THE DIAGNOSTIC VALUE OF IMMATURE GRANULOCYTE PERCENTAGE IN ACUTE APPENDICITIS PATIENTS

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Abstract

Studies have suggested that immature granulocyte percentage (IG%) levels may be an early marker of inflammation. In this study, it was tried to investigate whether there is a diagnostic value of IG% in appendicitis patients. This study was conducted retrospectively between the dates of 01.01.2018 and 01.05.2019 at Mersin University Faculty of Medicine, Department of Emergency Medicine. Patients over 18 years of age who were admitted to the emergency department and operated with the diagnosis of acute appendicitis were included in the study. The patients were classified into three groups as normal (control group), simple and complicated appendicitis. A total of 353 patients with a pre-diagnosis of appendicitis, 194 (55%) male, were included in the study. The mean age of the patients was calculated as 35.1 ± 14.0. The leukocyte parameter was found to be significant in distinguishing the disease; It was determined that the values of the patient group were higher than the values of the control group. (p values, respectively; 0.022 and 0.038). For CRP parameter; those with inflamed appendix were found to be lower than those with perforated appendicitis (p <0.001). When the neutrophil percentage parameter is examined; it was determined that patients with perforated appendicitis were higher than those with inflamed appendicitis (p <0.001). The immature granulocyte percentage (IG%) was found to have low power to distinguish disease and complications. For immature granulocyte percentage (IG%); AUC (Area under curve) was 0.505 and cut value was 0.2 while p value was 0.9128 in differentiating the disease. In distinguishing complications; AUC was 0.649 and cut value was 0.4 while p value was 0.0510. In our study, it was found that immature granulocyte percentage was statistically insignificant in distinguishing disease and complication. It was determined that the leukocyte value was significant in distinguishing the disease, and the CRP and percentage of neutrophils were statistically significant in distinguishing complications.

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1. Introduction

Acute appendicitis is a disease with inflammation of the appendix vermiformis and is the most common surgical cause of acute abdomen among patients with abdominal pain presenting to emergency services (1).

The lifetime risk of appendicitis is 7% worldwide, 8.6% for men and 6.7% for women. Appendectomy rates are 12% in men and 26% in women. It is thought that this rate is lower in Asian and African societies due to fiber diet (2).

The clinical diagnosis of acute appendicitis is often difficult because symptoms may be atypical due to other concomitant medical conditions (3). The main clinical decision in the diagnosis of a patient with suspected appendicitis is whether to have surgery. Ideally, the goal is to quickly treat all cases of appendicitis without unnecessary surgical intervention.

Anamnesis, physical examination, laboratory tests and imaging methods are used in the diagnosis and management of acute appendicitis. The tests used for diagnosis and determination of clinical severity are leukocyte count, C-reactive protein (CRP) level, procalcitonin, and total bilirubin (4-11). However, in the results obtained in the studies, we still do not have an easily accessible, reliable test that can be used to diagnose acute appendicitis and determine its severity. Immature granulocytes (IG) are cells released from the bone marrow into the blood during the early stages of the inflammatory process. In previous studies, it was found that the percentage of immature granulocytes was found to be high in peripheral blood samples of patients with septic disease (12-16.)

The cornerstone in the management of patients with acute appendicitis is to make an early diagnosis before serious complications develop and to find the balance between surgery and non-surgical recovery. In this study, our aim is to determine whether the percentage of IG can be used in clinical decision making in the diagnosis of acute appendicitis and determining its complications.

2. Materials and Methods

In our study, patients aged 18 years and older who applied to Mersin University Faculty of Medicine, Department of Emergency Medicine between 01.01.2018 and 01.05.2019 and were operated with the diagnosis of acute appendicitis were included. Before starting the study, approval was obtained with the decision of the Mersin University Clinical Research Ethics Committee dated 13/05/2020 and numbered 2020/358. The study was carried out retrospectively by scanning the hospital electronic medical information system. Demographic data, leukocyte count, CRP levels, immature granulocyte percentages and Neutrophil percentages of all patients were recorded. The patients were divided into three groups according to their histopathological findings: Control group; normal appendix, Appendicitis group; uncomplicated appendicitis (acute appendicitis), Complicated appendicitis group; complicated appendicitis (perforated, plastron).

