Eur. Cytokine Netw. Vol. 34, n° 3, *September 2023* 28-37

RESEARCH ARTICLE

Th1/Th2 cytokine profile in patients with acute and chronic calculus cholecystitis

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Article accepted on 12 September 2023

To cite this article: Chen L, Chen X. Th1/Th2 cytokine profile in patients with acute and chronic calculus cholecystitis. Eur. Cytokine Netw. 2023; 34(3): 28-37. doi: 10.1684/ecn.2023.0488

ABSTRACT. *Background:* Relatively little is known about the relationship between Th1/Th2 cytokines and calculus cholecystitis (CC). The purpose of this study was to investigate the correlation between serum Th1 and Th2 cytokine expression and CC, including both acute and chronic cases. *Methods:* In total, 102 patients with chronic calculous cholecystitis (CCC), 64 patients with acute calculous cholecystitis (ACC), and 55 healthy controls (HCs) were recruited for the study. Serum concentration of Th1 (IL-2, TNF-α, IFN-γ) and Th2 cytokines (IL-4, IL-6, IL-10) was measured at admission and on the fifth day after cholecystectomy using flow cytometry. In addition, the ratio of IL-6/IL-10 was calculated. Correlation of the corresponding factors was then analysed, and univariate and multivariate Cox regression analyses were performed to identify independent markers of ACC severity. *Results:* Compared to HCs, CCC patients exhibited significantly elevated expression levels of IL-6 and IL-10, while ACC patients demonstrated higher expression of IL-2, TNF-α, and IL-6/ IL-10 in addition to IL-6, and IL-10. In ACC patients, there was a strong positive correlation between IL-6 and IL-10 concentration, the expression of IL-2 was observed to positively correlate with serum ALT and AST concentration, and TNF-α expression positively correlated with the duration of hospitalization. Moreover, patients with moderate-to-severe ACC presented with higher expression of IL-10 compared to those with mild ACC. Cox regression analysis confirmed that IL-10 and IL-6 were independent factors for the severity of ACC. Following surgery, the levels of IL-6 and IL-6/IL-10 significantly decreased but did not fully return to baseline levels in ACC patients. *Conclusion:* Our study reveals atypical Th1/Th2 cytokine expression profiles in patients with acute and chronic CC, and further highlights the significant potential of these cytokines, particularly IL-6 and IL-10, in assessing the severity and progression of CC.

Key words: calculus cholecystitis, Th1/Th2 cytokines, IL-6, IL-10

Cholecystitis is a prominent diagnosis among inpatients for hepatobiliary surgery. The rise in the incidence of cholecystitis in numerous countries in recent decades may be attributed to improvement in living standards and the prevalence of poor dietary habits [1, 2]. Based on the content present in the gallbladder cavity, cholecystitis can be categorized into calculous cholecystitis (CC) and non-calculous cholecystitis. CC accounts for 90% of all cholecystitis cases. Additionally, CC can be subcategorized as chronic calculous cholecystitis (CCC) and acute calculous cholecystitis (ACC) based on the severity of clinical manifestations and the pace of disease progression.

The most widely accepted theory for the development of CC is that it occurs as a result of an obstruction in the cystic duct, caused by gallstones, sludge, or lithogenic bile. This obstruction subsequently leads to inflammation of the gallbladder through chemical or bacterial means. The severity and duration of this obstruction determine how quickly acute cholecystitis develops and the extent of inflammation in the gallbladder. The persistent obstruction of the cystic duct can lead to severe complications, such as gallbladder empyema, localized ulcer necrosis, and gallbladder perforation [3]. The acute phase of inflammation typically lasts for about one week to ten days. After 2-3 weeks, the purulent area is replaced with granulation tissue, leading to subacute cholecystitis. The repeated occurrence and regression of inflammation caused by the blockage of the gallbladder is one of the mechanisms that contribute to chronic cholecystitis [4, 5]. Some researchers propose that the occurrence of cholelithiasis and chronic cholecystitis is associated with an atypical makeup of bile, resulting in the formation of stones and chemical damage to the mucosal lining [6]. The pathophysiological mechanism of cholecystitis requires further study.

The immune system's inflammatory response is a crucial factor in the development of cholecystitis, which is regulated and mediated by several molecules [7].

doi: 10.1684/ecn.2023.0488

Cytokines are synthesized by almost all nuclear cells and function as key regulators of the immune response. Cytokines in the immune system form a representative universal media network between organs, tissue, and cells, which plays a key role in regulating the immune response, haematopoiesis, inflammation, and wound repair. Previous studies have indicated that an extensive array of cytokines is involved in the occurrence and development of cholecystitis, including IL-6, TNF-α, and IL-1α, which play an important role in the disease [8-10]. Treatment using biological agents targeted at these cytokines has demonstrated clinical effectiveness [11]. Cytokines produced by Th1 cells (IL-2, IFN-γ, TNF-α) perform a pivotal role in the cellular immune response, and cytokines produced by Th2 cells (IL-4, IL-6, IL-10) are indispensable for humoral or allergic responses. However, the Th1/Th2 cytokine spectrum and clinical significance in cholecystitis remain unclear. Thus, our study aimed to determine the variation in Th1/Th2 cytokine level in the serum of patients with acute and chronic CC and investigate the relationship between these cytokines and the occurrence, development, and severity of the disease, with a view to better understanding the potential immunopathological mechanisms involved in each clinical stage of CC.

