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ORIGINAL RESEARCH

Combination of CA19-9 and the Neutrophil-to-Lymphocyte Ratio for the Differential Diagnosis of Gallbladder Carcinoma

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Purpose: This study aimed to evaluate the efficiency of several parameters, including the neutrophil-to-lymphocyte ratio (NLR) obtained from preoperative routine blood examination, either alone or as an adjunct to the carbohydrate antigen 19-9 (CA19-9), for the diagnosis of gallbladder carcinoma (GBC).

Patients and Methods: Data from 123 patients with gallbladder cholesterol polyps (GCP), 80 with gallbladder adenoma (GA), and 103 with GBC were reviewed retrospectively. Receiver operating characteristic analysis was used to assess the sensitivity, specificity, and clinical value of the NLR, CA19-9, and their combination.

Results: Values of measured indicators were significantly higher in GBC patients than in GCP or GA patients but there were no significant differences between GCP and GA patients. The combination of NLR and CA19-9 had the best diagnostic efficiency for differentiating GBC from benign lesions with 74.8% sensitivity and 89.7% specificity. However, the NLR showed no significant difference between mid-to-advanced stage and early-to-mid stage GBC. The combination of NLR and CA19-9 (53.7% sensitivity and 88.9% specificity) did not reveal any advantages over CA19-9 alone (63.0% sensitivity and 89.0% specificity) in distinguishing different stages of GBC.

Conclusion: NLR and CA19-9, and their combination—parameters that are easily obtained preoperatively—have potential as diagnostic markers for GBC.

Keywords: gallbladder cancer, diagnosis, CA19-9, neutrophil-to-lymphocyte ratio

Introduction

Gallbladder carcinoma (GBC) is the most common cancer of the biliary system and the sixth most prevalent malignant tumour of the digestive system.[1] Patients with GBC have a poor prognosis and their five-year overall survival rate is less than 10%.[1,2] However, because of the lack of specific symptoms and impossibility of undergoing biopsy before an operation, accurate preoperative diagnoses for patients with GBC are challenging. Patients with GBC, especially in the early stages, are often misdiagnosed with benign neoplastic lesions such as adenomas, or non-neoplastic lesions such as cholesterol polyps.[3] Relatively conservative management should be performed for non-malignant lesions; therefore, the correct diagnosis of GBC is essential for developing an accurate therapeutic strategy.

Increasing evidence has shown that systemic inflammation and immune state are closely related to the genesis and progression of tumours.[4] A high neutrophil-to-lymphocyte ratio (NLR) as an inflammatory response-related indicator is associated

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with unfavourable prognosis in a variety of cancers, including GBC.[4–7] However, studies regarding the correlation between NLR and the correct diagnosis of GBC are rare. On the other hand, as a type of tumour-associated antigen, carbohydrate antigen 19-9 (CA19-9) is the most commonly used and studied tumour marker for evaluating the diagnosis and prognosis of pancreato-biliary tumours.[8] Wang et al indicated that the sensitivity and specificity of CA19-9 for the diagnosis of GBC was higher than that of other tumour markers.[9]

In the present study, various parameters including NLR and CA19-9 obtained from preoperative routine blood examination in different groups of patients with gallbladder cholesterol polyps (GCP), gallbladder adenoma (GA) and GBC were compared. The aim was to determine whether NLR alone or in combination with CA19-9 could better differentiate GBC from other gallbladder lesions, and thus increase the diagnosis accuracy.

Patients and Methods

Patients

This retrospective study was approved by the Institutional Ethics Committee of Peking University First Hospital and verbal informed consent was obtained from patients. A search of the clinicopathological database identified a total of 184 patients with GCP, 108 with GA and 139 with GBC from January 2008 to December 2018. The inclusion criteria were as follows: (a) patients had received operations and their final diagnoses were confirmed by pathological examinations; (b) patients had detailed routine blood and tumour marker examinations within 1 week prior to operation; (c) patients were without acute infectious or autoimmune disease; (d) patients had no other tumours; and, (e) patients did not have two or more of the above-mentioned lesions. Accordingly, the following patients were excluded: 42 patients with GCP, 23 with GA and 18 with GBC due to the lack of tumour marker examinations; 14 patients with GCP, 5 with GA and 16 with GBC due to the presence of acute infectious disease; 2 patients with both GBC and colorectal carcinoma; and, 5 patients with both GCP and GA. Finally, 123 patients with GCP, 80 with GA and 103 with GBC were included in this study.

