

Predictive value of immature granulocyte and delta neutrophil index in the diagnosis of complicated acute cholecystitis

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Abstract. – OBJECTIVE: The complicated gallbladder disorders are associated with increased mortality and morbidity. Thus, this study was aimed at evaluating the predictive value of immature granulocyte count and delta neutrophil index in the prediction of complicated cholecystitis.

PATIENTS AND METHODS: We retrospectively reviewed patients who underwent surgery for acute cholecystitis between January 2018 and April 2022. Overall, 351 patients fulfilling the inclusion criteria were included in the study. In all patients, demographic data, immature granulocyte count (IGC), delta neutrophil index (DNI), white blood cell (WBC) count, C-reactive protein (CRP), and albumin levels were recorded. Based on operative findings and histopathological examination, the patients were classified into 2 groups uncomplicated (group I) and complicated (e.g., perforation, gangrenous and emphysematous cholecystitis; group II) groups. The IBM SPSS version 26.0 (SPSS Corp, Armonk, NY, USA) was used to assess differences in blood parameters between groups. The predictive values of the parameters evaluated were estimated using ROC analysis. A p -value <0.05 was considered statistically significant.

RESULTS: Acute complicated cholecystitis was found in 138 of 351 patients. No significant difference was detected in age and gender distribution between groups ($p=0.352$ and $p=0.214$, respectively). When blood parameters were assessed, it was found that IGC, DNI, WBC, and CAR values were significantly higher in group II ($p<0.001$; $p<0.001$, $p<0.001$, and $p=0.036$, respectively), while there was no significant difference in CRP and albumin between groups ($p=0.099$ and $p=0.53$, respectively). In the ROC analysis, the highest AUC value was found for IG count and DNI (0.784 and 0.775, respectively). The sensitivity and specificity were found as 68.8% and 86.9% for IG count and 49.3% and 96.2% for DNI, respectively.

CONCLUSIONS: The IG count and DNI are two novel parameters with strong predictive value in

the early diagnosis of acute complicated cholecystitis, which may support clinical findings, imaging studies, and other laboratory parameters.

Key Words:

Cholecystitis, Immature granulocyte, Delta neutrophil index.

Introduction

Symptoms of gallbladder stones are the leading cause of presentation to the hospital. Again, acute cholecystitis due to obstruction of the biliary duct is also an important cause of emergency department (ED) visits^{1,2}. Although conservative treatment is generally sufficient in acute cholecystitis, emergent surgery may be required in complicated cases, such as those with perforation, gangrenous or emphysematous cholecystitis. Increased morbidity and mortality can be observed if emergent surgery is not performed^{3,4}.

The decision-making process for surgery may be challenging in the case of acute cholecystitis. Based on Tokyo criteria, cholecystectomy can be performed: if the patient is unresponsive to the conservative treatment given within the first 24 hours; if the time from symptom onset is no longer than 7 days; and if the patient has favorable general performance. Otherwise, external gallbladder drainage should be performed⁵. However, emergent surgery is indicated in case of complications such as perforation⁶.

The complete blood count is a simple, inexpensive, and readily available tool. Inflammatory biomarkers and parameters used to assess disease severity include WBC count, neutrophil-lymphocyte ratio, platelet count, immature granulocyte

count, and delta neutrophil index. Active bone marrow is indicated by a high delta neutrophil index (DNI) and immature granulocyte count (IGC). The alterations in WBC count are shown by the delta neutrophil index which reflects IG production during inflammatory and infectious processes⁷.

IGC can be readily measured using complete blood count in routine blood samples, owing to advances in automated analyzers. The IGC has been used as a biomarker in many studies^{8,9}. In addition, it was found that DNI was significantly increased in sepsis and infective conditions when compared to healthy individuals¹⁰⁻¹².

Our study was aimed at evaluating the effectiveness of IGC and DNI values, novel markers of inflammation, in distinguishing complicated and uncomplicated acute cholecystitis.

Patients and Methods

This retrospective, case-control study was approved by Keçiören Training and Research Hospital Clinical Research Ethics Committee (approval No: 2012-KAEK-15/2334, date: 22.06.2021). In the study, we retrospectively reviewed patients who underwent surgery for acute cholecystitis at T.R. Health Sciences University Keçiören Training and Research Hospital between January 2018 and April 2022. The demographic data and clinical characteristics were extracted from patient files and an electronic database. In patients who underwent surgery due to acute cholecystitis, the decisions regarding diagnosis and treatment were made based on physical examination, laboratory results, and sonography findings by the 2018 Tokyo criteria. The study was in accordance with the Helsinki Declaration.

