

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)**SciVerse ScienceDirect**journal homepage: <http://www.e-biomedicine.com>**Original article****Increased IL-8 and IL-1 $\beta$  in the bile of acute cholecystitis patients**

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Received 29 July 2013

Received in revised form

14 August 2013

Accepted 15 August 2013

Available online 7 October 2013

**Keywords:**

acute cholecystitis

bile

cytokines

**ABSTRACT**

**Background:** Acute cholecystitis is inflammation of the gallbladder, and a delayed diagnosis of this condition increases the incidence of patient complications and mortality. Although proinflammatory cytokines are reported to be critical mediators in hepatobiliary disease, little is known regarding the biliary cytokine profile in patients with acute cholecystitis.

**Methods:** Bile samples were collected from 13 patients with acute cholecystitis who underwent cholecystectomy and 16 healthy adults who underwent cholecystectomy as part of liver donation for transplant. Cytometric bead array immunoassays were performed to measure the levels of proinflammatory cytokines, including IL-8, IL-1 $\beta$ , IL-6, IL-10, TNF, and IL-12p70, in the bile samples.

**Results:** We found that biliary IL-8, IL-1 $\beta$ , and IL-6 levels in acute cholecystitis patients were significantly higher than those in healthy adults. A sequential measurement of biliary proinflammatory cytokines showed that IL-8 and IL-1 $\beta$  remained at significantly high levels and that this trend continued for approximately 4–6 days, whereas the IL-6 level varied.

**Conclusion:** Biliary IL-8 and IL-1 $\beta$ , but not IL-6, could serve as constant and reliable indicators for patients with acute cholecystitis. Because classical symptoms and blood tests are frequently underestimated or untypical in unconscious and elderly patients, these biliary biomarkers are valuable as a complement to current diagnostic criteria for correct diagnosis of acute cholecystitis.

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## 1. Introduction

Cytokines are small protein mediators involved in inflammatory, metabolic, and immunomodulatory functions [1]. They are not only produced by monocytes, lymphocytes, fibroblasts, and endothelial cells, but also in hepatocytes and the biliary epithelium [2]. Secreted inflammatory molecules, such as proinflammatory cytokines, are among the critical mediators of the altered processes implicated in hepatobiliary disease. High biliary levels of TNF- $\alpha$  and IL-6 were reported in patients with cholangitis, and IL-6 was specific for cholangitis (although the correlations between biliary cytokines and serum biochemical parameter were weak) [3]. Although increased cytokine mRNAs were detected upon treatment with lipopolysaccharide in cultured mouse gallbladder epithelial cells, the cytokine profile in the gallbladder for acute cholecystitis is still unclear [4].

Acute cholecystitis is an inflammation of the gallbladder that is due to biliary obstruction of the cystic duct by gallstones. This obstruction causes intraluminal pressure within the gallbladder and triggers an acute inflammatory response [5]. Approximately 10% of the adult population in the United States and 14.4% of adults in Taiwan [6] have cholelithiasis, and 1–3% of these patients develop acute cholecystitis or other severe complications, such as gallstone pancreatitis or cholangitis [7]. Current treatments for acute cholecystitis include medical management (antibiotics), gallbladder drainage (to drain infected bile), and cholecystectomy (to remove the gallbladder), depending on the patient's clinical condition [8]. Once suspected acute cholecystitis patients are referred to hospital and the diagnosis is confirmed, and they undergo early surgery within 72–96 hours, they have lower complication rates, lower conversion rates, and shorter hospital stays than patients who have delayed surgery [9,10]. More sequelae of acute cholecystitis, such as gangrene, pus formation, or perforation of the gallbladder can occur in instances of delayed diagnosis, and their resulting mortality rates range from 1.6% to 4.5% [11]. It is also difficult for diagnosis in elderly, unconscious, and critically ill patients, such as those with severe trauma and acute non-biliary illness; the morbidity rate of emergency cholecystectomy could be as high as 28.1% in these patient populations [12–14]. Thus, a more accurate early diagnostic marker of acute cholecystitis would be extremely valuable as a complementary indicator for instant diagnosis, especially for high risk patients.

