ORIGINAL ARTICLE

Laboratory markers used in the prediction of perforation in acute appendicitis

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ABSTRACT

BACKGROUND: In this study, we aimed to evaluate the laboratory markers used in the diagnosis of acute appendicitis (AA) and present the parameters that can be used to predict acute perforated appendicitis.

METHODS: The cases who underwent an appendectomy in our clinic between September 2018 and March 2020 were evaluated retrospectively. A total of 530 patients who met the study criteria were included in the study. Patients were divided into two groups according to the presence of an intraoperative perforation. Non-complicated appendicitis patients formed Group-1, and perforated appendicitis patients formed Group-2. The demographic, clinical, and laboratory characteristics of the patients were compared.

RESULTS: The number of non-complicated patients in Group 1 was 443, while there were 87 (16.4%) patients in Group 2 who had perforated appendicitis. The mean age of the patients in the normal appendicitis group was 29.90±10.51 years, and the mean age of the patients in the perforated appendicitis group was 36.32±14.58 years. In the normal appendicitis group, 257 (58%) of the patients were male, 186 (42%) were female, while in the perforated appendicitis group, 38 (43.7%) were male, 49 (56.3%) were female. In the perforated appendicitis group, white blood cell (WBC) value was 16.19±4.71 ($p<0.001$), C-reactive protein (CRP) value was 146.28±113.59 ($p<0.001$), total bilirubin value was 0.71±0.36 ($p<0.001$), and neutrophil-lymphocyte ratio (NLR) was 10.85±6.25 ($p<0.001$).

CONCLUSION: We believe that the WBC, total bilirubin, CRP, and NLR values obtained within this study, which is tested in the rapid and easily accessible blood tests in routine examinations that can contribute to the prediction of perforation.

Keywords: Bilirubin; C-reactive protein; neutrophil-lymphocyte ratio; perforated appendicitis; predictive factors; white blood cell.

INTRODUCTION

Acute appendicitis (AA) is one of the most common acute surgical conditions in general surgery clinics and accounts for approximately 2% of the patients presenting to the emergency department with acute abdominal pain.[1,2] There is an approximately 7% lifetime risk for AA.[3] The diagnostic process's first stage is evaluating the patient together with the anamnesis and physical examination findings, and the support with laboratory and imaging methods.[4] The aim should target to minimize unnecessary surgical intervention and possible complications. AA can be complicated in 18–34% of patients, and the most critical complication is perforation. Abscess formation is an important source of morbidity associated with wound infection and sepsis.[5]

Early detection of perforation is vital for the timely administration of specific antibiotic regimens and estimating the optimal time point for surgery.[6,7] The sensitivity of ultrasound and computed tomography scans in detecting perforated AA is low.[8] Therefore, a laboratory parameter is still needed to predict perforation, which is cheap, easily accessible, and fast, and can provide high sensitivity and specificity rates.[9] Although increased bilirubin and C-reactive protein (CRP) levels have been reported to be signs of perforation, it is not accurate enough.[10] The diagnostic accuracy of laboratory tests (especially the leukocyte count, CRP, and neutrophil percentage) has been reported to be 82.5%.[2] Mean platelet volume (MPV) is a marker derived from megakaryocytes associated with platelet (Plt) function and activation during Plt production. The roles of Plt in thrombosis and inflamma-

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tion have been investigated.[11,12] It has been found that the neutrophil-lymphocyte ratio (NLR), which is an inflammatory marker, is a predictor of the prognosis of colorectal cancer and cardiovascular diseases.[13,14]

Therefore, any factor that allows the prediction of perforation in AA contributes significantly to patient-specific treatment. Furthermore, the early diagnosis of perforation is likely to improve the results, allowing the surgeon to prepare for a relatively laborious operation, including laparoscopy or laparotomy. Our aim in this study is to evaluate the laboratory markers used in the diagnosis of AA and to develop parameters that can be used in predicting perforated AA, to guide the emergency room doctor first met with the patient and the general surgeon in the subsequent evaluation.

MATERIALS AND METHODS

Study Design and Patients

Patients who underwent an appendectomy in the General Surgery Clinic of Şanlıurfa Training and Research Hospital between September 2018 and March 2020 were retrospectively evaluated. The study was approved by the Harran University Ethics Committee with the decision of HRU/20.10.14 and was conducted under the Declaration of Helsinki. Written consent was obtained from all patients before surgery. Patients undergoing an elective appendectomy, patients younger than 18 years of age, and patients with incomplete file records were excluded from the study. Thus, 530 patients whose data were fully accessible through the hospital information system were included in the study. The patients were divided into two groups according to the presence of an intraoperative perforation. Group 1 consisted of non-complicated appendicitis patients (n=443) and Group 2 consisted of perforated appendicitis patients (n=87).