The inclusion criteria were age >18 years, presence of acute cholecystitis, lack of autoimmune or chronic disorders, no gallbladder cancer reported in final histopathology examination, symptom duration <7 days, unresponsiveness to medical therapy, and cholecystectomy due to failure of medical therapy. The exclusion criteria were age <18 years, presence of acalculous cholecystitis, presence of any chronic or autoimmune disease, and diagnosis of gallbladder cancer or another type of cancer. During the study period, 490 patients underwent surgery with an initial diagnosis of acute cholecystitis. Of these, 100 patients were excluded due to incomplete data whereas 17 patients due to the presence of acalculous chole-

cystitis, 16 patients due to comorbid diseases, 5 patients due to gallbladder adenocarcinoma, and one patient due to a different type of malignancy. Overall, 351 patients fulfilling inclusion criteria were analyzed. In patients who presented to ED, a complete blood count was performed following anamnesis, and physical examination findings favored acute cholecystitis. The data regarding WBC count, CRP and albumin levels, age, gender, delta neutrophil index (DNI), and IGC were extracted from an electronic database. The CRP/albumin index (CAR) was also calculated.

Based on surgical findings and histopathology reports, the patients were classified into two groups uncomplicated (group I) and complicated (perforated, gangrenous and emphysematous, abscess; group II).

Statistical Analysis

Data were analyzed using IBM SPSS for Windows version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics are presented as count (n), percent (%), mean±standard deviation (SD), and median (interquartile range (IQR)). Categorical variables were analyzed using Pearson's chi-square test and Fisher's exact test. Numerical variables were assessed for normal distribution using the Shapiro-Wilk test, normality test, and Q-Q plots. Independent samples *t*-test was used to compare variables with normal distribution while Mann-Whitney U test was used to compare variables with skewed distribution. Receiver operator characteristics (ROC) curve analysis was used to determine the success of laboratory parameters in predicting uncomplicated and complicated cases. The cut-off value was estimated using the Youden index. Specificity was calculated based on cut-off value estimations. A *p*-value<0.05 was considered statistically significant.

Results

Overall, 351 patients fulfilling the study criteria were included in the study (Figure 1). The mean age was found as 48.82±13.96 in the study population. There were 202 men (57.5%) and 149 women (42.5%) in the study. Based on the intraoperative assessment and histopathological examination results, 138 patients were classified as complicated. Of 138 patients with acute complicated cholecystitis, 64 were men and 74 were women. No significant difference was detected between male and female patients regarding acute

Table I. The comparison of laboratory findings between Groups.

Group	Group I median (IQR 25-75)	Group II median (IQR 25-75)	p-value
IGC	0.01 (0.00-0.01)	0.02 (0.01-0.03)	< 0.001*
DNI	0.1 (0.1-0.1)	0.2 (0.1-0.3)	< 0.001*
WBC	7.4 (6.2-8.6)	8.4 (6.9-10.8)	< 0.001*
CAR	2.04 (0.8-11.98)	3.73 (1.15-11.95)	0.036*
Albumin	4.1 (3.8-4.4)	4.05 (3.7-4.3)	0.099*
CRP	8.2 (3.3-50.9)	13.9 (4.95-46.6)	0.053*

IGC: Immature granulocyte count; DNI: delta neutrophil index; WBC: White blood cell; CRP: C-reactive pro-tein; CAR: CRP-to-albumin ratio. *Mann-Whitney U test, statistically significant values are indicated in bold.

complicated cholecystitis ($p=0.214$). The mean age was 47.52 ± 13.11 in group I and 50.83 ± 15.01 in group II. No significant difference was detected regarding age between groups ($p=0.088$; t -test). It was found that IGC, DNI, WBC, and CAR values were significantly higher in group II while there was no significant difference in CRP and albumin between groups (Table I). ROC curves were drawn to estimate the effectiveness of laboratory parameters (IGC, DNI, WBC count, CAR) in the diagnosis of acute complicated cholecystitis (Figure 2). All parameters were found to be significant for the diagnosis of acute complicated cholecystitis (Group II). However, the predictive value of IGC and DNI for group II was higher than the remaining parameters. Table II presents the results of the ROC analysis.

Discussion

The early diagnosis is important in acute complicated cholecystitis since its mortality is higher than its uncomplicated counterpart¹³. The IGC and DNI are simple, inexpensive, and readily available parameters that can be obtained from complete blood count in all patients with acute cholecystitis. In our study, it was concluded that elevated IGC and DNI were associated with risk for complications in acute cholecystitis. We think that they can be used to predict complications in patients with a definitive diagnosis of acute cholecystitis in any setting.

