute gastroenteritis.

Methods

Study design, setting, and participants

This single-center prospective observational cohort study was conducted among children hospitalized with acute gastroenteritis at Amir Kabir Hospital, Arak, Iran, from August 2024 to March 2025. The study protocol was approved by the Ethics Committee of Arak University of Medical Sciences (Ethics Code: IR.ARAKMU.REC.1403.127). Written informed consent was obtained from the parents or legal guardians of all participants before enrollment.

The required sample size was calculated using the standard formula for estimating a single population proportion with predefined absolute precision: $n = Z^2 \times p(1 - p) / d^2$. A two-sided α of 0.05, corresponding to $Z = 1.96$, and an absolute precision of 5% were assumed. Because no reliable local estimate was available for the proportion of culture-positive bacterial gastroenteritis among hospitalized children, the most conservative proportion of 0.50 was used, which maximizes the required sample size. This calculation yielded a minimum required sample size of 385 participants. The primary outcome for this sample-size calculation was the proportion of children with a positive stool culture. Eligible children were enrolled consecutively according to predefined inclusion and exclusion criteria. Participant enrolment and exclusions are summarized in

Figure 1. Because the sample size was calculated to estimate a proportion rather than to detect prespecified differences in CBC-derived inflammatory indices, subgroup, correlation, and regression analyses were regarded as exploratory.

Children aged < 18 years with clinically diagnosed acute gastroenteritis were eligible for inclusion. Acute gastroenteritis was defined as the passage of at least three loose or watery stools within 24 hours, with or without vomiting, fever, or abdominal pain, and a duration of less than 14 days. Patients with chronic inflammatory disorders, autoimmune diseases, hematologic conditions, immunodeficiency disorders, concomitant infections, corticosteroid therapy, or antibiotic use before admission were excluded.

Data collection and study variables

Demographic and clinical data, including age and sex, were recorded at hospital admission. Venous blood samples were obtained at admission and before the initiation of antibiotic therapy for complete blood count (CBC) assessment. All enrolled patients were followed prospectively throughout hospitalization until discharge. Length of hospital stay was defined as the interval from hospital admission to hospital discharge, measured in days. Discharge decisions were made according to routine clinical practice and the clinical judgment of the treating pediatric team, as no prospectively standardized discharge criteria were predefined for the study. The following CBC-derived inflammatory indices were calculated from admission CBC values: neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), eosinophil-to-lymphocyte ratio (ELR), eosinophil-to-neutrophil ratio (ENR), and mean platelet volume-to-platelet count ratio (MPV/PLT). Fresh stool specimens were collected before treatment initiation and processed in the hospital microbiology laboratory using routine culture methods for common bacterial enter pathogens. Culture evaluation included *Salmonella* spp., *Shigella* spp., and diarrheagenic *Escherichia coli*. A stool sample was classified as culture-positive when at least one pathogenic bacterial organism was isolated by routine microbiological culture; samples with no bacterial pathogen isolated were

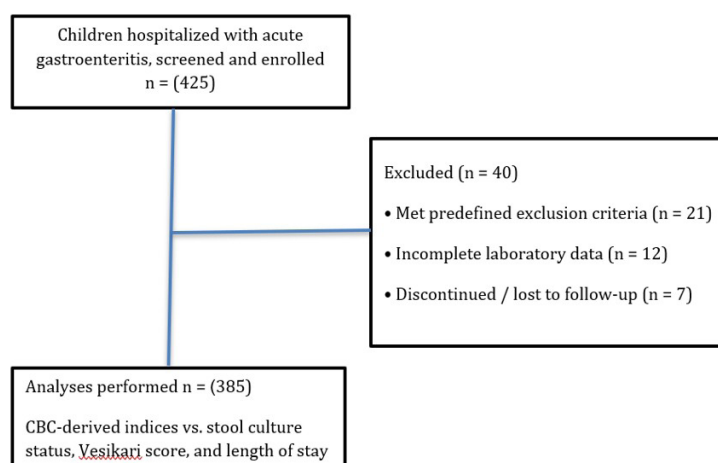


Figure 1. Participants flow diagram

classified as culture-negative. Viral antigen testing and molecular assays were not performed; therefore, culture-negative results were not considered confirmatory of viral gastroenteritis. Patients with positive stool culture results were categorized as having culture-positive bacterial gastroenteritis, whereas those with negative stool culture results were classified as having culture-negative gastroenteritis. This latter category was not presumed to represent a specific etiologic diagnosis.