Material and methods

Study subjects

Between June 2022 and May 2023, resected gallbladders of 240 patients, who underwent hepatobiliary surgery for cholecystectomy due to CCC or AAC, were sent for histopathological examination. Among them, 102 cases of CCC patients (male/female: 66/36; mean age: 50.62 ± 14.89 years) and 64 cases of ACC patients (male/female: 37/27; mean age: 52.06 ± 13.52 years) were diagnosed pathologically and enrolled in the study. The ACC patients underwent further classification using the Tokyo guidelines, classified as mild (19 cases), moderate (42 cases), and severe (three cases) ACC [12]. The criteria for exclusion were established as follows: (1) patients below 18 years of age or above 90 years of age; (2) complications with gallbladder polyp or gallbladder carcinoma; (3) complications with hepatitis C, intrahepatic or extrahepatic cholelithiasis; (4) complications with serious medical diseases such as heart, lung, brain or kidney; (5) complications with other autoimmune, tumoural, or inflammatory diseases; (6) patients with mental illness; (7) cholecystitis during pregnancy; and (8) non-calculous cholecystitis. Fifty-five age- and sex-matched healthy individuals (male/female: 35/20; mean age: 50.00 ± 15.53 years) were enrolled as healthy controls (HCs) from the Health Examination Center of the hospital during the same period. To further investigate fluctuations of Th1/Th2 cytokine levels following laparoscopic cholecystectomy, a cohort of 21 ACC patients, who did not experience any postoperative complications and did not require a switch from the laparoscopic to the open method, were selected at random on the fifth day after surgery to evaluate cytokine concentrations. The ratio of IL-6 to IL-10 was calculated to assess the underlying Th1/Th2 inflammatory response. The study protocol was approved by the institutional ethics committee (approval number: L2022-02-052). The comparative analysis of several important laboratory parameters between different groups is shown in *tables 1* and *2*.

Cytokine detection

Blood specimens were collected under sterile conditions when all subjects were admitted to the hospital before surgery and five days post-surgery. The serum concentrations of IFN-γ, TNF-α, IL-10, IL-6, IL-4, and IL-2 were measured utilizing the cytometric bead array (CBA) kit-BD™ CBA Human Th1/Th2 Cytokine Kit II (BD Biosciences, San Jose, CA, USA), which has been previously detailed in the literature [13]. To provide a brief overview, the CBA method employs six distinct populations of beads, each tagged with a unique capture antibody specific to a respective protein. The cytokine capture beads (using 25 μL) were combined with phycoerythrin-conjugated detection antibodies (25 μL), then incubated with recombinant standards or test samples (25 μL) at room temperature for 2.5 hours in the dark to form sandwich complexes. Subsequently, 1 mL phosphate buffer saline (PBS), pH 7.4, was utilized to eliminate residual unbound antibodies, followed by the acquisition of sample data using a FACSCanto II flow cytometer (Becton Dickinson, San Jose, CA, USA). The results were presented graphically and in tabular format using the FCAP Array Software version 3.0 (BD Biosciences, San Jose, CA, USA). Six standard curves were obtained from a single set of calibrators, and the concentrations of serum cytokines were calculated based on these standard curves. The linear range for the six cytokines was 0 to 2500.0 pg/mL. Moreover, the ratios of IL-6/IL-10 were calculated in order to assess the underlying Th1/Th2 inflammatory response.

Detection of other laboratory parameters

For the determination of routine blood counts, 2 ml of EDTA-anticoagulated blood samples was examined using a Sysmex XE-2100 Hematology System (East Asia Company, Japan). Peripheral blood (5 mL) was collected into pre-coagulation tubes and centrifuged to obtain serum, which was used to detect alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamine transferase (GGT), alkaline phosphatase (ALP), creatinine (Cr), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein AI (Apo AI), apolipoprotein B (Apo B), a-lipoprotein (Lp [a]) and C-reactive protein (CRP) using a Beckman Coulter AU5800 Automatic Biochemical Analyzer (Beckman Coulter Co. Ltd. USA). Sodium citrate-anticoagulation blood samples (5 mL) were used for international normalized ratio of prothrombin time (INR) analysis through the STA-R Evolution® Experts series (STAGO, France).

Statistical analysis

Comparisons between various individuals were performed using the Mann-Whitney U test or one-way

Table 1
Comparison of important laboratory parameters between the three groups.

Characteristics	HC (n=55)	CCC (n=102)	ACC (n=64)	p^a	p^b
Liver function indexes					
ALT (U/L)	12.791±4.942	59.642±25.903	80.731±24.522	<0.001*	0.009*
AST (U/L)	14.251±3.742	44.554±13.991	74.742±20.983	<0.001*	0.009*
GGT (U/L)	20.251±5.933	85.564±33.792	117.454±31.704	<0.001*	0.112
ALP (U/L)	91.542±53.261	107.246±42.634	112.6110±46.824	0.220	0.128
Serum lipid level					
TC (nmol/L)	4.611±0.68	4.96±1.012	4.422±1.041	0.371	<0.001*
TG (nmol/L)	1.172±0.37	1.76±1.251	1.221±0.753	0.091	<0.001*
HDL-C (nmol/L)	1.394±0.221	1.13±0.273	1.131±0.433	0.007*	0.908
LDL-C (nmol/L)	2.584±0.531	2.67±0.771	2.324±0.751	0.021*	<0.001*
Apo AI (g/L)	1.261±0.092	1.3±0.282	1.133±0.352	0.004*	<0.001*
Apo B (g/L)	0.783±0.111	0.81±0.231	0.741±0.241	0.121	0.001*
Lp (a) (mg/L)	224.144±160.070	223.7±246.620	168.811±104.242	0.002*	0.325
Complete blood cell count					
WBC ($\times10^9$/L)	5.832±1.091	7.09±2.570	11.653±5.701	0.003*	<0.001*
LYM (%)	35.651±8.342	28.61±10.801	18.941±2.602	<0.001*	<0.001*
NEUT (%)	55.351±8.431	63.10±11.712	75.641±11.122	<0.001*	<0.001*
NLR	1.672±0.682	3.06±2.631	8.462±6.761	<0.001*	<0.001*
PLT ($\times10^9$/L)	256.772±62.263	248.27±68.570	236.112±65.172	0.402	0.179
INR	1.110±0.11	1.121±0.311	1.181±0.111	0.302	0.223
CRP	3.026±2.851	10.39±13.06	133.02±101.84	<0.001*	<0.001*

p^a: comparison between the HC and CCC group; p^b: comparison between the CCC and ACC group. Data are presented as mean ± SD. ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; Apo AI: apolipoprotein AI; Apo B: apolipoprotein B; Lp (a): a-lipoprotein; WBC: white blood cell; LYM: lymphocyte; NEUT: neutrophil; NLR: neutrophil-to-lymphocyte ratio; PLT: platelet count; INR: international normalized ratio of prothrombin time; CRP: C-reactive protein; HC: healthy control; CCC: chronic calculous cholecystitis; ACC: acute calculous cholecystitis. * Statistically significant ($p < 0$ .05).