Several studies have demonstrated that the serum levels of the cytokines IL-6, IL-10, TNF $\alpha$ , and IL-8 were higher in patients with acute cholangitis or obstructive jaundice than in healthy individuals, although this elevation may be due to generalized neutrophil activation [15]. Because acute cholecystitis can progress from a local inflammation to a systemic inflammatory response syndrome, little is known regarding the biliary cytokine profile in patients with acute cholecystitis, or whether this profile causes the pathophysiology of acute cholecystitis. We investigated the profile of biliary proinflammatory cytokines by direct detection from bile samples in acute cholecystitis patients, to determine if biliary proinflammatory cytokines could serve as diagnostic markers for acute cholecystitis by directly reflecting the inflammatory status of the gallbladder.

## 2. Methods

### 2.1. Patients

Informed consent was obtained from each participant, and the study protocols were approved by the Institutional Review Board of Changhua Christian Hospital (091005 and 091006). The bile specimens were obtained during cholecystectomy from 13 patients who were diagnosed with acute cholecystitis (the study group, 6 men, 7 women, median age = 62 years, range = 40–83 years) and 16 healthy adults (the control group, 9 men, 7 women, median age = 28 years, range = 20–55 years) between 2010 and 2011 at Changhua Christian Hospital (Table 1). The patients in the study group who met the diagnostic criteria for acute cholecystitis (local and system signs of inflammation and confirmation with imaging) underwent cholecystectomy within 3–4 days after diagnosis [8]. Pathological examinations also confirmed the diagnosis as acute cholecystitis. The patients in the study group were further divided into two categories (Grade I and Grade II) according to severity using the Tokyo Guidelines [8]. The control group was made up of healthy adults who underwent cholecystectomy as living liver transplantation donors and were free of any signs or symptoms of cholecystitis.

### 2.2. Bile preparation

The protocol for bile preparation was modified according to the previous study [16]. Briefly, 1 mL of bile was centrifuged at 16,000  $\times$  g for 10 minutes at 4 °C. The supernatant was mixed with 250  $\mu$ L of Cleanascite HC (Ligo-Chem Inc., Fairfield, NJ, USA) and rotated for 1 hour at 4 °C. After centrifugation at 16,000  $\times$  g for 2 minutes, the supernatant was transferred to a pre-rinsed Vivaspin column (cut-off at 3 kDa) and centrifuged at 10,000  $\times$  g until most of the supernatant had passed through the filter. The column was washed with phosphate-buffered saline (PBS) and the samples were centrifuged to a final volume 250  $\mu$ L.

**Table 1 – The clinical presentations of acute cholecystitis patients in the study group.**

No.	RUQ pain	Fever	WBC (/ $\mu$ L)	Image	GB stones	Grade <sup>a</sup>
1	+	+	13,200	CT	+	I
2	+	+	12,000	Echo & CT	+	II
3	+	–	12,200	Echo	+	I
4	+	–	16,900	CT	+	I
5	+	+	13,300	CT	–	II
6	+	+	14,800	Echo & CT	+	II
7	+	+	9400	Echo	+	II
8	+	+	7900	Echo & CT	+	II
9	+	+	12,300	Echo	–	II
10	+	+	11,300	Echo & CT	+	I
11	+	+	5900	Echo & CT	+	I
12	+	+	11,200	Echo & CT	+	II
13	+	+	18,500	CT	+	II

CT = computed tomography; Echo = sonography; GB = gallbladder; RUQ = right upper quadrant.

<sup>a</sup> According to the Tokyo guidelines.

