

Endotoxin, cytokines, and endotoxin binding proteins in obstructive jaundice and after preoperative biliary drainage

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Abstract

Background—Obstructive jaundice is associated with postoperative complications related to increased endotoxaemia and the inflammatory response. In animals obstructive jaundice is associated with endotoxaemia and cytokine induction, which are reversed by internal biliary drainage.

Aims—To study endotoxaemia and the subsequent inflammatory response in obstructive jaundiced patients and after endoscopic biliary drainage.

Methods—In 15 patients with malignant distal obstructive jaundice, inflammatory and bacteriological parameters were assessed before endoscopic stent placement and after three weeks endoscopic drainage.

Results—Drainage reduced bilirubin from 252.5 to 45.1 $\mu\text{mol/l}$. At baseline low level endotoxaemia was detected (4.3 pg/ml) which was not affected after drainage (4.5 pg/ml). Serum interleukin 8 (IL-8) and endotoxin binding proteins were increased in jaundice and reduced after drainage (IL-8 113.6 to 20.7 pg/ml; lipopolysaccharide binding protein 24.2 to 16.5 $\mu\text{g/ml}$; sCD14 17.4 to 7.6 $\mu\text{g/ml}$; bactericidal/permeability increasing protein 2.9 to 1.8 ng/ml). Levels of other cytokines, augmented in animals, were only slightly increased and not changed after drainage (tumour necrosis factor (TNF): 21.7 and 18.4 pg/ml; sTNFr p55/75: 2.9/7.0 and 2.7/5.6 ng/ml; IL-6: 4.2 and 6.1 pg/ml; IL-10: 4.5 and 2.7 pg/ml). Elastase and lactoferrin tended towards reduction after drainage. All bile cultures were positive after stenting.

Conclusions—The effects of obstructive jaundice in humans on endotoxin and cytokines are different from those in animal models. Obstructive jaundice causes alterations in circulating endotoxin binding proteins and IL-8. Concentrations of other mediators (TNF, previously suggested as being responsible for systemic endotoxaemia effects) are low and not affected by drainage.

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Keywords: endotoxin; obstructive jaundice; cytokines; endotoxin binding proteins

Surgery in patients with obstructive jaundice is frequently associated with postoperative, mainly septic, complications. These complica-

tions have recently been associated with a proinflammatory state resulting from portal and systemic endotoxaemia, bacterial translocation, and subsequent activation of the inflammatory cascade leading to the sepsis syndrome.¹⁻⁴

Obstructive jaundice causes increased exposure to endotoxin by two different mechanisms. Firstly, the lack of bile in the gut lumen is thought to be responsible for an increase in translocation of endotoxins through the intestinal mucosa. Secondly, biliary obstruction causes a reduction in liver reticuloendothelial system function leading to a diminished clearance of endotoxin by Kupffer cells.⁵ Furthermore, it is very likely that the normal clearance of endotoxin to the bile will be decreased in biliary obstruction. Systemic endotoxaemia, and the additional inflammatory response caused by the operative trauma itself, have been associated with postoperative morbidity.

Experimental obstructive jaundice results in increased endotoxaemia, bacterial translocation, depressed cell mediated immunity, the induction of different cytokines, such as tumour necrosis factor (TNF) and interleukin 6 (IL-6), and release of soluble TNF receptors, thought to be responsible for the systemic inflammatory effects.^{4,6}

In experimental obstructive jaundice, the occurrence of endotoxaemia and subsequent cytokine induction, and bacterial translocation to mesenteric lymph nodes⁷ were significantly reduced by internal biliary drainage, and cellular immunity was restored towards normal.^{8,9} These effects translated into a reduction of postoperative mortality.^{10,11}

The cellular reaction to endotoxin depends partly on the presence of different endotoxin binding proteins, including CD14, the putative lipopolysaccharide (LPS) receptor, lipopolysaccharide binding protein (LBP), an LPS transport protein, and bactericidal/permeability increasing protein (BPI), which neutralises LPS activity.¹²⁻¹⁴ The sensitivity to the biological effects of endotoxin depends on the relative concentrations of, and interactions between, endotoxin and these proteins (and other factors, for example serum lipids).