Disease severity was assessed at the time of enrollment using the Vesikari scoring system, based on the clinical presentation at hospital admission. Vesikari scores were classified as mild for scores < 7, moderate for scores 7–10, and severe for scores ≥ 11. Because a separate dehydration variable was not consistently available, baseline Vesikari score, which incorporates dehydration-related clinical features, was used as the available measure of baseline clinical severity.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 27. The distribution of continuous variables was assessed using visual inspection of histograms and the Shapiro–Wilk test. Continuous variables are presented as mean ± standard deviation (SD) or median (interquartile range [IQR]), as appropriate, whereas categorical variables are presented as frequencies and percentages. Continuous variables with an approximately normal distribution were compared using the independent-samples t-test, whereas non-normally distributed variables were compared using the Mann–Whitney U test. Comparisons across the three Vesikari severity categories were performed using one-way analysis of variance or the Kruskal–Wallis test, as appropriate. Categorical variables were analyzed using the chi-square test or Fisher’s exact test according to expected cell counts. Associations between continuous variables were evaluated using Spearman’s rank correlation coefficient. Potential confounders were selected a priori on the basis of clinical relevance and findings from previous literature, rather than solely according to univariable statistical significance. The prespecified covariates were age, sex, baseline disease severity assessed using the Vesikari score, and stool culture status. Because the CBC-derived inflammatory indices are mathematically related and could show substantial multicollinearity, the five indices were not entered simultaneously into a single regression model. Instead, separate multivariable regression models were constructed for each inflammatory index.

Multivariable linear regression analyses were performed to explore the association between CBC-derived inflammatory indices and length of hospital stay. Each inflammatory index was evaluated separately as the primary independent variable and adjusted for age, sex, baseline disease severity assessed using the Vesikari score, and stool culture status. The CBC-derived indices were not entered simultaneously into a single model because of their mathematical interrelationships and shared CBC components. The regression findings were considered exploratory, and residual confounding could not be

excluded.

Disease severity was assessed according to the Vesikari score and categorized as mild, moderate, or severe. Because complete model output and diagnostic information for ordinal regression were not available for verification, ordinal logistic regression was not included in the final analysis. Accordingly, no adjusted associations between individual CBC-derived inflammatory indices and disease-severity categories were inferred. All statistical tests were two-sided, and a P value < 0.05 was considered statistically significant.

Results

A total of 425 children hospitalized with acute gastroenteritis were initially enrolled between August 2024 and March 2025. Of these, 40 participants were excluded from the final analysis: 21 met one or more predefined exclusion criteria, 12 had incomplete laboratory data, and 7 discontinued participation or were lost to follow-up. The participant flow is shown in Figure 1.

A total of 385 children with acute gastroenteritis were included in the analysis, comprising 224 (58.2%) males and 161 (41.8%) females. Based on routine stool culture results, 262 patients (68.1%) were classified as culture-negative and 123 (31.9%) as culture-positive. The mean age was 2.79 ± 0.71 years, and the mean duration of hospitalization was 3.11 ± 1.3 days.

Comparison between the culture-negative and culture-positive groups showed significantly higher values of NLR, PLR, ELR, ENR, and MPV/PLT in culture-negative patients (all $P < 0.01$) (Table 1). Spearman correlation analysis demonstrated significant inverse correlations between hospitalization duration and NLR and PLR (all $P < 0.01$). In contrast, ELR, ENR, and MPV/PLT were not significantly correlated with hospitalization duration (Table 2).

