Bilirubin, urobilinogen, pancreas elastase and bile acid in drain fluid. The GBUP-study: Analysis of biomarkers for a colorectal anastomotic leakage

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ABSTRACT

Purpose: A colorectal anastomotic leakage (CAL) is a major complication after colorectal surgery and leads to high rates of morbidity and prolonged hospital stay. The study aims to evaluate the benefit of using bilirubin, urobilinogen, pancreas elastase and bile acid in the drain fluid (DF) as a predictive marker for the CAL.
Methods: From June 2015 to October 2017 100 patients, who underwent left hemicolectomy (LH), sigma resection (SR), high anterior resection (HAR), low anterior resection (LAR) or reversal of Hartmann's Procedure (ROHP) were included in this monocentric non-randomized prospective clinical trial. During the first four postoperative days (POD) the concentration of bilirubin, urobilinogen, pancreas elastase and bile acid in the DF was measured.
Results: In total 100 patients were recruited. 17 were excluded due to intraoperative decisions to conduct a protective stoma. 6 patients had a CAL. The patients of the control group (n = 77) and the patients who suffered from a CAL (n = 6) had no increased concentration of urobilinogen and pancreas elastase in the DF. The concentration of bile acid in the DF of the patients who suffered from a CAL differed from those of the control group on the 4th POD (p = 0.055).

The concentration of bilirubin in the DF of the patients who suffered from a CAL significantly differed from those of the control group on the 1st POD (p = 0.031) and on the 3rd POD (p = 0.041).
Conclusion: Bilirubin and bile acid in the DF may function as a predictive marker for a CAL.

1. Introduction

A CAL is a major complication after colorectal surgery and leads to high rates of morbidity, reoperation, intervention and a prolonged hospital stay [1]. The International Study Group of Rectal Cancer defined in 2010 the CAL following anterior resection of the rectum as a defect of the intestinal wall integrity at the colorectal or colo-anal anastomotic site, which allows a connection between the extra- and intraluminal space. The authors differentiated between three grades of severity; grade A is a CAL requiring no active therapeutic intervention, grade B is a CAL requiring active therapeutic intervention without reoperation and grade C is a CAL requiring a reoperation [1]. The mortality rate lies between 10% and 20% [2]. Moreover a CAL has a

negative impact on the long-term cancer specific survival and recurrence rate [3].

Several studies have been carried out to circumscribe the risk factors for developing a CAL. In summary considered risk factors are: rectal resection, smoking, alcohol abuse, high body mass index (BMI), the male gender, treatment with steroids, radiotherapy and chemotherapy, a high American Society of Anesthesiologists (ASA) score, cardiac and vascular diseases, duration/length of surgery and emergency surgery [6–10].

The diagnosis of a CAL is mostly made between the 5th and 13th POD due to unspecific clinical signs and symptoms, which can mimic less serve infections (wound, urinary tract) [11–14]. In literature the computer tomography of the abdomen is described as being a sufficient

diagnostic approach to detect a CAL [11].

Viable predictive clinical diagnostics to detect a CAL are currently subject of research and due to morbidity and high rate of mortality urgently required. An endoscopic approach to treat small leaks would allow treatment without major surgery. We have therefore performed a prospective pilot study to evaluate the viability of the bilirubin, urobilinogen, pancreas elastase and bile acid concentration in drain fluids as a predictive marker. Pancreas elastase, bile acid and bilirubin as well as its metabolites like urobilinogen were excreted by bowel movement. We postulate that these metabolic products can leave the intraluminal space through the anastomotic leak. As in the beginning the leak is naturally a small one, we assume that pancreas elastase, bile acid, bilirubin and urobilinogen are detectable in the drain fluids even before the CAL is felt as abdominal pain due to peritonitis.

2. Methods

At Helios Hospital Berlin-Buch, Germany, the GBUP-study ("Gallensäure, Bilirubin, Urobilinogen, Pankreaselastase"-Studie) was conducted as a monocentric non-randomized prospective clinical trial from June 2015 until October 2017. The study was registered in the German Clinical Trial Register (DRKS-ID: DRKS00009738; https://drks-neu.uniklinik-freiburg.de/drks) and was approved by the medical ethical committee in accordance with the ethical standards of the Helsinki Declaration of 1975 in March 2015. All patients gave informed consent.