### 2.3. Cytometric bead array immunoassay

Proinflammatory cytokines in the bile samples were quantified using a human inflammation cytometric bead array kit (BD Biosciences, San Jose, CA, USA) following the manufacturer's instructions. In brief, 25 µL of purified bile samples was diluted to 50 µL with assay diluents and added to a mixture of six different antibody-coated beads. After the addition of 50 µL of a mixture of phycoerythrin (PE)-conjugated antibodies against the cytokines, the mixture was incubated for 2 hours at room temperature. The mixture was then analyzed using the BD FACSArray Bioanalyzer (BD Biosciences). The levels of IL-8, IL-1 $\beta$ , IL-6, IL-10, TNF $\alpha$ , and IL-12p70 were determined using the FACSArray software.

### 2.4. Statistical analysis

Statistical analyses of the two groups were performed using the Mann-Whitney U-test and SPSS software (version 12.0, SPSS Inc., Chicago, IL). A  $p$  value  $< 0.05$  was considered statistically significant.

## 3. Results

To investigate the biliary proinflammatory cytokine profile, bile samples from patients with acute cholecystitis and normal healthy adults were collected and purified for further analysis after cholecystectomy. All acute cholecystitis patients had clinical features, such as fever and right upper quadrant abdominal pain, and a confirmatory ultrasound or abdominal computed tomography (Table 1). Early inflammatory cytokines in acute cholecystitis patients were investigated by collecting bile samples that were analyzed for proinflammatory cytokines, such as IL-8, IL-1 $\beta$ , IL-6, IL-10, TNF $\alpha$ , and IL-12p70. In the acute cholecystitis group, the mean biliary IL-8, IL-1 $\beta$ , and IL-6 levels were 4194 pg/mL

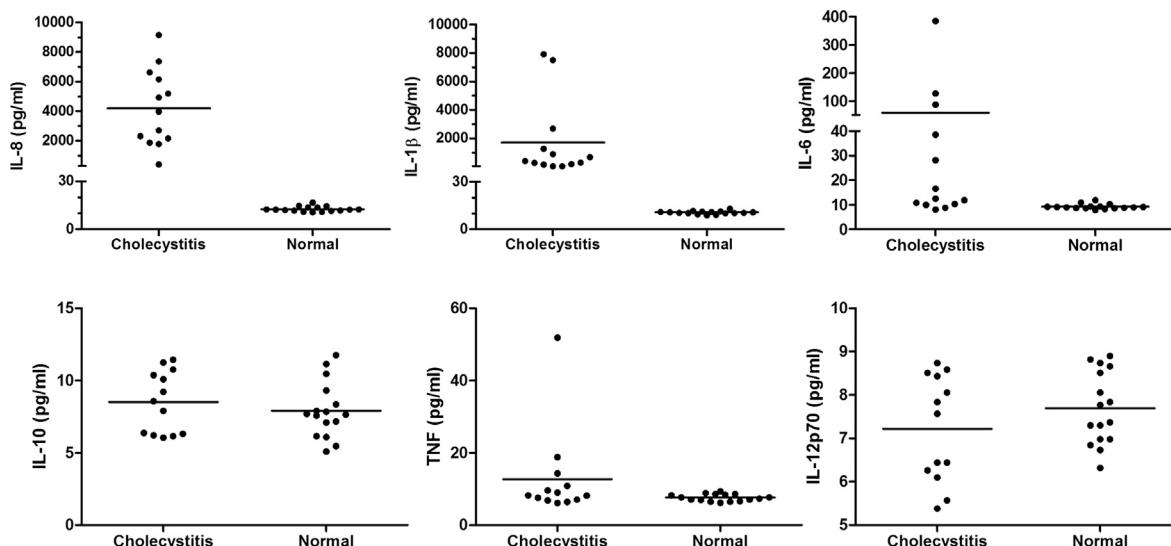
(range = 396–9140 pg/mL), 1723 pg/mL (range = 38–7915 pg/mL), and 58 pg/mL (range = 8–385 pg/mL), respectively. In the control group, the median biliary IL-8, IL-1 $\beta$ , and IL-6 levels were 12 pg/mL (range = 11–17 pg/mL), 11 pg/mL (range = 9–13 pg/mL), and 9 pg/mL (range = 8–12 pg/mL), respectively, and were significantly lower than those in the acute cholecystitis group ( $p < 0.01$ ) (Fig. 1). The differences between the two groups regarding their IL-8, IL-1 $\beta$ , and IL-6 levels were particularly striking, whereas the IL-10, TNF $\alpha$ , and IL-12p70 levels were very low in both groups (Fig. 1).