According to the Vesikari score classification, 77 patients (20.0%) had mild disease, 170 (44.2%) had moderate disease, and 138 (35.8%) had severe disease. Most CBC-derived inflammatory indices differed significantly across Vesikari disease-severity categories, with generally lower values observed in the moderate and severe groups than in the mild group. ENR was not significantly associated with disease severity (Table 1).

In exploratory multivariable linear regression analyses, each CBC-derived inflammatory index was evaluated separately after adjustment for age, sex, baseline disease severity assessed using the Vesikari score, and stool culture status. NLR remained significantly associated with hospitalization duration (standardized $\beta = -0.14$, $P = 0.01$), whereas the other evaluated indices did not show statistically significant associations. Given the exploratory nature of the analysis and the potential for residual confounding and interrelationships among CBC-derived indices, this finding was interpreted cautiously.

Discussion

The present study demonstrated significant associations between CBC-derived inflammatory indices and stool culture status, disease severity, and hospitalization

Table 1. Comparison of Hematological Ratios According to Vesikari Severity Score and Stool Culture Status in Pediatric Patients with Gastroenteritis

	Vesikari scoring			P value	Stool Culture Status		P value
	Mild (N=77)	Moderate (N=170)	Severe (N=138)		Negative Culture (N=262)	Positive Culture (N=123)	
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD		Mean $\pm$ SD	Mean $\pm$ SD	
NLR	2.83 $\pm$ 1.01	1.92 $\pm$ 0.99	1.90 $\pm$ 0.99	0.001	2.27 $\pm$ 1.07	1.72 $\pm$ 0.93	<0.01
PLR	162.84 $\pm$ 47.59	80.68 $\pm$ 35.58	88.83 $\pm$ 45.01	0.001	114.15 $\pm$ 58.10	69.96 $\pm$ 5.68	<0.01
ELR	0.079 $\pm$ 0.011	0.080 $\pm$ 0.011	0.068 $\pm$ 0.015	0.001	0.0796 $\pm$ 0.011	0.060 $\pm$ 0.020	<0.01
ENR	0.059 $\pm$ 0.005	0.060 $\pm$ 0.005	0.044 $\pm$ 0.011	0.181	0.059 $\pm$ 0.005	0.043 $\pm$ 0.011	<0.01
MPV/PLT	0.024 $\pm$ 0.008	0.018 $\pm$ 0.005	0.018 $\pm$ 0.006	0.001	0.0207 $\pm$ 0.007	0.0171 $\pm$ 0.004	<0.01

P-values for comparisons across Vesikari severity categories were obtained using one-way ANOVA or Kruskal–Wallis test, as appropriate. P-values for stool culture group comparisons were obtained using the independent-samples t-test or Mann–Whitney U test, as appropriate. NLR: Neutrophil-to-Lymphocyte Ratio; PLR: Platelet-to-Lymphocyte Ratio; ELR: Eosinophil-to-Lymphocyte Ratio; ENR: Eosinophil-to-Neutrophil Ratio; MPV/PLT: Mean Platelet Volume-to-Platelet Count Ratio; SD: Standard Deviation

Table 2. Correlation and Regression Analysis of Hematological Ratios with Length of Hospital Stay in Pediatric Patients with Gastroenteritis

	Correlation with Length of Hospital Stay			Regression model on length of hospital stays			
	Mean $\pm$ SD	Correlation Coefficient	P value	Regression Coefficient	CI 95%	Standardized Coefficient	P value
NLR	2.09 $\pm$ 1.08	-0.21	0.001	-0.165	-0.290 to -0.040	-0.14	0.01
PLR	100.04 $\pm$ 52.25	-0.208	<0.001	-0.001	-0.004 to 0.002	-0.04	0.52
ELR	0.075 $\pm$ 0.014	0.04	0.36	9.95	-2.94 to 22.84	0.11	0.13
ENR	0.054 $\pm$ 0.011	-0.02	0.65	-6.81	-24.52 to 10.90	-0.06	0.45
MPV/PLT	0.0196 $\pm$ 0.007	-0.093	0.07	2.11	-18.43 to 22.65	0.01	0.84

