Comparison of Eosinophil Values with Other Biomarkers in Predicting Perforation of Acute Appendicitis

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Abstract

Aim: Perforation is the most common complication of acute appendicitis (AA) and is also a significant cause of infertility among women. One significant challenge for accurate and timely diagnosis of AA before perforation occurs is the limited availability of sensitive and specific blood biomarkers. Although previous studies have proposed hyperbilirubinemia as a biomarker for predicting impending perforation, additional biomarkers with improved specificity and sensitivity are greatly needed. Recently, eosinopenia and altered neutrophil/leukocyte ratio have been proposed as candidate biomarkers for monitoring several emergency situations, such as sepsis. In this study, we aimed to determine whether several peripheral blood parameters, including bilirubin level, total numbers of eosinophils, platelets, and neutrophils, neutrophil/leukocyte ratio, and mean platelet volume, are predictive for impending perforation in patients with AA.

Materials and Methods: All cases with histopathologically confirmed AA who were admitted to our hospital between January 1, 2012 and December 31, 2013 were included in this retrospective study. The bilirubin levels, total numbers of eosinophils, platelets, and neutrophils, neutrophil/leukocyte ratios, and mean platelet volume levels were compared for non-perforated and perforated AA patients. To compare the groups, the post hoc Mann-Whitney-U test was used to analyze non-parametric continuous variables; also, the receiver operating characteristics (ROC) test was used for accuracy.

Results: Among the 590 patients who received a pathological diagnosis of AA, 10.8% progressed to perforation of the appendix. Significant differences in total leukocyte, neutrophil, and eosinophil counts, neutrophil/leukocyte ratios, and bilirubin levels were found between the non-perforated and perforated AA cohorts. The areas under the curve (AUCs) for each parameter were 0.64, 0.63, 0.66, 0.62, and 0.60, respectively. Neutrophil/leukocyte ratios ≥72.2% had the highest sensitivity (84.4%) and eosinophil counts of ≤20/mcl had the highest specificity (76.8%) in predicting perforation.

Conclusion: While eosinopenia alone does not appear to be a marker for perforation, eosinopenia accompanied by higher neutrophil and leukocyte counts, a higher neutrophil/leukocyte ratio, and a higher bilirubin level can be used as a biomarker panel for predicting perforation in cases of AA.

Keywords: Appendicitis, perforation, eosinopenia

Introduction

Acute appendicitis (AA) is a common cause of abdominal pain for patients presenting to emergency departments (EDs); it is the most common cause of acute abdomen-related operations (1). While the gold standard for the diagnosis of AA is physical examination and laboratory results (mainly leukocytosis), one major concern is that the symptoms and signs of AA frequently overlap those of several other acute abdominal emergencies. A delay in diagnosis and surgical intervention inevitably results in perforation, which is a leading cause of morbidity and mortality of AA. Furthermore, complications arising from AA, especially perforation, can result in dysfunction of the fallopian tubes; this usually leads to infertility (2). However, perforation in AA patients is usually diagnosed either intra-operatively by observation or post-operatively by histopathological examination. Thus, timely and accurate diagnosis of perforation is critical, as these complications can be prevented by surgical intervention and successful removal of the appendix.

Many studies have examined biomarkers as diagnostic tools for the diagnosis of appendicitis; however, the number of studies investigating candidate biomarkers for prediction of perforation is limited. Akyildiz et al. (3), in their study comparing perforated and non-perforated appendicitis patients failed to demonstrate differences in leucocyte counts. Studies suggested hyperbilirubinemia as a marker for

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Received: 04.12.2016 • **Accepted:** 23.01.2017

DOI: 10.5152/eajem.2017.99609

perforation (4-7). Sand et al. (4) showed that *Escherichia coli* endotoxin, upon entering the blood stream following perforation, can cause a reduction in bile flow *in vivo*, resulting in hepatocyte dysfunction and increased serum bilirubin levels.

Among other potential markers, Chaudhary et al. (7) have shown that increased leucocyte and neutrophil counts with neutrophil/leucocyte ratio are a poor predictor for perforation, while Abidi et al. (8) suggest that eosinopenia, a recently proposed marker for differential diagnosis of sepsis from systemic inflammatory response in ICU patients, may also be used to predict appendicitis-related perforation. In the study of Becchi et al. (9), furthermore, a change in mean platelet volume (MPV), an indicator of disruption of platelet production in bone marrow, has been shown to be a predictor of death in sepsis patients. In Narci et al. (10) and Tanrikulu et al. (11) studies, MPV has also been shown to decrease in AA cases compared to non-AA patients with acute abdominal symptoms; however, its utility in differentiating perforated AA from non-perforated AA has not yet been determined.