We further investigated whether the IL-8, IL-1 $\beta$ , and IL-6 levels correlated with the severity grading in acute cholecystitis patients. Our results revealed that IL-8 and IL-1 $\beta$  could not be used to discriminate the severity of disease, but the IL-6 levels were moderately increased in the Grade II group, although they were not significantly different between the patients with Grade I and Grade II cholecystitis (Fig. 2).

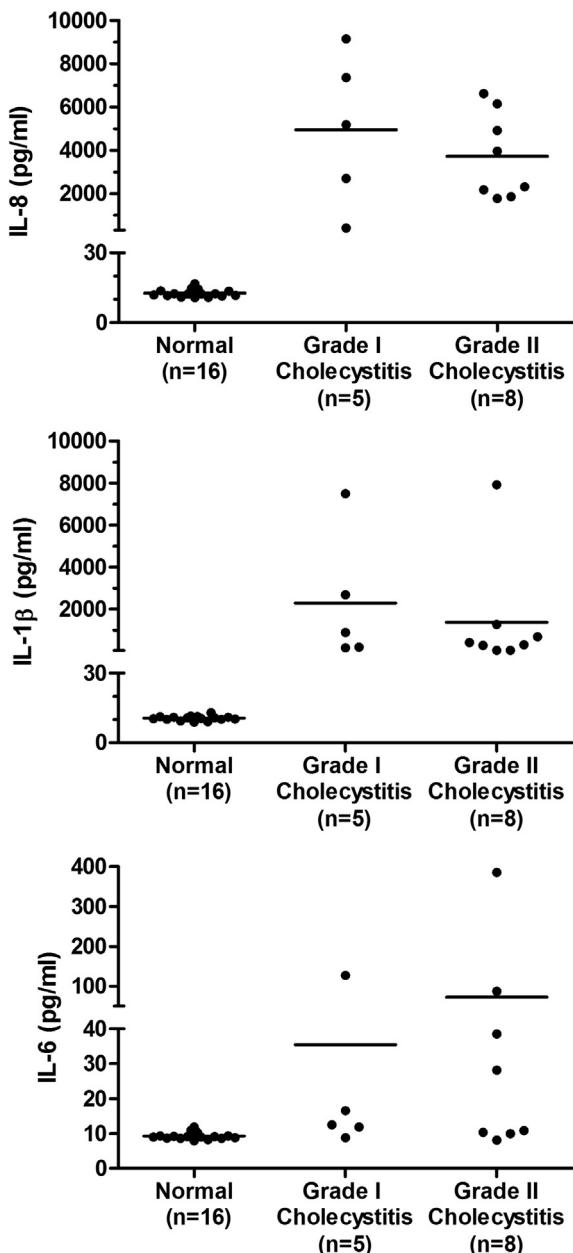
To further investigate whether the biliary proinflammatory cytokines consistently remained at high levels during the entire period of acute cholecystitis, sequential measurements of biliary proinflammatory cytokines from another three patients who had undergone gallbladder drainage were analyzed daily. The results showed that IL-8 and IL-1 $\beta$  remained at significantly high levels and remained this way for approximately 4–6 days, whereas their IL-6 levels varied (Fig. 3). The other cytokines remained at low levels similar to those in the normal control group (data not shown). These results indicated that high IL-8 and IL-1 $\beta$  levels as stable indicators for acute cholecystitis could be consistently detected in the gallbladder.

## 4. Discussion

Our results demonstrated that biliary IL-8, IL-1 $\beta$ , and IL-6 levels, especially IL-8 and IL-1 $\beta$ , were significantly higher in the patients with acute cholecystitis than in healthy adults.