Data Collection

The clinical and pathological characteristics of each patient including gender, age, presence of gallstones, symptoms, history of smoking, alcohol consumption and body mass index (IBM) were collected. The presence of gallstones was confirmed by preoperative imaging and postoperative pathological examinations. The parameters obtained from preoperative laboratory examinations such as white blood cell (WBC), neutrophil, lymphocyte and platelet counts, and CA19-9 levels were recorded. The NLR and the platelet-to-lymphocyte ratio (PLR) were defined as the absolute neutrophil and platelet counts divided by the absolute lymphocyte count, respectively. The combination value was defined as the serum CA19-9 level multiplied by NLR. For patients with GBC, the severity of the disease was evaluated using the American Joint Commission on Cancer (AJCC) staging system and patients were divided into the early-to-mid TNM stage group (stages I and II) or the mid-to-advanced TNM stage group (stages III and IV).[10]

Statistical Analysis

Univariate analyses using the *t*-test, chi-square test and Wilcoxon rank-sum test were performed to compare the clinical and pathological characteristics of the GBC group with those of the benign group (patients with GCP or GA). The receiver operating characteristic (ROC) curve was used to determine the efficiency of different parameters obtained from laboratory measurements for discriminating GBC from benign lesions.

The area under the ROC curve (AUC), sensitivity, and specificity were calculated as evaluation indicators. The mean values of the different laboratory parameters in the GBC, GCP and GA groups were compared via one-way analysis of variance, post hoc tests, or t-tests. The ROC analysis and Wilcoxon rank-sum test were also used for the efficiency evaluation of some related indicators in the early-to-mid stage group and the mid-to-advanced stage group of GBC patients.

Data management and analysis were performed using SPSS version 19.0 and a statistically significant difference was defined as a P value < 0.05.

Results

GBC versus Benign Lesion

Univariate analysis results comparing the clinicopathological findings of the benign and GBC groups are shown in Table 1. No significant differences were found between the two groups in terms of gender, history of smoking or drinking. However, the age of onset was significantly higher in patients with GBC than in the benign group (64.00 versus 50.00, P <

Table 1 Clinicopathological Data of Patients with Gallbladder Lesions

Criteria	Gallbladder Lesions[a,b]		P-value[c]
	Benign Group (n = 203)	GBC Group (n = 103)	
Age (years)	50.0 (39.0–59.0)	64.0 (55.5–73.0)	**<0.001**
Sex			0.218
Male	74 (36.5)	44(42.7)	
Female	129 (63.5)	59(57.3)	
Gallstones			**<0.001**
Absent	164 (80.8)	61 (59.2)	
Present	39(19.2)	42 (40.8)	
Presenting symptom			**<0.001**
None	159(78.3)	39(37.9)	
Abdominal pain	41(20.2)	48(46.6)	
Abdominal distension	2(1.0)	7(6.8)	
Others	1(0.5)	9(8.7)	
Smoking			0.377
Yes	40(19.7)	25(24.3)	
No	163(80.3)	78(75.7)	
Alcohol consumption			0.213
Yes	23(11.3)	17(16.5)	
No	180(88.7)	86(83.5)	
BMI	24.0(22.1–26.3)	23.1(21.1–25.5)	**0.004**

Notes: [a]For the qualitative analysis, data are numbers of patients with percentages in parentheses. [b]For the quantitative analysis, the normal distribution data is presented as mean±standard deviation; the non-normal distribution data is presented as median (interquartile range). [c]P-values written in bold indicate statistically significant difference.

0.001), the proportion of patients with gallstones in the GBC group was significantly higher than that in the benign group (40.8% versus 19.2%, P < 0.001), asymptomatic patients were significantly more common in the benign group than in patients with GBC (P < 0.001), and the BMI of patients with benign lesions was significantly higher than that of patients with GBC (24.0 versus 23.1, P = 0.004).

The AUC, cut-off value, specificity and sensitivity are recorded in Table 2. NLR, CA19-9 and their combination had higher AUC values, which exceeded 0.7, than those of the other parameters. For NLR, the AUC was 0.733 (95% CI 0.672–0.794) and the cut-off value was 2.28 with 74.8% sensitivity and a relatively low specificity of 64.0%. The AUC and cut-off value for CA19-9 were 0.840 (95% CI 0.788–0.892) and 26.80, respectively, with a relatively low sensitivity of 64.1% and 94.1% specificity. However, the combination integrated the advantages of NLR and CA19-9 and had the highest AUC of 0.868 (95% CI 0.819–0.916). The cut-off point for the combination value was 43.70 with relatively high sensitivity and specificity of 74.8% and 89.7%, respectively. Compared with CA19-9, the sensitivity and specificity of the combination value showed a 10.7% increase and 4.4% decrease, respectively.

