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Lipopolysaccharide-pathway proteins are associated with gallbladder cancer among adults in Shanghai, China with mediation by systemic inflammation

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Abstract

Purpose—We examined inflammation as a mediator of associations between bacterial infection markers and gallbladder cancer (GBC).

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Methods—Bacterial response proteins [lipopolysaccharide (LPS), soluble CD14 (sCD14), and LPS-binding protein (LBP)] were measured in 40 GBC cases and 126 gallstone controls with data on 63 serum inflammation markers. The relationships of LPS, LBP, and sCD14 with GBC were examined by logistic regression, which also was used to evaluate whether these associations are influenced by systemic inflammation as measured by a combinatorial inflammation score.

Results—The third versus the first tertiles of sCD14 and of LBP were associated with an increased GBC risk [Odds Ratio (95% Confidence Interval): 5.41 (2.00–16.75) for sCD14, and 6.49 (2.24–23.79) for LBP]. sCD14 and LBP were strongly associated with inflammation score (above versus below the median), which itself was associated with a >21-fold increased risk of GBC for the third vs. first tertiles. Associations between GBC and sCD14 and LBP were markedly attenuated when the inflammation score was included in the model. While LPS was not associated with GBC or inflammation, only 35% of cases and 22% of controls had detectable levels.

Conclusions—These findings suggest that these LPS-pathway proteins are associated with GBC via inflammation-related pathways.

Keywords

Gallbladder cancer; Lipopolysaccharide-pathway; Inflammation; Mediation analysis

INTRODUCTION

In 2012, gallbladder cancer (GBC) was the fifth leading gastrointestinal cancer, resulting in over 178,000 new cases and over 142,000 deaths globally [1, 2]. GBC is often diagnosed after regional spread or distant metastases have already occurred. With a five-year survival rate of <5% for metastatic disease, it is one of the deadliest cancers as a result of late detection [3].

Little is known about GBC pathogenesis. The key risk factor associated with GBC development is the presence of gallstones [4]. Regular use of aspirin and other nonsteroidal anti-inflammatory drugs has been associated with decreased GBC risk, suggesting that inflammation may play a role in GBC development [5]. Relationships between inflammatory gene polymorphisms and GBC, in particular an *IL8* SNP, have been reported [6]. Bacterial infections such as *Salmonella enterica* serovar Typhi (typhoid fever) and *Helicobacter* spp. also have been linked to GBC, although not all findings are consistent [7–14]. Moreover, Gram-negative bacteria have been detected in both gallstones and bile [15, 16]. We recently reported strong associations between GBC and local and systemic markers of inflammation [17], raising the possibility that chronic bacterial infection may play a role in GBC as these infections often induce chronic inflammation, systemically and locally.

Bacteria that have been linked to GBC are Gram-negative bacteria that have an outer membrane containing lipopolysaccharide (LPS). Thus, LPS-pathway proteins may help elucidate the role of bacterial infections related to GBC. LPS assays are available, but conflicting results on detectability and reproducibility have been reported [18]. However, new pre-treatment kits have become available to improve LPS detection. In addition, two LPS-pathway proteins, including cluster of differentiation 14 (CD14) and LPS-binding

protein (LBP), may serve as indirect measures of response to the LPS endotoxin [19, 20]. Thus, examining plasma levels of these LPS-pathway proteins may provide insight into the role of bacterial infection in GBC etiology.

In this study, we measured plasma levels of LPS, sCD14, and LBP in GBC cases and GS controls in a case-control study in Shanghai, China to evaluate the role of these markers in GBC and to assess the degree to which these relationships are mediated by systemic inflammation.