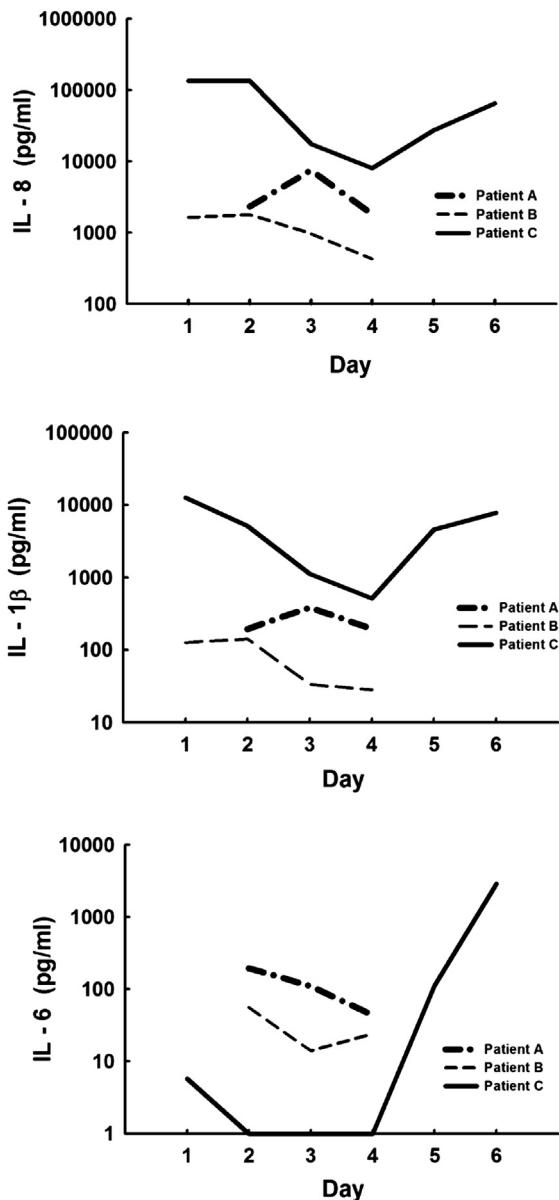


**Fig. 1 – Biliary cytokines of patients with acute cholecystitis and healthy adults. Cholecystitis refers to bile samples from patients with acute cholecystitis. Normal refers to bile samples from healthy individuals. The long bar represents the mean value of the cytokines in each group. The  $p$  values were calculated using the Mann-Whitney U-test.**



**Fig. 2 – Cytokine levels in patients with different grades of acute cholecystitis.** Five patients were diagnosed with Grade I and eight with Grade II. The long bar represents the mean value for each group.

These elevations may directly reflect the local inflammatory status of the gallbladder. In addition, IL-8 and IL-1 $\beta$  consistently remained at high levels during the entire period of acute cholecystitis. This suggests that IL-8 and IL-1 $\beta$ , but not IL-6, could serve as constant and reliable indicators for patients with acute cholecystitis. Although a significant correlation has been reported between the biliary IL-6 and TNF $\alpha$  levels in patients with cholangitis [3], we did not find a correlation between IL-6 and TNF $\alpha$  levels. However, we did find a moderate correlation between IL-8 and IL-1 $\beta$  levels ( $r = 0.37$ ) and IL-8 and TNF $\alpha$  levels ( $r = 0.37$ ) in the cholecystitis study. The differing cytokine elevations found



**Fig. 3 – Kinetic cytokine levels in the drained bile from acute cholecystitis patients.** Bile samples were collected at different time points from three patients with gallbladder drainage. The levels of IL-8, IL-1 $\beta$ , and IL-6 are shown here.

between cholangitis and cholecystitis may be due to the different pathophysiologies of the diseases, or different disease locations. Our study also demonstrates that the biliary cytokine profile *in vivo* of acute cholecystitis is different from that in bacterial infection *in vitro* [4], suggesting that IL-8 and IL-1 $\beta$  have roles in the development of acute cholecystitis in humans. Neutrophil activation is crucial in the pathogenesis of sepsis, and IL-8 is a key cytokine that stimulates neutrophil infiltration in *Shigella*-induced colitis [17,18]. Based on these studies, a high biliary IL-8 level may regulate neutrophil recruitment and could likely influence the pathogenesis of acute cholecystitis. However, these possibilities require further investigation.

