Thieme

White bile in patients with malignant biliary obstruction is an independent factor of poor survival

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ABSTRACT

Background and study aims White bile is defined as a colorless fluid occasionally found in the biliary tract of patients with bile duct obstruction. Its significance is not clearly established. Our objective was to analyze the prognostic value of white bile in a series of patients with biliary obstruction due to biliary or pancreatic cancer.

Patients and methods The study was conducted on a series of consecutive patients with malignant obstructive jaundice. They all underwent endoscopic retrograde cholangiopancreatography with collection of bile and biliary stent insertion. White bile was defined as bile duct fluid with bilirubin level <20 µmol/L. Univariate and multivariate analyses were performed to identify variables associated with overall survival (OS).

Results Seventy-three patients were included (32 pancreatic cancers, 41 bile duct cancers). Thirty-nine (53.4%) had white bile. The mean bile duct bilirubin level in this group was 4.2 ± 5.9 µmol/L vs 991 ± 1039 µmol/L in patients with colored bile (P < 0.0001). In the group of 54 patients not eligible for surgery, the multivariate analysis demonstrated an association between the presence of white bile and reduced OS (HR 2.3, 95%CI 1.1–4.7; P = 0.02). Other factors independently associated with OS were metastatic extension (HR 2.8, 95%CI 1.4–5.7) and serum total bilirubin (HR 1.003, 95%CI 1.001–1.006). There was a significant inverse correlation between serum and bile duct bilirubin levels (r = –0.43, P = 0.0001).

Conclusion White bile in patients with inoperable malignant biliary obstruction is an independent factor of poor survival.

Introduction

The presence of colorless and transparent fluid in the biliary tract of patients with bile duct obstruction has been reported as early as the beginning of the 20th century by surgeons [1, 2] and more recently by endoscopists [3–5]. The term "white bile" has been coined to describe this entity. Although a misnomer, this term is conventionally used to describe a translucent bilirubin-free fluid to be differentiated from the white-colored purulent bile [6, 7]. White bile is observed in patients with biliary obstruction of either benign or malignant origin. Biochemical analysis showed that white bile was nearly devoid of bilirubin and biliary acids [8–10].

The pathogenic mechanisms of white bile formation are still unclear. The initial hypothesis that white bile is observed only when the gallbladder is excluded [1, 11] has not been confirmed by subsequent clinical observations [3, 10]. Whether white bile reflects impaired hepatocellular function and is asso-

ciated with poorer prognosis is controversial [10, 12]. Until recently, most cases were anecdotal observations or small series. In the most recent and best documented study [3], white bile was associated with worse survival in patients with inoperable malignant biliary obstruction. However, only 18 patients with white bile, 15 of them with gallbladder carcinomas, were included in this study.

Given this, the main objective of the present study was to revisit the question of the prognostic significance of white bile in a larger series of patients with biliary obstruction due either to biliary or pancreatic cancer and undergoing endoscopic biliary drainage.

