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Relationship between blood parameters and *Clonorchis sinensis* infection: A retrospective single center study

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ABSTRACT

Our study aims to retrospectively investigate neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and eosinophil-to-lymphocyte ratio (ELR) in patients infected with *Clonorchis sinensis*. This study analyzes a total of 151 patients with *C. sinensis* infections and 53 healthy control patients from our hospital. We found close relationships between the three candidate markers and the stages of *C. sinensis* infection-related biliary obstruction. The NLRs, PLRs and ELRs of patients with *C. sinensis* infections were significantly higher than those of healthy individuals; of those, ELRs showed the most superior diagnostic accuracy (sensitivity = 62.9%, specificity = 92.5%). Further, we constituted a logistic regression prediction model; applying two variables (age and NLR) with a sensitivity of 88.89% and a specificity of 83.78% in differentiating *C. sinensis*-related cholelithiasis from *C. sinensis*-untreated patients. Cancer antigen 19-9 (CA19-9) is a commonly used marker in the diagnosis of cholangiocarcinoma. Significant correlation was observed between NLR and CA19-9 in patients with *C. sinensis*–related cholangiocarcinoma ($r = 0.590$, $P = 0.000$). In the receiver operating characteristic analysis for separating *C. sinensis*–related cholelithiasis and cholangiocarcinoma, the cutoff value of PLR was 145.14 with a sensitivity of 65.62% and a specificity of 68.89%; the sensitivity of CA19-9 was 75.00% with a specificity of 77.78%. PLR showed acceptable efficiency to separate *C. sinensis*-related cholelithiasis from cholangiocarcinoma. In conclusion, all of the candidate markers (PLRs, NLRs and ELRs) may act as the valuable supplement in detecting *C. sinensis* infections and diseases.

1. Introduction

Clonorchis sinensis is a food-borne zoonotic parasite, which causes clonorchiasis and is active principally in east Asia, including China, northern Vietnam, South Korea, et al. [1–3]. In China, an estimated 13 million people are infected with *Clonorchis sinensis* in 2004 [4,5]; especially in Guangxi and Guangdong provinces, the epidemiological data shows that clonorchiasis has become a common endemic [2,6,7]. *Clonorchis sinensis* infection often leads to biliary tract inflammation, which may lead to a higher risk of progressive cholelithiasis and cholangiocarcinoma (CCA) in substantial numbers of *Clonorchis sinensis* patients [4,5,8–11].

Recently, numerous studies have indicated that hematological markers—for instance, the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) — may act as the inflammatory markers. Their significances of prediction or prognosis for evaluating inflammatory diseases have been proved [12–15]. The levels of PLR and NLR have been shown to serve as predictive factors for cholangiocarcinoma prognosis and treatment outcomes [16–18]. In addition, eosinophil-to-lymphocyte ratio (ELR) has been evaluated in patients with nasal polyposis, allergic rhinitis, etc. and becomes another potential index of inflammation [19–22]. Nevertheless, It is still a scarcity in the diagnosis of *Clonorchis sinensis*-related diseases using these three blood parameters (NLRs, PLRs and ELRs).

Hence, a retrospective cohort study of *Clonorchis sinensis* infection was conducted. The present study aims to explore the predictive significances and diagnostic values of NLRs, PLRs and ELRs in patients with *Clonorchis sinensis* infection-related bile duct disease.

2. Patients and methods

2.1. Patients

The patients with *Clonorchis sinensis* infections enrolled in our study

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Table 1
Characteristics of different patient groups and controls.

Items	Patients infected with *C. sinensis*			Total patients	Healthy controls	P^a-value	P^b-value
	C.s–UT group	*C.s*–CIS group	*C.s*–CCA group				
Number	74	45	32	151	53	NA	NA
Male (n%)	53 (71.6%)	39 (86.7%)	28 (87.5%)	120 (79.5%)	40 (75.5%)	1.000	0.543
Age	39.96 ± 10.34	54.56 ± 7.83	55.47 ± 7.33	47.60 ± 11.73	44.49 ± 10.71	0.606	0.092
TBIL	10.85 (8.05, 15.03)	20.90 (10.30, 72.55)	17.10 (10.20, 104.68)	12.60 (8.80, 25.60)	9.90 (8.35, 12.95)	0.856	0.002
GGT	25.50 (19.75, 51.25)	274.00 (92.00, 392.50)	154.50 (76.50, 405.75)	70.00 (25.00, 274.00)	31.00 (20.00, 38.00)	0.605	0.000
ALP	63.00 (51.00, 80.50)	143.00 (91.00, 249.00)	150.00 (109.75, 349.00)	89.00 (63.00, 156.00)	75.00 (67.50, 86.00)	0.152	0.019
WBC	6.82 (5.76, 7.83)	8.82 (6.18, 10.25)	8.74 (6.55, 11.55)	7.10 (6.00, 9.45)	6.33 (5.66, 7.19)	0.441	0.000
PLT	228.87 ± 45.33	215.66 ± 52.87	271.09 ± 103.70	233.88 ± 66.70	236.63 ± 40.42	0.008	0.724
NEU	3.49 (2.79, 4.41)	4.63 (3.38, 6.78)	5.13 (3.89, 6.96)	3.92 (3.15, 5.47)	3.37 (2.94, 4.06)	0.251	0.002
EOS	0.26 (0.15, 0.46)	0.56 (0.16, 1.01)	0.57 (0.22, 1.37)	0.36 (0.17, 0.76)	0.18 (0.09, 0.25)	0.634	0.000
LYM	2.15 ± 0.52	1.85 ± 0.83	1.68 ± 0.79	1.96 ± 0.71	2.25 ± 0.50	0.401	0.002
NLR	1.53 (1.25, 2.16)	2.81 (1.66, 4.73)	2.98 (2.29, 6.32)	2.16 (1.42, 3.20)	1.49 (1.32, 1.85)	0.331	0.000
PLR	111.87 ± 33.88	141.58 ± 76.48	185.32 ± 93.89	136.29 ± 70.08	110.29 ± 30.71	0.027	0.000
ELR	0.12 (0.07, 0.22)	0.29 (0.14, 0.54)	0.38 (0.16, 0.84)	0.18 (0.09, 0.42)	0.07 (0.04, 0.11)	0.282	0.000
CA19-9	NA	17.54 (9.35, 68.49)	227.35 (42.87, 2143.00)	NA	NA	0.000	NA