Some studies showed that several serum cytokine levels were increased in patients with acute cholangitis compared to a normal group, such as TNF, IL-6, IL-8, and IL-10, although that would be related to the systemic inflammatory response [3,19]. In this study, we demonstrated that biliary IL-8, IL-1 $\beta$ , and IL-6 levels, especially IL-8 and IL-1 $\beta$ , were significantly higher in patients with acute cholecystitis than in healthy adults, which raises the question of whether the cytokine profiles in bile can be consistently presented in serum. From our observation in acute cholecystitis, albeit in one case, only IL-8 (242 pg/mL) and IL-6 (123 pg/mL) were detectable in serum, whereas the levels of IL-8 (70,156 pg/mL), IL-1 $\beta$  (564 pg/mL), and IL-6 (22 pg/mL) were high in bile samples. Consistent with the observation in patients with cholangitis [3], we found poor correlations between biliary and serum cytokines in acute cholecystitis, suggesting that systemic serum inflammatory cytokines are not proper indicators to present local inflammation, such as acute cholecystitis.

Although the current diagnosis of acute cholecystitis is accepted and widely used, the specificity of these criteria are lacking, especially in elderly and unconscious patients who may be unable to report pain [20]. Hepatobiliary scintigraphy has higher diagnostic accuracy than ultrasonography, but is not readily available in most hospitals [21]. This limitation has increased the annual incidence of acute cholecystitis in elderly patients over the last decade [22]; therefore, predictors of disease that can be used immediately in unconscious patients should be developed to prevent delayed diagnosis.

## 5. Conclusion

Our study shows that measuring the high levels of IL-8 and IL-1 $\beta$  cytokine in bile can assist the current diagnostic procedures for acute cholecystitis. Thus, these cytokines may help clinicians diagnose acute cholecystitis more accurately in the early phase of the disease, especially in unconscious or elderly patients.

## Authors' contributions

PYS, CYP, and YPS designed the study, SSW, YLC, and JRK collected the samples, SJL and YHC performed statistical analysis, and PYS and YPS drafted the manuscript. All authors read and approved the final manuscript.