2.1. Inclusion criteria

Patients undergoing (open/laparoscopic) left hemicolectomy (LH), (open/laparoscopic) sigmoid resection (SR), (open/laparoscopic) high anterior resection (HAR), (open/laparoscopic) low anterior resection (LAR), (open/laparoscopic) reversal of Hartmann's Procedure (ROHP) or (open/laparoscopic) colectomy (CT) were included.

Oncologic resections as well as resections for inflammatory disease and ischemic colitis were involved.

2.2. Exclusion criteria

Being under 18 years of age or pregnancy were criterions for exclusion. Patients who did not receive a drain as well as patients who got a protective ileostomy or colostomy were excluded.

2.3. Surgical approach

The surgical procedure and the technique for conducting the anastomosis (stapled/hand sewn) were left to the surgeon's discretion. The patients were operated by laparotomy or laparoscopy. All patients received preoperative antibiotic prophylaxis and an intra-abdominal drain.

To obtain the DF, a drain was placed at the anastomosis and was left in place during the first four postoperative days. We used the KapillardrainD12© (Dahlhausen, Köln, Germany) as a passive drainage systems.

When patients felt disturbed by the drain in terms of pain, movement limitation or paresthesia, it was removed earlier than planned.

2.4. Drain fluid

The DF was depleted once a day with intervals of 24 h, respecting rules of sterility.

2.5. Determination of bilirubin, urobilinogen, pancreas elastase, and bile acid

The determination of the total bilirubin, urobilinogen, pancreatic elastase and bile acid concentration was done via routine clinical chemistry assays. Total bilirubin was measured by using the third generation Bilirubin Total assay on a Cobas© c501 system (Roche Diagnostiscs, Mannheim, Germany). Urobilinogen was determined with a semi quantitative Combur© 10 test strip (Measurement: negative: $< 17\,\mu mol/l$; normal: $17–68\,\mu mol/l$; $+$: $68–135\,\mu mol/l$; $++$: $135–203\,\mu mol/l$; $+++ > 203\,\mu mol/l$). The Urisys© 1100 analyzer was used (Roche Diagnostiscs, Mannheim, Germany). The pancreatic elastase was measured by using a standard ELISA assay (ScheBo Pancreatic Elastase 1, ScheBo Biotech, Gießen, Germany) and bile acids by using an enzymatic Thio-NAD assay (Fifth Generation Bile Acid Assay, Labor + Technik, Berlin, Germany).

2.6. Definitions

The endpoint of the GBUP-study was the CAL. According to the recommendation of the International Study Group of Rectal Cancer in 2010, a CAL is defined as a defect of the intestinal wall at the anastomotic site which allows a connection between the extra- and intraluminal space. Radiologic confirmation of a CAL was defined as an extravasation of endoluminal contrast agent and/or significant perianastomotic air on computed tomography. The endoscopic confirmation of a CAL is defined as the visual detection of the leak.

Interventions to treat the CAL included the endoscopic negative pressure wound therapy, antibiotic therapy and the surgical approach disconnecting or sewing the anastomosis, constructing a new anastomosis via loop ileostomy or by conducting a colostomy via ROHP. Postoperative mortality is defined as the death of a patient within 30 days after surgery.

2.7. Data collection

Demographic data of the patients (age, ASA-score, BMI, gender), type of operation, postoperative complications, and follow-up data were collected through a standardized case record form. In the event of a CAL, the postoperative day of diagnosis, the diagnostic approach for detection and the treatment was documented.

2.8. Follow-up

To detect a CAL after the discharge from our hospital all patients were contacted after the 15th and 30th POD by phone or by mail.

2.9. Statistics

In order to compare the baseline data (age, gender, type of operation etc.) between the CAL and the no CAL groups, mean, standard deviation, minimum, maximum and median were calculated for continuous variables. Four categorical variables cross-tables were calculated.

Normality of continuous variables was tested with the Shapiro-Wilk-test. In case of normally distributed continuous variables t-tests for independent groups were used to compare the CAL-/no CAL-groups, otherwise Wilcoxon-tests were used. In case of categorical variables Fishers exact test was used.

A p-value < 0.05 was regarded as statistically significant.

Due to the small group of CAL of this investigation, no multivariate analysis was carried out. For the same reason as for the tests of differences (bilirubin and bile acid) at the 1st – 4th POD no alpha-correction was done.

All statistical analyses were carried out using R 3.2.1 ("World famous astronaut"). The sample size calculation was done by using PROC POWER in SAS 9.4.

3. Results

3.1. Baseline characteristics

From June 2015 to August 2017 100 patients were recruited for the GBUP-study at the HELIOS Hospital Berlin-Buch. 17 patients were excluded from the analysis due to intraoperative decisions to conduct a colo- or ileostomy. 83 patients were analyzed. Six patients suffered from a CAL.