Abbreviations used in this paper: BPI, bactericidal/permeability increasing protein; CT, computed tomography; ERCP, endoscopic retrograde cholangiopancreatography; IL, interleukin; LAL, Limulus amoebocyte lysate; LBP, lipopolysaccharide binding protein; LPS, lipopolysaccharide; TNF, tumour necrosis factor.

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As the relevance of data obtained in animal models remains unknown, the question arises whether the inflammatory status found in experimental obstructive jaundice also exists in the clinical situation. In addition, it remains uncertain whether preoperative drainage by endoscopic insertion of an endoprosthesis influences the inflammatory response. Recent data from the literature show conflicting results. One uncontrolled clinical study reported a reduction in morbidity,¹⁵ but a recent prospective randomised trial and another retrospective analysis showed no significant reduction of morbidity after preoperative drainage.^{16,17} Moreover, several complications are related to the endoscopic stent placement itself, such as clogging, cholangitis, and local inflammation of the bile duct.^{18,19} The present study was designed to characterise the circulating concentrations of endotoxin, endotoxin binding proteins, and different inflammatory mediators in patients with obstructive jaundice and to evaluate the effect of preoperative internal biliary drainage by endoscopic stent insertion.

Patients and methods

ELIGIBILITY CRITERIA

The study was approved by the research and ethical committees of the Academic Medical Centre. Patients were eligible for inclusion in the study when they presented with obstructive jaundice with bilirubin concentrations exceeding 100 $\mu\text{mol/l}$, caused by a malignant distal biliary obstruction. Patients were excluded if introduction of an endoprosthesis by endoscopic retrograde cholangiopancreatography (ERCP) was not possible, if they had undergone any previous ERCP attempt, or if they had clinical signs of cholangitis or were already treated with antibiotics. Patients had to be able to undergo a pancreatic resection, and preliminary diagnostic Doppler ultrasound examination and computed tomography scan had to be negative for signs of metastases. The selected group therefore comprised relatively fit patients without sepsis/cholangitis, preparing for pancreatic resection.

STUDY DESIGN

The study was designed so that all patients underwent an ERCP and stent placement followed within approximately three days (range 2–5 days) by a diagnostic laparoscopy (time point 0). The patients were then discharged, and underwent biliary drainage for approximately three weeks before returning to the hospital for surgery.

Blood and bile were obtained immediately prior to and during the ERCP. During laparoscopy biopsy specimens of a mesenteric lymph node and the liver were taken (all designated $t=0$). On readmission for the planned pancreaticoduodenectomy, all measurements were repeated ($t=3$ weeks).

SAMPLING

Blood was obtained in vacutainer tubes (Becton Dickinson, Mountain View, California, USA) containing 15% EDTA (K_3) for blood counts, and 30 units lithium heparin for

bilirubin, alkaline phosphatase, and other routine chemical measurements. For endotoxin assessment, blood was collected in sterile syringes and then immediately transferred into pyrogen free plastic tubes (Falcon 2063, Becton Dickinson, Mountain View, California) containing pyrogen free heparin (Thromboliquine, Organon, Oss, The Netherlands; final concentration 30 IU/ml) and immersed in melting ice. Platelet rich plasma (PRP) was prepared within 30 minutes after the blood collection, by centrifugation at 190 g for 10 minutes at 4°C; aliquots were then stored at -20°C until testing.

For bactericidal/permeability increasing protein (BPI), elastase- $\alpha 1$ -antitrypsin complexes, and lactoferrin, blood was collected in vacutainer tubes containing 3.8% sodium citrate. For cytokine, lipopolysaccharide binding protein, and soluble CD14 measurements, serum was prepared, following coagulation in vacutainer tubes, by centrifugation at 2000 g at room temperature for 20 minutes.

ASSAYS

Blood counts were determined routinely with the use of a flow cytometer (Technicon H1 system, Technicon instruments, Tarrytown, USA). Bilirubin and other routine biochemical measurements were determined routinely.

Endotoxin was assayed by the chromogenic *Limulus amoebocyte lysate* test (LAL) (Chromogenix, Amsterdam, The Netherlands), performed with minor modifications as described previously.²⁰ Inhibitors and activated clotting factors were removed by dilution and heating at 75°C for five minutes. Standard curves were made with *Escherichia coli* 055:B5 endotoxin (Mallinckrodt Inc., St Louis, Missouri, USA). The assay has a detection limit in blood of less than 35 EU LPS/l PRP (approximately 3.5 pg/ml). A plasma control of each sample was used to prevent false positive results owing to the intrinsic colour of jaundiced serum. Each sample was assayed either in duplicate or quadruplicate and the results were expressed as the mean of the two or four tests.

