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Can inflammatory biomarkers help in the diagnosis and prognosis of gangrenous acute cholecystitis? A prospective study

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ABSTRACT

Background: the diagnosis of gangrenous acute cholecystitis represents a diagnostic challenge for the physician and is rarely identified preoperatively.

Material and methods: we report a longitudinal prospective study in 180 patients who underwent cholecystectomy for acute cholecystitis. A ROC curve was obtained to determine the preoperative cut-off for various biomarkers (neutrophil to lymphocyte ratio [NLR], C-reactive protein [CRP], platelet to lymphocyte ratio [PLR], lactate and procalcitonin) and their association with both preoperative and postoperative findings.

Results: the area under the curve (AUC) for NLR, CRP, PLR, lactate and procalcitonin was 0.75, 0.8, 0.65 and 0.6, respectively.

Conclusion: NLR > 5 and CRP > 100 are still independent factors for gangrene (adjusted odds ratio [OR], 2 and 2.1, respectively).

Keywords: Cholecystitis. Biomarkers. Gangrene. Inflammation.

INTRODUCTION

Gangrenous acute cholecystitis is an evolutionary complication of acute cholecystitis, which is usually observed after a delayed diagnosis from symptom onset. The incidence reported by different studies oscillates between 2 % and 40 % (1). Diagnosis is rarely preoperative as both clinical and radiographic findings are usually nonspecific (2). However, in view of its associated morbidity and mortality, an early diagnosis with this type of cholecystitis would be of utmost clinical importance. A number of inflammation parameters have been used to aid in the diagnosis and prognosis of inflammatory and malignant conditions, including C-reactive protein (CRP), neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), lactate and procalcitonin (3,4). These are low-cost variables that can be obtained with a simple blood test. In the present study,

we propose a prospective assessment of five biomarkers (NLR, PLR, CRP, lactate and procalcitonin) as diagnostic and prognostic markers for patients with acute cholecystitis.

MATERIAL AND METHODS

Sample size

A sample size of 180 patients was estimated beforehand. A sensitivity and specificity of 70 % was used for the biomarkers according to the literature (5,6), a precision of 80 % and a 95 % confidence level.

Study design and case identification

An observational, longitudinal, prospective study was performed in a consecutive sample of patients diagnosed with acute cholecystitis (as per the Tokyo criteria) scheduled to undergo emergency surgery at the General Surgery Department, Hospital Peset, Valencia (Spain) within 24 hours after biomarker collection. Demographic and clinical data, symptom duration and lab results (including the above-mentioned inflammatory markers) were all collected both perioperatively and postoperatively. Disease severity was estimated based on the Tokyo guidelines (7). Complications within 30 days postoperatively were also recorded. Complications were classified as either minor or Clavien-Dindo I-II or major or Clavien-Dindo III-IV (8). The surgical specimen was evaluated by a single pathologist who made the definitive diagnosis.

Statistical analysis

A ROC curve analysis was used to establish the cut-off for NLR, PLR, CRP, lactate and procalcitonin for the diagnosis of gangrenous acute cholecystitis. After calculating the cut-offs for all five biomarkers, the sample was split into two groups, those with preoperative levels below or above the cut-off.

A univariate and multivariate analysis was performed to identify factors predictive of gangrene. A univariate analysis was also performed for the association between biomarkers and hospital stay, operative time, conversion during surgical procedure, the presence of postoperative complications and the presence of gangrene or gallbladder perforation.

The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA), version 15.0.1 was used for data description and analysis, considering p-values < 0.05 as significant. The study was approved by the hospital's Ethics Committee (CEIC: 120/12) and all included patients gave their informed consent.

RESULTS

The study started in January 2016 and ended in June 2018. During the study period, 376 patients were diagnosed with acute cholecystitis. A total of 281 patients underwent early cholecystectomy and 180 were eventually included in the study. The mean age was 66.1 (18-92) years and the study included 90 females (50 %) and 90 males (50 %).