The patients received neither neoadjuvant radiotherapy nor did they undergo a colectomy or subtotal colectomy.

Among the patients of the control group (n = 77) the mean age was 76.3 ± 12.3 (mean ± standard deviation), n = 37 (48.1%) were female and n = 40 (51.9%) were male. 22 (28,6%) patients had a BMI < 25, 32 (41,5%) patients had a BMI 25–30 and 23 (29.8%) patients had a BMI > 30. Concerning the ASA-score 13 (16.9%) patients had an ASA-score of I, 42 (54.5%) patients had an ASA-score of II, 21 (28.4%) patients had an ASA-score of III and 1 (1.3%) patient had an ASA-score of IV. The patients underwent surgery due to inflammatory disease (n = 41), malignancy (n = 35) and ischemic colitis (n = 1). 11 patients were operated in an open technique whereas 67 patients underwent a laparoscopic approach. As type of resection the LAR (n = 3), HAR (n = 13), LH (n = 10), SR (n = 44) or the ROPH (n = 7) was conducted. No patient underwent a colectomy or subtotal colectomy. The anastomosis was stapled in 66 cases and handsewn in 11 cases. The anastomosis was configured either end-to-end (n = 66), end-to-side (n = 2), side-to-side (n = 9) or side-to-end (n = 0) (Table 1).

Table 1
Univariate analysis of baseline characteristics of GBUP-study patients.

Variable	No AL (n = 77)	AL (n = 6)	*p* value
Age	67.0 ± 12.1	63.8 ± 11.4	0.470
Gender			
female	37 (48.1%)	2 (33.3%)	0.679
male	40 (51.9%)	4 (66.6%)	
BMI			
< 25	22 (28,6)	2 (33,3%)	0.999
25–30	32 (41,5%)	2 (33,3%)	
> 30	23 (29,9%)	2 (33,3%)	
ASA-score			
1	13 (16.9%)	1 (16.7%)	0.999
2	42 (54.5%)	3 (50.0%)	
3	21 (27.3%)	2 (33.3%)	
4	1 (1.3%)	0 (0.0%)	
Reason for surgery			
inflammatory disease	41 (53.2%)	2 (33.3%)	0.459
malignancy	35 (45.5%)	4 (66.7%)	
ischemic colitis	1 (1.3%)	0 (0.0%)	
Type of operation			
laparoscopic	66 (85.7%)	5 (83.3%)	0.999
open	11 (14.3%)	1 (16.7%)	
Type of resection			
LAR	3 (3.9%)	0 (0.0%)	0.999
HAR	12 (15.6%)	3 (50.0%)	
LH	10 (13.0%)	1 (16.7%)	
SR	45 (58.4%)	2 (33.3%)	
ROHP	7 (9.1%)	0 (0.0%)	
Construction anastomosis			
Stapled	66 (85.7%)	5 (83.3%)	0.999
Hand sewn	11 (14.3%)	1 (16.7%)	
Configuration anastomosis			
End-to-end	66 (85.7%)	5 (83.3%)	0.621
End-to-side	2 (2.6%)	0 (0.0%)	
Side-to-side	9 (11.7%)	1 (16.7%)	
Side-to-end	0 (0.0%)	0 (0.0%)	

AL: anastomotic leak; ASA-Score: American Society of Anesthesiologists-Score; BMI: Body-Mass-Index.
HAR: High anterior rectum resection; LAR: Low anterior rectum resection; LH: left hemicolectomy.
ROHP: Reversal of Hartmann's Procedure; SR: Sigmoid resection.

Six patients suffered from a CAL (Table 1 and Table 2). Their mean age was 63.8 ± 11.4). Two patients were female and four were male. Two patients had a BMI < 25, two patients had a BMI 25–30 and two patients had a > BMI 30. Concerning the ASA-score one patient had an ASA-score of I, three patients had an ASA-score of II and two patients had an ASA-score of III. These patients underwent surgery due to inflammatory disease (n = 2) and malignancy (n = 4). One patient was operated in an open technique whereas five patients underwent a laparoscopic approach. As type of resection the HAR (n = 3), the LH (n = 1) or the SR (n = 2) was conducted. The anastomosis was stapled in five cases and in one case it was handsewn. The anastomosis were configured as end-to-end (n = 5) and side-to-side (n = 1). In two cases the diagnosis of a CAL was made by computer tomography on the 4th (n = 1) and one on the15[th] POD (n = 1). In three cases a CAL was detected by endoscopy on the third day (n = 1), 6th (n = 1) or 13th POD (n = 1). In one case defecation was found in the drain fluid on the 5th POD (n = 1). All six patients were re-operated due to the CAL and survived this complication.

