

The role of biomarkers in predicting perforated cholecystitis cases: Can the c-reactive protein albumin ratio be a guide?

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ABSTRACT

BACKGROUND: Gallbladder perforation (GBP) is a rare but life-threatening complication of acute cholecystitis. Despite advancements in imaging technology and biochemical analysis, perforations are still diagnosed intraoperatively in some cases. This situation has revealed the need for new markers in the diagnosis of perforation. In this study, we aimed to analyze the role of biomarkers in the diagnosis of perforated cholecystitis cases.

METHODS: In this retrospective study, blood samples (white blood cells (WBC), hemoglobin, platelet count, C-reactive protein (CRP), albumin, CRP/albumin ratio (CAR), neutrophil-lymphocyte ratio (NLR), urea, creatinine, glucose, amylase, lipase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin, direct bilirubin) were analyzed in patients who were diagnosed with acute cholecystitis in the emergency department.

RESULTS: One hundred seventy patients were divided into two groups according to the presence or absence of gallbladder perforation. Sixty-three (37.1%) patients had perforation. Transition from laparoscopy to open operation, intensive care unit admission, length of hospital stay, and mortality were higher in the perforated group compared to the non-perforated group. When we analyzed the patients according to laboratory findings, there was a difference in WBC, NLR, CRP, albumin, and CAR parameters in the perforation group. In regression analysis, CRP and CAR performed better.

CONCLUSION: Our study showed that CRP and CAR may be diagnostic biomarkers with low specificity and sensitivity in predicting GBP in patients with acute cholecystitis. This marker is a low-cost and easily accessible parameter that may help clinicians make an early diagnosis and plan appropriate treatment for this condition with high morbidity and mortality.

Keywords: Acute cholecystitis; acute gallbladder perforation; c-reactive protein; biomarker.

INTRODUCTION

Acute cholecystitis, defined as acute inflammation of the gallbladder, is most commonly caused by the obstruction of the cystic duct by stones. The diagnosis is based on a careful history, physical examination, clinical and laboratory evidence of inflammation, and characteristic imaging findings. Abdominal ultrasonography is still considered the gold standard in diagnosing acute cholecystitis because it is non-invasive and easy

to perform. Additionally, contrast-enhanced computed tomography or magnetic resonance imaging are used to support the diagnosis or to rule out differential diagnoses. However, as these imaging techniques are not always available, it is important to use routine blood parameters as biomarkers. In acute cholecystitis, high white blood cell (WBC) counts and C-reactive protein (CRP) levels are observed, indicating systemic inflammation.^[1-3]

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Gallbladder perforation (GBP) is a rare but life-threatening complication of acute cholecystitis, occurring in 2-15% of patients diagnosed with the condition, often associated with the presence of stones.^[4] GBP sometimes presents clinically as uncomplicated acute cholecystitis and may cause high morbidity and mortality rates due to delays in diagnosis.^[5] Despite advancements in imaging and biochemical analysis, the diagnosis of perforation is still made at the time of surgery in some cases. This situation has underscored the need for new markers in the diagnosis of perforation.

This study was designed to determine the role of preoperative biomarkers in predicting GBP in patients admitted to the emergency department and undergoing surgery with a diagnosis of acute cholecystitis.

MATERIALS AND METHODS

Our retrospective study was approved by the local ethics committee. (Date: September 28, 2023; Decision No: 13/14). Patients who presented to the emergency department of our hospital between January 1, 2022 and August 31, 2023, and were operated on in our general surgery clinic with a diagnosis of acute cholecystitis were included in the study. Patients under 18 years of age and those with missing information in their records were excluded. Demographic data (age, sex, American Society of Anesthesiologists (ASA) score) and medical process data (type of surgery performed, pathology reports, complications, need for intensive care,

and mortality) of patients included in the study were collected by scanning the hospital digital database and physical patient records. Complications were categorized according to the Clavien-Dindo classification^[6]. Blood parameters assessed at the time of admission to the emergency department included WBC, hemoglobin (Hb), platelet count (PLT), CRP, albumin, CRP/albumin ratio (CAR), neutrophil/lymphocyte ratio (NLR), urea, creatinine, glucose, amylase, lipase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin, and direct bilirubin. The NLR was calculated by dividing the number of neutrophils by the number of lymphocytes, and the CAR was calculated by dividing the CRP level by the serum albumin level.