Correlation coefficients represent Spearman's rank correlation coefficients. Regression coefficients represent unstandardized coefficients, with 95% confidence intervals, derived from separate multivariable linear regression models for each CBC-derived index, adjusted for age, sex, baseline Vesikari score, and stool culture status. NLR: Neutrophil-to-Lymphocyte Ratio; PLR: Platelet-to-Lymphocyte Ratio; ELR: Eosinophil-to-Lymphocyte Ratio; ENR: Eosinophil-t -Neutrophil Ratio; MPV/PLT: Mean Platelet Volume-to-Platelet Count Ratio; SD: Standard Deviation

duration. In children with acute gastroenteritis, all evaluated CBC-derived inflammatory indices showed significantly higher values in the culture-negative group, while several indices were also associated with disease severity and hospitalization duration. These findings suggest that CBC-derived indices may provide potentially useful information during the initial clinical assessment of pediatric acute gastroenteritis; however, their associations should be interpreted in conjunction with clinical findings and other laboratory parameters rather than as standalone diagnostic or prognostic markers.^{5,7}

The predominance of culture-negative gastroenteritis observed in this cohort is generally consistent with previous epidemiological studies showing that many pediatric gastroenteritis cases are not associated with positive bacterial stool cultures.^{1,2} However, culture-negative stool results do not necessarily indicate a viral etiology. Some bacterial pathogens may remain undetected because of limited stool culture sensitivity, timing or quality of specimen collection, fastidious organisms, or the absence of molecular diagnostic methods. Therefore, the present findings should not be interpreted as evidence that culture-negative cases were predominantly viral.

Previous studies have reported elevated NLR and PLR in severe or systemic bacterial infections, including bacteremia^{7,10,11}. In contrast, these indices were higher in the culture-negative group in our study. This difference may reflect the distinct inflammatory characteristics of acute gastrointestinal infections compared with systemic or invasive bacterial infections. Nevertheless, because CBC-derived indices are indirect markers of systemic

inflammatory responses, their interpretation should remain cautious and should incorporate other clinical and laboratory findings. The observed pattern may also reflect differences in host response, disease stage, hydration status, or the spectrum of pathogens detected by routine stool culture.

Furthermore, NLR demonstrated an inverse relationship with disease severity and hospitalization duration in our cohort, while PLR showed a decreasing trend across severity categories. These findings differ from those reported in a retrospective study of 456 children with rotavirus gastroenteritis, in which NLR increased with increasing disease severity and severe cases had longer hospitalization durations. In that study, PLR was also higher in severe disease, although it did not show significant discriminatory performance in ROC analysis. The discrepancy between these findings and our results may reflect differences in etiologic composition, disease spectrum, timing of laboratory assessment, hydration status, and host immune response. Importantly, our cohort was not restricted to rotavirus infection, and stool culture-negative status could not be considered evidence of a viral etiology. Therefore, the observed inverse relationship between NLR and disease severity in our study should be interpreted cautiously and requires confirmation in larger, etiologically characterized pediatric cohorts.^{3,12} Potential explanations include differences in age-dependent immune responses, hydration status, nutritional status, pathogen spectrum, and the timing of laboratory evaluation relative to disease progression. One possible, but unconfirmed, explanation for the higher NLR values observed in culture-

negative gastroenteritis is transient lymphopenia associated with viral infection. Viral infections can alter circulating leukocyte profiles and potentially influence derived inflammatory ratios. However, because viral antigen testing or molecular pathogen detection was not performed in the present study, culture-negative status cannot be considered a proxy for viral gastroenteritis. Accordingly, this proposed mechanism remains hypothetical and requires confirmation in future studies incorporating pathogen-specific molecular or antigen-based testing.