The male patient (66 years of age, ASA-score of II, BMI 25,2), who suffered from a CAL on the 3rd POD after HAR immediately received a loop ileostomy and a transrectal Vacuum-assisted closure (VAC). He left the hospital after 28 days. The loop ileostomy was reversed after 208 days due to prior resection of liver metastasis.

The male patient (76 years of age, ASA-score of III, BMI 22,9), who developed a CAL following SR on the 4th POD, underwent an immediate open Hartmann's procedure and was discharged after 29 days.

The male patient (68 years of age, ASA-score of II, BMI 33,1), who suffered from a CAL on the 5th POD following HAR, underwent an open Hartmann's procedure. He left the hospital after 29 days.

The female patient (44 years of age, ASA-score of II, BMI 45,9), who suffered from a CAL on the 6th POD after SR, underwent immediate explorative laparoscopy with a handsewn anastomosis. An open Hartman's procedure was performed due to secondary AI 8 days after first revision. She was discharged after 53 days.

The female patient (56 years of age, ASA-score of I, BMI 21,5), who developed a CAL on the 13th POD after HAR received a loop ileostomy and a transrectal Vacuum-assisted closure (VAC) on the same day. She left the hospital after 33 days. The loop ileostomy was reversed after 87 days.

The male patient (69 years of age, ASA-score of III, BMI 25,1), who suffered from a CAL on the 15th POD following LH immediately underwent an open Hartmann's procedure and was discharged after 36 days.

3.2. Determination of pancreas elastase in the drain fluid

A semi-quantitative determination of the pancreas elastase in the DF was conducted on the 2nd and 4th POD. The patients of the control group (n = 77) and the patients who suffered from a CAL (n = 6) had no increased concentration of the pancreas elastase in the DF (> 60 μg/g).

3.3. Determination of urobilinogen in the drain fluid

A semi-quantitative determination of urobilinogen in the DF was conducted on the 1st, 2nd, 3rd and 4th POD. The patients of the control group (n = 77) and the patients who suffered from a CAL (n = 6) had no increased concentration of urobilinogen in the DF and it ranged between 17 μmol/l and 68 μmol/l.

3.4. Determination of bile acid in the drain fluid

A quantitative determination of bile acid was conducted on the 1st, 2nd, 3rd and 4th POD.

The control group (n = 77) had an average concentration of bile acid in the DF of 1.8 ± 2.3 μmol/l (mean ± standard deviation) on

Table 2
Characteristics of patients who suffered from a CAL.

Patients	Age	Gender	BMI	ASA-Score	TO	Detection of AL	RS	HD	Bilirubin 1st POD	Bilirubin 2nd POD	Bilirubin 3rd POD	Bilirubin 4th POD	Bile acid 1st POD	Bile acid 2nd POD	Bile acid 3rd POD	Bile acid 4th POD
	year		kg/m^2	I-IV		POD	days		μmol/	μmol/l	μmol/l	μmol/l	μmol/l	μmol/l	μmol/l	μmol/l
1	66	m	25,2	II	HAR	3rd POD[1]	M	28	10,7	12,5	25,6	NA	0,9	1,3	1,7	NA
2	76	m	22,9	III	SR	4th POD[2]	ID	29	11,5	NA	27,4	10,8	1	NA	1,3	11
3	68	m	33,1	II	HAR	5th POD[3]	M	29	9,6	9,8	16,6	12,1	5,6	6	7,8	15
4	44	f	45,9	II	SR	6th POD[2]	ID	53	14,6	12,1	10,8	20,7	1,4	2,2	2,6	4.2
5	56	m	21,5	I	HAR	13th POD[2]	M	33	16,5	16,3	25,6	49,1	1,4	0	2,1	2,5
6	69	m	25,1	III	LR	15th POD[1]	M	36	22,5	24,7	19,5	18,4	0	1,4	3,5	2,5

ASA-Score: American Society of Anesthesiologists-Score; BMI: Body-Mass-Index; CAL: Colorectal anastomotic leakage; HAR: High anterior rectum resection; HD; Hospital discharge.
ID: Inflammatory disease; LH: Left hemicolectomy; M: Malignancy; POD: Postoperative day; RS: Reason for surgery; SR: Sigmoid resection; TO: Type of operation.
[1]diagnosed by computer tomography; [2] diagnosed by endoscopy; [3]diagnosed by drain fluid content.

the 1st POD, 2.4 ± 2.6 μmol/l (mean ± standard deviation) on the 2nd POD, 2.9 ± 4.0 μmol/l (mean ± standard deviation) on the 3rd POD and 3.6 ± 5.8 μmol/l (mean ± standard deviation) on the 4th POD.