MATERIALS AND METHODS

Study Population and Data Collection

Details of the Shanghai Biliary Tract Cancer Study have been previously reported [5, 21]. Briefly, the study enrolled 368 GBC cases and 774 GS controls between June, 1997 and May, 2001. GBC cases with newly diagnosed GBC (ICD9 code: 156) between 18 and 75 years-old were ascertained from 42 hospitals in urban Shanghai, China, through a rapid reporting system to the Shanghai Cancer Registry. Case ascertainment rate (relative to all cases in Shanghai) and response rate of eligible cases were each over 90%. Control participants with documented gallstones who were undergoing either cholecystectomy or medical intervention in the same hospital as the index GBC case were enrolled and frequency-matched to GBC cases on 5-year age group and sex. Both cases and GS controls were permanent residents of urban Shanghai, China, without a prior history of cancer. The participation rate of eligible GS control patients was over 95%. This study was approved by the Institutional Review Boards at the National Cancer Institute and the Shanghai Cancer Institute, and informed consent was obtained prior to data collection. For the current analysis, we included 40 cases and 126 GS controls with previously-measured serum inflammation-related markers and available plasma for measurement of LPS, sCD14, and LBP levels.

Multiplex Immune-Related Marker Panel (MIP)

The multiplex Luminex-based system inflammation-related marker panel (MIP) has been previously described and validated [22]. The current study used data on 63 MIP markers measured in serum in a previous study utilizing the same population sample from the Shanghai Biliary Tract Cancer Study [23].

Detection of Plasma LPS, sCD14, and LBP

Plasma LPS and LPS-pathway protein levels were quantified at the HPV Immunology Laboratory at Leidos Biomedical Research, Inc. The plasma LPS levels were quantified (EU/mL) using the EndoLISA (Hyglos GmbH, Cat#609033) kit, according to the protocol provided. Prior to quantification, the samples were pretreated as follows: diluted 1:10 with cation buffer (Charles Rivers Laboratories EndoSafe, Cat# BC1000) and water. Following heat treatment at 80°C for 10 minutes, the 1:10 diluted samples were further diluted in EndoSpecific buffer (Charles Rivers Laboratories EndoSafe, Cat# BG120), yielding a final dilution of 1:50. The plasma sCD14 levels were quantified (pg/mL) using the Quantikine ELISA kit (R&D Systems Cat# DC140) according to the provided protocol. The samples

were diluted 1:800. The plasma LBP levels were quantified (pg/mL) using the Human LBP DuoSet ELISA kit (R&D Systems, Cat# DY870-05) according to the provided protocol. The samples were diluted 1:1500 with the exception of two samples that were diluted to 1:8000 because they exceeded the highest detectable level. For all three assays, the samples were assayed in duplicate; the concentration was calculated via either a four (LPS) or five (sCD14 and LBP) parameter logistic fit curve using the SoftMax Pro 6.3 (Molecular Devices, LLC) program. Blinded duplicates were included for 14 GBC cases and 20 GS controls.

Statistical Analysis

SAS V9.3 (Cary, North Carolina) was used for all analyses. Participant characteristics were compared between GBC cases and GS controls by univariate analysis using χ^2 tests for categorical variables, *t*-tests for normally distributed variables, and nonparametric Wilcoxon rank-sum tests for continuous variables with a skewed distribution. Percent agreement in detection (detectable vs. undetectable) between repeated measures of LPS among GBC cases and GS controls combined and among GS controls only was calculated as the percentage of duplicate measures that were both detectable or both undetectable divided by the total number of duplicate measures. For percent agreement between duplicate measures of sCD14 and LBP, the first and second measures were classified as tertiles, respectively; percent agreement was reported as the percentage of measurements that were in the same tertile for the two measurements. In addition to percent agreement, performance parameters of the sCD14 and LBP assays among GBC cases and GS controls and also among GS controls included: coefficients of variation (CV) and intraclass correlation coefficients (ICC) for natural log-transformed duplicate measures. These measures could not be calculated for LPS due to low detection.

χ^2 tests were used to examine the relationships between LPS (detectable vs. undetectable) and sCD14 and LBP. Pearson rho (ρ) was used to estimate the correlation between the natural log transformed values of sCD14 and LBP. Unconditional logistic regression was used to estimate unadjusted odds ratios (OR) and 95% confidence intervals (95% CI) of associations between GBC cases relative to GS controls and sCD14 and LBP categorized as tertiles referent to the first tertile (as defined among GS controls). Given the low proportion of detection (38% among cases vs. 26% among GS controls), LPS was evaluated as detectable versus undetectable. Because of concerns of collinearity, LPS-pathway proteins were not included together in the models for GBC. Models were adjusted for sex, age group (54, 55–65, 65), and batch.