Acute cholecystitis is an important cause of emergent surgical procedures¹⁴. A surgical or conservative approach can be preferred in the treatment of these patients¹⁴. The conservative treat-

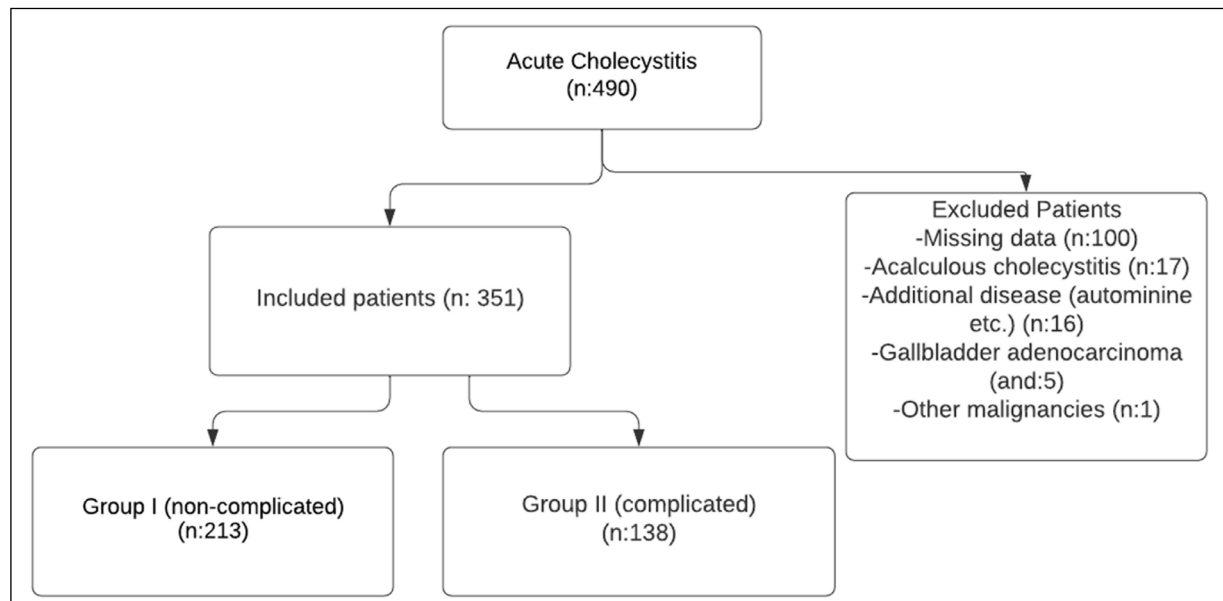


Figure 1. Flowchart of case distribution of the study.

Table II. ROC analysis of parameters in predicting complicated acute cholecystitis.

	Area under the curve	Cut-off value	Sensitivity	Specificity	95% confidence interval		Youden index
					Lower limit	Upper limit	
IGC	0.784	0.15	68.8	86.9	0.730	0.837	0.557
DNI	0.775	0.25	49.3	96.2	0.722	0.829	0.455
WBC	0.638	8.65	47.1	76.5	0.577	0.698	0.236
CAR	0.566	1.11	76.8	40.4	0.506	0.626	0.172

IGC: Immature granulocyte count; DNI: delta neutrophil index; WBC: White blood cell; CAR: CRP-to-albumin ratio.

ment is not an alternative for surgical treatment; rather, it aims to reduce inflammation in patients scheduled for surgical treatment⁶. The Tokyo criteria, established to standardize treatment choice in acute cholecystitis, have been updated over time¹⁵. Based on recent guidelines, surgery is indicated if the patient is unresponsive to conservative treatment given within first 24 hours; if time from symptom onset is no longer than 7 days and if the patient has favorable general performance. External drainage is performed in patients ineligible for surgical treatment due to comorbid diseases⁵.

In acute cholecystitis, major complications comprising risk are gallbladder perforation and gangrenous cholecystitis. In a study including 45,365 patients, Jansen et al¹⁶ found that gallbladder perforation was 12.7% in acute cholecystitis. Before this study, Pines and Rabinovitch¹⁷ reported the largest series of gallbladder perforation

(n=90). In this series, perforation incidence was 17.7%. In the case of perforation, surgical treatment is indicated. In the decision-making process for surgery, novel, imaging studies, and clinical findings should be supported by new, inexpensive and readily available methods. Although markers, such as WBC and CRP, have long been predictive, our study demonstrated the high predictive values of novel markers, such as IGC and DNI for complication development in acute cholecystitis. During evolution to a complication, immature granulocyte count will further increase since changes in granular leukocyte surface occur earlier than increase in WBC and neutrophil count and differentiation during infectious and inflammatory conditions start with granulocyte formation¹⁸. In addition, DNI and IGC are more valuable than CRP since CRP peak will occur 48 hours after symptom onset¹⁹.