Table 2
Laboratory data of ACC patients stratified according to severity grade.

Characteristics	M-ACC (n=19)	MS-ACC (n=45)	*p*
Liver function indexes			
ALT (U/L)	112.132±74.012	58.208±25.731	0.763
AST (U/L)	101.737±41.320	55.377±28.551	0.221
GGT (U/L)	106.235±34.661	126.64±30.752	0.621
ALP (U/L)	111.939±36.791	112.381±51.212	0.982
Serum lipid level			
TC (nmol/L)	4.654±1.074	4.248±1.007	0.077
TG (nmol/L)	1.360±0.864	1.111±0.644	0.132
HDL-C (nmol/L)	1.141±0.354	1.120±0.481	0.822
LDL-C (nmol/L)	2.555±0.751	2.153±0.712	0.011*
Apo AI(g/L)	1.217±0.332	1.061±0.353	0.036*
Apo B (g/L)	0.778±0.225	0.707±0.243	0.077
Lp (a) (mg/L)	177.368±161.658	162.679±70.481	0.744
Complete blood cell count			
WBC (×10/L)	8.429±3.033	13.957±6.056	<0.001*
LYM (%)	28.900±18.591	11.789±9.345	<0.001*
NEUT (%)	70.789±11.234	79.134±10.281	<0.001*
NLR	6.09±5.69	10.531±6.846	<0.001*
PLT (×10^9/L)	246.868±67.063	228.396±64.512	0.028
INR	1.121±0.073	1.191±0.123	0.224

Data are presented as mean ± SD. M-ACC: mild ACC group; MS-ACC: moderate-to-severe ACC group; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; Apo AI: apolipoprotein AI; Apo B: apolipoprotein B; Lp (a): a-lipoprotein; WBC: white blood cell; LYM: lymphocyte; NEUT: neutrophil; NLR: neutrophil-to-lymphocyte ratio; PLT: platelet count; INR: international normalized ratio of prothrombin time. *Statistically significant ($p < 0.05$).

analysis of variance (ANOVA), whereas comparisons between the same individuals were performed using the Wilcoxon matched-pair t-test or paired t-test, when appropriate. Correlations between variables were evaluated using the Spearman rank correlation test. To explore the association between cytokines and the NLR variable, first, a univariate linear regression was conducted, from which factors with p value less than 0.20 were included in a model. Quantitative variables were expressed as mean ± standard or median with an interquartile range (25^{th}-75^{th} percentile). A two-sided p value <0.05 was considered statistically. Statistical significance is indicated as follows: $***p < 0.001$, $**p < 0.01$, and $*p < 0.05$. All statistical analyses were performed using SPSS v.23 (SPSS Inc, Chicago, III).

Results

Th1 and Th2 cytokine expression in ACC and CCC patients

In patients with ACC, the expression levels of Th1 cytokines (including IL-2 and TNF-α) and Th2 cytokines (including IL-6 and IL-10) exhibited different degrees of increase compared with HCs, with a median concentration of 0.447 pg/mL IL-2 (0.00 to 1.800 pg/mL), 0.605 pg/mL TNF-α (0.00 to 69.51 pg/mL), 76.965 pg/mL IL-6 (1.89 to 1832.64 pg/mL), and 5.972 pg/mL IL-10 (1.038 to 208.75 pg/mL) (*figure 1A,C-E*). Similarly, in patients with CCC, the expression levels of Th2 cytokines (including IL-6 and IL-10) were also significantly increased with a median concentration of 2.569 pg/mL IL-6 (0.00 to 224.518 pg/mL) and 2.179 pg/mL IL-10 (0.00 to 33.576 pg/mL) compared to HCs (*figure 1 C,D*). The expression levels of IL-6 and IL-10 were notably higher in ACC patients in comparison to CCC patients. However, we did not find any notable variation in the levels of IL-2 and TNF-α between CCC patients and HCs (*figure 1A,E*). There were no significant differences observed in the expression of IL-4 and IFN-γ among the three groups (*figure 1B,F*). The cytokine values measured in the different groups are shown in *table 3*. Regarding inflammatory/anti-inflammatory balance, the ratio of IL-6 to IL-10 was observed to increase in patients with ACC compared to both HCs and CCC patients (*figure 1G*). It is noteworthy that there was no significant difference in the levels of IL-6/IL-10 between patients with CCC and HCs (*figure 1G*).