Patients and methods

Patients

This study was conducted on a series of consecutive patients with malignant obstructive jaundice undergoing diagnostic and therapeutic endoscopic retrograde cholangiopancreatography (ERCP) in the Hepato-Gastroenterology Unit of Reims University Hospital. Patients were initially referred for jaundice or cholangitis. They had a complete clinical, biological and imaging evaluation including liver tests, ultrasound, abdominal computed tomography scan and/or magnetic resonance cholangiopancreatography. The diagnosis of extrahepatic cholestasis was made and ERCP was performed to obtain a precise diagnosis of the nature of the biliary obstruction and to decompress the biliary tract. The examination was conducted with a TJF duodenoscope (Olympus Optical, Tokyo, Japan) under general anesthesia. Bile duct was cannulated using a sphincterotome (Autotome, Boston Scientific, Natick, Massachusetts, United States) mounted on a guide wire (Life Partners Europe, Bagnolet, France). After insertion of the guidewire across the stenosis, the cannula was positioned above the biliary obstruction and about 5 to 10 mL of fluid was collected for cytology by gentle suction with a syringe, before any injection of contrast agent. All patients with previous contrast agent injection, even mild, were excluded. Then, depending on the endoscopist's decision, brush cytology (Cook Medical, Bloomington, Indiana United States) and biopsy (Endojaw FB-211K, Olympus Optical) were also performed. Finally, biliary drainage was obtained by insertion of a 10 F or 11.5 F Teflon stent (Cook Medical) or a covered or uncovered metallic prosthesis (Boston Scientific). In some patients, echoendoscopy (Olympus Optical Co.) was coupled to ERCP and when appropriate, puncture of a solid mass was performed for cytological and/or histological examination. After biliary decompression, patients were followed clinically and serum bilirubin was measured at different intervals according to clinician decision. The following data were retrospectively collected from the medical files: age, sex, serum bilirubin, results of cytological and pathological examination, type of cancer, level of obstruction, presence of metastasis, history of cholecystectomy, and modalities of surgical and medical treatment. Patient records were anonymized and de-identified prior to analysis. The database was constituted in accordance with the reference methodology MR004 of the Commission Nationale de l'Informatique et des Libertés (n° 2206749, 13/09/2018).

Measurement of bile duct bilirubin

Bile duct fluid collected during ERCP was sent to the laboratory in the following hour to 2 hours. It was centrifuged at 1500 revolutions/min for 5 minutes and the precipitate was collected, smeared on slides, fixed in methanol and stained with the Papanicolaou method for cytological evaluation. The remaining supernatant was retrieved, frozen, and stored in the dark at –80 °C for further analysis. After thawing, total bilirubin in the bile duct fluid was measured by a diazo colorimetric method using the COBAS 8000 analyzer (Roche Diagnostics France). Previous studies on serum have reported that freezing at –80 °C did not affect total bilirubin measurement [13]. In addition, as bilirubin is sensitive to phototransformation, samples were sent to the laboratory and stored protected from light. White bile was defined by bile duct bilirubin level <20 µmol/L and colored bile was defined as bile duct bilirubin >20 µmol/L.

Follow-up and endpoints

Survival time was defined as the time between ERCP and death. The primary endpoint was overall survival (OS). Patients alive at the end of the follow-up were censored. Patients lost during follow-up were censored at the date of the last contact. Secondary endpoints were to analyze: 1) the relationship between the presence of white bile and the level of biliary obstruction below or above the cystic convergence in non-cholecystectomized patients; and 2) the relationship between serum bilirubin and bile duct bilirubin levels.

Statistical analysis

Quantitative variables were reported as mean ± standard deviation and qualitative data as number and percentage. OS was calculated from the date of ERCP to the date of death or censoring. The survival curves were established using the Kaplan-Meier method in population of patients with surgery and in population of patients without surgery. Variables associated with OS in the population of patients with surgery and in the population of patients without surgery were identified by univariate analyses using log rank tests and by multivariate analyses using a Cox proportional hazard model. Factors significant at the 0.10 level in univariate analysis were included in a stepwise regression multivariate analysis with entry and removal limits set at 0.10. The correlation between serum and bile duct bilirubin levels was estimated using the Pearson's correlation coefficient.

$P < 0.05$ was considered statistically significant. All statistical analyses were performed using SAS version 9.4 (SAS Inc, Cary, North Carolina, United States).

Results

Patient characteristics

A total of 73 patients were included. Thirty-two of them (43.8 %) had pancreatic cancer and 41 (56.2 %) bile duct cancer, including one with gallbladder cancer. The characteristics

► Table 1 Characteristics of patients with white bile (n = 39) and colored bile (n = 34).