2.1. Exclusion Criteria

• Having heart failure

• Presence of hematological disease

• Having cancer

• Presence of chronic infection

• Having liver disease

• Presence of vascular disease

• Unable to access file information

• Having an infection or inflammatory disease

For the determination of leukocyte, neutrophil percentage and immature granulocyte percentage, after blood was drawn into the tube with EDTA, the measurement was made using the electrical impedance method on the (Beckman Coulter LH 780) analyzer and the ratios were calculated. Serum CRP level was measured by turbidimetric method (Roche Cobas C 501). The normal reference values of the parameters in our study were as follows: Leukocyte (4500-10000/mm3), Neutrophil percentage (42.2%-75.2%), Immature granulocyte percentage (0-0.6), Serum CRP (0-5 mg/dL).

2.2. Statistical Analysis

Normality controls of continuous measurements were tested with the Shapiro Wilk test. Analysis of variance was used for group comparisons. Levene test was used for homogeneity of variances. One Way ANOVA was used for the differences between the group means in those whose homogeneity of variances condition was met, and the Welch test was used when the condition was not met. For pairwise comparisons, Bonferroni test was used when variances were homogeneous and Games Howell test was used when homogeneity is not supplied. Pearson correlation coefficient was used to control the relationship between continuous variables. Number and percentage values were given as descriptive statistics. $p < 0.05$ was taken as statistical significance.

3. Results

A total of 387 patients over the age of 18 who applied to Emergency Department between 01.01.2018 and 01.05.2019 were operated with a preliminary diagnosis of acute appendicitis, 34 patients were excluded from the study. A total of 353 appendiceal patients, 194 (55%) male and 159 (45%) female, were included in the study. Of the appendicitis patients included in the study, 50 of patients (14.2%) were in control group, 287 (81.3%)non-complicated appendicitis and 16 (4.5%) complicated appendicitis patients (Figure 1).

The mean age of the patients included in the study was calculated as 35.1 ± 14.0 years. The mean age of men was calculated as 33.7 ± 13.2, and the mean age of women as 36.9 ± 14.9, statistically significant difference was found between the mean age of the sexes (p=0.034).

For the leukocyte parameter; differences between groups were found to be significant (p=0.027). When the differences were examined, it was observed that the leukocyte values of only those with normal appendix were lower than those with non-complicated appendicitis (p=0.035). When the CRP parameter was examined, the differences between the groups were found to be significant (p=0.004). When the differences were examined, it was determined that the CRP values of those with complicated appendicitis were much higher than the CRP values of the control group and those with non-complicated
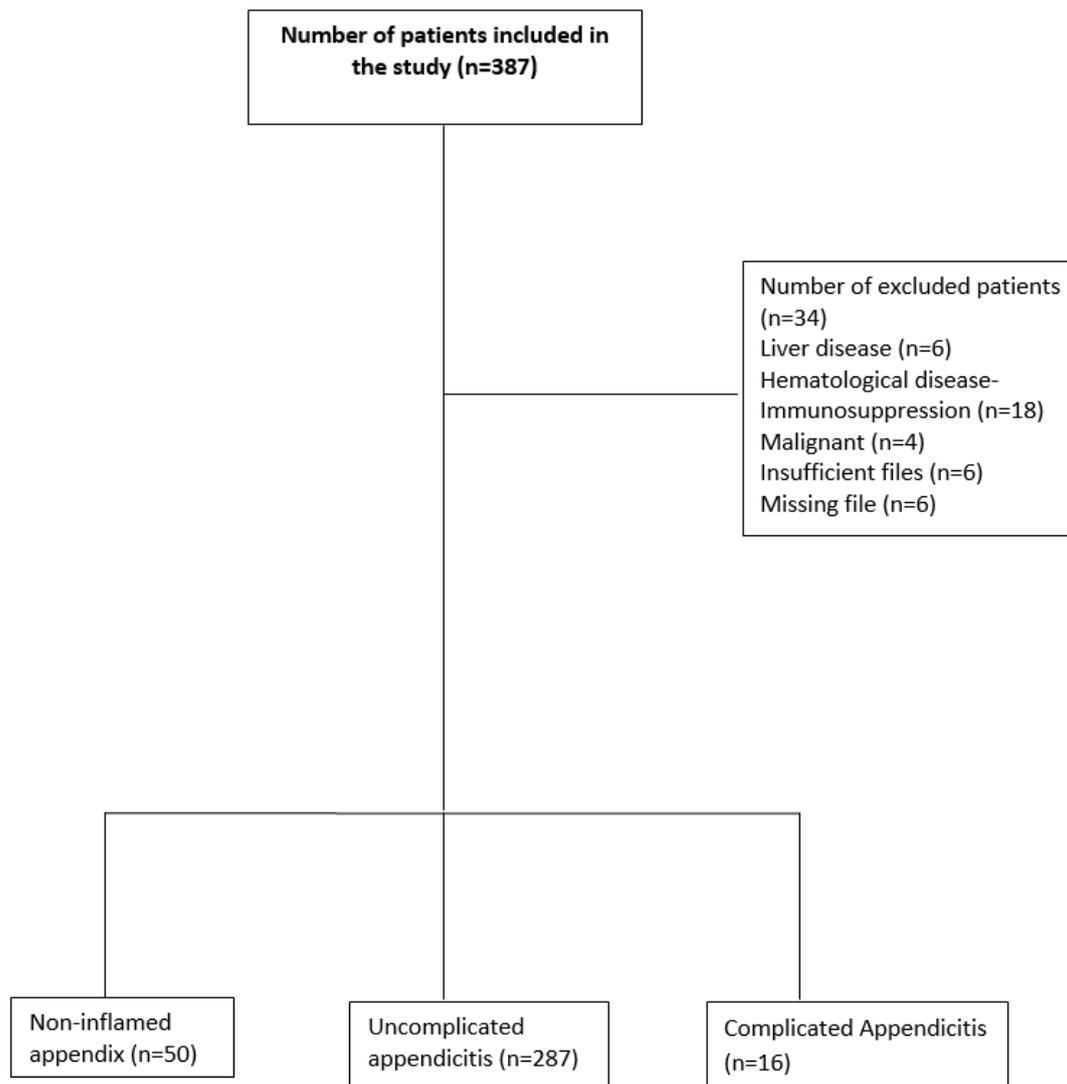
Figure 1. Flow chart of the patients included in the study