To summarize systemic inflammation, a score was calculated. The MIP markers to be included in the score were chosen as the 20 MIP markers that were statistically significantly associated with GBC at $P < 0.05$ in an independent case-control study conducted in Chile (Chilean GBC Study) [23]. This group of markers included 13 proteins associated with increased GBC risk (CXCL13, CCL19, CCL20, CRP, CXCL11, ICAM-1, IL29-IFNG, IL-8, CXCL10, resistin, SAA, sTNFRI, and VCAM-1) and seven markers associated with a decreased GBC risk (CCL11, CCL22, sEGFR, sIL-RII, sTNFRII, CCL17, and TRAIL). The score was defined as the weighted sum of the quartile value of the MIP marker in the Shangi

Biliary Tract Cancer Study (1, 2, 3, or 4). Weights assigned to the markers in the score calculation were -1 or $+1$ for odds ratios <1 and >1 , respectively, in the Chilean GBC Study.

Relationships between plasma LPS-pathway proteins and GBC and mediation by systemic inflammation were analyzed in a stepwise fashion via regression analysis (Figure 1)[24, 25]. First, unconditional logistic regression was used to investigate the relationships between plasma LPS-pathway proteins and GBC in separate models. Second, the relationship between each LPS-pathway protein (as tertiles) and systemic inflammation was analyzed by logistic regression (inflammation score as above vs. below median) adjusting for sex, age, and batch among GS controls only. Third, logistic regression was used to examine the relationship between inflammation score, as indicator variables (second and third tertiles vs. first tertile) or ordinal parameters (as tertiles), and GBC. Fourth, both the LPS-pathway marker and inflammation score were included in the model examining associations with GBC to evaluate mediation. The attenuation of associations between the LPS-pathway marker and GBC when inflammation score was entered into the model was considered *a priori* to be indicative of mediation of the associations between GBC and the LPS-markers by systemic inflammation. Significance of mediation was not assessed because of sample size limitations.

RESULTS

Participant Characteristics

GBC cases and GS controls did not differ significantly in terms of demographic characteristics or medical history (Table 1). There was a statistically nonsignificant trend for higher average smoking packyears among GS controls in comparison with GBC cases [(Mean (SD): 23.2 (15.2) vs. 15.2 (14.7); $P = 0.13$). The majority of the GBC cases were diagnosed at stage III or IV ($n = 24$, 60%) with lymph node ($n = 22$, 55%) metastases, and ~17% ($n = 7$) of them had distant metastases. The most common type of stone for GS controls was mixed type (cholesterol + pigment stones) [n (%): 66 (52%)]; whereas, the stone type was missing or unclear for the 33% of GBC cases ($n = 13$). Nine of these GBC cases without gallstone type information were stage III or above, and all of these cases either did not go to surgery or had no surgical information available.

Plasma LPS, sCD14, and LBP Characteristics

The percent agreement of LPS detection between duplicate measures was 53% assessed for GBC cases and GS controls combined ($n = 36$) or 59% when restricted to GS controls ($n = 22$). The percentages of agreement of replicate measures in tertiles among GBC cases and GS controls were 87% for sCD14 and 91% for LBP. Coefficients of variation were [mean (standard deviation)] 4.3% (5.3) for sCD14 and 4.3% (3.1) for LBP. When analyzed as natural log-transformed values between replicate measures among GS controls, sCD14 and LBP had ICCs of 0.96 and 0.99, respectively. While plasma natural log-transformed sCD14 and LBP levels were moderately correlated with each other (Pearson $\rho = 0.57$; $P < 0.0001$), neither marker (above vs. below the median) was associated with LPS detection on Chi-square analysis ($P = 0.33$ for sCD14 and 0.85 for LBP).