Data

Age, gender, type of surgery performed, pre-operative laboratory values (white blood cell (WBC), hematocrit (Hct), Plt, neutrophil, lymphocyte, monocyte, red cell distribution of width (RDW), MPV, CRP, total bilirubin, albumin, aspartate aminotransferase, and alanine aminotransferase values of the patients were recorded with histopathology results, and post-operative hospital stay duration through the hospital information system. NLR, platelet-to-lymphocyte ratio (PLR), RDW-Alb ratio, and CRP/Alb ratio values were calculated according to laboratory values.

Statistical Analysis

The Statistical Package for the Social Sciences 25 Inc., Chicago, IL, USA computer software was used for the biostatistical analyses. The data obtained from the participated patients were expressed as mean, standard deviation values, and as a percentage where necessary. The Kolmogorov–Smirnov test was used to examine the normal distribution of the data. Data with normal distribution were analyzed by student t-test. Group analysis of non-parametric data was performed with the Mann-Whitney U test. The logistic regression test was used for univariate analysis and to calculate odds ratios with a 95% confidence interval. Categorical groups were compared with the Chi-square test. Cutoff values were obtained by the receiver operating curve (ROC) analysis.

RESULTS

A total of 530 patients who met the study criteria were included in the study. The number of patients was 443 (83.6%) in Group–1 consisting of non-complicated patients, and 87 (16.4%) in Group–2 consisting of perforated appendicitis patients. The mean age of the patients in the normal appendicitis group was 29.90±10.51 years, and the mean age of the patients in the perforated appendicitis group was 36.32±14.58 years, and this difference was statistically significant (p<0.001). In the normal appendicitis group, 257 (58%) of the patients were male and 186 (42%) were female, while in the perforated appendicitis group, 38 (43.7%) were male and 49 (56.3%) were female. Female patients' rate was higher in the perforated patient group, and this difference was statistically significant (p=0.014). Open appendectomy was performed in 356 (80.4%) patients in Group–1, while laparoscopic appendectomy was performed in 87 (19.6%) patients. An open appendectomy was performed in 75 (86.2%) patients in Group–2, while laparoscopic appendectomy was performed in 12 (13.8%) patients. Although the rate of an open appendectomy was higher in the perforated appendicitis group, no statistically significant difference was found. When the pre-operative laboratory values of both groups were examined, the WBC value was 16.19±4.71 in the perforated appendicitis group and 13.05±3.70 in the non-complicated appendicitis group, the neutrophil value was 12.99±4.14 in the perforated appendicitis group and 9.53±3.65 in the non-complicated appendicitis group, monocyte value was 0.88±0.38 in the perforated appendicitis group and 0.77±0.28 in the non-complicated appendicitis group, and CRP value was 146.28±113.59 in the perforated appendicitis group and 24.12±36.23 in the non-complicated appendicitis group. The total bilirubin value was 0.71±0.36 in the perforated appendicitis group and 0.43±0.40 in the non-complicated appendicitis group. WBC (p<0.001), neutrophil (p<0.001), monocyte (p=0.003), CRP (p<0.001), and total bilirubin (p<0.001) values were higher in the perforated appendicitis group and this difference was found to be statistically significant. Hct value was 41.15±4.67 in Group–1 and 39.31±4.78 in Group–2, lymphocyte value was 2.27±0.83 in Group–1 and 1.44±0.64 in Group–2, and albumin value was 4.54±0.34 in Group–1 and 4.26±0.47 in Group–2. Hct (p=0.002), lymphocyte (p<0.001), and albumin (p<0.001) values were found to be lower in the perforated appendicitis group, and this difference was statistically significant. Other laboratory values of both groups were similar, and no statistically significant difference was found. NLR calculated over laboratory values was 10.85±6.25 in the perforated