appendicitis (p values; 0.038 and 0.013, respectively). For the neutrophil percentage parameter, the differences between the groups were significant (p=0.003), and when the differences were analyzed, it was determined that the mean of the complicated group was higher than the control group and the non-complicated group (p values; 0.002 and 0.014, respectively). (Table 1).

The difference between the groups for the percentage of immature granulocytes was not significant. It can be said that only the leukocyte parameter is effective in separating normal and sick individuals

(inflamed and complicated appendicitis) (p=0.0014, AUC=0.627). The cutoff point for the WBC parameter was calculated as 13.2. The positive predictive value (PPV) by cutoff point was 90.81 for WBC (Table 2). That means, 90.81% of what we call a acute appendicitis according to the WBC value is really acute appendicitis (without distinguishing between simple and complicated). However, values below the cut-off value determined for WBC were found to be low in exclusion of the disease. The other three laboratory values were not statistically significant in distinguishing sick individuals (Figure 2, Table 2).

Table 1. Comparison of laboratory values between groups. WBC; white blood cells, CRP; C-reactive protein, Neutrophil %; neutrophil percentage, IG %; immature granulocytes percentage.

	Non-inflame Appendix (n=50)	Un-complicated appendicitis (n=287)	Perfore Apandisit (n=16)	P
Age	37,5 ±15.2	33,9 ± 13,2	48.4±18,3†	**0,007**
WBC	12,11 ± 3,61	13,97±5,7*	15,23±6,1	**0,027**
CRP	52,10±75,2	31,37±49,2	163.31±158,9*,†	**0.004**
Neutrophil %	73,18±12,4	75,82±9,6	82,37±7,4*	**0,003**
IG%	0,42±0,2	0,43±0,1	0,5±0,2	0,201

Table 2. Comparison of laboratory values of all appendicitis patients with normal apendix. WBC; white blood cells, CRP; C-reactive protein, Neutrophil %; neutrophil percentage, IG %; immature granulocytes percentage