Variable[1]	White bile (n = 39)	Colored bile (n = 34)	P value
Age (years), mean ± SD	68.6 ± 10.8	68.6 ± 12.1	0.97
Sex ratio, M/F, n	22/17	19/15	0.96
History of cholecystectomy	3 (7.7)	7 (20.6)	0.17
Serum total bilirubin, µmol/L (md = 1)	345 ± 127	210 ± 134	< 0.0001
Bile duct total bilirubin, µmol/L	4.2 ± 5.9	991 ± 1040	< 0.0001
Level of obstruction in non-cholecystectomized patients (n = 63):			0.16
▪ Below cystic duct convergence	24/36 (66.7)	24/27 (88.9)	
▪ Above cystic duct convergence	5/36 (13.9)	1/27 (3.7)	
▪ Hilar	7/36 (19.4)	2/27 (7.4)	
Metastasis	24 (61.5)	13 (38.2)	0.047
Type of prosthesis			0.58
▪ Plastic	15	11	
▪ Metallic	24	23	
Surgical treatment	7 (18.0)	12 (35.3)	0.09
Chemotherapy	24 (61.5)	26 (76.5)	0.17

[1] Data are expressed using number (percentage) unless otherwise indicated.

of the whole population and the comparison between patients with pancreatic cancer and those with bile duct cancer are shown in the **Supplementary Table 1**.

All patients had jaundice at the time of ERCP, two of them with concomitant cholangitis (one patient with pancreatic cancer and colored bile and one with biliary cancer and white bile). The mean serum total bilirubin level was 283 ± 145 µmol/L. Thirty-nine patients (53.4 %) had white bile i. e., bile duct bilirubin < 20 µmol/L. The mean bile duct bilirubin level in this group was 4.2 ± 5.9 µmol/L compared to 991 ± 1040 µmol/L in the group of patients with colored bile (P < 0.0001). The characteristics of these two groups of patients are shown in **► Table 1**. Age and sex ratio were not significantly different between the two groups. Serum total bilirubin level was significantly higher in patients with white bile (345 ± 127 µmol/L) compared to patients with colored bile (210 ± 134 µmol/L, P < 0.0001). There was no significant difference in the type of prosthesis between patients with white bile and those with colored bile (P = 0.58). In five cases, initial endoscopic biliary drainage incompletely resolved jaundice, Percutaneous drainage was performed in three patients (2 patients with colored bile, 1 patient with white bile), a double surgical derivation in one (white bile patient), and a second procedure using metallic prosthesis to replace the biliary plastic prosthesis initially inserted in one (white bile patient). Patients with white bile more often had metastasis compared to patients with colored bile (61.5 % vs 38.2 %, P = 0.047). In the great majority of cases in both groups, evolution of cancer was the cause of death. In one patient with local recurrence of biliary cancer and peritoneal carcinosis, death was due to stroke (colored bile group). In another patient with biliary can-

cer, death was due to myocardial infarction (colored bile group). Two other patients with biliary cancer were lost of follow-up with no information on the precise cause of death (one patient in the colored bile group and one patient in the white bile group).

Survival analysis

Cumulative survival was significantly shorter in patients with pancreatic cancer compared to patients with bile duct cancer (P = 0.02, log rank test). Median survival was 9.6 months (95 % CI: 5.6–15) in the whole population and 15.2 months (95 % CI: 5.5–31.3) and 7.7 months (95 % CI: 4.1–10.1) in the groups of patients with bile duct cancer and pancreatic cancer, respectively (**► Fig. 1**).

Survival curves in the population of patients with intended curative surgery (n = 19) and those not eligible for surgery (n = 54) are presented in **► Fig. 2**. Median survival was 41.5 months (95 % CI: 18.7–94.8) in the population with surgery and 5.6 months (95 % CI: 3.3–8.9) in the population without surgery.

In the population with surgery, no factor was associated with OS (**► Table 2**). Median survival was 22.8 months (95 % CI: 10.8–94.8) in the "white bile" group (n = 7) and 26.5 months (95 % CI: 12.7–61.1) in the "colored bile" group (n = 12) (P = 0.96).