Table 1. Demographic, clinical and laboratory characteristics of the patients

Variables	Normal appendicitis (Mean±SD) (n=443, 83.6%)	Perforated Appendicitis (Mean±SD) (n=87, 16.4%)	p-value
Age	29.90±10.51	36.32±14.58	<0.001
Gender, n (%)			
Male	257 (58)	38 (43.7)	0.014
Female	186 (42)	49 (56.3)	
Operation type, n (%)			
Open	356 (80.4)	75 (86.2)	0.201
Laparoscopic	87 (19.6)	12 (13.8)	
WBC (x10^3/µl)	13.05±3.70	16.19±4.71	<0.001
Hct (%)	41.15±4.67	39.31±4.78	0.002
Platelet (x10^3/µl)	243.34±60.09	247.91±76.00	0.749
Neutrophil (x10^3/µl)	9.53±3.65	12.99±4.14	<0.001
Lymphocyte (x10^3/µl)	2.27±0.83	1.44±0.64	<0.001
Monocyte	0.77±0.28	0.88±0.38	0.003
RDW (%)	10.68±1.49	10.78±1.33	0.557
MPV (fL)	8.04±1.57	8.10±1.80	0.791
NLR	4.95±3.13	10.85±6.25	<0.001
PLR	121.79±57.74	220.73±195.82	<0.001
RDW/Alb	2.37±0.42	2.57±0.53	<0.001
CRP (mg/L)	24.12±36.23	146.28±113.59	<0.001
Total bilirubin (mg/dL)	0.43±0.40	0.71±0.36	<0.001
Albumin (g/dL)	4.54±0.34	4.26±0.47	<0.001
CRP/Alb	5.54±9.69	36.35±31.53	<0.001
Duration of hospitalization	1.68±0.77	5.44±1.53	<0.001

SD: Standard deviation; WBC: White blood cell; Htc: Hematocrit; MPV: Mean platelet volume; RDW: Red cell distribution of width; NLR: Neutrophil to lymphocyte ratio; PLR: Platelet to lymphocyte ratio; CRP: C-reactive protein.

appendicitis group and 4.95±3.13 in the non-complicated appendicitis group, PLR was 220.73±195.82 in the perforated appendicitis group and 121.79±57.74 in the non-complicated appendicitis group, RDW/Alb was 2.57±0.53 in the perforated appendicitis group and 2.37±0.42 in the non-complicated appendicitis group, and CRP/Alb was 36.35±31.53 in the perforated appendicitis group and 5.54±9.69 in the non-complicated appendicitis group. The elevations in the perforated appendicitis group were found to be statistically significant (p<0.001). Duration of hospitalization was 5.44±1.53 days in the perforated appendicitis group and 1.68±0.77 days in the non-complicated group, and the elevation in the perforated appendicitis group was statistically significant (p<0.001). The mean hospitalization in patients undergoing open surgery was 2.40±1.75 days. The mean hospitalization in patients who underwent laparoscopic surgery was 1.84±1.23 days (p=0.001). The demographic, clinical, and laboratory characteristics of the patients are summarized in Table 1.

In the univariate analysis performed, statistically significant demographic characteristics and pre-operative laboratory parameters between the two groups were taken into multivariate logistic regression analysis. In the logistic regression analysis, age (OR=2.037, 95 % CI=1.009–1.063, p=0.009), total bilirubin (OR=2.951, 95 % CI=1.515–5.747, p=0.001), CRP (OR=1.030, 95% CI=1.007–1.054, p=0.011), WBC (OR=1.213, 95 % CI=1.083–1.358, p=0.001), and NLR (OR=1.298, 95 % CI=1.107–1.522, p=0.001) were found as

Table 2. Independent variables for perforated appendicitis

Variables	OR	%95 CI	p-value
Age	2.037	1.009–1.063	0.009
Total Bilirubin	2.951	1.515–5.747	0.001
CRP	1.030	1.007–1.054	0.011
WBC	1.213	1.083–1.358	0.001
NLR	1.298	1.107–1.522	0.001

Logistic regression analysis statistically significant p<0.05. CRP: C-reactive protein; WBC: White blood cell; NLR: Neutrophil to lymphocyte ratio; OR: Odds ratios; CI: Confidence interval.