Plasma LPS-Pathway Proteins, Systemic Inflammation, and GBC

Plasma sCD14 and LBP levels (third versus the first tertiles) were strongly associated with GBC relative to GS controls after adjustment for sex, age, and batch with significant categorical trends in adjusted models; however, LPS detection was not associated with GBC (Table 2). While plasma sCD14 and LBP (second and third tertiles referent to the first tertile) were both associated with higher systemic inflammation score (above vs. below the median), LPS detection was not associated with systemic inflammation score (Table 3). The inflammation score was statistically significantly higher among GBC cases in comparison with GS controls [mean (standard deviation), range: 29 (9), 1 to 42 vs. 16 (8), 1 to 39; $P < 0.0001$]. Inflammation score (third vs. the first tertile) was associated with an over 21-fold (95% CI: 5.91–143.59) increased risk of GBC with a statistically significant trend for inflammation score as a categorical variable.

The relationships of plasma sCD14 and LBP with GBC were markedly attenuated and not significant after inflammation score (above vs. below the median) was included in the models (Table 2). The relationship between LPS detection and GBC remained null after inflammation score was included in Page 11 the model. In contrast, relationships between inflammation score (third vs. first tertile) and GBC were not altered substantively when plasma LPS detection was entered into the model or when sCD14 or LBP (above vs. below the median) was included in the model.

DISCUSSION

In summary, sCD14 and LBP were both associated with GBC and with a summary score of a panel of inflammation-related markers, associated with GBC in an independent study. This inflammation score strongly attenuated the associations between each protein and GBC, implying that inflammation mediates the relationships between these two LPS-pathway proteins and GBC. On the other hand, plasma LPS detection was not statistically significantly associated with GBC or systemic inflammation, and inclusion of inflammation score in the model did not markedly affect these null relationships. These findings argue that the relationships between sCD14 and LBP and GBC may be mediated by inflammation. The lack of a similar finding for LPS may suggest that sCD14 and LBP act through mechanisms independent of LPS, or may reflect misclassification in the LPS assay, which had a low level of detection.

Bacteria have been hypothesized to contribute to GBC etiology. Bacterial DNA has been detected by RT-PCR in approximately 80% of cholesterol gallstones [26], and Gram-negative bacteria may be the primary colonizers [16]. While meta-analyses of two other Gram-negative bacteria, *Salmonella enterica* serovar Typhi (typhoid fever) and *Helicobacter spp.*, showed overall associations with GBC and biliary tract cancer, the studies varied by method of carrier status detection [10, 14], and the association with *Salmonella* Typhi was extremely imprecise when the analysis was restricted to the cohort studies (summary estimate: 19.48, 95% CI: 0.27–1,418.18) [10]. Given these relationships between GBC and Gram-negative bacterial colonization and chronic bacterial infections, we studied LPS-pathway proteins as indirect measures of Gram-negative bacteria more broadly and found an increased risk of GBC with higher levels of sCD14 and LBP.

Little research has focused on the relationship between the LPS-pathway and GBC. The gastrointestinal literature more broadly, specifically regarding colorectal and gastric cancers, also provides support for the roles of bacteria and LPS-pathway signaling in cancer development. *CD14* and *LBP* functional genetic polymorphisms have been associated with colorectal cancer risk in a Chinese Han study population [27]. As described above, TLR4 is activated by LPS-pathway proteins, and *TLR4* polymorphisms, some of which are in coding regions, have also been associated with gastric, prostate, colorectal, nasopharyngeal, and gallbladder cancers [28, 29]. The LPS-pathway gene polymorphisms in *TLR2*, *TLR4*, *MD-2*, *LBP*, and *CD14* were associated with *Helicobacter pylori* infection and gastric cancer risk [30, 31]. Thus, there is evidence from other gastrointestinal cancers to suggest that the LPS-pathway may contribute to carcinogenesis through inflammatory processes.