The average concentration of bile acid in the DF of patients, who suffered from a CAL (n = 6), was 1.7 ± 2.0 μmol/l (mean ± standard deviation) on the 1st POD, 2.0 ± 2.1 μmol/l (mean ± standard deviation) on the 2nd POD, 3.5 ± 2.5 μmol/l (mean ± standard deviation) on the 3rd POD and 7.0 ± 5.7 μmol/l (mean ± standard deviation) on the 4th POD (Fig. 1, Table 3).

Since the distribution of bile acid is not normal, Wilcoxon tests were carried out, revealing no significant differences. However, the p-value is 0.055 on the 4th POD.

3.5. Determination of bilirubin in the drain fluid

A quantitative determination of bilirubin was conducted on the 1st, 2nd, 3rd and 4th POD.

The control group (n = 77) had an average concentration of bilirubin in the DF of 9.7 ± 5.1 μmol/l (mean ± standard deviation) on the 1st POD, 12.6 ± 6.8 μmol/l (mean ± standard deviation) on the 2nd POD, 14.9 ± 6.4 μmol/l (mean ± standard deviation) on the 3rd
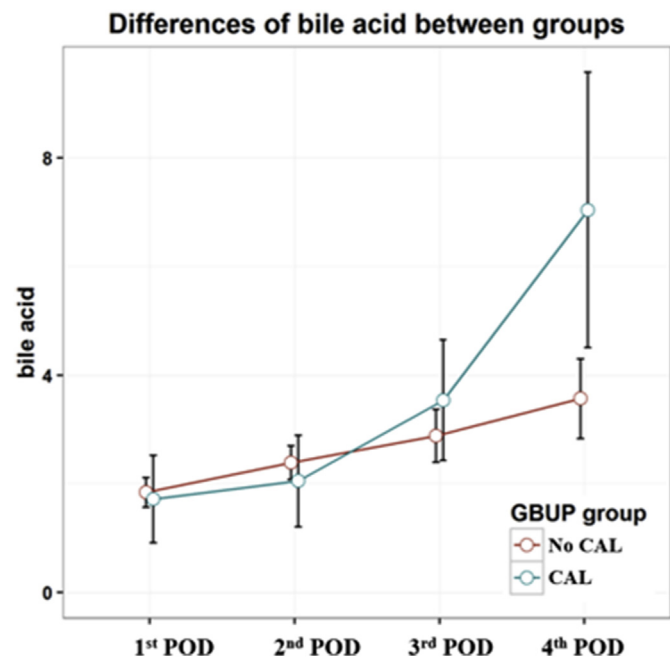
Table 3
Descriptive statistics of bile acid in the DF, SD: standard deviation.

Bileacid by groups	group	N	Miss	Mean	SD	Min	Median	Max
1st POD	no CAL	77	6	1.8	2.3	0.0	1.1	11.6
	CAL	6	0	1.7	2.0	0.0	1.2	5.6
	all	83	6	1.8	2.3	0.0	1.1	11.6
2nd POD	no CAL	77	7	2.4	2.6	0.0	2.0	15.3
	A CAL L	6	0	2.0	2.1	0.0	1.4	6.0
	all	83	7	2.4	2.5	0.0	2.0	15.3
3rd POD	no CAL	77	8	2.9	4.0	0.0	2.3	31.4
	CAL	6	1	3.5	2.5	1.7	2.6	7.8
	all	83	9	2.9	3.9	0.0	2.3	31.4
4th POD	no CAL	77	14	3.6	5.8	0.0	2.5	44.7
	CAL	6	1	7.0	5.7	2.5	4.2	15.0
	all	83	15	3.8	5.8	0.0	2.5	44.7

POD and 16.1 ± 12.4 μmol/l (mean ± standard deviation) on the 4th POD.