Serum concentrations of TNF were assessed by the highly sensitive TNF ELISA (Medgenix Diagnostics, Brussels, Belgium) according to the manufacturer's instructions. IL-6 and IL-8 were assayed by ELISA (Central Laboratory for the Blood Transfusion Service, CLB, Amsterdam, The Netherlands) according to the manufacturer's instructions. Briefly, for both IL-6 and IL-8, microtitre plates (Nunc, Maxisorp, Roskilde, Denmark) were coated with anti-human IL-6 or IL-8 overnight at room temperature on a plate shaker; after blocking, samples were added. The detecting antibody in both cases was biotinylated anti-human IL-6 or IL-8. Standard curves were made with a natural human IL-6 or IL-8 calibrated against the WHO Interim International Standard.

IL-10 was assessed by ELISA as described previously,²¹ using as coating antibody anti-IL-10 monoclonal clone JES3-9D7 (Pharmin-gen, San Diego, California, USA). Samples were diluted in ELISA buffer (CLB, Amster-

dam, The Netherlands); detection antibody was biotinylated IL-10 antibody monoclonal, clone JES3-12G8 (Pharmingen, San Diego, California, USA) and was conjugated with streptavidin-polyperoxidase (CLB, Amsterdam, The Netherlands).

The soluble TNF receptors sTNFrecP55 (P55) and sTNFrecP75 (P75) were measured by specific ELISA as described previously,²² using monoclonal antibodies (against p55 clone htr-20 or p75 clone utr-4), kindly provided by Dr H Gallati (Hoffmann-La Roche Ltd, Basel, Switzerland).

Bactericidal/permeability increasing protein (BPI; normal volunteers: ± 0.5 ng/ml²¹) and lipopolysaccharide binding protein (LBP; healthy individuals: $\pm 5-10$ μ g/ml) were determined as described previously^{23,24} using antibodies and standard recombinant proteins (rabbit anti-LBP, biotin-rabbit anti-LBP and rLBP₅₀; mouse anti-BPI monoclonal 6C2, biotin-rabbit anti-BPI and rBPI₅₀), kindly provided by Dr Stephen Carroll (Xoma Corporation, Berkeley, California).

Soluble CD14 (concentrations in healthy blood donors approximately 3 μ g/ml) was assessed by sCD14 ELISA according to the manufacturer's instructions (Medgenix Diagnostics, Brussels, Belgium).

Elastase- α 1-antitrypsin complexes and lactoferrin were both measured using radioimmunoassays (CLB, Amsterdam, The Netherlands).^{25,26}

Aerobic and anaerobic cultures were performed on blood, bile, mesenteric lymph nodes, and liver biopsy specimens by routine microbiological methods.

STATISTICAL ANALYSIS

Results are expressed as mean (SEM). Statistical differences between haematological, chemical, and cytokine data were analysed using the Wilcoxon rank sum test for two related samples. Correlations were performed with the two tailed Spearman test for non-parametric data. All statistics were carried out using the statistical SPSS for Windows 6.1 software (SPSS Inc., USA).

Results

Fifteen patients were included in the study. Eight patients underwent a pylorus preserving pancreaticoduodenectomy, all for pancreas head carcinoma. If, despite preoperative diagnostic work up, patients were found to have metastases or local ingrowth during operation, a palliative hepatojejunostomy and gastric bypass procedure was performed (n=7). There was no mortality following pancreaticoduodenectomy; one patient suffered from pancreatic leakage resulting in an abscess, which required reoperation with resection of the pancreas remnant. A second patient suffered from a postoperative septic period of unknown origin for four weeks, probably owing to a minimal (subclinical) leakage, but recovered without intervention.

