The role of immature granulocyte in the early prediction of acute perforated and nonperforated appendicitis in children

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ABSTRACT

BACKGROUND: Acute appendicitis (AA) is the most common reason for pediatric abdominal surgery in the world. Despite advances in science and technology, diagnosing AA is still difficult today, and complications are common as a result. The early prediction of complicated appendicitis is of great importance for the surgical planning, further treatments, and predicting the course of disease. The immature granulocyte (IG) is a new and more effective marker in predicting the severity of inflammation than traditional markers. Our aim is to determine the effectiveness of IG% in the diagnosis and severity of AA.

METHODS: Eighty-eight patients diagnosed with AA and a control group of fifty-eight healthy children were included in this prospective study. Patients with pathologically confirmed AA were divided into two subgroups: acute simple appendicitis (ASA) and acute perforated appendicitis (APA). The demographic characteristics, white blood cell (WBC) count, neutrophil-to-lymphocyte ratio (NLR), mean platelet volume (MPV), IG%, and C-reactive protein (CRP) values were analyzed. Receiver operating characteristics (ROC) analysis was used to compare the diagnostic accuracies and predictive performances.

RESULTS: Patients with AA had higher IG%, WBC count, NLR, and MPV value than control group (p=0.28, p=0.22, p<0.001, p=0.001, respectively). Only IG% showed statistically significant difference from other inflammatory markers evaluated in ASA and APA patients (p<0.001). ROC analysis showed that IG% is a good predictor for the presence of APA at an optimal cut-off for IG being 0.2% (sensitivity 81.8%, specificity 85.2%, area under the ROC curve 0.83).

CONCLUSION: In the present study, we demonstrated that AA patients with higher IG levels might be more likely to develop perforation. The IG values combined with a physical examination, imaging studies, and other laboratory tests may help clinicians to identify high-risk AA patients in the pediatric emergency department.

Keywords: Appendicitis; children; immature granulocytes.

INTRODUCTION

Acute appendicitis (AA) is the most common cause of abdominal surgical emergencies that present at the pediatric emergency department.¹ Early diagnosis of AA remains challenging due to atypical clinical features and the difficulty of obtaining a reliable history and physical examination.² Despite the development of diagnostic advances, approximately 30% of patients, in particular children under the age of 5 years, are revealed to have perforation at diagnosis, and 28–57% of older children present with missed and delayed diagnosis. Delay in the diagnosis and surgery for AA may lead to complications associated with perforation, gangrene, and intraabdominal abscess formation. Appendiceal perforation is associated with increased morbidity and mortality compared with non-perforating. It is difficult to make a correct diagnosis of AA due to the nonspecificity of symptoms and lack of specific biomarkers.³⁴ Although studies have been conducted on many markers such as white blood cell (WBC) count, platelet (PLT) count, neutrophil-to-lymphocyte ratio (NLR), mean platelet volume (MPV), C-reactive protein (CRP), procalcitonin, calprotectin for the diagnosis and prognosis of AA,
there is no definite marker yet. Therefore, biomarkers are needed for the early diagnosis of AA and preventing complications of AA.[1–8]

The immature granulocyte (IG) is a new inflammation marker that is not adequately known by most clinicians. The IG is a fast, easily available, and inexpensive parameter. The detection of IGs in peripheral blood, which do not normally occur in healthy people, is an indicator of a bone marrow activation and severe infection. An elevated IG% implies the enhancement of bone marrow activity to fight against sources of infections before leukocytosis is occurred. Recent studies have shown that IG% is a more effective marker in predicting the severity of inflammation than traditional markers such as the WBC count, CRP, and NLR.[9–12] The aim of this study was to demonstrate the role of IG%, which is a new inflammation marker, in both early diagnosing AA and discriminating between ASA and acute complicated appendicitis (ACA).