Statistical Analysis

During the relevant period, all patients who met the inclusion criteria were included in the study. The SPSS 17.0 statistical package (SPSS Inc., Chicago, IL) was used for all statistical analyses. All data were expressed as mean (standard deviation, SD) or number (percentage). The Kolmogorov-Smirnov test was used for the normality test of the data. In the comparison of cholecystectomy patients with or without perforation, the Mann-Whitney U-test was used for numerical variables that were not normally distributed, and Fisher's exact test was used for categorical variables. The T-test was used to compare normally distributed numerical data, and the Chi-square test was used to compare categorical variables. Receiver operating characteristic curve (ROC curve) analysis

Table 1. Comparison of study groups in terms of demographic and hospital data

	Perforation (-) (n=107)	Perforation (+) (n=63)	p value
Age (years)	56.5±14.2	65.3±16.4	<0.001
Sex (F/M)	44 (41.1)/63 (58.9)	23 (36.5)/40 (63.5)	0.552
Comorbidity			
Diabetes mellitus	25 (23.1)	23 (37.1)	0.076
Cardiac	48 (44.9)	26 (41.3)	0.648
Pulmonary	12 (11.2)	3 (4.8)	0.152
Renal	13 (12.1)	6 (9.5)	0.600
Others	36 (33.6)	20 (31.7)	0.799
ASA Class (I/II/III/IV)	51/44/12/0	32/13/17/1	0.006
Surgery type			
Laparoscopic surgery	90 (84.1)	37 (58.7)	<0.001
Conversion to open surgery	8 (7.5)	17 (27)	0.001
Open surgery	9 (8.4)	9 (14.3)	0.229
ICU admission	7 (6.5)	30 (47.6)	<0.001
ICU stay (days)	2.1±2.2	2.8±3.1	0.947
Hospital stay (days)	5.4±4	8±6.8	0.016
Mortality	0	10 (15.9)	<0.001

Data are expressed as mean±SD or n (%). ICU: Intensive Care Unit.

Table 2. Comparison of laboratory findings between study groups

	Perforation (-) (n=107)	Perforation (+) (n=63)	p value
White Blood Cell	11.5±5.9	14.3±5.6	0.003
Hemoglobin	13.4±1.9	13±2.1	0.246
Platelet	277.9±98.1	268.5±106.6	0.232
Neutrophil/Lymphocyte Ratio	6.9±7.9	9.3±7.1	<0.001
Serum Creatinine	1.1±0.8	1.1±0.6	0.149
Urea	15.4±8.4	16.1±5.9	0.084
CRP	67.6±97.5	158.9±130.3	<0.001
Albumin	36.4±5.2	33.5±21.7	<0.001
CRP/Albumin Ratio	2.1±3.1	5.5±4.7	<0.001
Glucose	125.3±51.7	141±74.2	0.096
AST	88±174.3	68.6±107	0.468
ALT	93.2±138.4	71.9±165.7	0.454
GGT	160.5±196.6	105.4±134.4	0.321
ALP	115.2±87.7	116.5±98.1	0.852
LDH	230±125.4	229±104.4	0.893
Total Bilirubin	1.3±1.1	1.6±1.8	0.176
Direct Bilirubin	0.5±0.7	0.6±1.1	0.070
Amylase	213.6±422.9	243.3±436.8	0.057
Lipase	355.5±995.7	220.2±495.3	0.144

Data are expressed as mean±SD.

was performed to determine the sensitivity and specificity of biomarkers (WBC, NLR, CRP, albumin, CRP/albumin ratio) that can be used in estimating gallbladder perforation. The optimal cutoff values for each parameter were considered at points on the ROC curve where the maximum of specificity and sensitivity was achieved. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 196 patients who underwent cholecystectomy surgery during the relevant dates were identified. However, 26 patients under the age of 18 who had missing data in their files were excluded from the study. The remaining 170 patients were divided into two groups according to whether they had gallbladder perforation or not. Perforation was present in 63 (37.1%) patients, 38 preoperatively and 25 intraoperatively.