Table 3. ROC curve analysis results

Variables	AUC	95% CI	p-value	Cut off value	Sensitivity %	Specificity %
Biluribin	0.769	0.714-0.824	<0.001	0.45	74.7	71.13
C-reactive protein	0.909	0.880-0.939	<0.001	25.5	92	72.5
White blood cell	0.697	0.635-0.759	<0.001	13610	70.1	53.7
Neutrophil to lymphocyte ratio	0.861	0.825-0.896	<0.001	6.69	77	76.3

ROC: Receiver operating curve; AUC: Area under the curve; CI: Confidence interval.

independent variables for the diagnosis of perforated appendicitis (Table 2). In the ROC curve analysis of these independent variables, the cutoff value of total bilirubin in the differential diagnosis of perforated appendicitis was: 0.45 (AUC:

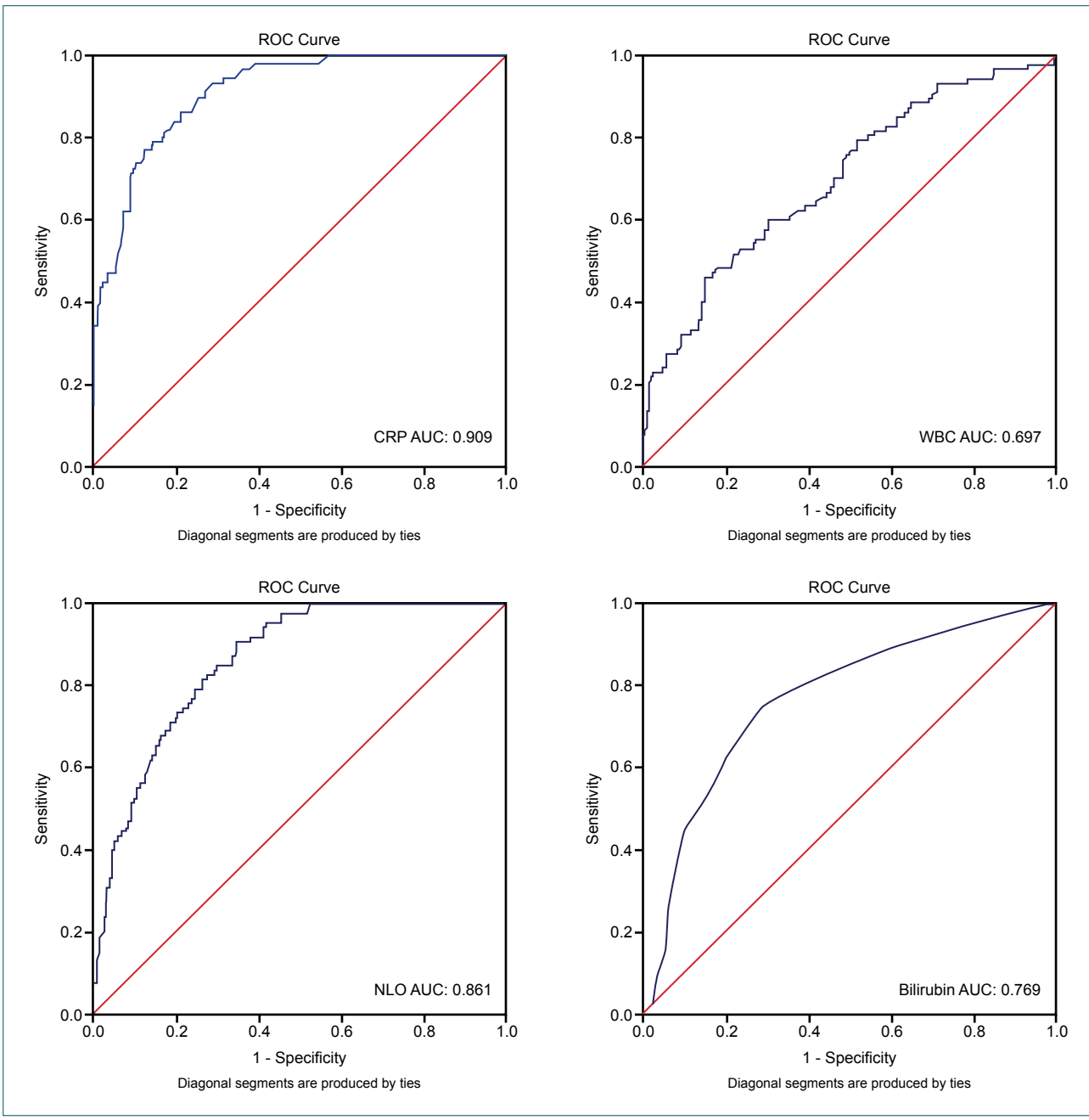

Figure 1. ROC curve analysis results.

0.769, 95% CI: 0.714–0.824, p<0.001), cutoff value of CRP was: 25.5 (AUC: 0.909, 95% CI: 0.880–0.939, p<0.001), cutoff value of WBC was: 13610 (AUC: 0.697, 95% CI: 0.635–0.759, p<0.001), and cutoff value of NLR was: 6.69 (AUC: 0.861, 95% CI: 0.825–0.896, p<0.001). ROC curve analysis results are summarized in Figure 1 and Table 3.