	Cut-off	AUC (p)	Sensitivity	Specificity	PPV	NPV	LR(+)	LR (-)
WBC	>13,2	**0,627 (0,0014)**	55,45 (49,65-61,13)	66,00 (51,23-78,79)	**90.81 (85.69-94.55)**	19.64 (13.93-26.47)	1,63 (1,3-2,0)	0,68 (0,4-1,0)
CRP	≤ 28	0,524 (0,5903)	70,30 (64,81-75,39)	44,00 (30,00-58,74)	88.38 (83.69-92.14)	19.64 (12.74-28.22)	1,26 (0,9-1,7)	0,68 (0,5-0,9)
NEU %	>71	0,575 (0,0719)	73,93 (68,60 - 78,78)	40,00 (26,41 - 54, 82)	88.19 (83.57-91.89)	20.20 (12.80-29.46)	1,23 (0,9-0,7)	0,65 (0,5-0,9)
Ig %	>0,2	0,505 (0,9128)	92,08 (88,44 - 94,86)	18,00 (8,59 - 31,44)	87.19 (83.02-90.65)	27.27 (13.33-45.53)	1,12 (0,6-2,0)	0,44 (0,3-0,7)

Figure 2. Comparative ROC analysis of disease discrimination characteristics of WBC(white blood cells) CRP(C-reactive protein), Neutr %(neutrophil percentage), IG%(immature granulocytes percentage)
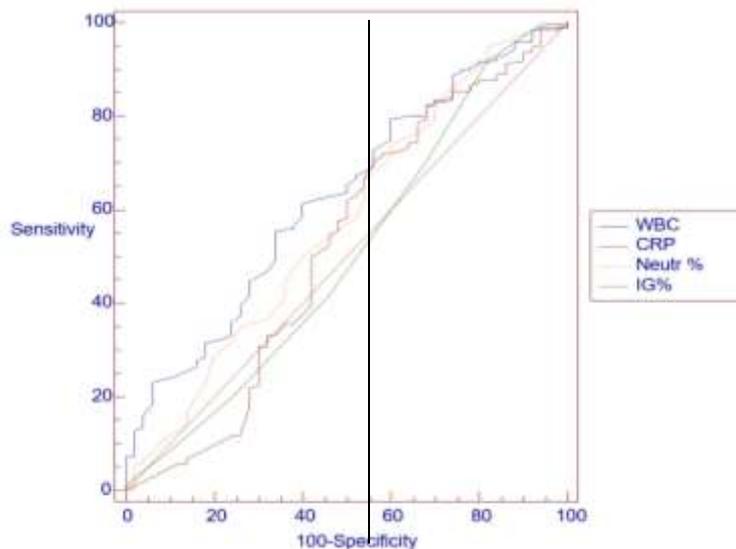
When Table 3 is examined, it is seen that the CRP and Neutrophil percentage parameters have a high statistical discrimination power. According to the table, non-complicated appendicitis patients are better differentiated by CRP values, while complicated appendicitis patients are better differentiated by Neutrophil parameter. For CRP; cut-off value was taken as 69 mg/L. It has been observed that the test's ability to distinguish patients with non-complicaed appendicitis is quite high; The selectivity value was calculated as 88.15 and the negative predictive value (NPV) as 97.68. This means: 88.15% of those with truly simple appendicitis have a CRP value of less than 69. In addition, 97.68% of individuals with a CRP below 69 actually have non-complicated appendicitis. Neutrophil % value, on the other hand, has a very high ability to distinguish complicated appendicitis. When the cut-off value of 81 for the neutrophil % value was taken, the selectivity in determining the complication was calculated as 88.15% and the negative predictive value as 98.48%; viz 98.48% of those with a neutrophil value above 81% have complicated appendicitis (Figure 3, Table 3).

Table 3. Comparison of laboratory values when patients were classified as simple and complicated. WBC; white blood cells, CRP; C-reactive protein, Neutrophil %; neutrophil percentage, IG ; immature granulocytes percentage

	Cut-off	AUC (p)	Sensitivity	Specificity	PPV	NPV	LR (+)	LR (-)
WBC	>14.4	0.546 (0.5430)	62.50 (35,47 - 84,71)	57.49 (51,55 - 63,28)	7.58 (3,70-13,49)	96,49 (92,52-98,69)	1,47 (1,0-2,2)	0,65 (0,3-1,2)
CRP	>69	**0.781** **(0.0001)**	62.50 (35,47 - 84,71)	**88.15** (83,84- 91,65)	22.73 (11.49-37,85)	**97,68** (95,02-99,14)	5,28 (3,6-7,7)	0,43 (0,2-0,9)
NEU %	>81	**0.701** **(0.0072)**	**81.25** (54,34 - 95,73)	67.60 (61,85 - 72,98)	12,26 (6,70-20,06)	**98,48** (95,61-99,67)	2,51 (2,0-3,2)	0,28 (0,1-0,8)
IG %	>0.4	0.649 (0.0510)	75.00 (47,63 - 92,58)	59.93 (54,01 - 65,65)	9,45 (4.98-15,93)	97,73 (94,28-99,36)	1,87 (1,4-2,5)	0,42 (0,2-1,0)

