J Hepatobiliary Pancreat Sci (2015) 22:491–497
DOI: 10.1002/jhbp.239

ORIGINAL ARTICLE

Intermittent Pringle maneuver is unlikely to induce bacterial translocation to the portal vein: a study using bacterium-specific ribosomal RNA-targeted reverse transcription-polymerase chain reaction

Naoya Yamaguchi · Yukihiro Yokoyama · Tomoki Ebata · Tsuyoshi Igami · Gen Sugawara · Takashi Asahara · Koji Nomoto · Masato Nagino

Published online: 17 March 2015

Abstract
Background The occurrence of bacterial translocation (BT) to the mesenteric lymph nodes following the Pringle maneuver is well established; however, the incidence of BT to the portal circulation remains unclear.
Methods Portal blood of patients with suspected hilar malignancy who underwent major hepatobiliary resection with cholangiojejunostomy was sampled three times during surgery: immediately after laparotomy (PV-1); before liver transection and after skeletonization of the hepatoduodenal ligament (PV-2); and after completion of the liver transection (PV-3). The samples were analyzed for microbes with a bacterium-specific ribosomal RNA-targeted reverse transcription-polymerase chain reaction method.
Results Fifty patients were enrolled in the study, with a mean total Pringle time of 86 min. Microbes in the portal blood were detected in 11 (22%) of the 50 patients. The occurrence of microbes was not different among the PV-1 samples (8% = 4/50), PV-2 samples (14% = 7/50), and PV-3 samples (14% = 7/50) ($P = 0.567$). Obligate anaerobes were predominantly detected. The positivity of the PV-3 samples showed no correlation with the total Pringle time or with the occurrence of postoperative infectious complications. The total Pringle time did not affect the surgical outcomes, including infectious complications, liver failure, or mortality. The concentrations of aspartate aminotransferase and alanine aminotransferase on postoperative day 1 significantly correlated with the total Pringle time.
Conclusions The intermittent Pringle maneuver is unlikely to induce BT to the portal circulation and is safe, even in difficult, complicated hepatobiliary resections requiring long clamping times.

Keywords Bacterial translocation · Portal vein · Pringle maneuver

N. Yamaguchi · Y. Yokoyama · T. Ebata · T. Igami · G. Sugawara · M. Nagino (✉)
Department of Surgery, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
e-mail: nagino@med.nagoya-u.ac.jp

T. Asahara · K. Nomoto
Yakult Central Institute for Microbiological Research, Tokyo, Japan

Introduction

The Pringle maneuver, developed by James Hogarth Pringle in 1908 [1], is very often used in liver surgery to minimize bleeding during liver transection. Many authors have demonstrated that the intermittent Pringle maneuver is safe and effective when used appropriately [2–5]. However, this cross-clamping technique blocks the mesenteric venous drainage and increases the pressure in the microvascular network of the intestine, which may induce bacterial translocation (BT) to the portal vein [6–9]. Positive cultures of portal blood sampled following the Pringle maneuver have been reported in experimental studies [8, 9] but not in clinical studies [10, 11]. Thus, the reality of BT to the portal vein following the Pringle maneuver remains unclear in humans.

In recent years, bacterial-specific ribosomal RNA (rRNA)-targeted reverse transcription-polymerase chain reaction (RT-qPCR) has been widely used to detect bacteria in place of conventional culture methods for the structural analysis of intestinal flora [12]. With this method [13, 14], intraoperative BT during major surgeries has been more precisely and quantitatively investigated [15–17].

The aim of the current study was to evaluate whether the intermittent Pringle maneuver truly induces BT to the portal vein (portal bacteremia) using a very sensitive quantitative detection method, i.e. bacterium-specific rRNA-targeted

RT-qPCR, and to review the safety of the Pringle maneuver in difficult clinical settings.

Patients and methods

Patients

This study involved patients with suspected hilar malignancy who were scheduled to undergo combined liver and extrahepatic bile duct resection with cholangiojejunostomy at the Nagoya University Hospital. Patients scheduled to undergo hepatopancreatoduodenectomy were excluded. Written informed consent for participation was obtained from each patient before enrollment, and this study was approved by the Human Research Review Committee of the Nagoya University Hospital. The protocol was registered in the University Hospital Medical Information Network (http://www.umin.ac.jp; registration number ID 000013987).

