

C-reactive protein to lymphocyte count ratio is a promising novel marker in hepatitis C infection: the clear hep-c study

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SUMMARY

OBJECTIVE: Chronic hepatitis C (CHC) is one of the most important health problems affecting the significant rate of world population and it may lead to cirrhosis and hepatocellular carcinoma. C-reactive protein to lymphocyte count ratio (CLR) is used in estimating inflammatory burden. Therefore, this study aimed to compare CLR values between CHC patients and healthy controls and between CHC patients with and without fibrosis.

METHODS: Patients with CHC infection who visited outpatient and inpatient internal medicine clinics of our institution between January 2021 and December 2021 were enrolled to this retrospective study. CLR of the patients with CHC and healthy controls were compared. We further compared CLR of CHC patients with and without fibrosis.

RESULTS: Median CLR of CHC and control subjects was 2.61 (5.13%) and 0.31 (0.37%), respectively. CLR of the CHC group was significantly increased compared to the CLR of the controls ($p < 0.001$). There was a significant positive correlation between CLR and APRI score ($r = 0.15$, $p = 0.04$). The sensitivity and specificity of CLR in determining CHC above 0.58% level were 84% and 82%, respectively (AUC: 0.884, $p < 0.001$, 95%CI 0.84–0.93). In subgroup analysis, CLR was 3.97 (6.6%) for CHC patients with fibrosis and 1.7 (4.4%) for CHC subjects without fibrosis ($p = 0.001$).

CONCLUSIONS: Increased CLR in patients with CHC may be an alarming finding of liver fibrosis, as CLR is associated with both CHC and hepatic fibrosis.

KEYWORDS: Inflammation. Chronic hepatitis C. Fibrosis. C-reactive protein to lymphocyte count ratio.

INTRODUCTION

Chronic hepatitis C (CHC) is one of the most important health issues affecting about 2–3% of the general population in the world¹. CHC may cause liver cirrhosis and hepatocellular carcinoma^{2,3}. Treatment of CHC is based on the data about the degree of the hepatic fibrosis, which is estimated by noninvasive aspartate-to-platelet ratio index (APRI) and FIB4 scores and by histopathological evaluation of the liver biopsy specimen, which is an invasive procedure^{4,5}. To estimate fibrosis in patients with CHC, novel, cost-effective, and noninvasive markers are needed.

Reports in literature introduced a novel biomarker, C-reactive protein to lymphocyte count ratio (CLR), to estimate inflammatory burden in certain conditions. Preliminary CLR studies were on cancer and suggested that CLR could be a reliable marker of prognosis in a variety of malignant conditions^{6,7}. CHC produces significant amount of inflammation as those malignancies do. Therefore, we designed this study to investigate the hypothesis that CLR may have a role in CHC and predict the degree of fibrosis. CLR values of the CHC patients were compared to those of healthy controls and also CHC patients with fibrosis to those without fibrosis.

METHODS

Study population

After study protocol was approved by institutional ethics committee (approval no: 2021/291, approval date: December 7, 2021), patients with CHC infection who visited outpatient and inpatient internal medicine clinics of our institution between January 2021 and December 2021 were enrolled in this retrospective study. Control subjects were healthy volunteers who visited our clinics for a routine checkup within the same time period. Subjects with malignancy, acute infection, and active inflammatory diseases were excluded from the study.

Anthropometric and laboratory analyses

Age and sex of the participants were recorded. White blood cell (WBC); neutrophil (neu), lymphocyte (lym), and platelet (PLT) counts; hemoglobin (Hb); hematocrit (Hct); aspartate (AST) and alanine (ALT) transaminases; blood urea; creatinine; and serum albumin levels were obtained from institutional database. Hemogram parameters were measured using the Abbott Cell-Dyn 3700 complete blood count device

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(Abbott Laboratories, Abbott Park, IL, USA). Serum biochemistry parameters were measured using an Abbott Architect C8000 auto-analyzer (Abbott Laboratories). APRI and FIB4 scores were calculated with the following formulas: $[(AST/\text{upper limit of the normal AST range}) \times 100]/PLT$ and $[(age \times AST) / (PLT \times \sqrt{ALT})]$, respectively. A CLR was calculated by dividing CRP by lym. Data of the patients with CHC and controls were compared. We further grouped CHC patients as fibrosis group and no-fibrosis group according to the HAI score in histopathological examination of liver biopsy. CLR and other variables of the fibrosis group and no-fibrosis group were compared.

Statistical analyses

Statistical analyses were conducted by using statistics software (SPSS version 16.0 for Windows; IBM Co., Armonk, NY, USA). Kolmogorov-Smirnov test was used to identify whether variables are normally distributed. Variables with normal distribution were compared with t-test and expressed as mean \pm standard deviation (SD). Mann-Whitney U test was used to compare non-normally distributed variables and those variables were expressed as median (interquartile range [IQR]). Categorical variables were compared with chi-square test and expressed as percentages and numbers. Correlation between CLR and other study variables was analyzed with Pearson's correlation test. The sensitivity and specificity of CLR and other variables in predicting CHC were obtained by receiver operative characteristics (ROC) test. Binary logistic regression analysis adjusted to age, AST, ALT, APRI, and FIB4 scores was performed to evaluate whether CLR was an independent risk factor for hepatic fibrosis in CHC patients. Statistical significance was considered at $p < 0.05$.