In this study, we aimed to determine whether peripheral blood levels of several biomarkers that were independently evaluated for AA diagnosis, including bilirubin level, total numbers of eosinophils, platelets, and neutrophils, neutrophil/leukocyte ratio, and MVP, can predict impending perforation among AA patients.

Materials and Methods

Patients admitted to a tertiary training hospital ED between January 1, 2012 and December 31, 2013 with operative and histopathological diagnoses of AA were included in this retrospective study. Approval was obtained from the Institutional Ethics Board prior to the start of the study. Laboratory values, pathology reports, operative diagnoses, and clinical data were obtained from patient files and the hospital electronic patient record system. The clinical diagnosis was established preoperatively by means of clinical examination of the attending general surgeon, laboratory results, and radiologic imaging by either ultrasound, performed by the attending radiologist, or intravenous contrast-enhanced computed tomography of the abdomen, interpreted by the attending radiologist.

All excised appendices were sent for pathological examination, and the definitive diagnosis was confirmed by histopathologic examination by the attending pathologist. Perforation was diagnosed either by disruption of the appendix wall intra-operatively in the presence or absence of abscesses or by observation of disruption of the appendix wall during histopathological examination. The exclusion criteria included patients younger than 14 years of age, patients with incomplete charts, patients transferred from another hospital with a diagnosis of AP, patients transferred to another hospital due to unavailable beds, and patients who left the ED or general surgery department on their own. Furthermore, patients with known liver and biliary diseases were excluded due to increased bilirubin levels in this patient group. In addition, patients receiving corticosteroid-containing therapies were excluded because corticosteroids may elevate leucocyte count. Finally, patients with allergic conditions were excluded because such patients may have higher eosinophil counts. Since this was a retrospective study, no informed consents were obtained.

Complete blood counts and full biochemistry panels for each patient were obtained during the initial physical examination by emergency physicians in the ED. The leucocyte and platelet counts, neutrophil and eosinophil differential counts, and MPV were measured using an automated hematology analyzer (BC 5800, Mindray, Shenzhen, China). The upper and lower limits of the reference intervals for leucocyte and platelet counts, neutrophil and eosinophil differential counts, neutrophil/leucocyte ratio, and MPV were 4 to $10x10^3$/mcl, 156 to $373x10^3$/mcl, 2.1 to $6.3x10^3$/mcl, 0 to 500/mcl, 41% to 73%, and 6.9 to 10.8 fl, respectively. Total bilirubin level was measured using a chemistry immune analyzer (AU 680, Olympus, Tokyo, Japan). The upper and lower limits of the reference interval for bilirubin were 0 to 1.2 mg/dL.

Statistical analysis was performed using Statistical Package for Social Sciences 21.0 for Windows (IBM Corp.; Armonk, NY, USA). The normality of distribution was assessed with Levene's test and the Kolmogorov-Smirnov test. To compare groups, the Mann-Whitney U test was used for analysis of non-parametric continuous variables. Continuous variables are presented as the mean and standard deviation, and the receiver operating characteristics (ROC) test was used to determine the accuracy of leucocyte, neutrophil, eosinophil, and platelet counts, neutrophil percentage, and MVP for predicting perforation of appendicitis. To find the optimal cutoff point, we used Youden's index to calculate sensitivity and specificity as well as positive and negative likelihood ratios and predictive values (12). ROC graphs were prepared using Medcalc 16.8 for Windows (Medcalc, Ostend, Belgium). For all statistical tests performed, $p<0.05$ was considered to be statistically significant.

Results

During the two-year period for which our study retrospectively analyzed data, 658 patients were admitted with an initial diagnosis of AA; of these, 590 patients who were conclusively diagnosed with AA based on histopathological findings were included in our study. 423 (71.7%) of patients were male, with an average age of 31.6±13.7 years. 64 (10.8%) patients had perforated appendicitis and 7 (1.2%) patients had wall disruption on histopathologic examination, meanwhile 42 (7.1%) received operative diagnoses and 15 (2.5%) received pathologic and operative diagnoses. When the ages and hospitalization times of patients with non-perforated AA were compared with those of patients with perforated AA, perforated AA patients were significantly older and had significantly longer hospital stays (30.5±12.6 vs 40.8±18.6 years, 2.7±1.4 vs 6.1±4.8 days, both $p<0.001$).

In this study, 142 (24.1%) patients had normal leucocyte counts, 118 (20.2%) had normal neutrophil counts, and 538 (91.2%) had normal total bilirubin counts. When we compared laboratory values between non-perforated and perforated AA patients, the differences in leucocyte, neutrophil, and eosinophil counts, neutrophil/leucocyte ratios, and total bilirubin levels were found to be significant between the two groups; however, the differences in platelet counts and MVP values were not found to be statistically significant when the two diagnosis groups were compared (Table 1).