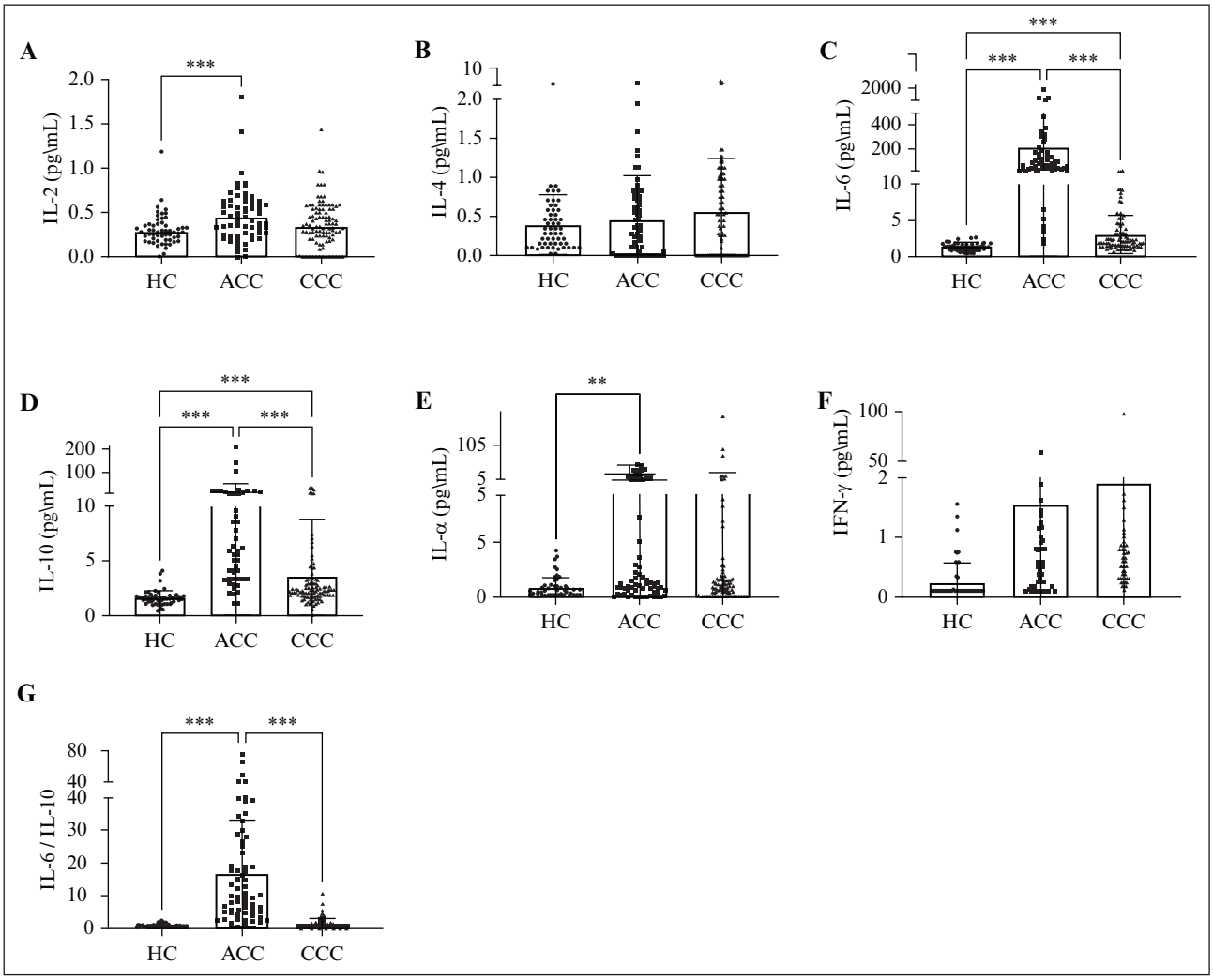

Figure 1
Comparison of Th1/Th2 cytokine expression between HCs, ACC, and CCC patients. **A)** IL-2. **B)** IL-4. **C)** IL-6. **D)** IL-10. **E)** TNF-α. **F)** IFN-γ. **G)** IL-6/IL-10. HC: healthy control; ACC: acute calculous cholecystitis; CCC: chronic calculous cholecystitis. The Kruskal-Wallis test was used to analyse the differences among groups. $*** p < 0.001$, $** p < 0.01$, and $* p < 0.05$.

Table 3
The level of serum cytokines (pg/mL) in different groups.

	Group	IL-2	IL-4	IL-6	IL-10	TNF-α	IFN-γ
Mean±SD	HC	0.303±0.179	0.392±0.386	1.372±0.559	1.607±0.655	0.436±0.505	0.238±0.325
	ACC	0.468±0.303	0.453±0.569	207.883±321.243	15.362±1.655	6.731±14.28	1.870±7.943
	CCC	0.454±0.722	0.551±0.686	13.491±33.248	3.571±2.655	5.311±22.434	1.941±10.16
	M-ACC	0.489±0.226	0.447±0.44	35.540±27.519	4.946±3.655	3.057±5.134	3.596±13.431
	MS-ACC	0.459±0.327	0.456±0.609	280.650±355.415	19.859±4.655	8.282±16.318	1.142±3.221
	Pre-ACC	0.436±0.306	0.603±0.792	201.100±224.7	23.150±5.655	4.508±11.98	0.740±1.058
	Post-ACC	0398±0.215	0.659±0.984	67.630±74.63	6.208±6.655	7.500±13.95	0.545±0.746
Median (25-75% percentile)	HC	0.28(0.19-0.36)	0.28(0.15-0.55)	1.38(0.99-1.69)	1.55(1.22-1.770)	0.25(0.1-0.52)	0.1(0.1-0.1)
	ACC	0.447(0.263-0.621)	0.278(0-0.728)	76.965(30.276-221.834)	5.972(3.407-11.437)	0.605(0.231-4.284)	0.242(0-0.829)
	CCC	0.339(0.204-0.543)	0.453(0-0.9)	2.569(1.646-6.4)	2.179(1.821-2.939)	0.386(0-0.845)	0.308(0-0.788)
	M-ACC	0.580(0.331-0.633)	0.38(0-0.751)	31.229(13.437-58.703)	4.3(2.886-6.72)	0.669(0.07-2.7)	0.442(0-0.579)
	MS-ACC	0.374(0.25-0.565)	0.276(0-0.702)	145.09(56.627-345.71)	8.534(4.053-19.600)	0.52(0.231-5.537)	0.176(0-0.998)
	Pre-ACC	0.38(0.24-0.585)	0.43(0-0.738)	94.58(49.39-420.6)	5.795(3.388-16.83)	0.34(0.01-1.31)	0.31(0-1.07)
	Post-ACC	0.345(0.275-0.588)	0.495(0.16-0.748)	42.41(21.98-79.43)	5.545(4.428-8.015)	1.14(0.635-4.525)	0.305(0-1.08)

Mean±SD: mean and standard deviation (SD); HC: healthy control; ACC: acute calculous cholecystitis; CCC: chronic calculous cholecystitis; M-ACC: mild ACC; MS-ACC: moderate-to-severe ACC; Pre-ACC: preoperative-ACC; Post-ACC: postoperative-ACC.