ROC curves were obtained to evaluate the efficiency of parameters obtained from preoperative laboratory examinations in differentiating the GBC from other lesions, with results of the analysis of the curves shown in Figure 1. Pairwise comparison of ROC curves was performed with Bonferroni correction. There were significant differences between the AUC of the combination value (0.868) and that of NLR (0.733, P < 0.001) and CA199 (0.840, P = 0.041) indicating that the combination value may have more diagnostic efficacy than either single value alone.

GCP, GA and GBC

The Bonferroni test was used to compare the difference in NLR, CA19-9 and their combination value between the GCP, GA and GBC groups. Table 3 shows that the

Table 2 Performance of Diagnostic Efficiency of NLR, CA19-9, Combination and Other Biochemical Indicators for Distinguishing GBC Patients Preoperatively

Variables	AUC	95% CI	Cutoff	Sensitivity	Specificity	P-value[a]
NLR	0.733	0.672–0.794	2.262	0.748	0.640	<0.001
CA19-9	0.840	0.788–0.892	26.52	0.641	0.941	<0.001
Combination	0.868	0.819–0.916	43.54	0.748	0.897	<0.001
WBC	0.609	0.540–0.678	5.69	0.583	0.626	0.002
Neutrophil	0.658	0.589–0.726	4.00	0.485	0.793	<0.001
Monocyte	0.629	0.565–0.693	0.30	0.641	0.591	<0.001
Platelet	0.539	0.467–0.611	274	0.320	0.818	0.266
PLR	0.654	0.589–0.719	160.49	0.631	0.636	<0.001

Note: [a]P-values written in bold indicate statistically significant difference.
Abbreviations: AUC, area under the curve; CI, confidence interval; WBC, white blood cells.

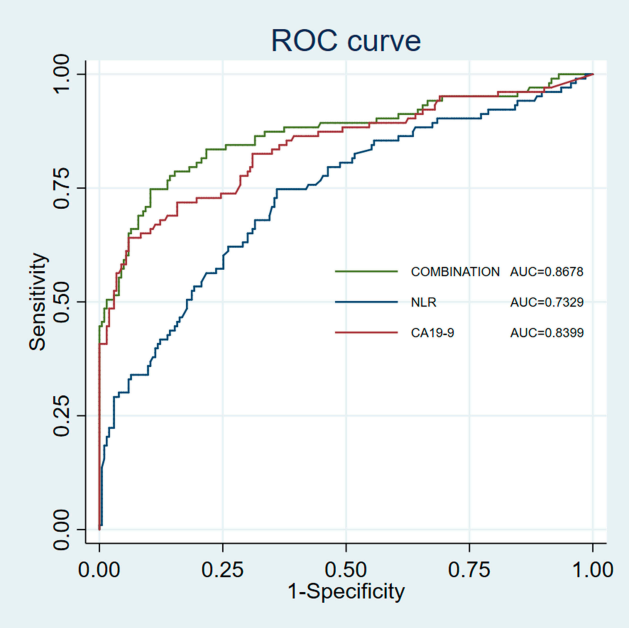

Figure 1 ROC curves of NLR, CA19-9 and the combination as biochemical indicators of GBC.

mean values of NLR, CA19-9 and their combination, with a 95% CI, were significantly higher in the GBC group than in the other groups (all P < 0.001). As shown in Figure 2, the NLR, CA19-9 and combination values were significantly higher in the GBC group than those in the GCP (P < 0.001) and GA (P < 0.001) groups; however, no significant difference was found between the GCP and GA groups.

GBC in Early-to-Mid and Mid-to-Advanced Stages

Results of the comparison of NLR, CA19-9 and their combination values between patients with early-to-mid and mid-to-advanced stage GBC are presented in Table 4. There was no significant difference in the NLR between the early-to-mid and mid-to-advanced groups (2.71 versus 3.00, P = 0.502). However, CA19-9 (16.08 versus 83.27, P < 0.001) and combination (55.91 versus 283.09, P < 0.001) values were significantly lower in the early-to-mid group than in the mid-to-advanced group. Table 5 and Figure 3 show that CA19-9 had the highest AUC (0.796, 95% CI 0.711–0.880) and its cut-off point was 40.79 with 63.0% sensitivity and 89.0% specificity. In contrast to results of previous analyses, the combination group had a lower AUC (0.758, 95% CI 0.666–0.850) than that of CA19-9 (0.796, 95% CI 0.711–0.880) and its 53.7% sensitivity and 88.9% specificity did not display any advantages in distinguishing early-stage GBC from advanced-stage GBC.