The ROC analyzes for the leucocyte, neutrophil, and eosinophil counts, total bilirubin, and neutrophil/leucocyte ratio revealed that none of these five values had high accuracy for the diagnosis of perforation in AA, as assessed by the areas under the curve (AUCs) (Table 2). The ROC curves of all five variables show similar accuracies for predicting perforation (Figure 1).

Finally, because all AA patients included in this study underwent operations and the histopathological examination was performed

within our institute, we evaluated the sensitivity and specificity, determined by Youden's index, of perforated appendicitis patients; we found that neutrophil/leucocyte ratios ≥72.2% had the highest sensitivity (84.4%), whereas eosinophil counts ≤20/mcl had the highest specificity (76.8%) in predicting perforation among AA patients (Table 3).

Discussion

The findings of our retrospective study suggest that eosinopenia accompanied with increased leucocyte and neutrophil counts, increased neutrophil/leucocyte ratio, and high total bilirubin level can be used as a predictor for perforation in patients with AA. The independent use of these biomarkers for predicting perforation is not supported statistically; the AUCs of the ROC curves for these values are between 0.6 and 0.7, indicating low accuracy.

We compared the eosinophil counts between the perforated versus non-perforated AA patient groups and found that perforated AA patients have significantly lower eosinophil counts compared to non-perforated patients. In their study, Bass et al. (13), eosinophils accounted for 1% to 3% of leucocytes, and the inflammatory cascade initiated eosinophilic response via adrenal corticosteroids and epinephrine. Same study investigating the utility of eosinophil count in diagnosing sepsis suggested that as the inflammation cascade builds up, chemotactic substances released from inflammatory cells during the inflammatory response lead to the sequestration of circulating eosinophils at the site of inflammation, thus decreasing the number of circulating eosinophils. During the course of AA, when perforation occurs, the infected tissue at the site of appendicitis spills into the peritoneal cavity, resulting in either a localized abscess or diffuse peritonitis; this site may become a focus for sequestration of circulating eosinophils, resulting in a decrease in eosinophil count. The optimal cutoff point for eosinopenia determined by Youden's index in this study is ≤20/mcl; while eosinopenia showed the lowest sensitivity among the five biomarkers included in our study, it showed the highest specificity (53.1% and 76.8%, respectively).

Although other biomarkers were statistically different between perforated and non-perforated cases, we failed to find statistical differences between platelet counts and MPV. In the study of Sevinc et al. (14), authors evaluated the aforementioned markers and found that although differences in both markers exist for perforated and non-perforated AA, only MPV is different between AA and non-AA patients. However, the low AUC values of these

Table 1. Comparison of laboratory values between perforated and non-perforated appendicitis

Laboratory marker	Non-perforated appendicitis	Perforated appendicitis	p
Leucocyte count	13.1±4.3x10^3	15.4±4.5x10^3	<0.001
Neutrophil count	10.1±4.3x10^3	12.2±4.1x10^3	0.010
Neutrophil/leucocyte count	75.0±116%	79.1±8.8%	0.010
Eosinophil count	124.0±133.7	78.1±117.8	<0.001
Platelet count	249.2±60.3x10^3	256.3±75.4x10^3	0.859
Mean platelet volume	9.2±1.3	9.2±1.4	0.565
Total bilirubin	0.7±0.5	0.8±0.5	0.003

Table 2. Areas under the curve (AUCs) of laboratory values for leucocyte, neutrophil, and eosinophil counts, total bilirubin, and neutrophil/leucocyte ratio

Laboratory marker	AUC (95% CI)
Leucocyte count	0.64±0.03 (0.57-0.70)
Neutrophil count	0.63±0.03 (0.57-0.7)
Neutrophil/Leucocyte	0.60±0.03 (0.53-0.67)
Eosinophil count	0.66±0.04 (0.58-0.74)
Total Bilirubin count	0.62±0.03 (0.55-0.69)