RESULTS

A total of 198 subjects were enrolled in the study, with 132 in CHC group and 66 in control group. Median age was 55 (23) years for CHC group and 52.5 (5) years for controls ($p = 0.067$). In CHC group, 68 (51.5%) were women and 64 (48.5%) were men, while 25 (38%) were women and 41 (62%) were men in control group ($p = 0.07$).

Table 1 shows characteristics and laboratory data of the CHC and control groups. Median CLR of CHC and control subjects was 2.61 (5.13%) and 0.31 (0.37%), respectively. Therefore, CLR of the CHC group was significantly higher than that of the controls ($p < 0.001$).

There was a significant positive correlation between CLR and APRI score ($r = 0.15$, $p = 0.04$).

The sensitivity and specificity of CLR in determining CHC above 0.58% level were 84 and 82%, respectively (AUC: 0.884, $p < 0.001$, 95%CI 0.84–0.93). APRI score > 0.23 with 81% sensitivity and 82% specificity indicates CHC (AUC: 0.871, $p < 0.001$, 95%CI 0.82–0.92), while FIB4 score > 1 with 75% sensitivity and 73% specificity indicates CHC (AUC: 0.807, $p < 0.001$, 95%CI 0.75–0.87). Figure 1 shows the ROC curves of CLR, APRI, and FIB4 scores in determining CHC subjects.

We further compared CHC patients with fibrosis to those without fibrosis in histopathological evaluation. Age ($p = 0.42$), WBC ($p = 0.4$), neu ($p = 0.75$), lym ($p = 0.22$), Hb ($p = 0.06$), Hct ($p = 0.06$), PLT ($p = 0.21$), ALT ($p = 0.22$), urea ($p = 0.57$), and creatinine ($p = 0.08$) levels were not statistically different between CHC subjects with and without fibrosis. CRP ($p < 0.001$), APRI score ($p = 0.002$), FIB4 score ($p = 0.001$), AST ($p = 0.007$), albumin ($p = 0.01$), and HAI score ($p < 0.001$) of the CHC patients with and without fibrosis were significantly different from each other. CLR of the CHC patients with fibrosis was 3.97 (6.6%) and for those without fibrosis was 1.7 (4.4%), with statistically significant difference ($p = 0.001$).

Table 1. Summary of the data of study population.

Variable	CHC group	Control group	p
Sex			
Male (n, %)	64 (48,5)	41 (62)	0.07
Female (n, %)	68 (51,5)	25 (38)	
Mean \pm SD			
Lym (k/mm ³)	1.9 \pm 0.86	2.1 \pm 0.61	0.104
Hb (g/dL)	13.5 \pm 2.2	14.6 \pm 1.5	<0.001
Hct (%)	40.4 \pm 6.5	44 \pm 4.5	<0.001
PLT (k/mm ³)	199 \pm 77	240 \pm 44	<0.001
Median (IQR)			
Age (years)	55 (23)	52.5 (5)	0.067
CLR (%)	2.61 (5.13)	0.31 (0.37)	<0.001
CRP (mg/L)	4.6 (9.2)	0.7 (0.8)	<0.001
WBC (k/mm ³)	6.5 (2.5)	7 (3)	0.06
Neu (k/mm ³)	3.25 (1.5)	3.9 (1.6)	0.071
APRI (%)	0.53 (0.68)	0.19 (0.07)	<0.001
FIB4 (%)	1.79 (2.43)	0.87 (0.37)	<0.001
AST (U/L)	39.5 (42.5)	17 (5.3)	<0.001
ALT (U/L)	42 (51)	18 (8.5)	<0.001
Blood urea (mg/dL)	30 (15)	24.5 (11)	<0.001
Creatinine (mg/dL)	0.883 (0.38)	0.75 (0.12)	0.002
Serum albumin (g/L)	41 (7)	45 (1)	<0.001

Bold indicates significant p-value.

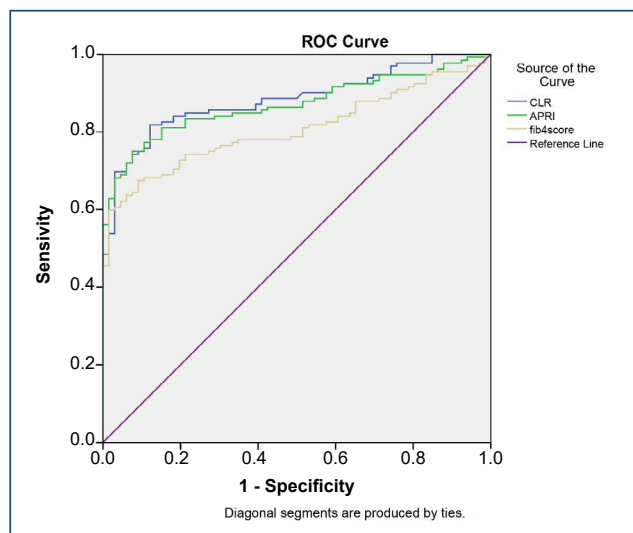


Figure 1. Receiver operating characteristic curves of C-reactive protein to lymphocyte count ratio, aspartate-to-platelet ratio index, and fibrosis-4 scores in determining chronic hepatitis C.