Discussion

Even if radical surgeries are performed, patients with GBC still have a low overall five-year survival rate and poor prognosis.[11,12] The importance of distinguishing GBC from benign lesions preoperatively cannot be overemphasized, as this is essential for establishing the correct surgical treatment. The majority of patients with GBC are diagnosed in the mild-to-advanced stage without any symptoms. The lower BMI in patients with GBC in the present study may be due to its being a chronic wasting malignant disease. Also in the present study, gallstones were more commonly seen in patients with GBC than in patients with benign lesions; this result confirmed findings of prior studies that the malignant transformation of gallbladder epithelium has a close relationship with the chronic inflammation caused by the stimulation of gallstones.[6,13] Therefore, GBC is one type of inflammation-associated malignant disease.[14]

NLR and PLR are known indicators of systematic inflammation and are closely associated with the prognosis of various malignant tumours, including GBC.[4–7,15,16] In contrast to numerous studies focusing on the influence of NLR on the prognosis of cancer patients, there are limited studies that have focused on the use of increasing NLR values for the accurate diagnosis of GBC. In the present study, when compared with PLR and other differential WBC counts, NLR had superior diagnostic efficiency with the highest AUC of 0.733.

As described in previous studies, increasing neutrophil levels have a significant influence on the tumour

Table 3 Mean Values of NLR, CA19-9, and Combination in Study Subjects

Variables	GCP (n=123)	GA (n=80)	GBC (n=103)	P-value[a]
NLR	2.21 ± 1.22	2.35 ± 0.81	3.54 ± 2.59	<0.001
CA19-9	9.65 ± 7.93	13.02 ± 9.57	180.26 ± 315.80	<0.001
Combination	20.00 ± 18.05	31.14 ± 27.99	704.74 ± 1540.76	**<0.001**

Notes: Values are presented as mean±standard deviation. [a]P-values written in bold indicate statistically significant difference.