To further study the relationship between cytokine expression and the severity of ACC, we compared the different degrees of severity among ACC patients. In patients with moderate-to-severe acute calculous cholecystitis (MS-ACC), IL-10 expression was significantly increased compared to mild acute calculous cholecystitis (M-ACC) patients (*figure 2D*). Although the concentrations of IL-2 and IL-6 and the IL-6/IL-10 ratio were markedly elevated both in M-ACC and MS-ACC patients compared to HCs, no significant difference was observed between the two patient groups (*figure 2A,C,G*). Moreover, we found that in patients with MS-ACC, the TNF-α concentration was elevated, compared with HCs (*figure 2E*), with a median of 0.520 pg/mL (range from 0.00 to 69.510 pg/mL). There was no significant difference in expression of IL-4 or IFN-γ among the three groups (*figure 2B,F*).

Correlation between cytokines and cholecystitis-related markers in ACC patients

To identify cytokines that are similarly regulated during the perioperative period, we examined the inter-relationship among cytokines in patients with ACC, who exhibit significant alterations in cytokine levels. IL-10 was found to positively correlate with IL-6 ($r = 0.399$, $p = 0.001$), as shown in *figure 3D*.

To investigate alterations in serum cytokine expression in patients with ACC, we analysed the relationship between cytokine concentration (including IL-2, IL-10, IL-6, and TNF-α) and clinical markers closely related to ACC (including ALT, AST, and the neutrophil-to-lymphocyte ratio [NLR]). NLR has been identified as a biomarker of systemic inflammation and has demonstrated superiority over leukocytes in the assessment of ACC severity, as previously reported [14]. We found that in ACC patients, IL-2 expression positively correlated with the concentration of ALT ($r = 0.309$, $p = 0.013$) and AST ($r = 0.275$, $p = 0.028$) (*figure 3A,B*), and the concentration of TNF-α significantly positively correlated with the length of hospital stay ($r = 0.328$, $p = 0.008$) (*figure 3C*). Additionally, both IL-6 ($r = 0.492$, $p < 0.001$) and IL-10 ($r = 0.5225$, $p < 0.001$) showed a highly positive relationship with NLR (*figure 3E,F*).

To determine independent associations between cytokines and NLR, a linear regression analysis was performed using only the significant cytokines ($p<0.20$). In the final model, IL-10 ($p = 0.001$, 95% confidence interval = 0.032-0.122) and IL-6 ($p = 0.004$, 95% confidence interval = 0.002-0.011) were identified as independent factors contributing to an increase in NLR (*table 4*).

Change in cytokine expression after laparoscopic surgery in ACC patients

On the fifth day after the laparoscopic cholecystectomy procedure in patients with ACC, there was a clear decrease in the level of IL-6 and the IL-6/IL-10 ratio, as depicted in Figure 4C, G. However, these levels remained significantly higher than the initial baseline levels. Conversely, the levels of IL-2 and TNF-α remained elevated. (*figure 4A,E*).