In the population without surgery, as shown in **► Fig. 3**, cumulative survival was significantly poorer in patients with white bile (n = 32) compared with patients with colored bile (n = 22) (P = 0.003, log rank test). Median survival was 2.9 months (95 % CI: 0.2–25.9) in the "white bile" group and 9.1 months (95 % CI: 1.1–55.2) in the "colored bile" group. In univariate analysis based on the log rank test (**► Table 3**), other factors associated
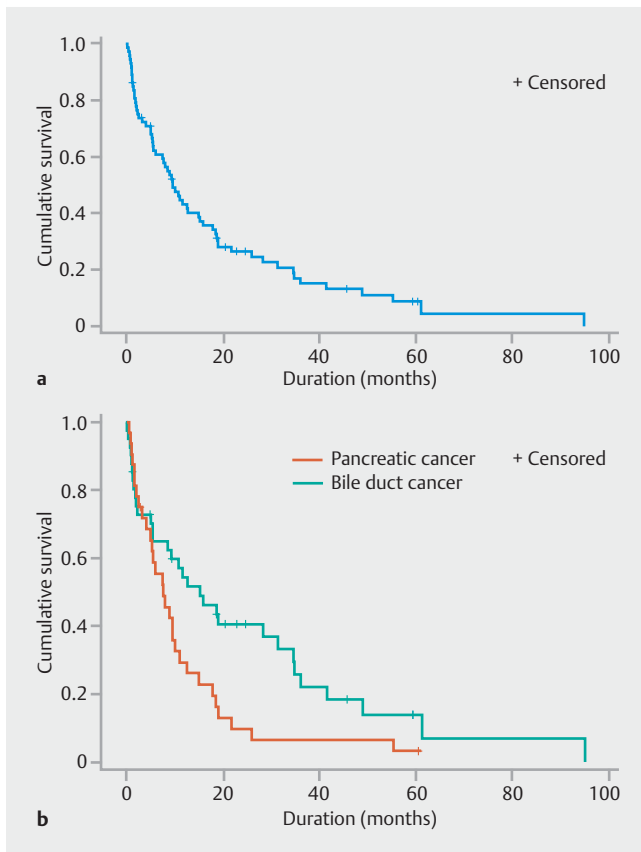
▶ Fig. 1 Kaplan-Meier survival curves. a For the whole population (n = 73). b For patients with biliary (n = 41) and pancreatic cancer (n = 32) (P = 0.02, log rank test).
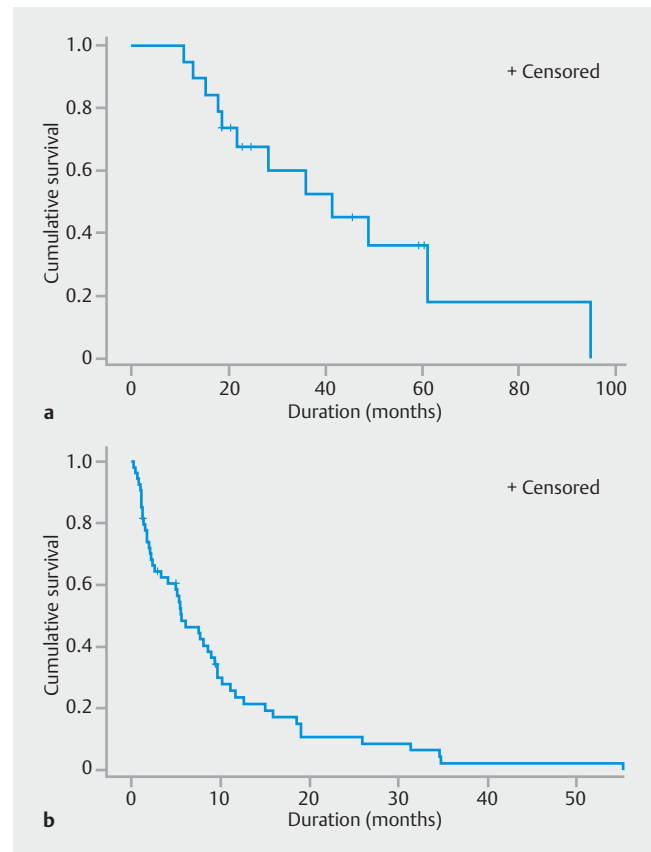
▶ Fig. 2 Kaplan-Meier survival curves. a In patients eligible for surgery (n = 19, median survival 41.5 months; 95 % CI: 18.7–94.8). b In inoperable patients (n = 54, median survival 5.6 months; 95 % CI: 3.3–8.9).