Laparoscopic cholecystectomy was performed in 168 patients (93.3 %), with a conversion rate of 6.6 % (12 patients). Forty-one (22 %) patients developed complications; 17 major complications (five intra-abdominal abscesses, four self-limited biliary fistulae, three sustained sepsis, one choleperitoneum, one bile duct lesion and one hemoperitoneum) and 15 minor complications (six wound infections, four pneumoniae, one paralytic ileus and two urinary tract infections). Nine patients were readmitted (four intra-abdominal abscesses, two cholangitides and one liver abscess). In the final pathology assessment, 53 (29.4 %) patients were diagnosed with non-gangrenous acute cholecystitis and 127 (70.6 %) with gangrenous acute cholecystitis.

Table 1. *Area under the curve, cut-off, sensitivity and specificity of inflammatory biomarkers*

	Area under the curve	Cut-off	Sensitivity	Specificity
NLR	0.75	5	75 %	69 %
CRP	0.8	100	69 %	73 %
Lactate	0.55	1.4	61 %	55 %
Procalcitonin	0.6	1.7	55 %	65 %
PLR	0.65	175	60 %	60 %

NLR: neutrophil to lymphocyte ratio; CRP: C-reactive protein; PLR: platelet to lymphocyte ratio.

Table 2. *Correlation of demographic data and biomarkers with the histological diagnosis of gangrenous acute cholecystitis. Results of the univariate and multivariate analyses*

	Non-gangrenous cholecystitis (n = 53)	Gangrenous cholecystitis (n = 127)	Univariate OR (95 % CI)	p	Multivariate OR (95 % CI)	p
Sex	Male 23 Female 30	Male 67 Female 60		0.25		
Age > 65	25 (44)	83 (68)	2.1 (1.1-4.1)	*0.001*		0.6
Tokyo II-III	29 (51)	108 (87)	4.5 (2.2-9.3)	*< 0.001*	2.4 (1.3-5.2)	*0.03*
DM	8 (14)	40 (32)	2.5 (1.1-5.8)	*0.027*		0.1
Symptom duration > 72 h	17 (31)	50 (41)		0.3		
CRP > 100	16 (30)	74 (58)	3.1 (1.5-6.2)	*0.002*	2.1 (1.2-4.9)	*0.04*
NLR > 5	31 (58)	105 (83)	3.9 (1.8-8.3)	*0.00*	2 (1.2-4.7)	*0.03*
PLR > 175	30 (57)	67 (52)		0.7		
Lactate > 1.4	36 (67)	76 (60)		0.5		
Procalcitonin > 1.7	32 (60)	64 (51)		0.6		

DM: diabetes mellitus; NLR: neutrophil to lymphocyte ratio; CRP: C-reactive protein; PLR: platelet to lymphocyte ratio.

The area under the ROC curve was 0.75, 0.8, 0.65, 0.55 and 0.6 for NLR, CRP, PLR, lactate and procalcitonin, respectively. The cut-off, sensitivity and specificity values for all five markers are listed in table 1. Table 2 shows the relationship between demographic data and inflammatory biomarkers with the presence of gangrenous or non-gangrenous acute cholecystitis. Differences between non-gangrenous and gangrenous cholecystitis were only found for patients with NLR > 5 and CRP > 100 (p = 0.00, OR = 3.9; p = 0.002, OR = 3.1). In the multivariate analysis, only the Tokyo II/III variable, NLR > 5 and CRP > 100 remained in the model with an adjusted OR of 2.5, 2 and 2.1, respectively.

NLR > 5 was associated with a longer operative time (79 *vs* 91 minutes), hospital stay (4 *vs* 7 days) and the presence of macroscopic gangrene (OR = 2.1) (p = 0.001, 0.01 and 0.03, respectively) (Table 3). CRP > 100 was associated with a higher rate of major (OR = 4.2) and minor (OR = 3.8) complications and an increased rate of perforation (OR = 2.5) (p = 0.02, 0.03 and 0.01, respectively). Similarly, CRP > 100 was associated with a longer operative time (83 *vs* 103 minutes) and postoperative stay (4 *vs* 7 days). PLR > 175 was only associated with a higher rate of gallbladder perforation (OR = 1.6) and a longer hospital stay (4.5 *vs* 6 days). No differences were found for lactate and procalcitonin and the presence of intra- or postoperative findings.