Fifty-four patients (85.7%) in whom perforation was detected underwent laparoscopic cholecystectomy surgery. However, 17 (27%) patients were converted to open surgery. While 7 (6.5%) of the patients in the non-perforation group were admitted to the intensive care unit (ICU), this number was 30 (47.6%) in the perforated group ($p<0.001$). In addition, the length of hospital stay was longer in the perforation group (5.4 ± 4 vs. 8 ± 6.8 days) ($p=0.016$). While no patients died in the non-perforation group, the mortality rate was 15.9% in

the perforation group (Table 1).

When examining the patients according to laboratory findings, there was a difference between the groups in terms of WBC, NLR, CRP, albumin, and CAR parameters (Table 2). When the pathology results of the patients were examined, chronic stony cholecystitis was the most common diagnosis ($n=76, 44.7\%$) (Table 3).

The distribution of postoperative complications according to the Clavien-Dindo classification is given in Table 4. According to the ROC curve analysis, CRP and CAR parameters outperformed other parameters (WBC, NLR) with area under the ROC curve (AUROC) values of 0.734 and 0.748, sensitivity of 71.4 and 71.4, and specificity of 68.2 and 66.4, respectively. (Table 5, Fig. 1).

DISCUSSION

The main finding from this study is that CRP and CAR may be biomarkers for predicting perforation in patients diagnosed with acute cholecystitis. CRP is a pentraxin synthesized by the liver in response to inflammatory mediators such as interleukin 6, tumor necrosis factor alpha, and interleukin 1 β . The serum level of this acute-phase protein increases in response to infections, inflammatory diseases, trauma, and cancer.^[7] With a half-life of 19 hours, this protein activates the

Table 3. Distribution of pathology results of patients

	Perforation (-) (n=107)	Perforation (+) (n=63)	Total (n=170)
Chronic cholecystitis with cholelithiasis	63 (58.9)	13 (20.6)	76 (44.7)
Acute on chronic cholecystitis with cholelithiasis	17 (15.9)	17 (27)	34 (20)
Gangrenous cholecystitis	11 (10.3)	16 (25.4)	27 (15.9)
Acute cholecystitis	11 (10.3)	7 (11.1)	18 (10.6)
Necrotizing cholecystitis	3 (2.8)	7 (11.1)	10 (5.9)
Xanthogranulomatous cholecystitis	2 (1.9)	2 (3.2)	4 (2.4)
Adenocarcinoma	0	1 (1.6)	1 (0.6)

Data are expressed as n (%).

Table 4. Distribution of postoperative complications according to Clavien-Dindo classification

Grade	Definition	Non-perforated (n=107)	Perforated (n=63)	Total		
I	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic, and radiological interventions	Pseudocyst	9	18	27	
		Atelectasis	1	Subcutaneous abscess drainage	1	
		Wound infection	2	Pleural effusion	3	
		Pleural effusion	1	Atelectasis	1	
		Liver enzyme elevation	1	Pulmonary edema	1	
		Biliary fistula	2	Liver enzyme elevation	1	
		Hydronephrosis	1	Biliary fistula	7	
2	Requiring pharmacological treatment with drugs other than those allowed for Grade I complications	Blood and blood product transfusion	5	Blood and blood product transfusion	17	22
			3	Requiring surgical, endoscopic, or radiological intervention	Intervention not under general anesthesia	Percutaneous drainage of perihepatic abscess
Percutaneous drainage of biliary fistula	1					
Percutaneous drainage of perihepatic abscess	1					
3b	Intervention under general anesthesia	Evisceration	1		1	
4	Life-threatening complication requiring intensive care (IC) or intensive care unit (ICU) management					
4a	Single organ dysfunction		7	30	37	
4b	Multiorgan dysfunction					
5	Mortality			10	10	
Total			23	78	101	

classical complement cascade and stimulates phagocytosis.^[8] Albumin, the most abundant plasma protein in the blood, is synthesized in the liver and is a negative acute-phase reactant used as a prognostic marker in the intensive care setting.

This protein is downregulated by inflammatory signals, and low levels have been associated with a severe inflammatory response and increased short-term mortality.^[9-11] CAR is a novel inflammatory marker that can be easily calculated from

Table 5. Area under the curve (AUC) and receiver operating characteristic (ROC) of biomarkers

	AUC	Sensitivity %	Specificity %	Cut-off Value	95% CI Lower - Upper
CRP	0.734	71.4	68.2	50.1 mg/L	0.657 - 0.812
CRP/Albumin	0.748	71.4	66.4	1.4	0.672 - 0.824
WBC	0.664	65.1	59.8	11550/mm ³	0.581 - 0.747
NLR	0.663	68.3	60.7	5.11	0.581 - 0.746

WBC: White Blood Cell; NLR: Neutrophil/Lymphocyte Ratio.