The average concentration of bilirubin in the DF of patients, who suffered from a CAL (n = 6), was 14.2 ± 4.8 μmol/l (mean ± standard deviation) on the 1st POD, 15.1 ± 5.9 μmol/l (mean ± standard deviation) on the 2nd POD, 20.9 ± 6.5 μmol/l (mean ± standard deviation) on the 3rd POD and 22.2 ± 15.6 μmol/l (mean ± standard deviation) on the 4th POD (Fig. 2, Table 4).

Since the distribution of bilirubin is not normal, Wilcoxon tests were carried out, revealing significant differences on the 1st POD (p = 0.031) and on the 3rd POD (p = 0.041).

4. Discussion

The CAL is a major complication after colorectal surgery and leads to high rates of morbidity, reoperation, intervention and a prolonged hospital stay with a mortality rate between 10% and 20% [2,3].

In some cases patients who suffered from a minor CAL (grade A + B) without generalized peritonitis and sepsis can be treated conservatively with antibiotics or endoscopically by conducting a transrectal vacuum-assisted closure (VAC). The VAC treatment may in comparison to a conservative approach possibly shorten the time of wound healing of anastomotic leakages after colorectal surgery but the evidence in literature is low [4]. Accordingly Nagell et al. performed a clinical trial comparing the median time of wound healing of the CAL after conservative treatment (n = 10) to the time of wound healing after endoscopic transrectal VAC (n = 4) following rectal resection. The authors recorded a median time of wound healing of 51 days when the CAL was treated by VAC and a median time of wound healing of 336 days when the leak was treated conservatively [4]. Therefore to benefit from an endoscopic approach, in terms of shortened time of wound healing and reduced duration of hospital stay, a predictive diagnosis for a minor CAL may possibly be helpful. Hence, reoperations would be

Fig. 1. Mean and standard error of bile acid in the DF(x-axis:POD[days]; y-axis: bile acid[μmol/1]).
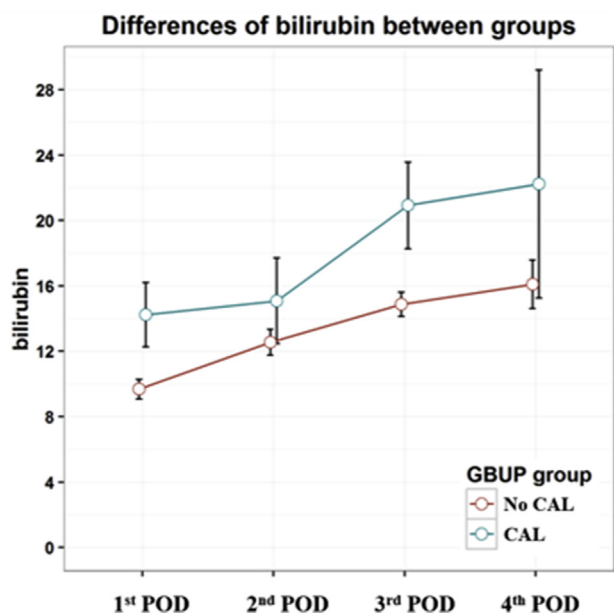
Differences of bilirubin between groups

Fig. 2. Mean and standard error of bilirubin in the DF (x-axis:POD[days]; y-axis:bilirubin [μmol/1]).

Table 4
Descriptive statistics of bilirubin in the DF, SD: standard deviation.

Bilirubin by groups	group	N	Miss	Mean	SD	Min	Median	Max
1st POD	no CAL	77	6	9.7	5.1	0.0	8.9	25.3
	CAL	6	0	14.2	4.8	9.6	13.1	22.5
	all	83	6	10.0	5.2	0.0	9.3	25.3
2nd POD	no CAL	77	4	12.6	6.8	0.0	11.4	53.7
	CAL	6	1	15.1	5.9	9.8	12.5	24.7
	all	83	5	12.7	6.7	0.0	11.5	53.7
3rd POD	no CAL	77	4	14.9	6.4	4.2	13.7	34.4
	CAL	6	0	20.9	6.5	10.8	22.6	27.4
	all	83	4	15.3	6.5	4.2	14.2	34.4
4th POD	no CAL	77	8	16.1	12.4	0.0	12.6	88.0
	CAL	6	1	22.2	15.6	10.8	18.4	49.1
	all	83	9	16.5	12.6	0.0	13.1	88.0

performed earlier and could therefore most probably reduce morbidity.

Until now current diagnostic approaches cannot predict a CAL [1].