C.s–UT, C. sinensis untreated patients; *C.s*–CIS, *C. sinensis*-related cholelithiasis patients; *C.s*–CCA, *C. sinensis*-related cholangiocarcinoma patients; TBIL, total bilirubin; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; WBC, white blood cells; PLT, platelets; NEU, neutrophils; EOS, eosinophils; LYM, lymphocytes; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; ELR, eosinophil-to-lymphocyte ratio; CA19-9, cancer antigen 19-9; NA, not applicable; P^a-value, *P* value for the comparison of patients with *C. sinensis*-related cholelithiasis and *C. sinensis*-related cholangiocarcinoma; P^b-value, *P* value for the comparison of *C. sinensis* infections and healthy control data.

were selected from the medical database of the First Affiliated Hospital of Guangxi Medical University from 2012 to 2017. This study conformed to the ethics of the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University. Written informed consent was acquired from all participators. A total of 151 patients with *C. sinensis* infections were included in the research. We categorized them into three groups: 74 *C. sinensis* untreated patients (*C.s*–UT group), 45 *C. sinensis*-related cholelithiasis patients (*C.s*–CIS group), and 32 *C. sinensis*-related cholangiocarcinoma patients (*C.s*–CCA group). *Clonorchis sinensis* infection was diagnosed in each patient by microscopy, serological test or imaging. All of the *C.s*–UT patients were first diagnosed as clonorchiasis; each of them had never undergone an anti-parasitic treatment before; there were no any clinical features or imaging characteristics for their progress of cholelithiasis or cholangiocarcinoma. Cholelithiasis patients were diagnosed through radiological evidence, and the diagnoses of all enrolled patients with cholangiocarcinoma were verified by histopathology. There were three *C.s*–CIS patients and six *C.s*–CCA patients who had been diagnosed as clonorchiasis before living in our hospital. Of those, only one of *C.s*–CCA patients had received an anti-parasitic treatment in other hospital four months ago; however, we fail to get more information about the specific drugs and treatment cycle.

Patients were excluded if they met any of the following conditions: (I) HBV, HCV, HIV or other virus infection; (II) autoimmune disorder; (III) hematologic disease; (IV) malignant disease; (V) blood transfusion and organ transplantation; (VI) nephropathy; (VII) diabetes; (VII) cardiovascular disease; or (IX) co-infection with another parasite.

In addition, the control group included 53 healthy individuals matching with *C. sinensis* infected patients in sex and age, selected from individuals who had come to the Physical Examination Center of the hospital for a health examination.

2.2. Data collection

We extracted the following data for each of the healthy control and test group patients before treatment: gender, age, total bilirubin (TBIL), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), white blood cells (WBC), platelets (PLT), neutrophils (NEU), eosinophils (EOS), lymphocytes (LYM). Additionally, the results of cancer antigen 19-9 (CA19-9) for patients with cholelithiasis and CCA were also collected. NLR is the ratio of the patient's neutrophil count to his or her lymphocyte count, PLR is the ratio of the platelet count to the lymphocyte count, and ELR is the ratio of eosinophil count to the lymphocyte count.

2.3. Laboratory methods

TBIL, GGT and ALP were determined using a Hitachi 7600 chemistry analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). The blood parameters were tested using a Beckman Coulter LH 780 blood analyzer (Beckman Coulter, Brea, CA). The level of CA19-9 was measured using a Roche E6000 analyzer (Roche Diagnostics, Basel, Switzerland).

2.4. Statistical analysis

Quantitative data were presented in two forms: value ± standard deviation (mean ± SD) for normal distribution data and median value with the inter quartile range (IQR) for non-normal distribution. Variable distributions were performed using the Kolmogor–Smirnov test. Qualitative data were described in terms of frequency and percentage. Analysis of the statistical differences of the two groups was conducted using a Student *t*-test, Mann–Whitney *U* test or X^2 test, and comparison of more than two groups was performed applying a one-way ANOVA or Kruskal–Wallis *H* test, as appropriate. The diagnostic efficiency of candidate markers was assessed employing sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, the receiver operating characteristic (ROC) curve and the area under the ROC curve (AUROC). Comparison between the area under the ROC curves was performed utilizing a z-test. The optimal cutoff value was determined by the Youden index. Univariate and multivariate analyses were performed to identify the independent risk factors of *C. sinensis*-related cholelithiasis. The Spearman correlation analysis was used for two continuous variables. All data analyses were conducted employing IBM SPSS (version 20) and MedCalc statistical software (version 11.3.8.0). A two-tailed test was employed, using a significant level of 0.05.

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