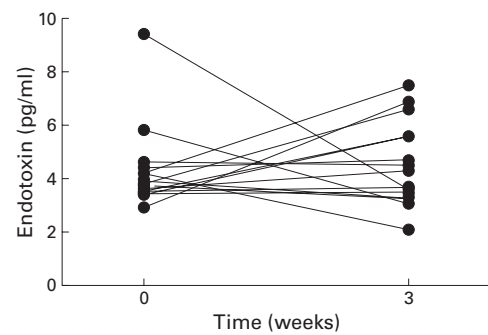


Figure 1 Endotoxin concentrations in systemic blood as measured by the LAL assay. $p=0.7$.

ROUTINE MEASUREMENTS

Internal biliary drainage by endoprosthesis for a mean of 3.8 (0.3) weeks resulted in adequate biliary drainage with a significant reduction in bilirubin concentrations from 252.5 (30.8) to 45.1 (17.3) μ mol/l ($p=0.003$). Other parameters of biliary obstruction, such as alkaline phosphatase, were also significantly reduced after three weeks (390.7 (43.6) to 171.9 (23.1) U/l; $p=0.003$). On the other hand, no effect of drainage was observed on serum creatinine (68.1 (6.1) to 65.9 (9.9) μ mol/l), serum albumin (42.4 (1.4) to 38.8 (1.9) g/l), or haemoglobin concentrations (8.2 (0.26) to 7.5 (0.3) mmol/l).

LEUCOCYTE COUNTS

Leucocyte counts were $8.1 (0.8) \times 10^9/l$ at $t=0$ and were not significantly different after three weeks ($7.5 (0.6) \times 10^9/l$). Although neutrophil percentages varied, absolute neutrophil numbers were not significantly different ($5.6 (0.7) \times 10^9/l$ at $t=0$ and $4.7 (0.6) \times 10^9/l$ at $t=3$; $p=0.1$). The changes in circulating lymphocyte and monocyte percentages or numbers were also not significant.

ENDOTOXIN IN PLASMA

Endotoxin concentrations at $t=0$ in systemic blood, as measured by the LAL assay, were found to be 0.043 (0.005) U/ml (4.3 pg/ml), which is marginally above the cut off for normal concentrations. After biliary drainage no reduction in endotoxin concentrations was found (0.045 (0.005) U/ml; 4.5 pg/ml; $p=0.7$; fig 1).

CYTOKINES AND ENDOTOXIN BINDING PROTEINS

Obstructive jaundice was found to be associated with a significant increase of endotoxin binding proteins (table 1). The concentration of lipopolysaccharide binding protein was dramatically increased and significantly reduced after drainage ($p=0.01$; fig 2A). Similar data were obtained for soluble CD14 concentrations ($p=0.002$; fig 2B).

The plasma BPI concentration was measured in only eight patients.²⁷ Concentrations of BPI were increased at baseline and reduced after drainage from 2.9 (0.5) to 1.8 (0.5) ng/ml. This did not reach significance, probably owing to the limited sample size.

Circulating TNF concentrations were increased in all patients at baseline and showed no reduction after three weeks. Serum concen-

Table 1 Cytokine and endotoxin binding protein concentrations in obstructive jaundice and after preoperative drainage

Mediator	t=0	t=3	p Value
LBP ($\mu\text{g/ml}$)	24.2 (2.8)	16.5 (4.3)	0.01*
sCD14 ($\mu\text{g/ml}$)	17.4 (2.1)	7.6 (1.4)	0.002*
BPI (ng/ml) (n=8)	2.9 (0.5)	1.8 (0.5)	0.1
TNF (pg/ml)	21.7 (3.1)	18.4 (4.2)	0.2
sTNF P55 (ng/ml)	2.9 (0.4)	2.7 (0.7)	0.2
sTNF P75 (ng/ml)	7.0 (0.9)	5.6 (1.2)	0.2
IL-6 (pg/ml)	4.2 (0.9)	6.1 (2.6)	0.9
IL-10 (pg/ml)	4.5 (1.4)	2.7 (0.8)	0.055
IL-8 (pg/ml)	113.6 (18.5)	20.7 (6.5)	0.001*

Cytokine and endotoxin binding protein concentrations in obstructive jaundice at t=0 and after biliary drainage at t=3 weeks (n=15). Results are given as mean (SEM). Endotoxin binding proteins are present at increased concentrations and are reduced after drainage. The reduction in bactericidal/permeability increasing protein (BPI) did not reach significance owing to the smaller number of samples (n=8). Of the cytokines, a significant reduction is only present in interleukin (IL) 8, although there is a nearly significant trend for IL-10.