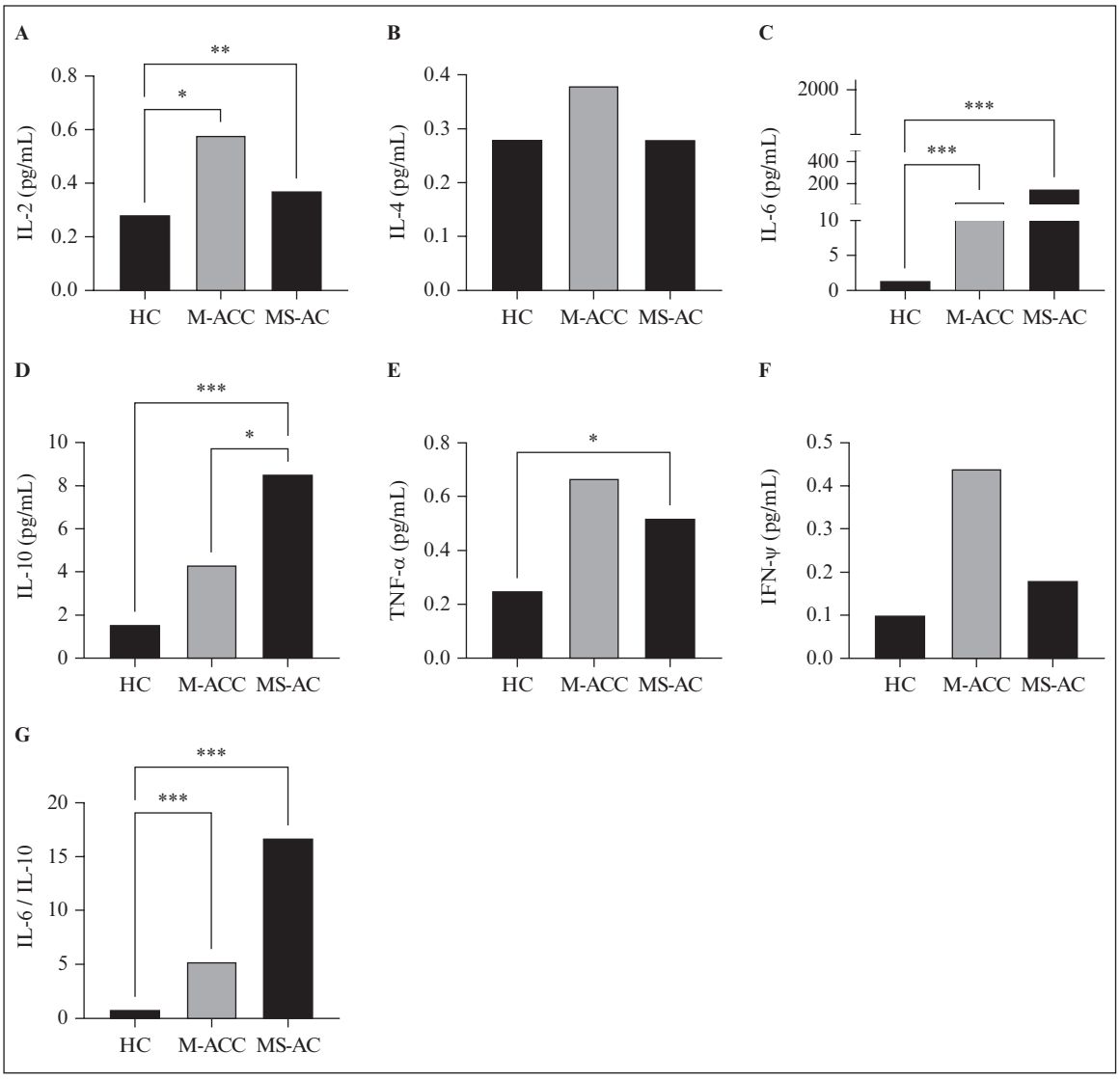

Figure 2
Comparison of cytokine expression between HCs, M-ACC, and MS-ACC patients. **A)** IL-2. **B)** IL-4. **C)** IL-6. **D)** IL-10. **E)** TNF-α. **F)** IFN-γ. **G)** IL-6/IL-10. M-ACC: mild acute calculous cholecystitis; MS-ACC: moderate-to-severe acute calculous cholecystitis. The Kruskal-Wallis test was used to analyse the differences among groups. $*** p < 0.001$, $** p < 0.01$, and $* p < 0.05$.

Discussion

Cholecystitis is a prevalent condition of the digestive system that induces acute and chronic inflammation in the gallbladder, typically triggered by gallstones or other aetiologies. Histologically, acute cholecystitis is characterized by diffuse poly-morphonuclear infiltration in the mucosa, submucosa, or muscular region, and chronic cholecystitis is identified by the presence of increased lymphocytes, with or without mural thickening or fibrosis. Cytokines play an integral role as inflammatory mediators, and their measurement in blood samples can yield valuable insights into immune cell functionality, the severity of inflammation, and disease prognosis. Th1 and Th2 cytokines mediate cellular immunity and humoral immunity, respectively, which are important components of the cytokine network of the body, and may therefore be used to evaluate the immune function of patients. In our study, we focussed on the secretion profile and clinical significance of Th1 and Th2 cytokines in patients with acute and chronic cholecystitis.

Our study revealed that the levels of IL-6 and IL-10 in the serum of CCC patients were significantly higher, indicating clear TH2 dominance. On the other hand, the levels of IL-6, IL-10, IL-2, and TNF-α in ACC patients were significantly elevated but did not show a clear TH1 or TH2 bias. Instead, a mixed cytokine expression profile, encompassing both TH1 and TH2, was observed. IL-10 and IL-6 were confirmed as independent factors for an increase in NLR value, which has been identified as a useful biomarker for assessing the severity of ACC.

Our investigation revealed that IL-6 and IL-10 were the most variable cytokines in both acute and chronic cholecystitis patients. IL-6 is considered a major mediator of immune and inflammatory responses, primarily produced by fibroblasts, mononuclear macrophages, lymphocytes, epithelial cells, keratinocytes, and a variety of tumour cells. IL-6 can act directly on vascular endothelial cells, increasing their permeability and promoting differentiation and the production of antibodies by beta-cells, and induce and regulate the

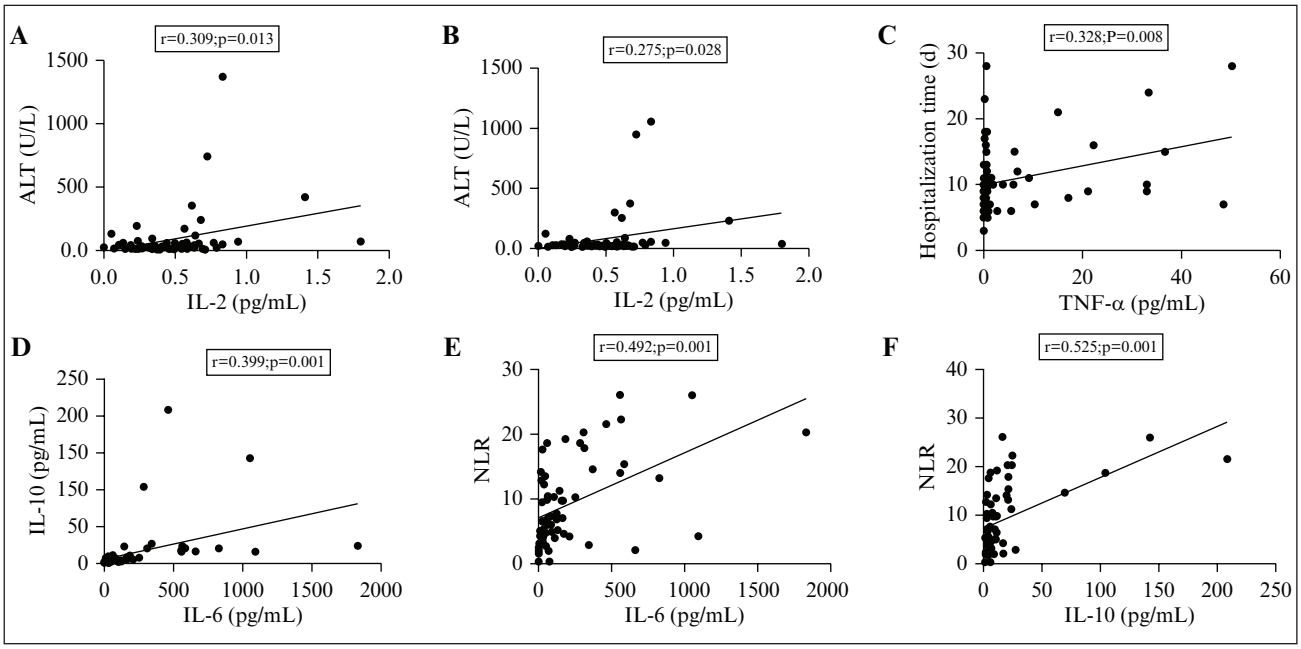

Figure 3
Correlation between IL-2 and ALT **(A)**, IL-2 and AST **(B)**, TNF-α and hospitalization time **(C)**, IL-6 and IL-10 **(D)**, IL-6 and NLR **(E)**, and IL-10 and NLR **(F)**. NLR: neutrophil-to-lymphocyte ratio.