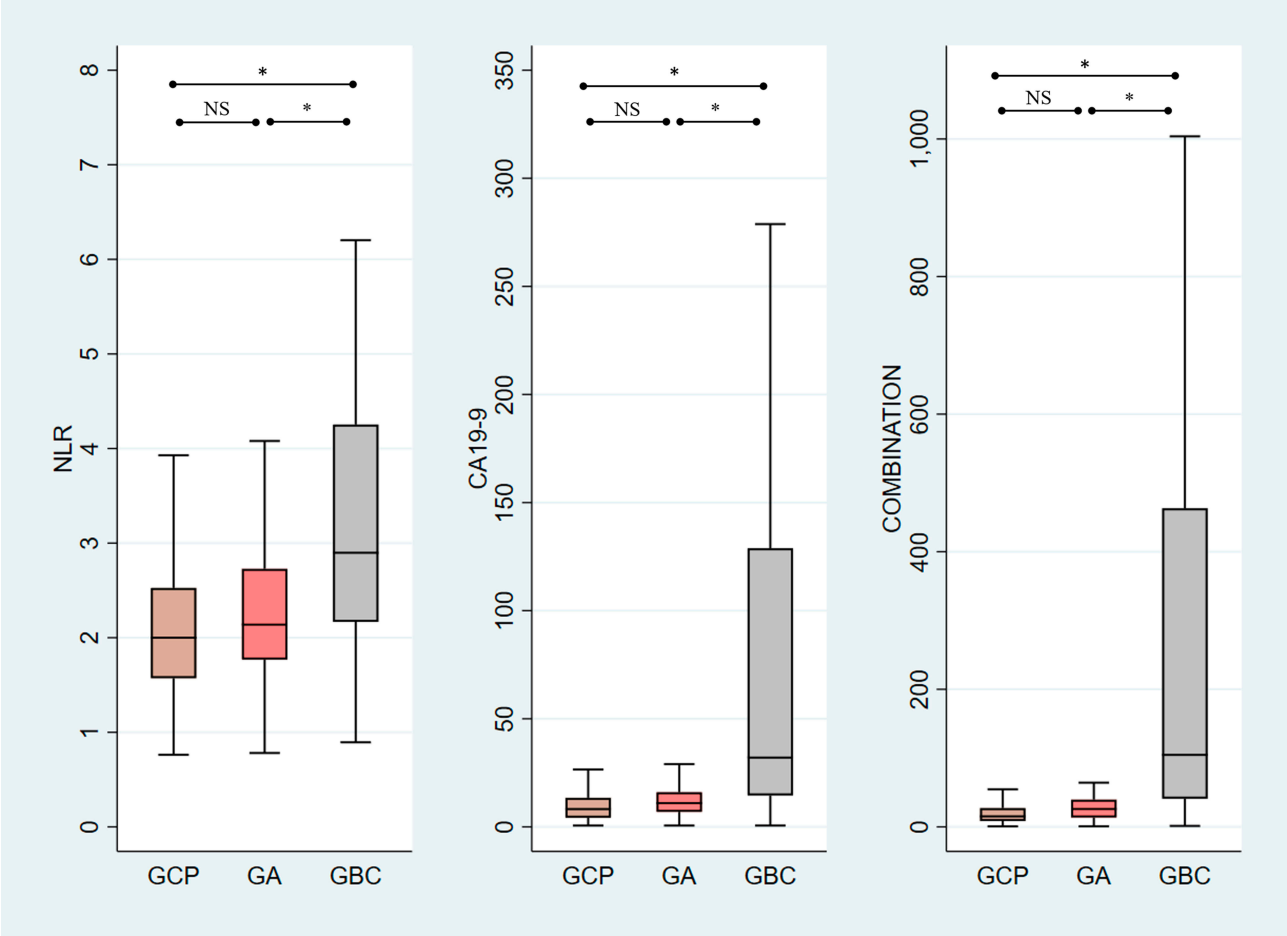

Figure 2 Comparison of NLR, CA19-9 and the combination in GCP, GA and GBC groups. There was no statistically significant difference between the GCP and GBA groups with NLR, CA19-9 or the combination. All three indicators showed a significant difference for GBA vs GBC and GCP vs GBC. NS ($P > 0.05$). *$P < 0.001$.

microenvironment by inducing various cytokines and chemokines, which accelerate the proliferation and metastasis of tumour cells.[17–20] Lymphocytes, on the other hand, which have immunosurveillance and suppression effects on the maturation of tumour cells, have been found to be present at lower levels in patients with malignancy, making lymphocyte levels a poor prognosis indicator.[21,22] In accordance with previous studies, the present study found that NLR caused by neutrophilia and lymphocytopenia was significantly higher in patients with GBC than in patients with benign lesions. Additionally, the present study found that CA19-9, which has been shown to have the best diagnostic efficacy compared to other tumour markers in the screening of GBC,[9] was significantly higher in patients with GBC. However, NLR had ideal sensitivity but low specificity, at 74.8% and 64.0%, respectively. In contrast, CA19-9 had low sensitivity of 64.1% but a specificity over 90%.

To combine the advantages of NLR and CA19-9, a combination value was calculated by multiplying the

Table 4 Comparison of Values of NLR, CA19-9, and Combination in Patients with GBC

	Early-to-Mid (Stages I–II) (n=36)[a]	Mid-to-Advanced (Stages III–IV) (n=67)[a]	P-value[b]
NLR	2.71 (2.08–4.52)	3.00(2.30–4.18)	0.502
CA19-9	16.08 (9.56–30.74)	83.27(22.54–344.00)	P<0.001
Combination	55.91(17.65–123.86)	283.09(51.77–1143.70)	**P<0.001**

Notes: [a]The non-normal distribution data are presented as median (interquartile range). [b]P-value written in bold indicates a statistically significant difference.

Table 5 Performance of Diagnostic Sensitivity and Specificity of NLR, CA19-9, and Combination for Distinguishing GBC Patients with Stage III–IV from I–II

Variables	AUC	95% CI	Cutoff	Sensitivity	Specificity	P-value[a]
NLR	0.540	0.415–0.666	2.46	71.64	47.22	0.530
CA19-9	0.796	0.711–0.880	40.25	62.69	88.89	<0.001
Combination	0.758	0.666–0.850	227.65	53.73	88.89	**<0.001**

Note: [a]P-values written in bold indicate statistically significant difference.
Abbreviations: AUC, area under the curve; CI, confidence interval.

serum CA19-9 values by the NLR values. This combination, which had the highest AUC, also had higher sensitivity and specificity than that of either NLR or CA19-9 alone. Cho et al indicated that a combined marker (serum CA-125 levels multiplied by NLR) had a better diagnostic value than CA-125 or NLR alone for distinguishing endometriosis in patients from those without endometriosis.[23] This was a similar result to the present study, wherein the combination of CA19-9 and NLR had the highest diagnostic efficiency in differentiating GBC from benign lesions than that of CA19-9 or NLR alone.