Among the evaluated parameters, NLR was the only index that remained significantly associated with hospitalization duration in the exploratory multivariable analysis. However, this finding should be interpreted cautiously because the evaluated CBC-derived indices are mathematically interrelated and share common hematological components. For example, NLR and PLR share lymphocyte count in their denominators, while ELR and ENR are also mathematically related through their shared cellular components. Such interrelationships may introduce multicollinearity when multiple derived indices are considered simultaneously and may affect the stability and interpretation of individual regression coefficients. Therefore, the observed association between NLR and hospitalization duration should not be interpreted as definitive evidence of an independent clinical effect.

Regarding disease severity, most CBC-derived indices showed decreasing values across increasing Vesikari severity categories in the present cohort. However, these observed associations should be interpreted cautiously, as the present analysis does not establish an independent or adjusted effect of any individual index on disease severity. The multifactorial nature of acute gastroenteritis severity, which may be influenced by hydration status, nutritional condition, age, and pathogen-related factors, should therefore be considered.^{3,12,13} Additional biomarkers, such as interleukin-6 and interleukin-8, may provide complementary information for prognostic stratification in future studies.^{14,15} Overall, the present findings support further investigation of CBC-derived inflammatory indices as readily available markers associated with clinical characteristics of pediatric acute gastroenteritis, while their diagnostic, prognostic, and clinical utility should be established in prospective studies incorporating comprehensive pathogen detection and validated predictive analyses.

This study has several strengths, including the prospective cohort design, the simultaneous evaluation of multiple readily available CBC-derived inflammatory indices, a relatively adequate sample size, and the assessment of clinically relevant outcomes, including stool culture status, disease severity, and length of hospital stay.

Multivariable regression analyses were adjusted for prespecified covariates, including age, sex, baseline disease severity (Vesikari score), and stool culture status. These variables were selected a priori as clinically relevant potential confounders because they could influence both CBC-derived inflammatory indices and clinical outcomes.

Several limitations should be acknowledged. First, the single-center design may limit the generalizability of the findings. Second, residual confounding remains possible because some clinical and socioeconomic factors, including an independently documented measure of dehydration, were unavailable. Third, etiologic classification relied on routine stool culture without molecular or viral testing; therefore, culture-negative cases could not be definitively classified as viral gastroenteritis. Fourth, specific inflammatory biomarkers were not measured, precluding direct comparison with established inflammatory markers. Fifth, associations with disease severity were based on unadjusted comparisons, and the mathematical overlap among CBC-derived indices may introduce multicollinearity and affect coefficient interpretation. Sixth, multivariable linear regression was used for exploratory analysis; however, hospitalization duration is a positive outcome with potential right-skewness, and complete residual diagnostic information was not available for independent verification. Therefore, the observed associations between CBC-derived indices and hospitalization duration, including the association with NLR, should be considered exploratory and confirmed using appropriate distribution-based models in future studies. Finally, the study was not designed to assess predictive performance or clinical utility, and exclusion due to incomplete data may have introduced selection bias. Larger multicenter studies with comprehensive etiologic testing and appropriate multivariable modeling are warranted.

Conclusion

CBC-derived inflammatory indices, including NLR, were associated with stool culture status and hospitalization duration in children with acute gastroenteritis. However, their associations with disease severity were limited, and these indices should not be used as standalone diagnostic or prognostic tools. Given their low cost and wide availability, they may warrant further investigation as adjunctive markers for early clinical assessment. Multicenter prospective studies incorporating molecular pathogen detection and formal evaluation of predictive performance are needed before their clinical utility can be established.

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Artificial Intelligence Use Disclosure

ChatGPT was used for language editing, including correction of grammar, spelling, punctuation, and clarity.

Authors' Contribution

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Competing Interests

The authors declare that they have no competing interests.

Ethical Approval

The study protocol was approved by the Ethics Committee of Arak University of Medical Sciences, Arak, Iran (Ethics Code: IR.ARAKMU.REC.1403.127). Written informed consent was obtained from the parents or legal guardians of all participants before enrollment. The study was conducted in accordance with the principles of the Declaration of Helsinki.

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