MATERIALS AND METHODS

This is a prospective case-control study which is conducted between January and December 2019. Demographic characteristics, clinical, laboratory, and radiological findings of the patients were recorded. The pediatric appendicitis score (PAS) of the entire patient group was ≥7.[13] Ultrasound (US) was performed by a radiologist in all patients with suspected AA, and computed tomography (CT) was performed in cases that could not be detected by the US. The final diagnosis of appendicitis was determined by pathology and operation reports. Patients with appendicitis (pathologically confirmed) were divided into subgroups: Acute simple appendicitis (ASA) and acute perforated appendicitis (APA). Patients with any infectious disease, chronic systemic disease, cardiovascular disease, ischemic disease, chronic gastrointestinal disease, trauma, who had negative appendicitis surgery, who had not undergone appendicitis surgery and followed-up only, and those older than 18 years were not included in the study. The control group comprised 58 healthy children (with similar age/gender) presented to pediatric general policlinics for routine health control or vaccination. The study was performed in accordance with the guidelines of the Declaration of Helsinki, and written informed consent was obtained from all patients before participating in the study. The study was approved by the Ethics Committee.

Biochemical Analysis

Preoperative blood samples were taken from the patients, who were strongly suspected to have AA after physical examination, radiological imaging (USG, CT), and clinical history, and after being evaluated by the pediatric surgery. IG, WBC, NLR, MPV parameters were investigated in CBC. CBC parameters (IG%, WBC count, NLR, PLT count, MPV) were measured using an automated by the Sysmex XE 2100 automated hematologic analyzer and CRP analysis with Cobas Integra 800. The IG measurement includes promyelocytes, myelocytes, and metamyelocytes, but not bands or blasts. It was performed in differential channels of the analyzer. IG values are routinely measured in the laboratory of our university. Therefore, we did not receive any financial support from any institution or organization for the study. Imaging methods were done by a same pediatric radiologist. The informed consent form was filled in by informing the participants about the study. The ethical aspects were respected and the research was approved by the ethics and research committee (Decision number:2019/512, Date: 10.07.2019).

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA. Released 2013). The Shapiro–Wilks test was used to evaluate the normality of the parametric data. Numerical variables are presented as mean±SD or median (Q1–Q3). The Mann–Whitney U-test was used to compare non-normally distributed groups. Comparisons between groups for data that did not show a normal distribution were performed using the Kruskal–Wallis test. The receiver operating characteristic (ROC) curve was used to evaluate the optimal cutoff points for parameters for which significant differences were found. The Youden J index was used to estimate the best cutoff values. Sensitivity, specificity, cutoff points, negative predictive value (NPV), positive predictive value (PPV), and the area under the ROC curve (AUC) were calculated for these parameters. The results are reported with 95% confidence intervals. Values of p<0.05 and AUC >0.600 were considered statistically significant.

RESULTS

Participants included 88 patients who were admitted to the pediatric emergency department between January and December 2019 and were diagnosed with AA based on pathology. The control group included 58 healthy children similar to the patients in terms of age and sex. Of the 88 patients, 77 had ASA, and 11 had ACA. The median age of the patients was 12 (9–17) years, and that of the control group was 11 (10–14) years. Of the 88 patients, 56 (65.9%) were male and 32 (34.1%) were female. Of the control group, 31 (53.4%) were male and 27 (46.6%) were female. The patient and control groups did not differ significantly in terms of age or sex (p=0.22, p=0.39, respectively) or in terms of WBC count and IG% values (p=0.25, p=0.28, respectively). The NLR and MPV values of the patient group was significantly higher than that of the control group (p<0.001, p=0.001, respectively) (Table 1). There was no statistically significant difference between ASA and APA in terms of age, sex, WBC count, MPV value, NLR, and CRP value (p>0.05). Only IG% was statistically significantly higher in APA group (p=0.001) (Table 2). The ability of the biomarkers (IG, WBC, NLR, MPV) to discriminate between AA patients and healthy controls were evaluated by
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ROC curve analysis, which yielded AUC values of 0.55 for IG, 0.54 for WBC, 0.71 for NLR, and 0.65 for MPV. The ROC curve showed significant fair sensitivity and high specificity for NLR among patients with AA (61.6%, 88.5%, respectively) (Table 3 and Fig. 1).