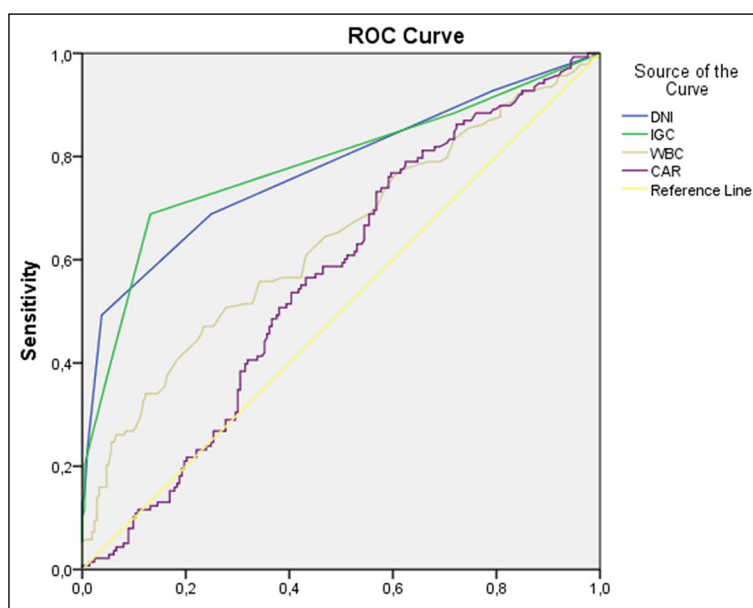


Figure 2. Complicated acute cholecystitis group: ROC curves. IGC: Immature granulocyte count; DNI: delta neutrophil index; WBC: White blood cell; CAR: CRP-to-albumin ratio; ROC: Receiver operating characteristics

In Tokyo 2018 criteria, WBC count was incorporated into discriminative criteria used for assessment of disease severity⁵. Although WBC count was identified as predictive factor for acute complicated cholecystitis, its predictive ability was significantly lower than DNI and IGC. In addition, leukocyte count may be decreased by advancing inflammation. In some patients, there may be no WBC response. Thus, some limitations may exist to accepting WBC count as predictive.

The level of CRP, produced in the liver in case of infection, was not a significant marker for the prediction of acute complicated cholecystitis in our study. In a few studies, increased CRP was shown to be a good marker for severe inflammation²⁰⁻²². The contradictory finding for CRP in our study may be because CRP is a reliable marker 48 hours after symptom onset; thus, CRP response was lacking. In addition, although there are studies suggesting that serum albumin is an important factor that predicts gangrenous cholecystitis, it showed no independent predictive ability despite marked difference in serum albumin levels between groups in our study²³. In previous studies²⁴, the CAR was shown to be a better marker for inflammation when compared to isolated CRP and albumin levels. The CRP is a positive acute-phase reactant that increases in response to inflammation while serum albumin is a negative acute phase reactant that decreases in response to inflammation²⁵. Thus, it could be better to use CRP: albumin ratio rather than using these parameters separately. In our study, there was a significant difference in CAR between the two groups, but the predictive value was lower than DNI and IGC.

There are some studies²⁶ demonstrating that acute complicated cholecystitis is more prevalent among elder individuals. It is thought that this is due to a reduction in physiological defense mechanisms against inflammation in these patients. Moreover, ED presentation may be delayed due to atypical manifestation^{27,28}. In our study, there was no significant difference in age between groups although patients with acute complicated cholecystitis were slightly older.

Limitations

This study has some limitations. The major limitation is the retrospective nature of the study. In addition, it was conducted in a single center with a relatively small sample size. Moreover, the exclusion of some patients may lead to selection bias. Thus, we made effort to include a maximum

number of patients in the study. In addition, IGC and DNI were not a part of routine CBC until recently. Thus, we could not include patients from past years. Also, we could not assess DNI and IGC responses after treatment.

Despite these limitations, this study is one of the limited studies evaluating the predictive value of DNI and IGC in acute complicated cholecystitis. The ability to obtain these parameters from routine laboratory tests rapidly is the strength of our study. In addition, we think that these parameters can prevent unnecessary surgeries, as well as overlook complicated conditions in centers without access to advanced tests (e.g., computerized tomography). This study is also important in shedding light to further prospective studies.

Conclusions

IGC and DNI are simple, inexpensive parameters that can be obtained from routine blood parameters. Acute complicated cholecystitis is associated with high morbidity and mortality if not diagnosed early. Thus, DNI and IGC are two novel parameters with high predictive value which may be supportive to clinical findings, imaging studies and other laboratory tests in the early diagnosis of acute complicated cholecystitis.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Ethics Approval

This study was approved by Keçiören Training and Research Hospital Clinical Research Ethics Committee (approval No: 2012-KAEK-15/2334, date: 22.06.2021).

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