with survival were metastatic extension of cancer (P = 0.0002) and serum total bilirubin (P < 0.0001). In multivariate analysis (▶ Table 3), all these factors remained significant independent factors associated with OS: white bile (HR 2.3, 95 % CI: 1.1–4.7, P = 0.02), metastatic extension (HR 2.8, 95 % CI: 1.4–5., P = 0.003). and serum total bilirubin (HR 1.003, 95 % CI: 1.001–1.006, P = 0.01).

Impact of the level of bile duct obstruction on white bile formation

White bile was observed both in cholecystectomized and non-cholecystectomized patients (3/10 and 36/63 respectively). The relationship between the level of biliary obstruction and white bile formation was analyzed in the subgroup of non-cholecystectomized patients (n = 63). Fifty percent of patients (24/48) with distal obstruction below the cystic convergence (i. e., with gallbladder connected to occluded biliary tract), presented with white bile at the time of ERCP versus 12/15 (80.0 %) patients with biliary obstruction above the cystic convergence (P = 0.04).

Correlation between serum and bile duct bilirubin levels

Concentration of serum bilirubin was significantly higher in white bile patients than in patients with colored bile (345 ± 127 μmol/L versus 210 ± 134 μmol/L, P < 0.0001) and there was a significant inverse correlation between serum and bile duct bilirubin levels (Pearson's correlation coefficient r = −0.43; P = 0.0001) (▶ Fig. 4).

Discussion

The prognostic significance of white bile as well as the pathogenic mechanisms involved in its formation are a matter of debate. Our study, the largest to date, shows that white bile in patients with inoperable malignant biliary obstruction is an independent factor associated with poorer OS. In addition, our analysis confirms that a functioning gallbladder does not prevent the formation of white bile in bile ducts. The inverse correlation between serum and bile duct bilirubin concentrations indicates that white bile in bile ducts appears progressively according to the degree of cholestasis induced by complete and prolonged obstruction.
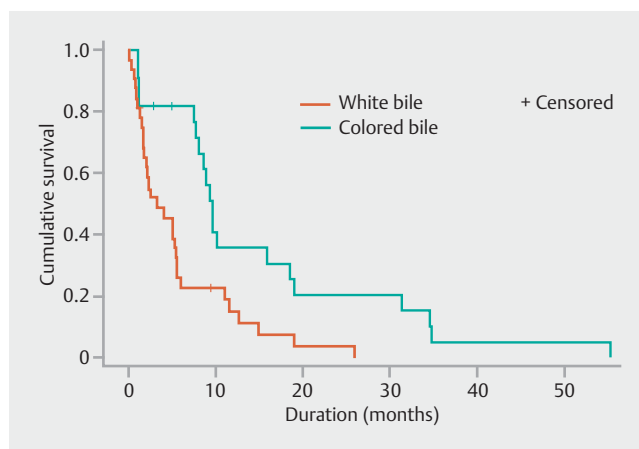
▶ **Table 2** Univariate analysis of factors associated with survival in the population of patients with malignant biliary obstruction eligible for surgery (n = 19).