Nevertheless. in literature different diagnostic approaches using DF diagnostics to predict the CAL can be found; still the evidence is still low due to lack of prospective clinical trials with an appropriate high number of patients suffering from a CAL. For example Komen et al. (2014) performed a multicenter prospective non-RCT analyzing real-time polymerase chain reaction (RT-PCR) to detect *Escherichia coli* and *Enterococcus faecalis* and the level of C-reactive protein (CRP), lipopolysaccharide-binding protein (LBP), and procalcitonin (PCT) in the DF of 243 patients following colorectal surgery. An increased level of LBP was significantly associated to a higher chance of developing a CAL [5]. Furthermore several authors investigated cytokine levels in the DF following colorectal surgery. Most of those trials revealed an increased concentration of cytokineon the 5th to 20th POD [6–8]. Moreover some authors postulated that the measurement of pH and matrix metalloproteinases in the DFmay be a diagnostic approach to predict the CAL [9,10]. The risk of a CAL was 22 times higher when the anastomotic intramucosal pH was less than 7.28 in the first 24 h [10]. Some authors examined the role of macroscopic changes in the DF as an early predictor for the CAL. Furthermore Tsujinaka et al. diagnosed and treated a CAL of 21 patients who underwent elective anterior resection because of rectal cancer. The authors observed changes in pelvic drain content of 15 patients suspecting a CAL, 11 of them remaining asymptomatic

[11,12]. These findings suggest that macroscopic changes in the DF may also predica CAL. On the contrary in our trial only one patient developing a CAL had pelvic drain content (defecation) suspecting a CAL.

Moreover in a retrospective clinical trial analyzing 546 patients undergoing laparoscopic-assisted right hemicolectomy Wu et al. detected increased concentration of amylase and bilirubin in the DF of four patients, who suffered from a CAL [13]. Unfortunately the full publication is not available in English.

So far the role of bilirubin, urobilinogen, pancreas elastase and bile acid as predictive markers for a CAL of patients undergoing LH, SR, HAR, LAR and ROHP has not been examined in prospective clinical trials. Those metabolites can pass a CAL and leave the intraluminal space. Therefore we conducted this trial to measure average concentrations of these metabolites and to observe their possible increase in the DF due to a CAL. We diagnosed 6 CALs. 17 patients of the total number were excluded due to intraoperative decision to conduct a protective stoma. The control group included 77 patients.

Due to costs and availability we only semi-quantitative measured the concentration of the pancreas elastase (2nd and 4th POD) and urobilinogen (1st – 4th POD) in the DF. The concentration of urobilinogen in the DF of all patients ranged (n = 83) between 17 μmol/l and 68 μmol/l. The patients of the control group (n = 77) and the patients who suffered from a CAL (n = 6) had no increased concentrations of pancreas elastase (> 60 μg/g) in the DF.

To further analyse whether or not our choosen components have the possibility of being early predictive markers for a CAL, it is necessary to conduct a clinical trial with a quantitative determination of the urobilinogen and pancreas elastase concentration in the DF and in addition to detect the dynamics of the increase. Generally the measured average concentrations of the metabolit products bilirubin and bile acid in the ascites may be helpful for further investigations in the field of intraabdominal diseases.

Concerning the detection of bile acid in the DF, the patients who suffered from a CAL showed increase concentration of bile acid on the 4th POD (p = 0.055) (Fig. 1, Table 2). On the 1st, 2nd and 3rd POD the concentration of bile acid in the DF was not statistical significantly different to the one of the control group. This may be explained by the assumption that at this time the stapled or handsewn anastomosis were completely intact. No leak at this time allows the bile acid to leave the intraluminal space especially in a late appearing CAL. To that 83% (5/ 6) of the patients who suffered from a CAL showed a continuously increase of bile acid in DF from the 1st to the 4th POD, whereas only 42% (33/77) of the patients of the control group indicated this continuous increase. On the other hand three patients had only moderate concentrations of bile acid in the DF on the 4th POD (Table 3; Patient 1, Patient 5, Patient 6). But patient 5 and 6 did suffer from a late occurring CAL (Patient 5 on 13th POD; Patient 6 on 15th POD). One may assume that this is because on the 4th POD the anastomosis was still intact and therefore the bile acid could not enter the extraluminal space. In summary the concentration of the bile acid in the drain fluid on the 4th POD may be a predictive marker especially for early occurring CAL. For the bile acid in the DF a sample size calculation based on the results obtained in the study was done. Under the assumption of a mean difference of 3 μmol/l, standard deviation of 5, alpha = 5%, power = 80% and an assumed ratio of CAL/no CAL = 1/10 a sample size of n = 275 (25 AI cases and 250 controls) was calculated [14,15]. This sample size would be necessary to elevate the hypothesis when conducting an additional clinical trial.