AUC: area under curve; CI: confidence interval

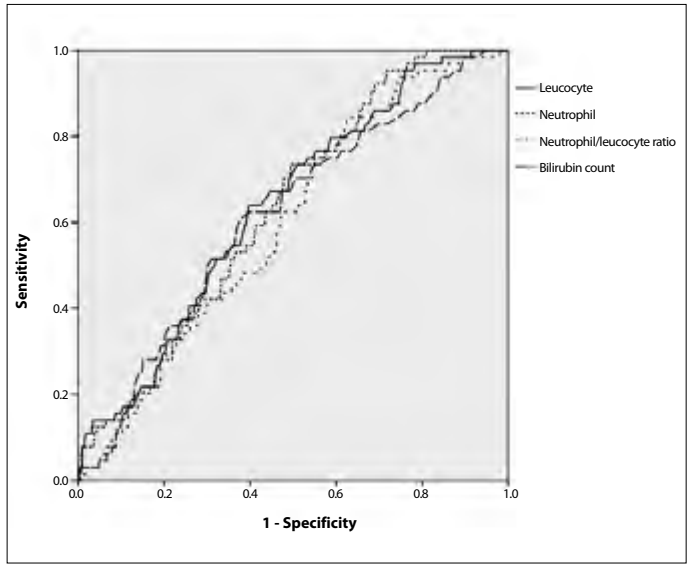

Figure 1. ROC curves of leucocyte, neutrophil, and bilirubin counts, eosinophil count, and neutrophil/leucocyte ratio

Table 3. Sensitivity and specificity for leucocyte, neutrophil, and eosinophil counts, total bilirubin, and neutrophil/leucocyte count

Laboratory marker	Laboratory value	Sensitivity	Specificity	+LR	-LR	+PV	-PV
Leucocyte count	13.900	64.1% (51.1-75.7%)	60.3% (55.9-64.5 %)	1.61	0.60	16.4	93.2
Neutrophil count	9.950	73.4% (60.9-83.7%)	50.6% (46.2-54.9%)	1.49	0.53	15.3	94
Neutrophil/leucocyte count	72.2 %	84.4% (73.1-92.2%)	37.6 (33.5- 41.9%)	1.35	0.42	14.1	95.2
Eosinophil count	20	53.1% (40.2-65.7%)	76.8 (73.0- 80.3%)	2.29	0.61	21.8	93.1
Total bilirubin	0.62	60.9% (47.9-72.9%)	62.0% (57.7- 66.1%)	1.60	0.63	16.3	92.9

+LR: positive likelihood ratio; -LR: negative likelihood ratio; +PV: positive predictive value; -LR: negative predictive value

biomarkers, between 0.5 and 0.6, limit their use. In Temple et al. (15) and Sahm et al. (16) studies, the perforation rates were similar to our study.

Several modalities are available for the diagnosis of AA in ED, including biochemistry panels, urinalysis, and radiologic imaging. While computerized tomography (CT) is highly accurate in detecting appendicitis, the presence of five key signs of perforation (abscess, phlegmon, extraluminal air, appendicolith, and focal defects in the appendiceal wall) can vary between patients. In the study of Horrow et al. (17), in several patients, some or all of these signs may be absent, thus decreasing the sensitivity and the specificity of CT. The lack of access to CT in certain settings, concerns regarding radiation, and potentially inadequate interpretation of CT are among the limitations of CT for the diagnosis of perforation. We strongly believe that eosinopenia along with increased leucocyte and neutrophil counts, increased total bilirubin level, and increased neutrophil/leucocyte ratio can predict perforation as a biomarker panel, especially in settings where CT use is limited or unavailable.

Study limitations

The limitations of this study are shared by other retrospective studies; specifically, we failed to define possible confounding variables and sources of bias. First, our hospital lacks an obstetrics and gynecology department, and most female patients with right lower quadrant pain are transferred to the nearest hospital for gynecological evaluation; this may have resulted in an unequal number of male patients with AA as a selection bias. In addition, the exclusion of patients who were managed non-operatively and the exclusion of patients who were transferred to other hospitals before the completion of treatment should be considered as additional selection biases. Furthermore, all patients with right lower quadrant pain with suspected AA who underwent operations and patients with pathologically proven appendicitis were included; thus, patients with negative laparotomy results were excluded from our study. The retrospective nature of the study limits the definition of confounding variables. We believe a prospective study will overcome these limitations.

Conclusion

We would like to note that while AA patients with eosinopenia prior to operation are more likely to suffer perforation, eosinopenia is not an absolute marker for perforation. Our results support that perforation remains an operative or histopathological diagnosis. Patients with decreased eosinophil counts prior to surgery accompanied by increases in leucocyte and neutrophil counts, with high total bilirubin level and high neutrophil/leucocyte ratio, have an increased risk of perforation. Therefore, meticulous efforts should be made to select the surgical procedure and post-operative clinical course for these patients.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Izmir Bozyaka Training and Research Hospital (13.01.2015, Decision No: 001).

Informed Consent: Informed Consent: Informed consent is not necessary due to the retrospective nature of this study.

Peer-review: Externally peer-reviewed.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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