In ROC curve analysis, IG, WBC, NLR, MPV, and CRP turned out to have a predictive value for appendiceal perforation. However, the predictive value of WBC, NLR, MPV, and CRP for appendiceal perforation was fair (AUC: 0.61, 0.51, 0.58, 0.62, respectively). The AUC of the IG was the highest among all assessed markers (Fig. 2). The AUC for the ability of the IG to predict the presence of APA was 0.83. Best cut-off value of IG% was ≥0.2 with a sensitivity of 81.8% and a specificity of 85.2%, NPV of 97.4%, and PPV of 40.9% (Table 4).

### Table 1. Demographic and clinical data for patients with acute appendicitis and control group

|                  | Patients | Control | p   |
|------------------|----------|---------|-----|
| Age (year)       | 12 (9–17)| 11 (10–14) | 0.22|
| WBC (10³/µL)     | 12.5 (9.4–18.4) | 9.81 (8.80–16.52) | 0.25|
| MPV (fL)         | 9.8 (9.3–10.4) | 9.4 (8.4–10.15) | 0.001|
| NLR              | 5.02 (2.38–10.55) | 2.5 (1.8–4.0) | <0.001|
| IG (%)           | 0.06 (0.03–0.15) | 0.03 (0.02–0.25) | 0.28|

WBC: White blood cell; MPV: Mean platelet volume; NLR: Neutrophil-to-lymphocyte ratio; IG: Immature granulocyte.

### Table 2. The comparison of demographic data and laboratory findings between acute simple and perforated appendicitis groups

|                  | ASA     | APA     | p   |
|------------------|---------|---------|-----|
| Age (year)       | 12 (9–17)| 12 (10–13) | 0.78|
| Sex, male (%)    | 50 (59) | 6 (54.5) | 0.41|
| WBC (10³/µL)     | 12.2 (9.25–18.41) | 9.8 (7.4–14.2) | 0.13|
| MPV (fL)         | 9.7 (9.3–10.4) | 9.8 (9.4–10.7) | 0.36|
| NLR              | 5.02 (2.78–10.29) | 8.66 (1.97–12.7) | 0.89|
| CRP (mg/L)       | 14.12 (7.69–21.87) | 20.24 (16.1–34.8) | 0.23|
| IG (%)           | 0.04 (0.02–0.11) | 0.3 (0.2–0.5) | 0.001|

ASA: Acute simple appendicitis; APA: Acute perforated appendicitis; WBC: White blood cell; MPV: Mean platelet volume; NLR: Neutrophil-to-lymphocyte ratio; CRP: C-reactive protein; IG: Immature granulocyte.

### Table 3. ROC analysis of inflammatory markers for discrimination between acute appendicitis group and control group

|                  | Cut-off | Sensitivity % | Specificity % | AUC | 95% CI         | p   |
|------------------|---------|---------------|---------------|-----|----------------|-----|
| IG (%)           | >0.03   | 69.7          | 55.7          | 0.55| 0.46–0.62      | 0.31|
| WBC (10³/µL)     | >9.78   | 59.3          | 51.1          | 0.54| 0.46–0.62      | 0.22|
| NLR              | >4.5    | 61.6          | 88.5          | 0.71| 0.64–0.78      | <0.001|
| MPV (fL)         | >8.6    | 98.0          | 32.8          | 0.65| 0.57–0.72      | 0.001|

IG: Immature granulocyte; WBC: White blood cell; MPV: Mean platelet volume; NLR: Neutrophil-to-lymphocyte ratio; CRP: C-reactive protein; AUC: Area under the curve; CI: Confidence interval; ROC: Receiver operating characteristic.
DISCUSSION

AA is the most common reason for pediatric abdominal surgery in the world. Laboratory tests, scoring systems and imaging methods are used in AA diagnosis, as well as the clinical history and physical examination. Despite all of the developments in diagnosis and treatment methods, high perforation rates have still been reported. ACA is a serious problem that increases the risk of postoperative complications, delays recovery, and increases the length of hospital stay and medical costs. Early diagnosis of AA is important to prevent the risk of complications such as abscess, perforation, and gangrene formation. The diagnosis of AA can be difficult due to the absence of a pathognomonic symptoms or sign and the poor predictive value of associated laboratory testing. Therefore, new and specific biomarkers are needed and researches are being conducted on this subject.