Variables	n	Survival time (mo)[1]	Univariate analysis P value
Age			0.61
Sex			0.08
▪ Male	13	21.8 [10.8–94.8]	
▪ Female	6	47.3 [18.7–61.1]	
Type of cancer			0.71
▪ Bile duct	16	26.5 [10.8–94.8]	
▪ Pancreatic	3	21.8 [17.9–60.4]	
Hilar obstruction			0.42
▪ Yes	3	59.3 [41.5–61.1]	
▪ No	16	22.3 [10.8–94.8]	
Serum total bilirubin			0.81
White bile			0.96
▪ Yes	7	22.8 [10.8–94.8]	
▪ No	12	26.5 [12.7–61.1]	

[1] Data are presented as median [range].

▶ **Fig. 3** Kaplan-Meier survival curves in patients not eligible for surgery with white bile (n = 32) and with colored bile (n = 22) (*P* = 0.003, log rank test).

Although initial observations of white bile date back a century [1, 2], data about its clinical significance are scarce and controversial [3, 10, 13]. Most reports are anecdotal or concern small numbers of patients. The largest series was reported by Ahuja et al [3] in 2002, who compared 18 white bile patients with 17 patients with colored bile. Among patients with white bile, 15 had gallbladder cancer and three had pancreatic cancer. White bile was defined visually as translucent bile duct fluid. The mean concentration of bile duct bilirubin in these patients was 8.2 ± 0.07 μmol/L. Although the number of patients was small, reported survival was significantly worse in white bile patients [3]. In another study published in Korean in 2008, Jung et al [14] analyzed a series of 16 patients with white bile and 44 patients with colored bile. Data from the English abstract indicate that all patients had malignant biliary obstruction. White bile was defined as bile duct fluid with bilirubin <25 μmol/L. Survival analysis conducted in a subgroup of 35 inoperable patients did not show any significant difference between patients with and without white bile.

Our study was conducted on the largest population of white bile patients so far. In contrast with Ahuja et al's study [3], in which most patients had gallbladder cancer (83%), our series included only one patient with gallbladder cancer and an approximately equal distribution of patients with bile duct cancer and pancreatic cancer. In the small subgroup of 19 patients eligible for surgery, OS was not significantly different between patients with white bile and those with colored bile. In contrast, in the group of 54 patients with inoperable malignant biliary obstruction, the multivariate analysis demonstrated that the presence of white bile in the bile ducts was an independent factor associated with poorer OS (HR 2.3, 95% CI: 1.1–4.7, P = 0.02) as it may reflect more severe and prolonged cholestasis due to locally advanced cancer. Other factors associated with poorer survival were the presence of mestatasis at diagnosis, which reflects advanced stage disease (HR 2.8, 95% CI: 1.4–5.7, P = 0.003) and higher serum total bilirubin (HR 1.003, , 95% CI: 1.001–1.006, P = 0.01).

The mechanisms of white bile formation have long been controversial. As reviewed by Elmslie et al [10], old studies pointed to the role of the gallbladder, claiming that gallbladder distal to the obstruction (outside the obstructed tract) or not functioning was a prerequisite for formation of white bile due to the lack of absorptive functions by the gallbladder [10]. Old studies in animals supported this hypothesis. In several animal models, Rous and Mc Master [11] reported that white bile appeared after prolonged common bile duct obstruction associated with obstruction of the neck of the gallbladder. The stasis bile was initially pigmented and then became progressively clear and finally colorless. In contrast, when obstructed ducts were still connected with a functioning gallbladder, stasis bile remained colored. They concluded that white bile was formed in obstructed bile ducts only in case of excluded or non-functioning gallbladder. Although clinical studies initially supported this hypothesis [1], later studies showed that white bile may appear in obstructed bile ducts with or without a functioning gallbladder [3, 10] and this was confirmed in our series. White bile was observed in cholecystectomized patients and in patients with gallbladder in situ, whatever the level of obstruction, above or below the cystic convergence, indicating that a functioning gallbladder does not prevent the formation of white bile in patients with malignant biliary obstruction. The inverse correlation between serum bilirubin and bile duct bilirubin suggests that the severity of cholestasis, i.e., the degree of obstruction, plays an important role in white bile formation. This

▶ **Table 3** Univariate and multivariate analysis of factors associated with survival in the population of patients with inoperable malignant biliary obstruction without surgery (n = 54).

