Author’s response to reviews

Title: A retrospective clinical and microbial analysis of 32 patients with bilomas

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Please see uploaded file "Answers to Reviewer comments" as the table below is not correctly displayed. Thank you.

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A retrospectice clinical and microbial analysis of 32 patients with bilomas

BMC Gastroenterology

Silvia Würstle; A. Göß; Christoph D. Spinner, PD Dr.; Wolfgang Huber, Prof. Dr.; Hana Algül, Prof. Dr.; Christoph Schlag, PD Dr.; Roland M. Schmid, Prof. Dr.; Andreas Weber, Prof. Dr.; Andreas Obermeier, M.sc.; Jochen Schneider, PD Dr.

Response to Reviewers Comments
Evangelos Kalaitzakis (Reviewer 1): Würstle et al have performed a case-series study aiming to investigate the etiology, clinical presentation, pathogen spectrum and treatment of patients with bilomas. They retrospectively enrolled 32 patients with bilomas (identified by means of a search in their endoscopy database amongst patients having undergone ERCP or PTC). Most patients (66%) developed bilomas following surgery. 34% of patients had multiple bilomas. Ten out of 32 patients had infected bilomas (31%) according to clinical criteria (fever and CRP elevation and/or leukocytosis). Bile cultures were performed in 25/32 patients (78%) and 23/25 had a positive culture. Multiresistant bacteria were isolated from 8/32 (25% of patients) or 8/25 (32%) of those cultured. Most patients received interventional treatment. 80% of patients receiving interventional treatment as well as 80% of those treated in a conservative fashion achieved complete biloma resolution during follow-up.

Major concerns

1. Microbial pathogens were isolated from the bile of patients without clinically infected bilomas. The authors, however, do not provide data on isolates from infected bilomas which would probably be very meaningful. Also, do the authors feel that isolates from non-clinically infected patients were clinically relevant?

We appreciate this helpful comment. We absolutely agree with the Reviewer that isolation of bacteria from non-infected biloma are not clinically relevant. According to the Standard Operating Procedure (SOP) of our department of gastroenterology, bile collection is only performed if infection of the biliary tract system is suspected. Overall, biloma infection was suspected in 25 patients. Therefore, bile collection was performed in 25/32 (78.1%) patients. However, according to our study criteria for “infected biloma” (see below), 10/25 (40%) proved to suffer from infected bilomas. This subgroup revealed positive microbiology in 8 cases and negative microbiology in 2 cases. 15/25 patients with bile culture (60%) did not fulfil our study criteria for “infected biloma”, mainly because a concurrent infection focus like urinary tract infection or pneumonia was diagnosed or could not be ruled out. None of these bile cultures were sterile. As illustrated in the following table (Table Reviews 1), no significant difference in pathogen spectrum between infected and colonized biloma was found.

Study criteria for infected biloma:

Fever (>38.5 °C) with CRP-elevation (>5 mg/dl) or leucocytosis (>12.0 G/l) and no indications for other focus of infection.

Table Reviews 1: Pathogen spectrum of infected and colonized biloma

<table>
<thead>
<tr>
<th>Pathogen spectrum</th>
<th>Infected biloma</th>
<th>Non-clinically infected biloma (colonized)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(two-sided Fisher’s exact test)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobe gram-positive bacteria</td>
<td>7/10 (70%)</td>
<td>10/15 (67%)</td>
<td>1</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>------------</td>
<td>-------------</td>
<td>------------</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>2/10 (20%)</td>
<td>4/15 (27%)</td>
<td>1</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>6/10 (60%)</td>
<td>9/15 (60%)</td>
<td>1</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>3/10 (30%)</td>
<td>3/15 (20%)</td>
<td>0.6532</td>
</tr>
<tr>
<td>Enterococcus spp. susceptible to Ampicillin</td>
<td>2/10 (20%)</td>
<td>3/15 (20%)</td>
<td>1</td>
</tr>
<tr>
<td>Coagulase negative staphylococcus</td>
<td>0/10 (0%)</td>
<td>2/15 (13%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1/10 (10%)</td>
<td>1/15 (7%)</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aerobe gram-negative bacteria</th>
<th>5/10 (50%)</th>
<th>10/15 (67%)</th>
<th>0.4422</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>1/10 (10%)</td>
<td>5/15 (33%)</td>
<td>0.3449</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>4/10 (40%)</td>
<td>2/15 (13%)</td>
<td>0.1753</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>4/10 (40%)</td>
<td>6/15 (40%)</td>
<td>1</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td>3/10 (30%)</td>
<td>2/15 (13%)</td>
<td>0.3577</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0/10 (0%)</td>
<td>1/15 (7%)</td>
<td>1</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>1/10 (10%)</td>
<td>0/15 (0%)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

| Anaerobe bacteria                           | 0/10 (0%)  | 1/15 (7%)   | 1          |