Although a careful abdominal examination is key in the diagnosis of pediatric appendicitis, clinicians can develop diagnostic tools using auxiliary laboratory parameters for final decision. We intended to evaluate the laboratory values that aided us the most in diagnosing and prognosing AA in our pediatric patients and assessed whether IG% would add additional diagnostic benefit in these patients.

Inflammation plays an important role in the pathology of AA. Laboratory indicators that have been associated with AA include WBC count, NLR, left shift, and elevated markers of inflammation such as CRP and erythrocyte sedimentation rate. The complete blood count (CBC) is the most frequently used and easily found baseline hematological parameter in clinical laboratories. The parameters of leukocyte count, neutrophil percentage, thrombocyte count, MPV, NLR, red cell distribution width, platelet-to-lymphocyte ratio (PLR), and platelet distribution width have been studied for use in the diagnosis and prediction of AA. However, their role in the diagnosis of AA has yielded diverse and controversial results. Several reports have suggested that elevated WBC count is typically the first laboratory measure to indicate inflammation of the appendix, and most patients with AA present with leukocytosis. However, an elevated WBC count has a low predictive value because the WBC is also elevated in up to 70% of patients with other causes of right lower abdominal quadrant pain. However, the use of VBC is often to support suspected clinical situations and is neither sensitive nor specific for the diagnosis of AA. Similar to the literature, in our study, the WBC count was higher in the patient group compared to the healthy group, but there was no significant difference. Systemic inflammatory response can also cause neutrophilia and lymphocytopenia, resulting in an increase in the NLR a sign of inflammation in AA. The physiological response of leukocytes in circulation to stress leads to an increase in neutrophil numbers and a decrease in lymphocyte numbers. Thus, NLR is used as a parameter of inflammation.

In a study it was determined that NLR was a useful parameter for diagnosing AA and discriminating between ASA and ACA. Yazici et al. found that a NLR of 3.5 may be considered as a diagnostic cut-off value in children with AA. Celik et al. found that NLR had a better AUC (0.717) compared with PLR, neutrophil percentage or WBC for predicting complicated/severe appendicitis and a reasonable sensitivity and specificity. However, according to other studies, NLR is not more sensitive or specific than that of WBC or CRP. In our study, NLR was significantly higher in the patient group than in the healthy group (p<0.001). In addition, NLR was higher in the ACA group compared to the ASA group, but there was no significant difference. NLR above 4.5 for the diagnosis of AA shows high specificity (88.5%) and low sensitivity (61.6%). Since there are very variable results about NLR for the diagnosis of AA, larger studies are needed to use it as a diagnostic marker for AA.

Many thrombocyte markers, including MPV, have been related to thrombosis and inflammation. It is known that thrombocytes have proinflammatory activities thanks to the bioactive molecules in their granules, and these molecules are rapidly secreted by the activation of thrombocytes. In some studies in which MPV was tested as a simple inflammatory marker, MPV was reported to have been affected by inflammation, and that it increases significantly in sepsis and some inflammatory diseases. In our study, MPV values were significantly higher in the patient group compared to the healthy group (p<0.001), but there was no significant difference between the APA and ASA groups (p=0.36). Few studies found no

### Table 4. ROC results of inflammatory markers for discrimination of acute complicated from uncomplicated appendicitis

| Cut-off     | Sensitivity % | Specificity % | AUC  | 95% CI     | p     |
|-------------|--------------|---------------|------|-----------|-------|
| IG (%)      | 81.8         | 85.2          | 0.83 | 0.75–0.90 | <0.001|
| WBC (10³/µL)| >15.46       | 100           | 0.61 | 0.51–0.73 | 0.08  |
| NLR         | >8.6         | 54.5          | 0.51 | 0.40–0.61 | 0.91  |
| MPV (fL)    | >9.1         | 100.0         | 0.58 | 0.47–0.68 | 0.33  |
| CRP (mg/L)  | >18.3        | 71.1          | 0.62 | 0.54–0.73 | 0.12  |

IG: Immature granulocyte; WBC: White blood cell; MPV: Mean platelet volume; NLR: Neutrophil-to-lymphocyte ratio; CRP: C-reactive protein; AUC: Area under the curve; CI: Confidence interval; ROC: Receiver operating characteristic.
significant difference between the appendicitis cases (complicated or uncomplicated) and non-appendicitis cases with respect to MPV levels.\textsuperscript{[24,28,29]} Another study found a significantly higher MPV level in cases with AA compared to the control group.\textsuperscript{[30]} Another study found the MPV values were significantly lower in the AA group compared to the control group \((p<0.001)\). These variable results suggest that MPV is not a reliable marker in the diagnosis of AA.