Table 3. *Association of NLR > 5, CRP > 100 and PLR > 175 with intra- and postoperative findings*

	NLR < 5 (n = 40)	NLR > 5 (n = 140)	OR	p
Mean operative time (min)	79.1 ± 28.8	98.9 ± 38.6		*0.001*
Laparoscopy	36 (90)	130 (92)		0.8
Conversion	2 (5)	10 (7)		0.63
Macroscopic gangrene	14 (35)	74 (53)	2.1 (1.1-4.5)	*0.03*
Perforation	3 (8)	16 (11)		0.4
Hospital stay (days)	4 ± 1.9	7 ± 4.6		*0.01*
Major complications	2 (5)	15 (11)		0.27
Minor complications	3 (8)	12 (9)		0.8
Readmissions	2 (5)	7 (5)		0.8
	CRP < 100 (n = 78)	CRP > 100 (n = 102)	OR	p
Mean operative time (min)	83.3 ± 27.3	103.3 ± 40.6		*0.01*
Laparoscopy	77 (99)	89 (87)		0.9
Conversion	4 (5)	8 (8)		0.5
Macroscopic gangrene	32 (41)	56 (54)	2.0 (1.08-3.7)	*0.02*
Perforation	3 (4)	16 (15)	2.5 (1.5-4.7)	*0.01*
Postoperative stay (days)	4 ± 1.9	7 ± 4.6		*0.00*
Major complications	3 (4)	14 (13)	4.2 (1.1-15.4)	*0.02*
Minor complications	3 (4)	12 (11)	3.8 (1.1-14.1)	*0.03*
Readmissions	4 (5)	5 (5)		0.5
	PLR < 175 (n = 80)	PLR > 175 (n = 100)	OR	p
Mean operative time (min)	92.2 ± 35.3	96.9 ± 43.6		0.4
Laparoscopy	74 (93)	92 (92)		0.1
Conversion	5 (6)	7 (7)		0.9
Macroscopic gangrene	37 (46)	51 (51)		0.6
Perforation	3 (4)	16 (16)	1.6 (1.2-3.7)	*0.008*
Postoperative stay (days)	4.5 ± 2.9	6.5 ± 4.6		*0.02*
Major complications	4 (5)	13 (13)		0.07
Minor complications	5 (6)	10 (10)		0.3
Readmissions	4 (5)	5 (5)		0.5

NLR: neutrophil to lymphocyte ratio; CRP: C-reactive protein; PLR: platelet to lymphocyte ratio.

DISCUSSION

Acute cholecystitis was diagnosed based on a combination of clinical, laboratory and radiographic findings. Early diagnosis is crucial when facing an emergency surgical procedure as gangrenous acute cholecystitis is associated with increased morbidity and mortality. It represents a diagnostic challenge for physicians, which is only rarely overcome preoperatively. Diagnosis relies on imaging findings (computed tomography [CT] or abdominal ultrasounds). However, these tests are also plagued with a high failure rate for the identification of gangrene (9-11).

The present study shows that an NLR greater than 5 and CRP greater than 100 are useful to differentiate between gangrenous and non-gangrenous acute cholecystitis. NLR and RCP are easy-to-determine parameters that may guide us toward better therapeutic decisions for each patient at an earlier time point. Early laparoscopic cholecystectomy is the treatment of choice for this condition (12). However, many hospitals do not perform the procedure as an emergency (13).

In this study, high NLR and CRP levels were identified as independent predictors of gangrene. They also exhibited the highest diagnostic sensitivity and specificity. Hence, preoperative NLR and CRP may help reduce postoperative morbidity and hospital stay. They are also related to increased surgical time and in the case of CRP, with an increased rate of both major and minor complications. This may help us to foretell which procedures will be more challenging.

This is the first prospective study to relate different biomarkers with the presence of gangrenous acute cholecystitis. Surgery was performed within 24 hours after diagnosis, as any delay would result in different condition stages. We found a high incidence of gangrenous acute cholecystitis (70 %), consistent with recently reported prospective studies (14,15).

AUTHOR STATEMENTS

This study was approved by the Ethics Committee at Hospital Doctor Peset, Valencia, Spain (CEIC: 120/12). All patients included in the study provided their informed consent.

AUTHOR CONTRIBUTIONS

Dr. Díez Ares (first author) was responsible for managing the project, performing the statistical analysis and writing the manuscript. Dr. Peris, Dr. Estellés and Dr. Valenzuela were responsible for collecting the data. Dr. Ripollés, Dr. Martínez-García and Dr. Planells were responsible for designing the study and writing the protocol.

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