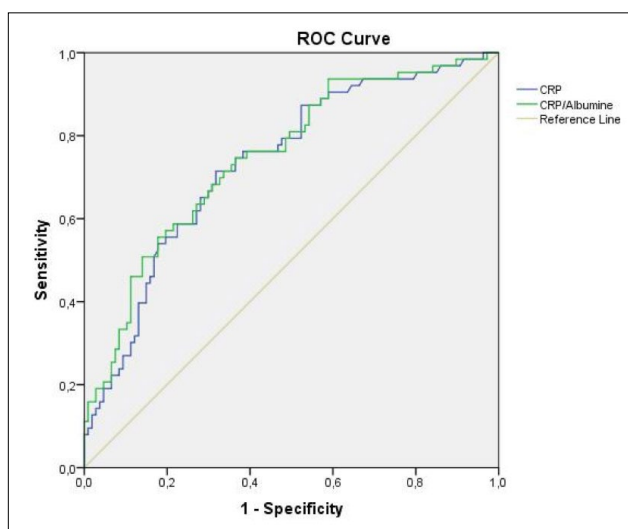


Figure 1. Receiver operating characteristic curve for CRP and CRP/Albumine

biochemical outcomes, predicting the prognosis of low-cost, critically ill patients and various cancers. Piñerúa-González et al. analyzed 722 patients with acute pancreatitis and reported that, despite the low sensitivity and specificity of CAR, it can be used as a complementary marker to classical scores in the initial assessment of acute pancreatitis prognosis.^[12] When the literature was examined, studies in which the CRP level was analyzed in predicting acute cholecystitis severity and CRP and WBC were compared in determining this severity drew attention.^[13,14] Sato et al. found that CAR was significantly higher in patients with acute cholecystitis in relation to the degree of severity.^[15] However, in this study, the degree of severity was determined according to the Tokyo Guideline I3, whereas in our study, the differentiation of cases was determined according to the presence of perforation in intraoperative and pathological reporting.

WBC is an indicator of inflammation, similar to CRP, and is employed in the examination of non-specific systemic inflammation and infection. An increase in WBC is a frequently used biomarker in the evaluation of acute cholecystitis.^[3] Studies have reported that high WBC values are due to the intense inflammatory response to necrotic changes in

the gallbladder wall rather than the severity of bacteremia.^[16] Teefey et al. reported the relationship between elevated WBC values and the development of necrotizing gangrenous cholecystitis as WBC count greater than 17,000.^[17] In a different study, the authors reported this value as 12,400 with elevated CRP, whereas in another study, the WBC count was reported as greater than 9,000.^[18,19] In a retrospective study of 600 patients by Mahmood et al., 169 patients underwent emergency cholecystectomy. The study reported that CRP, NLR, and age were independent factors related to the severity of acute cholecystitis. The authors suggested that CRP and NLR can be used as biomarkers to predict patients at risk of complicated acute cholecystitis.^[20] Uludağ et al. analyzed 250 patients who were admitted to the emergency department and operated on with a diagnosis of acute cholecystitis over a 5-year period. In this study, the gallbladder was evaluated histopathologically, and the cases were divided into two groups: acute cholecystitis and complicated cholecystitis. As a result, WBC, CRP, and NLR were reported as valuable biomarkers in predicting complicated cholecystitis.^[19] Recently, many studies have been published investigating the role of the ratios of blood parameters to each other, such as NLR, in determining the diagnosis and severity of the disease. Kler et al. observed that NLR can predict the diagnosis of acute cholecystitis (AC), yet no studies have the capacity to discern the severity of the disease.^[21] Despite this finding, other studies have concluded that an NLR of 4.18 can predict severe cholecystitis, with a sensitivity of 78.3% and a specificity of 74.3%.^[22,23]

In our study, preoperative levels of NLR and WBC were found to be higher than the normal range, consistent with systemic inflammation, but were not identified as biomarkers predictive of perforation in the regression analysis. As these analyzed values were in the blood sample taken at the time of initial admission to the emergency department, different results may have been obtained in the aforementioned studies.