CRP is an acute-phase protein and can be used as a marker and diagnostic tool in some inflammations and attacks of some disorders.\textsuperscript{[31]} A large body of studies has shown that CRP levels increase in parallel with the severity of the inflammatory response in AA. Among various inflammatory markers, CRP is a well-known biomarker for predicting complicated appendicitis because its level begins to rise 8–12 h after the initiation of the inflammation process and maintains for 24–48 h.\textsuperscript{[19,32]} A study found that there was not any definite level of CRP that will diagnose AA, but its elevated level may suggest abscess formation in AA cases.\textsuperscript{[19]} One study reported that CRP level was higher in perforated appendix compared to normal appendix, but there were no significant differences between uncomplicated appendicitis and non-appendicitis cases.\textsuperscript{[32]} In our study, the CRP value of the APA group was higher than the ASA group, but it was not statistically significant \((p=0.23)\). The reported sensitivities and specificities for markers (CRP, MPV, NLR, WBC count) are highly variable and cannot be independently relied upon to accurately exclude, confirm, or differentiate between acute and perforated appendicitis accurately.\textsuperscript{[33,34]}

IG is generated and differentiated in bone marrow, and their presence in peripheral granulocytes circulation indicates greatly increased bone marrow activation due to an infectious condition. Therefore, it is suggested that IG can be considered as a new early diagnosis and prognostic marker in infectious diseases. In recent years, it has been possible to detect the percentage and number of IG due to technical developments in automated hematological analyzers. Studies have shown that IG count and percentage (IG\%\bigtextsuperscript{)}\ are significantly increased in sepsis and infections when compared with healthy individuals.\textsuperscript{[9–12,32]} Pavare et al.\textsuperscript{[36]} found that IG percentage is a useful early predictor for the severity of bacterial infection. Senthilnayagam et al.\textsuperscript{[9]} found that IG percentage of blood culture positive children patients were significantly higher than in culture-negative patients. In a study, the IG\% as a routinely obtained marker appeared to be a promising, independent biomarker and was a better predictor of early prognosis in severe acute pancreatitis.\textsuperscript{[33]} IG\% is elevated in sepsis and is associated with bloodstream infections, but there are few studies that investigated the association of IG with AA in children.\textsuperscript{[37–39]} Shin et al.\textsuperscript{[37]} found that the delta neutrophil index (DNI), the IG fraction in circulation, was a reliable marker in discriminating between acute non-perforated appendicitis and APA among elderly individuals. Receiver operator characteristic curve analysis showed that DNI is a good predictor for the presence of appendiceal perforation at an optimal cut-off for DNI being 1.4\% (sensitivity 67.7\%, specificity 90.0\%, AUC 0.807). Mathews et al.\textsuperscript{[38]} reported that IG\% can distinguish perforated appendicitis in pediatric patients but that it has no additional benefit for detecting perforated appendicitis when combined with classic inflammatory markers, including WBC count, a left shift in neutrophils, and CRP. In another study the DNI was significantly higher in the ACA group than in the ASA, and the DNI was an independent predictor of ACA in adults. They showed that a DNI >2 could be a reliable parameter for AA and DNI >6 could identify ACA.\textsuperscript{[39]} In a study the designed by Ünal. IG\% had a greater ability to predict ACA than the other parameters (AUROC: 0.979, sensitivity: 94.4\%, specificity: 97.9\%).\textsuperscript{[32]}

In our study, the IG level was higher in the AA group compared to the healthy group, but it was not statistically significant \((p=0.28)\). According to us, this means that the appendicitis, one of local inflammation, do not induce differentiation or proliferation of IG in the early stages. Additionally, the IG\% was statistically significantly higher in the ACA group than in the ASA group. In ROC curve analysis, IG turned out to be a good predictor of appendiceal perforation and had largest AUC among studied parameters (AUC: 0.83, sensitivity: 81.8\%, specificity: 85.2\%, \(p<0.001\)). Furthermore, we found out that IG\% >0.2 is a reliable predictor of appendiceal perforation among in children. This situation can be explained by the increase of IG in the circulation with the progression of the disease due to the inflammation of the appendix and peri-appendix structures as a result of perforation.

