Journal of Infection (2015) 70, 483–490

BIAMM British Infection Association

www.elsevierhealth.com/journals/jinf

Microbiological analysis of bile and its impact in critically ill patients with secondary sclerosing cholangitis

Torsten Voigtländer [a], Ensieh Leuchs [a], Ralf-Peter Vonberg [b], Philipp Solbach [a], Michael P. Manns [a], Sebastian Suerbaum [b], Tim O. Lankisch [a,*]

[a] *Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany*
[b] *Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Hannover, Germany*

Accepted 24 January 2015
Available online 7 February 2015

KEYWORDS
Bile;
Microbiology;
Secondary sclerosing cholangitis;
Critically ill patients;
Multi drug resistance;
Primary sclerosing cholangitis

Summary *Objectives:* Secondary sclerosing cholangitis in critically ill patients (SSC-CIP) is an emerging disease entity with unfavourable outcome. Our aim was to analyze the microbial spectrum in bile of patients with SSC-CIP and to evaluate the potential impact on the empiric antibiotic treatment in these patients.
Methods: 169 patients (72 patients with SSC-CIP and 97 patients with primary sclerosing cholangitis (PSC)) were included in a prospective observational study between 2010 and 2013. Bile was obtained during endoscopic retrograde cholangiography (ERC) and microbiologically analyzed.
Results: Patients with SSC displayed a significantly different microbiological profile in bile. *Enterococcus faecium*, *Pseudomonas aeruginosa* and *non-albicans species of Candida* were more frequent in SSC compared to patients with PSC ($p < 0.05$). Patients with SSC showed a higher incidence of drug or multi-drug resistant organisms in bile ($p = 0.001$). The antimicrobial therapy was adjusted in 64% of patients due to resistance or presence of microorganisms not covered by the initial therapy regimen.
Conclusions: Patients with SSC-CIP have a distinct microbial profile in bile. Difficult to treat organisms are frequent and an ERC with bile fluid collection for microbiological analysis should be considered in case of insufficient antimicrobial treatment.

* Corresponding author. Department of Gastroenterology, Hepatology and Endocrinology, Medical School Hannover, Carl-Neuberg Str. 1, 30625 Hannover, Germany. Tel.: +49 511 532 2406; fax: +49 511 532 3351.
E-mail address: lankisch.tim@mh-hannover.de (T.O. Lankisch).

http://dx.doi.org/10.1016/j.jinf.2015.01.013

Introduction

Secondary sclerosing cholangitis (SSC) in critically ill patients (SSC-CIP) is an emerging disease entity with unfavourable outcome.[1] Recently, a growing number of patients with SSC during or after intensive care unit treatment are reported.[1–5] The exact mechanisms contributing to this form of cholangiopathy are not fully understood. Ischemic injury to the bile ducts may be one of the first events which triggers the degeneration of the biliary tree.[2] Moreover, biliary tract infection seems to play a critical role in the pathogenesis as repetitive episodes of cholangitis deteriorate the bile duct integrity.[1,2]

In general, bile is sterile in healthy humans. Cholangitis results from a combination of biliary obstruction and bacterial or fungal growth in bile.[6–8] Patients with structural abnormalities of the biliary system (e.g. SSC and primary sclerosing cholangitis (PSC)) are at special risk of this potentially life-threatening condition.[1,2,9] Empiric antibiotic or antimycotic therapy and eventual biliary drainage are the treatment of choice.[10,11] However, an identification of the causative pathogen is of great importance in order to establish the best therapy possible especially in patients with SSC who often display refractory cholangitis episodes.[1] Blood cultures are regularly used to identify the causative organism, but even in febrile patients with cholangitis, blood cultures remain negative in more than 50% of cases.[7,12,13] Consequently, a microbiological fluid analysis obtained at site of the inflammatory process seems reasonable. In former studies we showed that routine bile collection during endoscopic retrograde cholangiography (ERC) to perform microbiological analysis of the bile and subsequent adaption of the antibiotic treatment regimen according to the microbiological profile is a valuable tool in selected patients.[14]

These findings prompted us to analyze the microbial spectrum in bile of patients with SSC-CIP and to evaluate the potential impact on the empiric antibiotic treatment in patients with SSC.

Patients and methods

Patients

Consecutive patients with SSC-CIP and PSC presenting for ERC at the endoscopic unit of Hannover Medical School between January 2010 and December 2013 were enrolled in a prospective observational study. The diagnosis of SSC-CIP was based on typical cholangiographic findings such as strictures or irregularity of intrahepatic and/or extrahepatic bile ducts with presence of biliary casts. All patients with SSC were treated at least for 5 day at the intensive care unit (ICU). Before ICU treatment no patient with SSC had a history of pre-existing liver disease. PSC was diagnosed in patients with a characteristic patient history and elevated cholestatic parameters with multifocal typical strictures of the biliary tree in ERC and exclusion of secondary causes of sclerosing cholangitis. Demographic data and laboratory values at day of bile collection were recorded. Patients with repetitive ERC were only included at first presentation.

Methods

All duodenoscopes (Olympus, Hamburg, Germany) were disinfected before use according to the guidelines of the Robert Koch Institute.[15] Bile samples were aspirated by placing a single-use (5 F), standard ERC catheter (without flushing or guidewire cannulation) into the bile duct before contrast injection. Bile was obtained through the catheter with a 10 ml sterile syringe. Approximately 0.5 ml—5 ml of bile was collected and transferred into a sterile tube. Bile specimens were delivered to the microbiological laboratory within 2 h at room temperature. Samples were cultured under aerobic conditions (36 °C for at least 48 h) on 5% Columbia sheep blood agar (Becton Dickinson GmbH, Heidelberg, Germany), McConkey agar (Oxoid GmbH, Wesel, Germany) with first reading after 24 h. Anaerobic growth was observed by the use of Schaedler agar (Becton Dickinson GmbH) at 36 °C for the same time period. Species differentiation was then performed according to standard operation protocols of internal guidelines that had been certified by the German Accreditation Group "Deutsche Akkreditierungsstelle" (DAkkS) according to German laboratory practice guideline DIN EN ISO 15189. Species identification and antibiotic susceptibility testing of most bacteria were performed using the commercially available VITEK-2-XL (bioMerieux, Nuertingen, Germany) system. For antibiotic susceptibility testing of slow-growing Gram negative bacteria the commercially available Merlin MICRONAUT Sprint Dispenser automated broth micro-titer system (Genzyme Virotech, Ruesselsheim, Germany) was used according to the instructions of the manufacturer. Micro-titer plates of 96 wells were used as recommended by the German Network for the Antimicrobial Resistance Surveillance (GENARS) for susceptibility testing of Gram negative bacteria. The detection of more than 10^4 organisms/ml of bile was defined as significant bacterial growth.

The study protocol was approved by the local institutional ethics review board and is in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.

Statistical analysis

Group data are presented as number/percentages or median with interquartile range (IQR). All data were tested for normality (Shapiro-Wilk test, Kolmogorov-Smirnov test). Continuous data of the study groups were compared using Kruskal Wallis test. In case of statistical differences, Mann—Whitney or Wilcoxon test was used to compare groups. P values <0.05 were considered statistically significant. The software used was the SPSS Statistical Package (version 19.0, SPSS Inc, USA) and GraphPad Prism (version 6.01, GraphPad Inc, USA).

Results

Study cohort and general microbiological characteristics

Bile samples from 169 patients (72 patients with SSC-CIP and 97 patients with PSC as a control group) were included