Table 4
Univariate and multivariate linear regression analysis of mean NLR.

	Univariate regression				Multivariate regression			
		95% CI				95% CI		
	β	Lower	Upper	*p* value	β	Lower	Upper	*p* value
IL-2	-0.154	-8.799	2.112	0.225				
IL-4	-0.071	-3.763	2.107	0.575		-		
IL-6	0.492	0.006	0.015	0.000	0.336	0.002	0.011	0.004
IL-10	0.518	0.060	0.148	0.000	0.384	0.032	0.122	0.001
TNF-α	0.032	-0.102	0.132	0.803				
IFN-γ	-0.050	-0.225	0.169	0.694				
Age (y)	0.065	-0.082	0.139	0.609				
Sex (%)	-0.149	-5.3.3	1.348	0.239				

β: regression coefficients; CI: confidence interval. ∗Parameters with p value <0.20 were included in the multivariate analysis. Level of significance was accepted at $p < 0.05$.

synthesis of acute-phase proteins, which further promote the inflammatory immune response. Nonetheless, IL-6 can also stimulate the production of immunosuppressive cytokines, such as IL-10, to suppress inflammation [15]. Previous studies have indicated that IL-6 plays an important role in the pathogenesis of ACC. The level of IL-6 is closely correlated with the severity of ACC [16]. The present study showed that the IL-6 level of the ACC group was higher than that of the healthy control group. However, there was no noteworthy difference observed between the M-ACC and MS-ACC groups. This suggests that the elevated expression of IL-6 in serum before operation might indicate a strong inflammatory status in patients with ACC, however, this cannot be used as a biomarker for distinguishing the severity of ACC. This could be a result of cytokines causing a cascade of inflammation-related amplification effects, and the secretion of IL-6 might be affected by many factors.

TNF-α is a pro-inflammatory cytokine released early in the acute phase of the inflammatory response that is primarily generated by activated monocytes and macrophages. TNF-α can initiate a series of cytokine responses and stimulate the production of interleukins and other secondary inflammatory mediators. TNF-α has been demonstrated to work in partnership with IFN-γ to instigate and attract additional immune cell populations to foci of inflammation. Further, TNF-α has the potential to enhance the expression of chemokines and adhesion molecules, including RANTES and E-selectin, which are integral to the management of leukocyte-endothelial cell interactions and vascular endothelium activation. Previous research has indicated that in ACC patients, TNF-α expression in the gallbladder is significantly elevated, with increased TNF-α expression positively correlating with greater gallbladder inflammation [8]. In our study, TNF-α expression positively correlated with hospital stay duration in ACC