*Significant.

LBP, lipopolysaccharide binding protein; TNF, tumour necrosis factor.

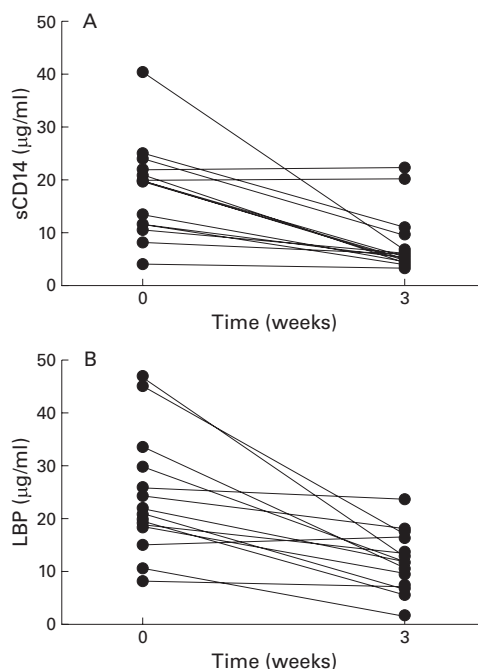


Figure 2 (A) Lipopolysaccharide binding protein (LBP) and (B) soluble CD14 concentrations in obstructive jaundice and after biliary drainage.

concentrations of the soluble TNF receptors before drainage were 2.9 (0.40) ng/ml for P55 and 7.0 (0.9) ng/ml for P75, which is somewhat higher than concentrations found in healthy humans; concentrations were not significantly affected by biliary drainage.

Interleukin 6 concentration was 4.2 (0.9) pg/ml at time point 0 and 6.1 (2.6) after three weeks drainage. Very low concentrations of interleukin 10 were detectable at time point 0 (4.5 (1.4) pg/ml) and after three weeks drainage (2.7 (0.8); $p=0.055$). Finally, circulating interleukin 8 concentrations were substantially increased before drainage (113.6 (18.5) pg/ml) and were reduced significantly after drainage (20.7 (6.5) pg/ml; $p<0.001$; fig 3).

NEUTROPHIL ACTIVATION

The elastase- α 1-antitrypsin complex concentration was 76.9 (22.9) ng/ml at t=0 and 50.4 (11.9) ng/ml after drainage ($p=0.16$). Lactoferrin concentrations were 155.1 (29.8) and 113 (17.5) ng/ml respectively ($p=0.16$).

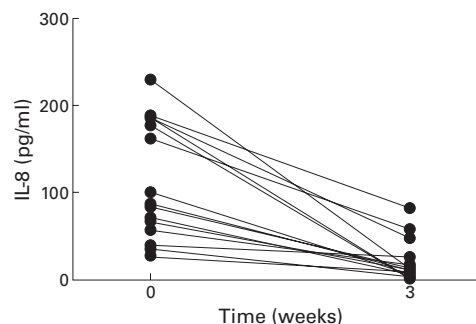


Figure 3 Interleukin (IL) 8 concentrations before and after biliary drainage. $p<0.001$.

CULTURES

Bile cultures, obtained during endoscopic stenting, were positive in two of 15 samples at time point 0, but all 15 samples were positive after three weeks of stenting ($p<0.001$). The cultured microorganisms included *E coli* (n=8) and enterococcus, streptococcus, klebsiella, and pseudomonas species.

Cultures of mesenteric lymph nodes did not reveal any growth unless prolonged culture was performed. Bacteria were then cultured in two of nine at t=0 and two of 15 after three weeks at operation. The isolated bacteria included *E coli* (n=1), streptococcus (n=1), klebsiella (n=1), and bacillus species (n=1). All liver biopsy cultures remained sterile.

CORRELATIONS

A significant correlation was found between leucocyte numbers and BPI concentrations after three weeks ($r=0.76$; $p=0.001$). Relevant correlations were also found at both time points between elastase and lactoferrin concentrations, between BPI and elastase, and between BPI and lactoferrin. Between bilirubin concentrations at t=3 and both creatinine and P55, significant correlations were also found (respectively, $r=0.67$; $p=0.01$ and $r=0.75$; $p=0.002$), and also with IL-8 ($r=0.65$; $p=0.01$).