The mechanism underlying this inflammation-cancer link is complex, making it challenging to segregate acute and chronic inflammation stimulating cancer initiation and promotion from inflammation stimulated by the tumor itself. In addition, gallstones may induce gallbladder mucosal injury leading to inflammation and/or gallbladder mucosa bacterial colonization with subsequent innate immune system activation. Also, chronic infections may stimulate alteration of bile salts and proinflammatory and innate immune responses that together facilitate gallstone formation, as has been shown in animal and cell line studies [15, 32–34]. While it is possible that the presence of a tumor may stimulate bacterial growth, the results of the current study could not have been due to an effect of infections on gallstone development since we compared GBC cases to patients with gallstones. In addition, lymphoid infiltrate and precancerous histologic findings in the gallbladder, including mucosal hyperplasia, metaplasia and dysplasia have been more frequently observed in patients with *Helicobacter pylori* infection arguing for a role of bacterial involvement in GBC development [35]. When considered in the context of these findings, the relationship between the responses to Gram-negative bacteria and GBC warrants further exploration.

This study has several notable strengths. It includes a unique analysis of circulating LPS and LPS-pathway proteins as indirect measures of chronic infection and gallbladder carcinogenesis utilizing well-characterized epidemiologic data. This study used GS controls as the reference group, allowing for the evaluation of GBC risk compared with patients with gallstones, the primary GBC risk factor. While systemic LPS showed marked inter-assay variability, both plasma sCD14 and LBP demonstrated strong reliability. Further the use of mediation analysis allowed for the examination of the impact of measures of systemic inflammation on these relationships to illuminate potential biological mechanisms in the GBC developmental process.

Limitations of this study should be noted. The small sample size led to imprecise estimates and limited the type of mediation analysis that could be conducted; however, we were able to see significant risk estimates. This study population has been shown to have a low exposure to *Salmonella* Typhi, which has been hypothesized to increase GBC risk [12]; however, the measurement of LPS, sCD14, and LBP also served as broader markers of bacterial infection and of response to bacterial infection. Potentially the low level of LPS assay detection, which could contribute to misclassification bias, may have led to an underestimation of true associations with disease. Additionally, examination of circulating

LPS-pathway proteins may not reflect factors acting in the gallbladder tissue itself to cause GBC. Similarly, our measurement of systemic inflammatory markers may reflect an etiologic association with GBC or stimulation of expression of systemic inflammatory markers by the tumor itself that is best followed up by analysis of measurements from a prospective cohort study.

A number of future studies may be proposed from the findings in this project. In addition to optimization of LPS, LBP, and sCD14 measurements in bile, the plasma LPS endotoxin assay should be further developed for improved reproducibility and sensitivity. LPS, sCD14, LBP, and bacteria could be measured in gallbladder tissue via immunohistochemistry and/or PCR, and then be correlated with local inflammatory infiltrate, inflammatory markers in serum and bile, and corresponding pathology. Finally, a prospective approach to a cohort study is necessary to disentangle the temporality of these relationships between infection, inflammation, and gallbladder cancer.

CONCLUSIONS

These results suggest that systemic inflammation may mediate relationships between GBC and sCD14 and LBP; however, the impact of the tumor on systemic inflammation and LPS-pathway proteins cannot be ruled out. Collectively, these findings indicate that sCD14 and LBP may be contributing to GBC as sequela of bacterial infections or via independent mechanisms.

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LIST OF ABBREVIATIONS

CCL11	C-C motif ligand 11
CCL17	C-C motif ligand 17
CCL19	C-C motif ligand 19
CCL20	C-C motif ligand 20
CCL22	C-C motif ligand 22
CI	Confidence interval
COX-2	Cyclooxygenase-2
CRP	C reactive protein
CXCL10	C-X-C motif ligand 10