Following passage was added to results of the manuscript – results, Microbiological analysis of bilomas on page 8, line 178:

According to the Standard Operating Procedure (SOP) of our department of gastroenterology, bile collection is only performed if infection of the biliary tract system is suspected. Overall, biloma infection was suspected in 25 patients. Therefore, bile collection was performed in 25/32 (78.1%) patients. However, according to our study criteria for infected biloma, 10/25 (40%) proved to suffer from infected biloma. This subgroup revealed positive microbiology in 8 cases
and sterile microbiology in 2 cases. 15/25 patients with bile culture (60%) did not fulfil our study criteria for infected biloma, mainly because a concurrent infection focus like urinary tract infection or pneumonia was diagnosed or could not be ruled out. None of these bile cultures were sterile. No significant difference in pathogen spectrum between infected and colonized biloma was found.

2. A large proportion of patients in the study received antibiotics (p.10, l. 204; 11/32 piperacillin/tazobactam and 18/32 ciprofloxacin). It is unclear why this is the case. Was this post-operative/post-transplant rather than due to a diagnosed infection/infected biloma? If yes, may this have prevented the diagnosis of further "clinically" infected bilomas?

Thank you for this valuable comment. We are in complete agreement that the proportion of patients with antibiotic therapy is high in our study population. We explain this with a high proportion of multimorbid patients in our study cohort. For instance, 21/32 patients (65.6%) underwent surgery before developing bilomas including liver transplantation (7/32, 21.9%) or partial liver resection (5/32, 15.6%). 17/32 (53.1%) patients suffered from a malignant disease like hepatocellular, cholangiocellular, colorectal or pancreatic carcinoma. One patient developed a biloma based on a stab wound injuring the right lobe of the liver, the small intestine, and the transverse colon.

In detail, 27/32 patients received antibiotic therapy. 10/27 with antibiotic therapy fulfilled the study criteria for an infected biloma. 17/27 patients with antibiotic therapy did not fulfil the study criteria for an infected biloma. These 17 patients received antibiotic treatment due to another source of infection (i.e. urinary tract infection, post-operative wound infections, prostatitis or pneumonia) or got antibiotic prophylaxis after aortic homograft change or to prevent Post-ERC(P) cholangitis. Indeed, due to the high rate of non-biloma associated infections, we cannot rule out that the incidence of infected biloma was underestimated in our study cohort.

Following limitation was added to the discussion, page 13, line 291:

Furthermore, due to the high proportion of patients with antibiotic treatment initiated either because of a concurrent, non-biloma associated infection focus (i.e. pneumonia, urinary tract infection) or prophylactic reasons (i.e. endocarditis prophylaxis), the incidence of infected biloma might be underestimated in our study cohort.

3. As the authors acknowledge, patients were identified through an endoscopy database; thus, they were more likely to require interventional therapy. Also, 37% of patients developed bilomas after liver transplantation or liver resection. This may have influenced the generalizability of the results of the study.

Yes, this is absolutely true. The patient recruitment via the endoscopy database has to be emphasized as possible bias. In general, patients with biloma undergoing ERC(P) present frequently a worse condition compared to patients with biloma not requiring endoscopy. As
stated in the limitation of the study, this might limit the generalizability of our study. However, one major aim of the study was to show that endoscopic therapy of bilomas is safe and efficient. Therefore, we chose patient recruitment using an endoscopic database.

We added following limitation to the manuscript on the discussion, page13, line 284:

Limitations of this study include the recruitment of patients based on an endoscopic database and hereby selecting more patients with extensive morbidity, which may have influenced the generalizability of the results of the study. Patients with extensive (co)morbidities are more prone to infections, may receive more frequently antibiotic treatment and therefore have a higher risk of acquiring multiresistant bacteria than patients with less comorbidities. However, one major aim of the study was to show that endoscopic treatment is safe and efficient for patients with bilomas.