As postoperative complications occurred in only two patients, an analysis of the correlation with circulating cytokine concentrations was meaningless.

Discussion

The results from this study in obstructive jaundiced patients undergoing adequate routine preoperative endoscopic internal biliary drainage show an increase in endotoxin binding proteins, IL-8, and neutrophil activation during severe jaundice, which were all reduced after biliary drainage. Furthermore, the study indicates that clinical obstructive jaundice seems to be associated with only low level endotoxaemia, and various proinflammatory cytokines, that have been associated with adverse outcome in animal models, were detected at only low concentrations.

Routine parameters, such as serum albumin or creatinine, perceived previously to be risk factors for postoperative morbidity,²⁸ were in the normal range before drainage and, there-

Table 2 Cytokine and endotoxin binding protein concentrations in healthy volunteers, and patients with obstructive jaundice or cholangitis

Mediator	Healthy volunteers* (n=8)	Obstructive jaundice* (n=15)	Cholangitis† (n=10)
TNF (pg/ml)	0.3	22	113
sTNFr P55 (ng/ml)	1	2.9	7
sTNFr P75 (ng/ml)	5	7	17
IL-6 (pg/ml)	<5	4	690
IL-8 (pg/ml)	<5	114	201
IL-10 (pg/ml)	<5	5	602
BPI (ng/ml)	0.5	3	9
LBP (µg/ml)	5–10	24	47
sCD14 (µg/ml)	3	17	17

*von der Möhlen *et al.*³³

†Kimmings *et al.*⁴²

BPI, bactericidal/permeability increasing protein; IL, interleukin; LBP, lipopolysaccharide binding protein; TNF, tumour necrosis factor.

fore, did not improve by relief of jaundice. This was probably owing to selection of patients in a relatively good general condition to undergo major surgery.

Endotoxin concentrations in the present study were low. When assessing endotoxin concentrations, it should be recognised that the endotoxin test used is a chromogenic assay that is potentially influenced by the yellow colour of jaundiced plasma. We have noted this problem before and have used each sample measured chromogenically for endotoxin as its own control. It is unclear whether such controls have always consistently been performed in previous studies, and this could account for the high endotoxin concentrations reported in some.¹ Moreover, the significant reduction in endotoxin concentrations after biliary drainage that occurs in animal studies was not observed here.^{6–10}

The increased concentrations of endotoxin binding proteins observed in this study, as well as the reduction after stenting, have not been previously described in obstructive jaundice. These proteins play important roles in regulating the reaction to endotoxin, either by facilitating cell activation in response to endotoxin, or by neutralising endotoxin released from bacteria. LBP is an acute phase protein produced by hepatocytes. It binds to the lipid A moiety of LPS, acting as a carrier that presents endotoxin to other molecules, such as CD14.^{12–29–30} Soluble CD14 is the 48 kD soluble form of membrane bound CD14, the putative LPS receptor. Soluble CD14 plays a role in LPS induced activation of cells lacking membrane CD14, or at high concentrations may cause endotoxin neutralisation by competing with cell bound CD14 or by shuttling LPS to lipoproteins.^{15–31–32} BPI is a 55 kD protein, produced in primary granules of neutrophils and released, *in vitro*, on neutrophil activation. Binding of BPI to LPS leads to neutralisation of LPS activity.^{14–33}

The concentrations of LBP and soluble CD14 observed at $t=0$ were significantly higher than those described for healthy individuals or patients with non-infectious diseases.^{24–31} The significant reduction of LBP and sCD14 detected after drainage indicates an attenuation of the hepatic acute phase response and the inflammatory reaction in general.

Baseline concentrations of BPI at $t=0$ were comparable to those found after low dose

endotoxin challenge in human volunteers³³ and were reduced by drainage to concentrations approaching those found in controls (table 2). It should be noted that these concentrations of BPI are considered far too low to neutralise endotoxin effectively. In the present study BPI correlated with leucocyte numbers, but the BPI/neutrophil ratio (ng BPI/ 10^6 neutrophils), reflecting BPI concentrations independent of neutrophil counts,³⁴ was also reduced by biliary drainage. Hence the effect of endoscopic stenting on BPI concentrations was not a consequence of a mere reduction in neutrophil counts.