CXCL11	C-X-C motif ligand 11
CXCL13	C-X-C motif ligand 13
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EndoLISA	Endotoxin-linked immunosorbent assay
GBC	Gallbladder cancer
GS	Gallstone
ICAM-1	Intercellular adhesion molecule-1
ICC	Intraclass correlation coefficient
IL-8	Interleukin 8
IL-29	Interleukin 29
LBP	LPS binding protein
MD-2	Myeloid differentiation protein-2
MIP	Multiplex Luminex-based system inflammation-related marker panel
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
OR	Odds ratio
PGE₂	Prostaglandin E ₂
RT-PCR	Reverse transcriptase polymerase chain reaction
SAA	Serum amyloid A
sCD14	Soluble cluster of differentiation 14
sEGFR	Soluble epidermal growth factor receptor
sIL-RII	Soluble interleukin receptor 2
SNP	Single nucleotide polymorphism
sTNFRI	Soluble tumor necrosis factor receptor 1
sTNFRII	Soluble tumor necrosis factor receptor 2
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
TLR	Toll like receptor
VCAM-1	Vascular cell adhesion molecule-1

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STUDY HIGHLIGHTS**1) WHAT IS CURRENT KNOWLEDGE**

- Gallbladder cancer has been associated with infections and inflammation.

2) NOVEL FINDINGS

- Lipopolysaccharide-pathway proteins are associated with gallbladder cancer.
- Inflammation may mediate these associations.

FIGURE 1. Stepwise mediation testing with regression analysis
The stepwise mediation testing via logistic regression analysis is outlined according to step and corresponding table where results are reported. Abbreviations:
LPS=Lipopolysaccharide, GBC=gallbladder cancer, sCD14=soluble cluster of differentiation 14, LBP=lipopolysaccharide binding protein.

Table 1

Shanghai Study Participant Characteristics by Gallbladder Case-Gallstone Control Status

Characteristic	Gallbladder Cancer Cases	Gallstone Controls
N (%)	40 (24)	126 (76)
Sex		
Women	30 (75)	90 (71)
Men	10 (25)	36 (29)
Age at Interview in Years: mean (SD)^a	65.2 (7.6)	63.0 (9.2)
Age at Interview Group		
54	4 (10)	18 (14)
55–65	13 (33)	37 (29)
66	23 (58)	71 (56)
Highest Level of Education		
None	15 (38)	46 (37)
Primary or Junior Middle School	11 (28)	28 (22)
Senior Middle School	5 (13)	32 (25)
Community College/University	9 (23)	20 (16)
Gallstone Type		
Cholesterol	7 (18)	34 (27)
Pigment	1 (3)	14 (11)
Mixed	7 (18)	66 (52)
Unclear	12 (30)	12 (10)
Missing ²	13 (33)	0
History of Inflammatory Bowel Disease (IBD)		
No	40 (100)	124 (98)
History of Inflammatory Bowel Disease (IBD)		
Yes	0	2 (2)
<i>Crohn Disease</i>	0	0
<i>Ulcerative Colitis</i>	0	1 (50)
<i>Other IBD</i>	0	1 (50)
Diabetes Mellitus		
No	36 (90)	107 (85)
Yes	4 (10)	19 (15)
<i>Type I</i>	0	0
<i>Type II</i>	4 (100)	16 (84)
<i>Unknown</i>	0	2 (11)
<i>Not Ascertained</i>	0	1 (5)
Cigarette Smoking in Pack-years among Smokers: mean (SD)^a	15.2 (14.7)	23.2 (15.2)
Cigarette Smoking Status		

Characteristic	Gallbladder Cancer Cases	Gallstone Controls
Never	31 (78)	98 (78)
Former	6 (15)	10 (8)
Current	3 (8)	17 (14)
BMI 5 Years Ago in kg/m²: mean (SD)	24.5 (3.0)	24.0 (3.0)
BMI 5 Years Ago Category (kg/m²)		
Underweight: 15.5 to 18.5	1 (3)	3 (2)
Normal: >18.5 to <25	20 (51)	82 (67)
Overweight: 25 to <30	17 (44)	33 (27)
Obese: 30+	1 (3)	4 (3)

^aAnalyzed by nonparametric univariate Wilcoxon rank-sum test.

²Nine of 13 (69%) of the cases where gallstone type was missing were stage III and above. Data on surgical information was either missing or indicated that surgery had not been conducted for all of the cases where gallstone type was missing.