Figure 3. ROC analysis of four parameters by disease subgroups comparatively. WBC; white blood cells, CRP; C-reactive protein, Neutr %; neutrophil percentage, IG %; immature granulocytes percentage
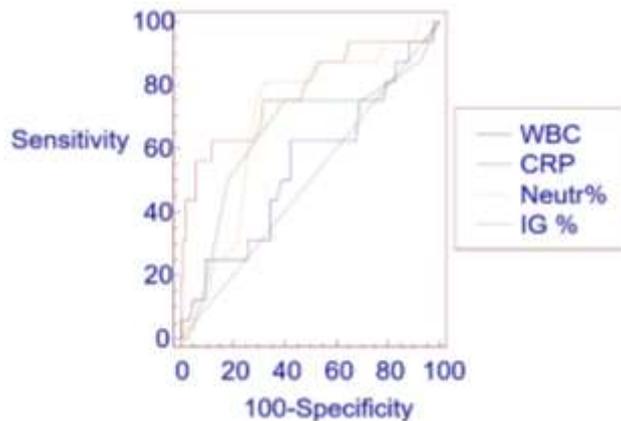
4. Discussion

The lifetime risk of appendicitis is 8.6% in men and 6.7% in women. Appendectomy rates are 12% in men and 26% in women (2). The reason for the high rate in women is thought to be the inability of definitive diagnostic confirmation and the confusion of pelvic pathologies with appendicitis in women17. Similarly, in our study, the rate of negative appendectomy was found to be higher in female patients; It was determined that 73.7% of men had inflamed appendicitis, while 74% of women had normal appendix.

Clinical findings, laboratory tests and imaging methods are used together in the diagnosis of acute appendicitis (1-3). Many biomarkers have been used in the diagnosis of acute appendicitis for a cost-effective approach, to shorten the hospital stay of patients, and to detect complications early (4-11).

As demonstrated in studies, it has been observed that inflammatory markers are important in the diagnosis of acute appendicitis, but other factors that increase inflammation reduce the diagnostic value (1-3). In addition, it is stated in studies that laboratory findings should be evaluated together with clinical findings (1-3). However, supportive tests are still needed for accurate diagnosis and early detection of complications (1-3).

The leukocyte value is used as a diagnostic parameter in patients with acute appendicitis. High leukocyte values support the diagnosis. However, it should be kept in mind that it also increases in other inflammatory conditions and the diagnosis of acute appendicitis cannot be excluded in values below the cut-off value. In a meta-analysis study conducted by Andersson in 2004, the sensitivity of the white blood cell count (WBC) in the diagnosis of acute appendicitis was found to be 83% and the specificity 67%, and it was shown that the predictive values increased as the white blood cell count increased (3). In the same study, the positive likelihood ratio (LR+) of high WBC values in predicting perforation was found to be 7.263. In the study conducted by Suat Ulukent et al., the sensitivity for the WBC cutoff value of 8650 was 76%, the specificity was 94%, and the AUC was 0.911. However, there was no analysis for complicated appendicitis in this study()18.

In the study of Zuhoor K Al-gaithy, when normal appendix and appendicitis patients were compared, WBC sensitivity was 76.8%, specificity was 65.5%, and AUC was 0.701. When the complicated and normal group were compared, the AUC was 0.76, the sensitivity 76.6, the specificity 72.4, the positive predictive value was 97%, and the negative predictive value was 16.1%()19. In a study conducted by Dong Hyuk Shin et al., the sensitivity of the WBC value was 70.9%, the specificity 65.7%, and the AUC value was 0.687()20. In our study, the literature is supported, and it was found that a high WBC value has a high ability to distinguish sick individuals. According to the result of our study, when the cut-off value was 13.200/mm3 in distinguishing sick individuals, the AUC value was found to be 0.627, and the sensitivity was found to be 55.45% and the selectivity as 66%. The positive predictive value, which is statistically significant in distinguishing sick individuals, is 90.81%. However, in our study, it was revealed that WBC did not determine the risk of complications of the disease.