The overall effect of the general increase in endotoxin binding proteins would be expected to increase sensitivity to endotoxin in patients with obstructive jaundice. Further studies are required to characterise the effect of biliary drainage on endotoxin sensitivity in humans.

In previous studies of biliary obstruction in animals significant concentrations of circulating cytokines were described. Immunologically detectable, but not biologically active, TNF⁴ and interleukin 6 were found, both important proinflammatory cytokines. Concentrations of soluble TNF receptors, the proteolytically cleaved cell membrane receptors,^{35–36} correlated with postoperative mortality.⁶ In our study population only low serum concentrations of proinflammatory cytokines were detected and no significant change was observed. TNF was detected by immunoassay, and the concentrations of P55 and P75 were somewhat higher than those reported in normal individuals. However, these parameters were not influenced by drainage, indicating ongoing release of TNF and its receptors. In a recent study a similar increase in sTNFr-P55 and P75 was reported and again activation of the TNF/sTNFr complex remained unchanged after drainage.³⁷ In our study both IL-6 and IL-10, an anti-inflammatory cytokine, were only slightly increased compared with normal (table 2). Again, no significant reduction was observed after drainage. Others also found increased concentrations of inflammatory cytokines.^{38–41} In particular, increased IL-6 concentrations were found in patients suffering from cholangitis.^{38–41} In another study from our institution high concentrations of IL-6 were found in patients with bile duct stones related cholangitis.⁴²

The chemokine interleukin 8 was present at a high concentration at baseline and its circulating concentration was dramatically reduced after drainage. The finding of increased circulating IL-8 concentrations is of importance in patients with biliary obstruction because this cytokine is involved in activation of neutrophils.⁴³ High tissue concentrations of IL-8 cause recruitment of neutrophils, whereas high serum concentrations have been reported to cause a reduction in the ability of neutrophils to transmigrate through the endothelium.⁴⁴ Indeed, neutrophil adhesion has been reported to be impaired in the presence of obstructive jaundiced serum,⁴⁵ but in this study any relation to IL-8 was not assessed.

Yanagitani *et al* also reported that in vitro chemotaxis of peripheral neutrophils to IL-8 was significantly increased in bile duct ligated rats.⁴⁶

For further characterisation of neutrophil activation, elastase and lactoferrin concentrations were measured and showed a trend towards higher concentrations during severe jaundice. BPI and elastase are released from the same neutrophil granules and in the present study a very strong correlation was found. In conjunction with the lactoferrin concentrations, which also correlated, these data indicate that in patients with obstructive jaundice a generalised state of neutrophil activation exists, that can be reversed by drainage.

Why were TNF concentrations unaffected by biliary drainage? One explanation is that the insertion of an endoprosthesis leads to infected bile with Gram negative bacteria and endotoxins and moreover induces local inflammation of the bile duct wall.¹⁹ It could be speculated that these factors could also be responsible for low enduring production of certain inflammatory factors, separate from production caused by obstructive jaundice. This would imply that routine preoperative drainage may not be indicated, especially as the overall clinical benefit has still to be elucidated, and recent data from the literature have also not yet shown whether preoperative biliary drainage by endoprosthesis really benefits the patient.¹⁵⁻¹⁷ As reported in other studies, TNF concentrations are much higher in patients with cholangitis and a decrease was found after drainage.^{38 42}

In conclusion, this study shows that obstructive jaundice causes profound alterations in circulating concentrations of endotoxin binding proteins. In addition, we found a generalised state of neutrophil activation and increased concentrations of IL-8. On the other hand, the concentrations of many of the previously investigated mediators, such as TNF and TNF receptors, are not as high. Biliary drainage did significantly reduce important inflammatory mediators (IL-8 and endotoxin binding proteins). Conversely it did not change many of the mediators suggested to be responsible for mortality in animal experiments, possibly owing to low concentrations and to an inflammatory effect of the endoprosthesis itself. Hence, the generalised inflammatory state in patients with obstructive jaundice differs profoundly from findings in animal models of biliary obstruction as well as in patients with severe cholangitis. Future studies are needed to investigate whether the changes in endotoxin binding proteins and the reduction of neutrophil activation by biliary drainage outweigh the less beneficial effects of an endoprosthesis.

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