DISCUSSION

The frequency of appendix perforation in patients undergoing surgery for AA varies between 12 and 25%.[10,15] Some publications mention rates over 35%.[16] It is important to determine the presence of perforation before the surgery. Although non-perforated AA can be treated simply by an appendectomy, gangrenous, or perforated appendicitis which can cause various complications that may result in life-threatening conditions in some cases.[17] Despite radiological developments, differentiating subgroups of patients with severe disease can be difficult for the surgeon. Some studies have been carried out on various biomarkers or imaging methods to predict pre-operative perforation and reduce morbidity and mortality in AA cases. Interdependent parameters such as leukocyte, neutrophil, and lymphocyte count and NLR have been examined in many studies as inflammatory markers that are easily accessible and can be rapidly evaluated. In recent years, appropriate threshold values for these markers have been dwelled on and the performances of the markers at different threshold values have been investigated.[18,19]

In our study, in the first complete blood count and biochemistry values of the patients in the emergency department, WBC, neutrophil, monocyte, CRP, and total bilirubin values were statistically significant in the perforated appendicitis group. High Hct, lymphocyte, and albumin levels in the non-perforated group were found to be statistically significant. NLR, PLR, RDW/Alb, and CRP/Alb values were higher in the perforated appendicitis group and were found to be statistically significant.

Leukocytosis is an expected laboratory finding in AA and is valuable for diagnosis, but it does not stand out with its sensitivity and specificity. WBC is not a specific marker and is commonly elevated in other inflammatory diseases included in the differential diagnosis. In the study of Tamanna et al.[20] conducted with 116 patients, they reported a sensitivity of 89.6% and a specificity of 73.5% according to the 10.7x103 leukocyte breakpoint (AUC; 0.822) determined by the ROC curve. Sevinç et al.[21] reported 71% sensitivity and 68% specificity with a cutoff value of 11,900/mm^3 in their study. In a meta-analysis of 3382 patients, leukocytosis's sensitivity and specificity (leukocyte count >10,000/mm^3) were 83% and 67%, respectively. The sensitivity of neutrophilia (neutrophil count >6500/mm^3) presence has been reported as 71–89%, and the specificity as 48–80%.[22] In our study, 70.1% sensitivity and 53.7% specificity were found for WBC with a cutoff value of 13,610/mm^3; the AUC value was 0.697. Due to the low AUC value, it seems weak alone in predicting perforation; we think that this marker would be valuable with other markers. The number of non-perforated appendix patients with WBC elevation according to the cutoff value of 13,610/mm^3 alone was 206 (57.8%). This shows that WBC alone is a weak indicator.

CRP, which is an acute-phase reactant, increases later than the leukocyte count. CRP begins to increase at 8–12th h of the inflammatory process and reaches its highest levels later than WBC at 24–48th h.[7] Although McGowan et al.[10] found that all biochemical markers were significantly high in perforation, it was suggested that especially serum bilirubin and CRP elevation could be used as biomarkers for appendix perforation. It has been determined that the sensitivity of CRP is 78.57%, specificity is 63.01%, the sensitivity of bilirubin is 62.96%, and specificity is 88.31%. Xharra ±.[23] reported that the elevated CRP level is directly related to the severity of inflammation. In a meta-analysis of 12 studies examining CRP levels in the diagnosis of appendicitis, the elevated CRP value's sensitivity for the diagnosis of AA was reported as 65–85% and the specificity as 59–73%.[7] Burcharth et al.[18] reported that hyperbilirubinemia's specificity in the diagnosis of pre-operative appendix perforation is high, but its sensitivity is low. Sevinç et al.[21] confirmed that serum bilirubin level above 1 mg/dl has a highly specific and significant relationship with diagnoses of AA (92.4% specificity) and perforated appendicitis (81.4% specificity); however, the sensitivity of this test was found to be relatively low, 19% for AA, and 34% for perforated appendicitis. In our study, the sensitivity of 92% and specificity of 72.5% were found with a cutoff value of 25.5 for CRP; the AUC value was 0.909. The sensitivity was 74.7%, and specificity was 71.13%, with a cutoff value of 0.45 for bilirubin. CRP with a high AUC value is a valuable parameter in predicting perforation.