The patients who suffered from a CAL showed – in comparison to the control group - a statistically significant increase of the bilirubin concentration in the DF on the 1st POD (p = 0.031) and on the 3rd POD (p = 0.041). (Fig. 2, Table 4). Concerning bilirubin in the DF a sample size calculation based on the results obtained in the study was done. Under the assumption of a mean difference of 15 μmol/l of bilirubin, a standard deviation of 20, alpha = 5%, power = 80% and an assumed

ratio of CAL/no CAL = 1/10 a total sample size of n = 176 (16 CAL cases and 160 controls) was calculated [14,15]. This sample size would be necessary to elevate the hypothesis when conducting an additional clinical trial.

In reference to the increase of the bilirubin concentration in the DF of patients with a CAL it is thinkable that an increase of other enteral products of the bilirubin metabolism just like urobilin, mesobilirubin, mesobilirubinogen, stercobilinogen or stercobilin could also be found in the DF due to their passage through the leak [16,17]. Thus those metabolites may also work as predictive biomarkers for the CAL. Again, clinical trials are mandatory to confirm this hypothesis.

We measured the bilirubin total. Further investigations should measure the unconjugated and conjugated bilirubin in DF. Bilirubin, which is conjugated with glucuronic acid by the enzyme glucuronyl-transferase in the liver and excreted together with the bile is naturally defined as the conjugated bilirubin. So it is thinkable that in cases of a CAL the conjugated bilirubin increases in the ascites due to their passage through the leak as we postulated before.

In literature it is known that the unconjugated bilirubin in the blood stream is linked to immune-modulatory and antioxidant effects [18,19]. Accordingly Aziz et al. conducted a case-controlled study having patients with familial adenomatous polyposis. The authors observed an increased concentration of unconjugated bilirubin in the blood of patients who developed a CAL following laparoscopic total colectomy [20]. In case the CAL is the result of a wound healing disorder with inflammation in the area of the anastomosis, it is imaginable, that the unconjugated bilirubin increases in the blood stream and diffuses into the intraabdominal space. There, in the intraabdominal space, it could be detectable, which would be an explanation for the statistically significant increase of the bilirubin concentration on the 1st POD of our patients, who suffered from a CAL. Further investigations with an appropriate amount of patients should be conducted to elevate the hypothesis.

Naturally our results need to be critically discussed. Only 6 patients suffered from a CAL.

We did not reduce the rate of reoperation but the average concentration of bilirubin, urobilinogen, pancreas elastase and bile acid were not known before.

The patients had differing indications for surgery and underwent different surgical approaches. Furthermore there is the possibility of a false increase of the bilirubin concentration and bile acid due to forgotten daily removal of the DF.

Moreover an intraoperative drainage of the abdominal cavity may be leave a lot of irrigation fluid and leads to dilution.

The bilirubin concentration in the blood stream differs individually. To reveal more valuable results following clinical trials should take the calculation of the quotient of the bilirubin concentration in the serum and in the DF into consideration.

5. Conclusion

The measured average concentrations of the metabolite products bilirubin and bile acid in the ascites may be helpful for further investigations on the field of intraabdominal diseases.

Bile acid (4th POD: p = 0.055) and bilirubin (1st POD: p = 0.031; 3rd POD: p = 0.041) in the DF may be predictive markers for especially early occurring CAL after colorectal surgery. To that early prediction of an AI may prevent major surgery due to prior endoscopic treatment.

According to that the enteral products of the bilirubin metabolism like urobilin, mesobilirubin, mesobilirubinogen, stercobilinogen and stercobilin may be also increased in the DF due to their passage through the leak. Thus these metabolites may also be possible predictive biomarkers for the CAL. Further investigations with an appropriate amount of patients should be conducted to elevate this hypothesis.

Author contribution

Dr. med. Christoph Paasch (corresponding author):
Contribution to the paper: author, data collection, data analysis and interpretation, writing the paper, examination and treatment of the patient.

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Contribution to the paper: data analysis, examination and treatment of the patient.

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Ulrich Gauger (co-author).
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Contribution to the paper: data analysis.

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Contribution to the paper: data analysis, examination and surgical treatment of the patients.

Conflicts of interest

None.

Research registration number

The study was registered in the German Clinical Trial Register (DRKS-ID: DRKS00009738; https://drks-neu.uniklinik-freiburg.de/drks).

Guarantor

Dr. med. Christoph Paasch.

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