## REFERENCES

- [1] Tilg H, Wilmer A, Vogel W, Herold M, Nolchen B, Judmaier G, et al. Serum levels of cytokines in chronic liver diseases. *Gastroenterology* 1992;103:264–74.
- [2] Matsumoto K, Fujii H, Michalopoulos G, Fung JJ, Demetris AJ. Human biliary epithelial cells secrete and respond to cytokines and hepatocyte growth factors in vitro: interleukin-6, hepatocyte growth factor and epidermal growth factor promote DNA synthesis in vitro. *Hepatology* 1994;20:376–82.
- [3] Rosen HR, Winkle PJ, Kendall BJ, Diehl DL. Biliary interleukin-6 and tumor necrosis factor-alpha in patients undergoing endoscopic retrograde cholangiopancreatography. *Dig Dis Sci* 1997;42:1290–4.
- [4] Savard CE, Blinman TA, Choi HS, Lee SK, Pandol SJ, Lee SP. Expression of cytokine and chemokine mRNA and secretion of tumor necrosis factor-alpha by gallbladder epithelial cells: response to bacterial lipopolysaccharides. *BMC Gastroenterol* 2002;2:23.
- [5] Strasberg SM. Acute calculous cholecystitis. *New Engl J Med* 2008;358:2804–11.
- [6] Liu CM, Tung TH, Liu JH, Lee WL, Chou P. A community-based epidemiologic study on gallstone disease among type 2 diabetics in Kinmen, Taiwan. *Dig Dis* 2004;22:87–91.
- [7] Friedman GD. Natural history of asymptomatic and symptomatic gallstones. *Am J Surg* 1993;165:399–404.
- [8] Hirota M, Takada T, Kawarada Y, Nimura Y, Miura F, Hirata K, et al. Diagnostic criteria and severity assessment of acute cholecystitis: Tokyo Guidelines. *J Hepatobiliary Pancreat Surg* 2007;14:78–82.
- [9] Pessaux P, Tuech JJ, Rouge C, Duplessis R, Cervi C, Arnaud JP. Laparoscopic cholecystectomy in acute cholecystitis. A prospective comparative study in patients with acute vs. chronic cholecystitis. *Surg Endosc* 2000;14:358–61.
- [10] Eldar S, Eitan A, Bickel A, Sabo E, Cohen A, Abrahamson J, et al. The impact of patient delay and physician delay on the outcome of laparoscopic cholecystectomy for acute cholecystitis. *Am J Surg* 1999;178:303–7.
- [11] Winbladh A, Gullstrand P, Svanvik J, Sandstrom P. Systematic review of cholecystostomy as a treatment option in acute cholecystitis. *HPB (Oxford)* 2009;11:183–93.
- [12] Parker LJ, Vukov LF, Wollan PC. Emergency department evaluation of geriatric patients with acute cholecystitis. *Acad Emerg Med* 1997;4:51–5.
- [13] Rodriguez-Sanjuan JC, Arruabarrena A, Sanchez-Moreno L, Gonzalez-Sanchez F, Herrera LA, Gomez-Fleitas M. Acute cholecystitis in high surgical risk patients: percutaneous cholecystostomy or emergency cholecystectomy? *Am J Surg* 2012;204:54–9.
- [14] Hatzidakis AA, Prassopoulos P, Petinarakis I, Sanidas E, Chrysos E, Chalkiadakis G, et al. Acute cholecystitis in high-risk patients: percutaneous cholecystostomy vs conservative treatment. *Eur Radiol* 2002;12:1778–84.
- [15] Kimmings AN, van Deventer SJ, Obertop H, Rauws EA, Huibregtse K, Gouma DJ. Endotoxin, cytokines, and endotoxin binding proteins in obstructive jaundice and after preoperative biliary drainage. *Gut* 2000;46:725–31.
- [16] Kristiansen TZ. A proteomic analysis of human bile. *Mol Cell Proteomics* 2004;3:715–28.
- [17] Singer M, Sansonetti PJ. IL-8 is a key chemokine regulating neutrophil recruitment in a new mouse model of *Shigella*-induced colitis. *J Immunol* 2004;173:4197–206.
- [18] Livaditi O, Kotanidou A, Psarra A, Dimopoulou I, Sotiropoulou C, Augustatou K, et al. Neutrophil CD64 expression and serum IL-8: sensitive early markers of severity and outcome in sepsis. *Cytokine* 2006;36:283–90.
- [19] Kimmings AN, van Deventer SJ, Rauws EAJ, Huibregtse K, Gouma DJ. Systemic inflammatory response in acute cholangitis and after subsequent treatment. *Eur J Surg* 2000;166:700–5.
- [20] Ahvenjarvi L, Koivukangas V, Jartti A, Ohtonen P, Saarnio J, Syrjala H, et al. Diagnostic accuracy of computed tomography imaging of surgically treated acute acalculous cholecystitis in critically ill patients. *J Trauma* 2011;70:183–8.
- [21] Chatzioannou SN, Moore WH, Ford PV, RD D. Hepatobiliary scintigraphy is superior to abdominal ultrasonography in suspected acute cholecystitis. *Surgery* 2000;127:609–13.
- [22] Lillemoe KD. Surgical treatment of biliary tract infections. *Am Surg* 2000;66:138–44.