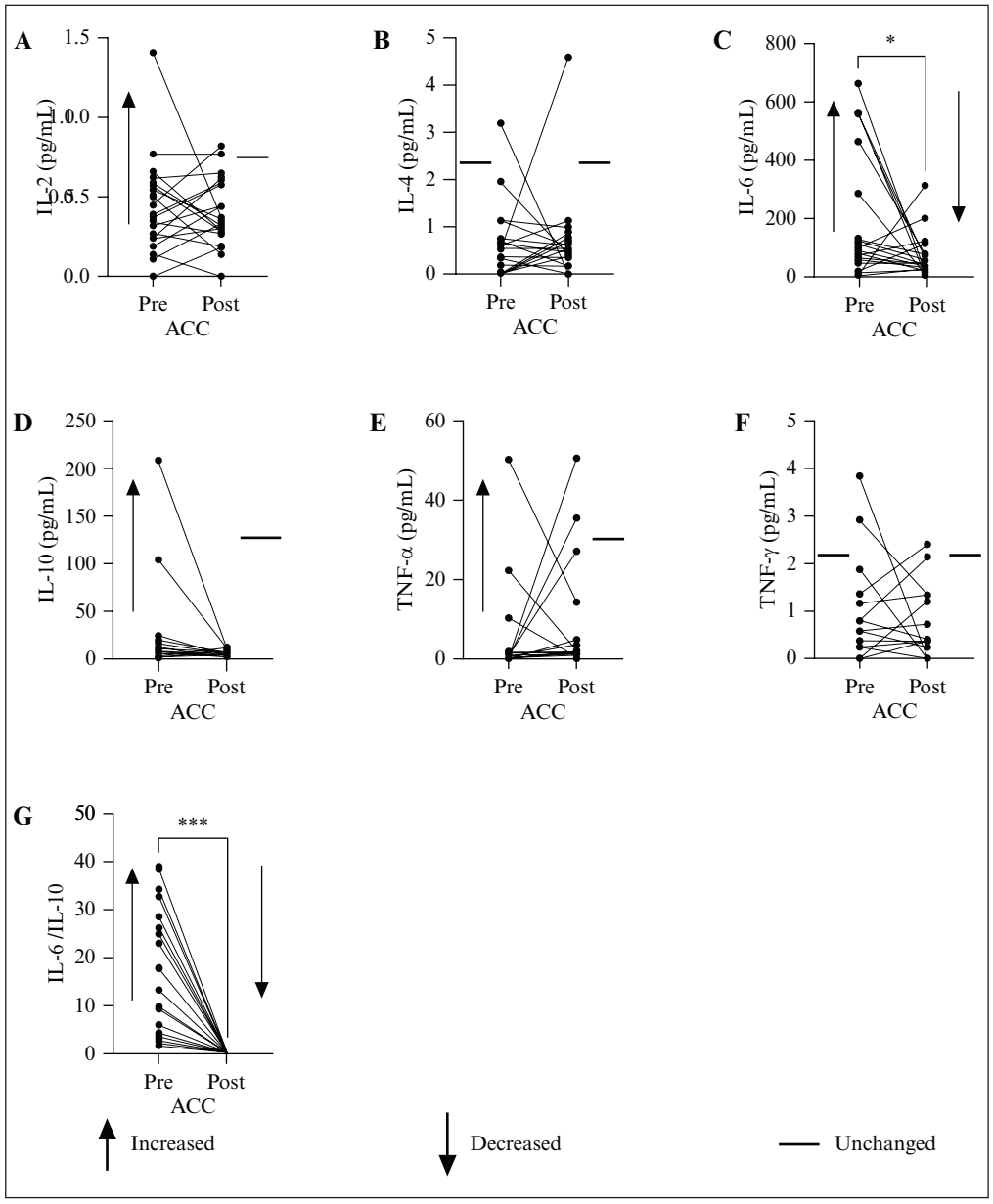

Figure 4
Change in cytokine expression before and after laparoscopic surgery in patients with ACC. Pre: preoperative average; Post: postoperative average. **A)** IL-2. **B)** IL-4. **C)** IL-6. **D)** IL-10. **E)** TNF-α. **F)** IFN-γ. **G)** IL-6/IL-10. The arrow on the left of each graph represents the trend in cytokine expression when comparing Pre among HC. The arrow on the right of each graph represents the trend in cytokine expression for Pre relative to Post.

patients, particularly in MS-ACC patients who have high TNF-α expression. TNF-α appears to play a critical role in determining the prognoses of ACC patients, which warrants further investigation.

IL-10 is arguably the most potent anti-inflammatory Th2 cytokine, produced by almost all innate and adaptive immune cells. It primarily targets antigen-presenting cells, such as monocytes and macrophages, to mitigate inflammation by inhibiting the release of pro-inflammatory factors, including, but not limited to, TNF-α, IL-1β, IL-6, IL-8, granulocyte-colony stimulating factor, and granulocyte-macrophage colony stimulating factor. In addition to its suppression of pro-inflammatory factors, IL-10 has been shown to enhance the release of anti-inflammatory factors, such as IL-1 receptor antagonists, and inhibit TNF-α receptors. Our study showed that changes in IL-6 and IL-10 occurred at the same time in ACC and CCC patients, although the expression of IL-6 was significantly higher than that of IL-10. It is plausible that IL-10 secretion serves as a negative feedback mechanism to regulate increased IL-6 expression, further limiting the potentially harmful effects of sustained or excess expression of pro-inflammatory factors. The positive correlation between IL-10 and IL-6 in ACC supports the anti-regulatory activity of IL-10 as an anti-inflammatory cytokine against IL-6. Furthermore, we identified that IL-10 is the only cytokine that shows differential expression among M-ACC and MS-ACC patients. This result can be interpreted as IL-10 playing a strong regulatory role in the body's immune response, thereby contributing to restricting the spread of a severe inflammatory reaction and promoting healing and repair of tissue. We hypothesize that the

inflammatory response observed in ACC is a result of excessive expression of pro-inflammatory cytokines (IL-6 and TNF-α) and insufficient release of anti-inflammatory cytokines (IL-10). In cases where treatment is delayed, the inflammatory response of CC could escalate, leading to gallbladder pus, necrosis, and even perforation due to an uncontrolled and imbalanced expression of inflammatory factors.

IL-2, also known as T-cell growth factor, can promote a T-cell-dependent immune response, boost the cytolytic capacity of natural killer cells and tumour-infiltrating lymphocytes, stimulate immunoglobulin production by activating B cells, facilitate the homeostatic proliferation of Treg cells, and modulate effector T-cell differentiation. Our study indicates that IL-2 expression is significantly increased in ACC patients. Furthermore, the expression of IL-2 positively correlated with the concentration of ALT and AST. IL-2 expression is reported to be associated with ALT concentration in patients with acute liver injury [17], and antibodies targeting IL-2 can provide protection against liver injury by reducing the number of CD4+ T cells [18]. Moreover, IL-2 is capable of stimulating the proliferation of Treg cells and inducing apoptosis of activated T cells, curbing the immune response during liver damage [19]. Based on these findings, it appears that excessive IL-2 secretion may be the primary cause of increased ALT and AST in ACC patients, and IL-2 antagonists may represent an effective treatment for ACC patients with liver injury.

In the current study, we observed a decline in IL-6, and sustained elevation of IL-10, IL-2, and TNF levels was not significantly decreased following cholecystectomy in ACC patients. This indicates that surgery can diminish the inflammatory response, but the body's equilibrium is not fully restored to its normal state. Surgical intervention does not correct cytokine imbalance. Therefore, adequate pharmacological correction is required.

We also determined cytokine ratios, as the relative concentrations of antagonizing cytokines are more physiologically relevant than individual cytokine levels. The balance between proinflammatory (IL-6) and anti-inflammatory (IL-10) cytokines is crucial in regulating the immune response. The ratio of IL-6/IL-10 provides an index of the net inflammatory milieu and immune dysregulation in the body, and displays significant variability in CC patients. We observed a higher IL-6/IL-10 ratio that could reflect an unbalanced over-production of IL-6 (and other pro-inflammatory cytokines) and insufficient secretion of IL-10 (and perhaps other anti-inflammatory cytokines) in ACC patients who were in a state of significant inflammation. These results are consistent with the known pathophysiology. Interestingly, there were no differences in the IL-6/IL-10 ratio between M-ACC and MS-ACC patients. This might be due to the variation of IL-6, which was one order of magnitude greater than that of IL-10.

Conclusion

In this study, we examined Th1 and Th2 cytokine profiles in patients with acute and chronic CC. Our findings demonstrate that CCC patients exhibit significantly elevated expression levels of IL-6 and IL-10 in serum, whereas ACC patients demonstrate higher expression of IL-2 and TNF-α, in addition to IL-6 and IL-10. The expression of IL-6 and IL-10 correlated with disease progression and severity. Further research is needed to uncover the significant functions and immunological mechanisms associated with these cytokines, particularly IL-6 and IL-10, in modulating the progression of cholecystitis.

Disclosure. Financial support: none. Conflicts of interest: none.

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