While in acute cholecystitis the pathological diagnosis of chronic calculous cholecystitis and acute exacerbation of chronic calculous cholecystitis are in the forefront, in cases of perforated gallbladder the pathological diagnosis of gangrenous and necrotizing cholecystitis is more frequently ob-

served.^[5] The results of our study were found to be consistent with the literature. Morbidity (pleural effusion, biliary fistula, etc.) and mortality are higher in cases of perforated cholecystitis than in cases of acute cholecystitis.^[4,24] The morbidity and mortality rates found in our study are consistent with those reported in the literature. Therefore, it is important to identify cases of perforation at the earliest stage and to determine early surgical strategies.^[25]

The main limitation of our study is that it is a retrospective analysis from a single center. In addition, as the study was conducted in a tertiary healthcare institution, the referral of patients with complicated clinical conditions from other healthcare institutions also contributes to the limitation. This limits the general applicability of the study results.

CONCLUSION

Both the severity of complications and the high mortality rates demonstrate the need for early diagnosis, close, and multidisciplinary management of acute cholecystitis and perforated cholecystitis. Our study showed that CRP and CAR may be useful biomarkers in the diagnosis of patients with acute cholecystitis, although their specificity and sensitivity in predicting GBP are low. These markers are inexpensive and easily accessible parameters that may help clinicians to plan appropriate treatment by early diagnosis of this condition with high morbidity and mortality. However, for these biomarkers to be reliable indicators of GBP, large-scale studies are needed to test their validity and establish cutoff values.

Ethics Committee Approval: This study was approved by the Antalya Research And Training Hospital Ethics Committee (Date: 28.09.2023, Decision No: 13-14).

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ORJİNAL ÇALIŞMA - ÖZ

Perfore kolesistit olgularını öngörmede biyobelirteçlerin rolü: C- reaktif protein albumin oranı yol gösterici olabilir mi?

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AMAÇ: Safra kesesi perforasyonu (SKP), akut kolesistitin nadir fakat yaşamı tehdit eden bir komplikasyondur. Ancak teknolojik görüntülemeler ve biyokimyasal analizlere rağmen bazı olgularda perforasyon tanısı halen operasyon esnasında konulmaktadır. Bu durum perforasyon tanısında yeni belirteçler ihtiyacı olduğunu ortaya koymuştur. Bu çalışmayla perfore kolesistit olgularının tanısında biyobelirteçlerin rolünü analiz etmeyi amaçladık.

GEREÇ VE YÖNTEM: Bu retrospektif çalışmada acil serviste akut kolesistit tanısı konularak ameliyat edilen hastalarda çalışılan kan örnekleri (beyaz kan hücresi, hemoglobin, trombosit sayısı, C-reaktif protein (CRP), albümin, CRP /Albümin oranı (CAR), nötrofil lenfosit oranı (NLR), üre, kreatinin, glukoz, amilaz, lipaz, AST, ALT, ALP, GGT, total bilirubin, direk bilirubin) analiz edildi.

BULGULAR: 170 hasta safra kesesi perforasyonu olup olmamasına göre iki gruba ayrıldı. Hastaların 63'ünde (%37.1) perforasyon mevcut idi. Perforasyon tespit edilen grupta laparoskopiden açık operasyona geçiş, yoğun bakıma yatış, hastanede yatış süresi ve mortalite perfore olmayan gruba göre yüksekti. Hastaları laboratuvar bulgularına göre incelediğimizde; gruplar arasında beyaz kan hücresi, NLR, CRP, albümin ve CAR parametreleri açısından fark tespit edilmiştir. Regresyon analizinde ise CRP ve CAR daha iyi performans gösterdi.

SONUÇ: Çalışmamız CRP ve CAR'nın akut kolesistit tanılı hastalarda SKP'ü öngörmede özgüllük ve duyarlılığı düşük olmasına rağmen tanıya yardımcı bir biyobelirteç olabileceğini göstermiştir. Bu belirteç klinisyenlerin yüksek morbidite ve mortaliteye sahip bu tabloya erken tanı koyarak uygun tedaviyi planlamalarına yardımcı olabilecek düşük maliyetli ve kolay ulaşılabilen bir parametredir.

Anahtar sözcükler: Akut kolesistit; akut safra kesesi perforasyonu; c-reaktif protein; biyobelirteç.

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