Our study had some limitations. Conducting in a single center was the main limitation of our study. That may limit the generalization of the results. It would be more interesting if we included patients who had abdominal pain in the right lower quadrant but did not develop appendicitis.

**Conclusion**

Our study revealed that high IG levels might help identify those who are more likely to develop complications in patients with AA. An IG\% value of >0.2 in routine CBC in a patient who is with suspected AA may be an important and early indicator of APA. Moreover, these parameters are not expensive to measure, are easily available, and the short time required for analysis is valuable in the emergency department. We recommend that clinicians use the IG values combined with the results of a physical examination, imaging studies, and other laboratory tests to help identify high-risk AA patients in the emergency department.

**Ethics Committee Approval:** This study was approved by the Erciyes University Clinical Research Ethics Committee. (Date: 10.07.2019, Decision No: 219/512).

**Peer-review:** Internally peer-reviewed.
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Çocuklardaki akut perfore ve nonperfore apandisitin erken tahmininde immatür granülositin rolü

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AMAC: Dünyada çocuklardaki abdominal cerrahinin en sık nedeni akut apandisittir (AA). Bilim ve teknolojideki gelişmelerle rağmen günümüzde hala AA teşhisi zordur ve sonuçta komplikasyonlar da sıkıt. Komplike apandisitin erken tespit edilmesi, cerrahi planlanması, sonraki tedaviler ve hastalığın seyri tahmin etmede büyük önem taşmaktadır. İmmatür granülosit (IG), enfiamasyon ciddiyetini tahmin etmede geleneksel enfjamasyon belirteçlerine göre yeni ve daha etkin bir belirteçtir. Amacımız AA tanı ve şiddetinde IG etkinliğini belirlemektir.

GEREC VE YÖNTEM: Bu ileriye yönelik çalışmamıza AA teşhis edilen 88 hasta grubu ve 58 sağlıklı çocuktadan oluşan kontrol grubu dahil edildi. Patolojik olarak doğrulanmış AA hastaları akut basit apandisit (ABA) ve akut perfore apandisit (APA) olarak iki alt gruba ayrıldı. Grupların demografik özellikleri, beyaz kan hücreleri (WBC), nötrofil-lenfosit oranı (NLO), ortalama trombosit hacmi (MPV), IG% ve C-reaktif protein (CRP) değerleri analiz edildi. Parametrelerin tanı doğruluklarını ve öngörü performanslarını karşılaştırmak için receiver operating characteristics (ROC) analizi kullanıldı.

BULGULAR: AA hastalarının IG, WBC sayısı, NLO ve MPV değerleri kontrol grubuna göre daha yüksekti (sirala, p=0.28, p=0.22, p<0.001, p=0.001). ABA ile APA arasında bakılan enfiamatuvar belirteçlerden sadece IG% diğer belirteçlere göre statistiksel olarak anlamli şekilde farklılık gösteriyordu (p<0.001). ROC analizinde IG’nin APA varlığı için en uygun kestirim değeriinin >%0.2 olarak tespit edildi (duyarlılık %81.8, özgüllük %85.2, EAA: 0.83).

TARTIŞMA: Bu çalışmada, daha yüksek IG düzeylerine sahip AA’lı hastalarda perforasyon gelişme olasılığının daha yüksek olabileceği gösterildi. Klinisyenlere çocuk acil servisinde yüksek riskli AA hastalarının tespit etmelerine yardımcı olmak için fizik muayene, görüntüleme çalışmaları ve laboratuvar testleriyle birlikte IG değerlerini kullanmasına öneriz.

Anahtar sözcükler: Apandisit, çocuklar, immatür granülosit.

Ulus Travma Acil Cerrahi Derg 2022;28(3):375-381 doi: 10.14744/tjtes.2021.41347