Additionally, the mean values of NLR, CA19-9 and their combination in patients with GCP, GA, and GBC were determined. These values were not significantly increased in the GCP and GA groups. Therefore, these indexes are not suitable biomarkers for the diagnosis of gallbladder cholesterol polyps and adenoma.

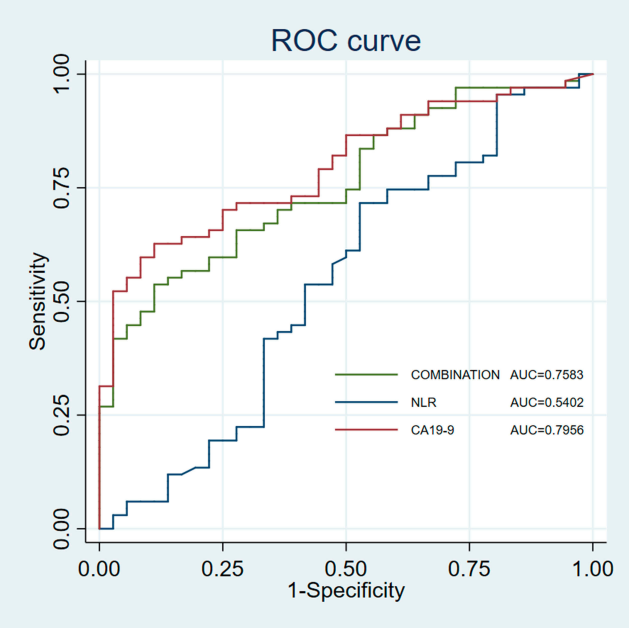

Figure 3 ROC curves of the diagnostic efficiency of NLR, CA19-9 and the combination for separating groups of GBC patients with different degrees of TNM stages (I–II vs III–IV).

Based on the AJCC staging system, which is an ideal tool for assessing the prognosis of GBC, patients with GBC were divided into early-to-mid and mid-to-advanced groups. Several studies have shown that high NLR values have a close relationship with poor prognosis in GBC.[5,6] However, the findings of the present study did not support these previous findings: the mean NLR values of the two groups were close, without any significant difference. The increase in CA19-9, which has been well documented as a clear indicator of poor prognosis and low respectability of GBC,[24] was significant in the mid-to-advanced group in the present study. Despite the increase in CA19-9, the undifferentiated values of NLR in the two groups meant that the combination did not show any advantages over CA19-9 alone in differentiating between the stages of GBC. The similar NLR values in the two groups indicated that the NLR increased significantly even in early-to-mid stage GBC patients. Therefore, NLR has potential as a biomarker for use in the screening of early-stage GBC.

This study had several limitations. First, it was a retrospective study instead of a prospective study. Second, because of the limited number of patients with gallbladder lesions and complete clinical data in a single centre, the sample size was not large. Third, owing to the correlation of inflammation and NLR, some acute inflammatory processes were excluded before NLR-related parameters could be used in the preoperative diagnosis of GBC. Notwithstanding these limitations, this study revealed several parameters that were easily obtained from preoperative routine laboratory blood examinations for the differential diagnosis of GBC with poor prognosis. Comparison of these parameters from GBC patients to those from GA or GCP patients, all of which could have similar gallbladder imaging features, provided practical diagnostic results especially when preoperative imaging diagnosis was controversial.

In summary, NLR, CA19-9 and their combination were significantly higher in patients with GBC than those with GCP or GA and have the potential to become indicators for the preoperative diagnosis of GBC. The combination of NLR and CA19-9 had the best diagnostic efficiency with the highest AUC and relatively high sensitivity and specificity. NLR could not efficiently differentiate early-to-mid stage GBC from mid-to-advanced stage GBC. CA19-9 was significantly higher in patients with mid-to-advanced stage GBC; however, the combination value did not reveal any superiority over CA19-9 in distinguishing GBC at different stages. While these parameters are easily acquired and show promise as accurate tools for the preoperative diagnosis of GBC, the generalisability of these findings should be confirmed in a larger cohort of patients.

Disclosure

The authors report no conflicts of interest in this work.

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