Variables	n	Survival time (mo)[1]	Univariate analysis P value	Multivariate analysis[2]	
				HR (95 % CI)	P value
Age			0.69		
Sex			0.11		
▪ Male	28	6.7 [0.2–55.2]			
▪ Female	26	5.0 [0.6–34.6]			
Metastasis			0.002		0.003
▪ Yes	37	3.3 [0.2–34.6]		2.8 [1.4–5.7]	
▪ No	17	11.6 [1.2–55.2]		1	
Type of cancer			0.98		
▪ Bile duct	25	5.0 [0.2–34.7]			
▪ Pancreatic	29	6.1 [0.6–55.2]			
Hilar obstruction			0.66		
▪ Yes	7	5.1 [0.4–34.7]			
▪ No	47	5.4 [0.2–55.2]			
Serum total bilirubin			<0.0001	1.003 [1.001–1.006]	0.01
White bile			0.003		0.02
▪ Yes	32	2.9 [0.2–25.9]		2.3 [1.1–4.7]	
▪ No	22	9.1 [1.1–55.2]		1	

[1] Data are presented as median [range].
[2] Variables included in multivariate analysis were white bile, metastasis, and serum total bilirubin
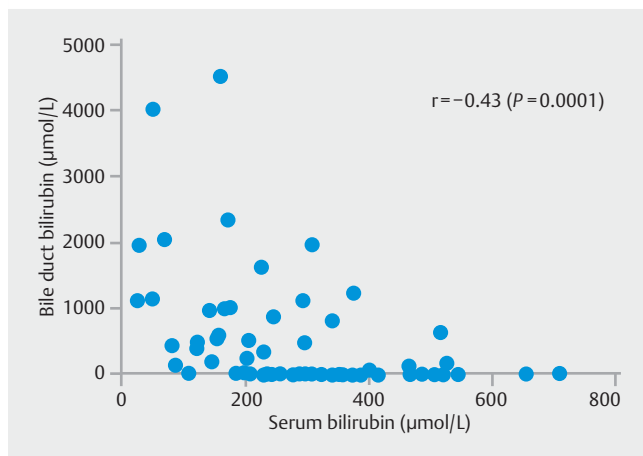
▶ **Fig. 4** Correlation between serum total bilirubin and bile duct total bilirubin.

should be brought in line with the role of the pressure within obstructed bile ducts. Experimental studies in animal models have reported that bile flow in obstructed bile ducts is main-tained up to a certain level of pressure variable according to animal species. Above this level, bile flow decreases and rapidly stops, resulting in a reverse flow and regurgitation into the blood and lymphatics. At that point, the colored bile is replaced by the colorless fluid secreted by cholangiocytes, which is devoid of bilirubin and biliary acids [15].

The main limitation of this study is inherent in its retrospective nature. The risk of bias is reduced by multivariate analysis but it cannot be completely neutralized. The lack of standardized monitoring of serum bilirubin levels after biliary decompression did not allow us to study the kinetics of bilirubin decrement, which may be impaired or slowed down in patients with white bile [3].

Conclusion

In conclusion, this study demonstrated that the presence of white bile within the bile ducts of patients with inoperable malignant biliary obstruction is an independent factor associated with OS. The presence of white bile in these patients reflects severe cholestasis due to a prolonged and tight biliary obstruction. The lack of a gallbladder is not a prerequisite for the for-

mation of white bile, which may be observed whatever the level of biliary obstruction.

Competing interests

The authors declare that they have no conflict of interest.

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