Neutrophil and lymphocyte counts play an important role in the systemic inflammatory response and indicate the severity of the response. In the study of Yardımcı et al.,[24] although neutrophil and lymphocyte values were found to be high in the perforated appendicitis group, they were found to be statistically insignificant. However, the NLR cutoff value of 7.95 has been shown to distinguish perforation with a sensitivity of 78% and a specificity of 67%. In the study of Markar et al.,[25] conducted with 1117 diseases, they reported that NLR had a statistically higher diagnostic sensitivity when compared to leukocyte and CRP values. In the study of Eren et al.,[26] it was shown that high NLR values are significantly associated with the diagnosis of AA and that the risk of gangrene/perforation is 3.2 times higher in cases with NLR ≥4.5. In the study of Ishizuka et al.,[27] in which they examined diagnostic tests in 222 patients who underwent appendectomy, they reported that high NLR was the most effective variable in predicting gangrene/perforated appendicitis. Kapçı et al.[28] reported NLR threshold value of 3.9; for AUC: 0745, the sensitivity of 70.9% and specificity of 75%. In our study, 77% sensitivity and

76.3% specificity, and the AUC value of 0.861 was found with the cutoff value of 6.69. We believe that NLR is a valuable parameter in predicting perforation due to the high AUC value. Like NLR, high PLR, RDW/Alb, and CRP/Alb values are also valuable in predicting perforated appendicitis.

Our study has some limitations. First of all, the study is retrospctive and has a single-center structure with a low number of patients. To support the physical examination findings with laboratory values, especially in centers that do not have emergency radiological imaging methods in peripheral hospitals. WBC, NLR, and CRP are non-specific inflammatory markers, these markers may be elevated in AA. According to the results of our study, we believe that evaluating total bilirubin, WBC, NLR, and CRP values together will be more valuable in determining the severity of AA and predicting perforation.

Ethics Committee Approval: This study was approved by the Harran University Faculty of Medicine Clinical Research Ethics Committee (Date: 01.06.2020, Decision No: HRU/20.10.14).

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ORİJİNAL ÇALIŞMA - *ÖZ*

Akut apandisitte perforasyonu öngörmede kullanılan laboratuvar belirteçleri

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AMAÇ: Bu çalışmada, akut apandisit tanısında kullanılan laboratuvar belirteçlerin değerlendirmesini yapmak ve perfore akut apandisiti öngörmede kullanılabilecek parametreleri sunmayı amaçladık.

GEREÇ VE YÖNTEM: Eylül 2018 ile Mart 2020 tarihleri arasında kliniğimizde apendektomi yapılan olgular geriye dönük olarak değerlendirildi. Çalışma kriterlerine uyan toplam 530 hasta çalışmaya alındı. Hastalar intraoperatif perforasyon varlığına göre iki gruba ayrıldı. Non-komplike apandisit hastaları Grup 1 ve perfore apandisit hastaları Grup 2 oluşturdu. Hastaların demografik, klinik ve laboratuvar özellikleri karşılaştırıldı.

BULGULAR: Non-komplike hastalardan oluşan Grup 1'de hasta sayısı 443 (%83.6), perfore apandisit hastalarından oluşan Grup 2'de hasta sayısı 87 (%16.4) idi. Normal apandisit grubu hastaların ortalama yaşı 29.90±10.51 yıl, perfore apandisit grubu hastaların ortalama yaşı 36.32±14.58 yıl idi. Normal apandisit grubunda hastaların 257'si (%58) erkek, 186'sı (%42) kadın, perfore apandisit grubunda 38'i (%43.7) erkek, 49'u (%56.3) kadın idi. Perfore apandisit grubunda WBC değeri16.19±4.71 ($p<0.001$), CRP değeri 146.28±113.59 ($p<0.001$), total bilirubin değeri 0.71±0.36 ($p<0.001$), NLO perfore apandisit grubunda 10.85±6.25 ($p<0.001$) olarak bulundu.

TARTIŞMA: Akut apandisit hastalarına acil servislerde rutin bakılan hızlı ve kolay ulaşılabilir kan tetkikindeki belirteçlerle yapılan bu çalışma ile elde edilen WBC, total biluribin, CRP ve NLO değerlerinin perforasyonu öngörmede katkı sağlayabileceği kanaatindeyiz.

Anahtar sözcükler: Beyaz küre; bilirubin; C-reaktif protein; nötrofil lenfosit oranı; öngörücü faktörler; perfore apandisit.

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