C-Reactive Protein (CRP) is an acute phase reactant that starts to rise at 8-12 hours of inflammation and peaks at 24-48 hours. In the study conducted by Ghimire, it was shown that there is a clinical correlation between the high CRP value and the probability of acute appendicitis()21. In the study conducted by Amalesh et al., it is stated that CRP has no place in the diagnosis; In this study, the sensitivity was 91%, while the specificity was 42%22. In a study conducted with 542 patients, the AUC value of CRP in acute appendicitis patients was 0.60 on the first day, 0.77 on the second day and 0.88 on the third day; in the case of perforation, the AUC value is 0.90 on the first day()23. According to a meta-analysis, the sensitivity of CRP was found to be 65-85%, and the specificity as 59-73%()24. In our study, on the other hand, CRP value was found to be an important marker in determining complications in correlation with the general studies. For CRP; Below 69 mg/L is indicative of uncomplicated appendicitis; The selectivity value was 88.15, the sensitivity value was 62.5%, the NPV value was 97.68, and the PPV was 22.7%.

The percentage of neutrophils has become a hemogram parameter that has started to be included in the statistics in the differential diagnosis of diseases in recent years. In a retrospective study conducted on 897 patients with suspected acute appendicitis, the sensitivity was 87.2% and the specificity was 26.1% when the Neutrophil % cut-off value was 74% and above. In the same study, the combined specificity of CRP, WBC and Neutrophil percentage values was found to be quite low as 6.1%()25. In the study conducted by Xharra et al., when the cut-off value of the neutrophil percentage was taken as 75% and above, the sensitivity of the neutrophil percentage alone was 79.1%, and the specificity was 61%. In the combination of WBC, CRP and neutrophil percentage, the sensitivity was 95.3% and the specificity was 91.9%()26. In the study conducted by Andersson et al., it was stated that the Neutrophil % value could be used as a tool in determining complications()27. In our study, it was observed that the % Neutrophil was not statistically significant in diagnosing, however, it was an important laboratory value in determining complications. When the cut-off value was taken as 81 for the neutrophil % value, the selectivity was calculated as 88.15% and the negative predictive value as 98.48%.

Immature granulocyte cells are a collection of precursor cells formed by promyelocyte, myolocyte, and metamyolocytes that are not normally found in peripheral blood()28. Studies show that immature granulocytes can be used as an early inflammation marker in the presence of inflammation()12-16. There are studies showing that immature granulocyte amounts can be significantly high in many inflammatory conditions such as liver abscess, infective complications after cardiac surgery, sepsis or acute abdomen, and therefore it can be used as an early diagnosis tool()12-16. Ayres et al. stated that they excluded the diagnosis of sepsis with a specificity of 90.9% and a sensitivity of 38.5% in patients with a cut-off value of 2% for IG%, and stated that IG% is a marker that can be used in early diagnosis in patients with sepsis()29. In their study, Park et al. found that the sensitivity of IG% for sepsis was 81.3% and the specificity was 91%()30. In the study conducted by Ünal Y. on 438 patients, the cut-off value for the percentage

of IG was 0.4, the sensitivity was 47%, and the specificity was 88.4%. When the cut-off value was 0.6 in patients with complicated appendicitis, the sensitivity was 94.4% and the specificity was 97.9%()12. In the study of Jae-Sang Park et al., the cut-off value was 0.3, the sensitivity was 68% and the specificity was 44%. In the same study, other inflammatory markers were found to be more significant than the percentage of immature granulocytes in the ROC curve analysis()31. In our study, when the sick and non-patient group was examined, when the cut-off value was 0.2 for IG%, the sensitivity was 92.08% and the selectivity was 18%. It was observed that it was not statisically significantand other parameters were significantin the comparative ROC analysis. When the simple and complicated groups were compared, the sensitivity was 75% and the selectivity was 59.93% for the cut-off value of 0.4.

The limitations of our study are that it is single-centered and the temporal relationship between clinical findings and laboratory values is not mentioned.

5. Conclusion

As a result, CRP, WBC and neutrophil percentage have been found to have diagnostic value in the evaluation of patients with suspected acute appendicitis. It has been revealed in our study that the percentage of immature granulocytes, which has been suggested as a new marker of inflammation in recent years, has no place in the diagnostic evaluation of acute appendicitis. When evaluated together with previous studies, it was concluded that there is still no reliable test in the management of acute appendicitis, and future studies are important.

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