LPS, sCD14, and LBP, Systemic Inflammation, and Gallbladder Cancer Relative to Gallstone Controls

Table 2

LPS ^a	Cases N (%)	Controls N (%)	Sex, Age & Batch Adjusted OR (95% CI)	Sex, Age, Batch, & Inflammation Score ^b Adjusted OR (95% CI)	
Undetectable	26 (65)	98 (78)	1.00	1.00	
Detectable	14 (35)	28 (22)	1.94 (0.88–4.22)	1.74 (0.71–4.25)	
sCD14	Cases N (%)	Controls N (%)	Sex, Age & Batch Adjusted OR (95% CI)	Sex, Age, Batch, & Inflammation Score ^b Adjusted OR (95% CI)	
Tertiles:					
1 st	6 (15)	40 (32)	1.00	1.00	
2 nd	7 (18)	43 (34)	1.19 (0.36–4.05)	0.57 (0.14–2.21)	
3 rd	27 (68)	43 (34)	5.41 (2.00–16.75)	1.53 (0.44–5.53)	
		<i>P</i> -trend	0.0006	0.26	
LBP	Cases N (%)	Controls N (%)	Sex, Age & Batch Adjusted OR (95% CI)	Sex, Age, Batch, & Inflammation Score ^b Adjusted OR (95% CI)	
Tertiles:					
1 st	4 (10)	43 (34)	1.00	1.00	
2 nd	10 (25)	41 (33)	2.57 (0.76–10.29)	0.99 (0.24–4.50)	
3 rd	26 (65)	42 (33)	6.49 (2.24–23.79)	1.97 (0.53–8.30)	
		<i>P</i> -trend	0.0004	0.12	
Inflammation Score	Cases N (%)	Controls N (%)	Age & Sex Adjusted OR (95% CI)	Sex, Age, LPS, & Batch ^a Adjusted OR (95% CI)	Sex, Age, LBP ^c , & Batch Adjusted OR (95% CI)
Tertiles:					
1 st	2 (5)	40 (34)	1.00	1.00	1.00
2 nd	3 (8)	42 (36)	1.82 (0.29–14.46)	2.00 (0.31–16.08)	1.71 (0.27–13.69)
3 rd	35 (88)	34 (29)	21.86 (5.91–143.59)	22.57 (5.99–150.22)	21.77 (5.41–151.20)
		<i>P</i> -trend	<0.0001	<0.0001	<0.0001

^a LPS was analyzed as detectable versus undetectable levels.

^b Inflammation score analyzed as above versus below median.

^c sCD14 and LBP were analyzed as above versus below median.

Table 3

LPS and LPS-Pathway Proteins and Inflammation Score among Gallstone Controls

Marker	Inflammation Score (Above vs. Below Median)		
	Above Median N (%)	Below Median N (%)	Age, Sex & Batch Adjusted OR (95% CI)
LPS:			
Undetectable Level	43 (78)	55 (77)	1.00
Detectable Level	12 (22)	16 (23)	0.96 (0.39–2.33)
sCD14^a	Above Median N (%)	Below Median N (%)	Age, Sex & Batch Adjusted OR (95% CI)
Tertiles:			
1 st	7 (13)	33 (46)	1.00
2 nd	18 (42)	25 (35)	3.93 (1.35–12.71)
3 rd	30 (55)	13 (18)	19.04 (5.69–76.34)
<i>P</i> -trend	<0.0001		
LBP^b	Above Median N (%)	Below Median N (%)	Age, Sex & Batch Adjusted OR (95% CI)
Tertiles:			
1 st	6 (11)	37 (52)	1.00
2 nd	23 (42)	18 (25)	6.72 (2.24–23.12)
3 rd	26 (47)	16 (23)	11.48 (3.90–39.49)
<i>P</i> -trend	<0.0001		

^a sCD14 Cutoffs among GS controls: Tertiles <842760 (1st), 842760–1066040 (2nd), >1066040 (3rd)^b LBP Cutoffs among GS controls: Tertiles: <9866475 (1st), 9866475–13396905 (2nd), >13396905 (3rd)