Binary logistic regression analysis adjusted to age, AST, ALT, APRI, and FIB4 scores showed that CLR was an independent risk factor for fibrosis in CHC patients ($p=0.01$, OR: 0.88, 95%CI 0.79–0.98).

DISCUSSION

Results of this study show that CLR could be associated with the presence of CHC and may be a marker of fibrosis in this population. In addition, CLR has significant correlation with APRI score, another predictor of fibrosis in chronic hepatitis subjects. Finally, we showed that CLR has better sensitivity and specificity than both APRI and FIB4 scores in determining subjects with CHC.

A study on CRP and lymphocyte count in heart failure patients found that CRP was increased and lymphocyte count was reduced (meaning elevated CLR) in heart failure compared to healthy individuals⁸. Moreover, a recent study in patients with incarcerated hernia reported increased CRP and decreased lymphocyte count were associated with intestinal ischemia in that population⁹. Thus, both intestinal ischemia and heart failure are associated with some degree of inflammatory burden. Similarly, CHC is associated with inflammation; thus, a similar elevation in CLR was found in CHC patients in this study.

Association between CLR and inflammatory conditions has been well established. In a study investigating the prognostic role of CLR in patients undergoing esophagogastric resection for esophageal cancer, it was found that CLR is a useful marker in the prediction of major morbidity after esophagogastric resection

surgery⁷. A recent work that studied CLR in pancreatic cancer revealed that it was better than any other prognostic indicators in predicting survival of those patients⁶. Subsequently, their findings were supported in another study by Fan et al., which reported CLR as a useful prognostic marker¹⁰. Pancreatic cancer, like all malignancies, induces significant inflammation. In contrast, inflammation also plays an important role in surgical procedures. Since inflammation is a common pathway in those conditions and in CHC, this study also reported elevated CLR.

The prognostic role of CLR has been studied in other malignancies, too. According to a study which observed CLR in oral malignancy, elevated level of CLR was associated with better prognostic performance compared to other inflammatory markers in subjects with squamous cell carcinoma¹¹. Similarly, Meng et al. investigated CLR in patients with colorectal cancer and reported that patients with high CLR had shorter overall survival than those with low CLR levels¹². Subsequently, Mungan et al.¹³ confirmed the results of Meng et al.¹². In addition, prognostic value of elevated CLR has also been shown in patients with osteosarcoma¹⁴, cholangiocarcinoma¹⁵, and lung cancer¹⁶. It is a fact that malignant conditions are associated with increased inflammatory burden¹⁷, as seen in patients with CHC. Therefore, increased CLR levels in CHC and further higher CLR in subjects with hepatic fibrosis, which reported in this study, are the findings consistent with literature knowledge.

Hepatic fibrosis in CHC is associated with increased inflammatory burden^{18,19}. Hepatic stellate cells are responsible of accumulation of extracellular matrix proteins (i.e., collagen) in liver in patients with CHC. Inflammatory cytokines and chemokines which produced and released by hepatitis C-infected hepatocytes trigger the activation of hepatic stellate cells²⁰. Indeed, we found higher CLR levels in CHC patients with fibrosis compared to the CHC subjects without liver fibrosis. Inexpensive, easy-to-assess, and noninvasive nature are advantages of CLR over other fibrosis markers in CHC.

There are three main limitations of this study: retrospective design, relatively small study cohort, and single-center nature of the conducted work. Due to single-center nature, the association between CHC and CLR may not be a global association. However, to the best of our knowledge, this is the first study in literature to report elevated CLR in CHC patients and even higher levels of CLR in those with liver fibrosis compared to the subjects without fibrosis.

CONCLUSIONS

Increased CLR in patients with CHC may be an alarming finding of liver fibrosis since CLR is associated with both CHC and hepatic

fibrosis. Therefore, inexpensive and easy-to-assess nature of CLR make it a useful marker in clinical follow-up of this population.

AUTHORS' CONTRIBUTIONS

MED: Conceptualization, Data curation, Writing – review & editing. **GA:** Conceptualization, Formal analysis, Supervision,

Writing – original draft, Writing – review & editing. **SB:** Conceptualization, Methodology, Software. **GK:** Data curation, Writing – original draft, Writing – review & editing. **OK:** Data curation, Writing – original draft, Writing – review & editing. **BMA:** Data curation, Formal analysis, Writing – review & editing. **TTD:** Conceptualization, Data curation, Writing – review & editing.

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