Minor concerns

1. Please provide tables at the end of the paper and do not embed them in the text.

As suggested by the reviewer, we have changed the location of the tables.

2. Table 4, 3rd and 5th column: Please change "Number of patients (%)…” to "Proportion of patients (%)…”

The correction has been made. The column headings now read “Proportion of patients (%) during initial hospital stay” and “Proportion of patients (%) during clinical follow-up”.

3. The paper would probably benefit from a grammar check.

As suggested by the reviewer, we have rechecked the grammar spelling with due diligence and changed the English (US) to English (UK) spelling. In detail, the following grammar changes have been made:

Page 4, line 89: leucocytosis instead of leucocytosis

Page 5, line 103: a Chiba needle was inserted into the biliary system instead of a Chiba needle was inserted into biliary system

Page 12, line 272: tumour instead of tumor

Page 13, line 283: Only one patient instead of only one patients

Page 13, line 296: single-centre instead of single-center
Jianfeng Yang (Reviewer 2): Overall impression:

This paper has a complete structure, clear thinking and novel theme

Specific comments:

1. Whether to use antibiotics before collecting bile, how to ensure the reliability of results if antibiotics are used?

We appreciate the positive feedback. Due to the retrospective study design we had no influence on the begin of antibiotic therapy. Referring to the Standard Operating Procedure (SOP) of our department of gastroenterology, antibiotic therapy was initiated after bile collection, provided that the patient was not septic.

Following passage was added to the method section, Patients, page 4, line 90:

Referring to the Standard Operating Procedure (SOP) of our department of gastroenterology, antibiotic therapy was initiated after bile collection, provided that the patient was not septic.

2. It is suggested to quantify the microorganism in bile and determine the number of microorganisms

At our department, bile samples are injected in a culture flask to decrease preanalytical problems like long transportation. Particularly vulnerable bacteria like anaerobes are better protected. However, quantification of bacteria in culture flask is not possible.

Following passage was added to the method section, Microbiological processing, page 5, line 112:

Bile was injected into aerobic/anaerobic blood culture flasks and subsequently incubated for 5 days at 37 °C. Each flask was subcultivated under aerobic (chocolate agar in 5% CO2) and anaerobic conditions (Schaedler anaerobic agar) at the end of the incubation period for control and to exclude failure of automatic detection of the BacTec system (BD Becton Dickinson, Franklin Lakes, USA). If bacterial growth was detected in a culture flask, 50–100 µL of bile was transferred onto solid culture media (Columbia sheep blood agar, chocolate agar, MacConkey
agar, Schaedler anaerobic agar, Schaedler KV anaerobic agar and Sabouraud agar) and subsequently cultured for at least 48 hours at 37.8 °C.

3. It is generally considered that normal human bile is sterile. Why are there bacteria in the bile of patients with bilomas?

We absolutely agree with you on that point. The biliary tract is usually sterile in humans. The continuous flow of bile, the bacteriostatic effects of bile salts, anatomical barriers such as the sphincter of Oddi and potent immunological defence mechanisms protect the biliary system from bacterial invasion. In general, microbial colonization of the biliary tract occurs mainly due to a disruption of the protective anatomic barrier of the Sphincter Oddi (i.e. biliodigestive anastomosis, bile duct stenting, papillotomy …). In case of a disruption of the anatomic barrier of the Sphincter Oddi, bacteria can easily ascend from the duodenum and colonize the biliary system. In our study cohort, most patients had an endoscopic or surgical intervention on the biliary tract system prior the diagnosis of biloma.

4. Why are there drug-resistant bacteria in patients with bilomas?

Thank you for this interesting question. We explain this high rate of multi-resistant bacteria with the high proportion of multimorbid patients in our study cohort requiring antimicrobial treatment either due to infected bilomas or another infection focus. The majority of our study population suffered from a serious illness, which had demanded several hospitalisations in the past (e.g. liver transplantation).

We added following limitation to the discussion, page 13, line 284:

Limitations of this study include the recruitment of patients based on an endoscopic database and hereby selecting more patients with extensive morbidity, which may have influenced the generalizability of the results of the study. Patients with extensive (co)morbidities are more prone to infections, may receive more frequently antibiotic treatment and therefore have a higher risk of acquiring multiresistant bacteria than patients with less comorbidities. However, one major aim of the study was to show that endoscopic treatment is safe and efficient for patients with bilomas.