Perioperative patient management

All patients received a regular diet preoperatively, and none received parenteral or enteral nutritional supplement before surgery. Pre- and postoperative synbiotic treatment was used in all patients, as described in our previous reports [18, 19]. Patients with obstructive jaundice routinely underwent biliary drainage before surgery. All of the bile drained externally from the biliary drainage catheter was replaced orally or via a nasoduodenal tube to maintain the intestinal integrity, as previously reported [20]. All patients underwent intestinal preparation with an iso-osmotic solution (2 L) administered the day before surgery and received antibiotic prophylaxis as a single intravenous drip 30 min before the operation.

An 8-F catheter for postoperative enteral feeding was placed through a jejunal limb during surgery. The enteral feeding began on postoperative day 1 at a rate of 100 kcal/day and was increased gradually to 400 kcal/day by day 5. Patients usually began oral feeding on day 4 or 5, and enteral feeding was gradually decreased as oral intake increased. Total parenteral nutrition was not used, and the central venous catheter inserted in the operating room was removed 2–4 days after surgery.

Surgery

All hepatectomies were performed once serum total bilirubin concentrations had decreased to < 2 mg/dL. The parenchymal transection was performed using a Cavitron ultrasonic surgical aspirator under both hepatic artery and portal vein clamping for 15 or 20 min at 5-min intervals. All patients underwent regional lymph node dissection. Vascular resections were performed only when the vessel adhered to and could not be freed from the tumor during skeletonization resection of the hepatoduodenal ligament [21]. Bilioenteric continuity was re-established by Roux-en-Y cholangiojejunostomy, as previously reported [22].

Sampling of portal blood

Portal blood was sampled from the patients three times during the operation using a 27-gauge fine needle. The first blood sample (PV-1) was taken from the branch of the superior mesenteric vein during the laparotomy before surgical mobilization. The second blood sample (PV-2) was taken from the skeletonized main portal trunk before liver transection and after skeletonization of the hepatoduodenal ligament. The third blood sample (PV-3) was taken from the skeletonized main portal trunk before the preparation for the cholangiojejunal reconstruction and after the completion of the liver transection, i.e. removal of the tumor. To avoid bacterial contamination during the sampling of PV-2 and PV-3, the surgical field was completely irrigated with warm saline.

Detection of microorganisms

Microorganisms in the portal blood were detected using sensitive quantitative detection with bacterium-specific rRNA-targeted RT-qPCR (Yakult Intestinal Flora Scan); the details of this method have been described in previous studies [13–17]. Briefly, 1 ml of blood was added to 2 ml of RNA protect bacterial reagent (QIAGEN GmbH, Hilden, Germany) immediately after collection and stored at –80°C. The blood samples were transported at –20°C to the Yakult Central Institute for Microbiological Research. Blood samples were thawed at room temperature, centrifuged at 5000 *g* at 4°C for 10 min, and the supernatant was carefully removed. The RNA in the blood was isolated using methods described elsewhere [12]. Finally, the nucleic acid fraction was suspended in 100 μL of nuclease-free water (Ambion, Austin, TX, USA). A standard curve was generated with the RT-qPCR data using the threshold cycle values for the dilution series of the standard strains as described elsewhere [13, 14, 23, 24]. To quantify the bacteria present in the samples, three serial dilutions of an extracted RNA sample were used for RT-qPCR. The threshold cycle values in the linear range of the assay were applied to the standard curve generated in the same experiment to obtain the corresponding bacterial cell count in each nucleic acid sample; this cell count was then converted to the number of bacteria per sample. The specificity of the RT-qPCR assay using the group-, genus-, or species-specific primers was determined as described previously [13, 14, 23, 24]. Group-, genus-, and species-specific primer sets for the predominant pathogenic obligate anaerobes and facultative anaerobes in the human intestinal

flora and the pathogenic aerobes in the compromised host were used for the RT-qPCR assay.

Recording of infectious complications

A detailed daily postoperative course was recorded for up to 30 days after surgery. Wound infection was defined as a spontaneous or surgically-released purulent discharge from the wound with positive cultures. An intra-abdominal abscess was defined as a purulent discharge with positive cultures from the abdominal drains placed during surgery or as fluid collection requiring a drainage procedure postoperatively. Pneumonia was defined as a characteristic pulmonary infiltrate on a chest radiograph accompanied by leukocytosis. Blood was obtained for cultures if a patient developed a fever exceeding 38.5°C at any time after the operation, independent of the presence or absence of other infectious sources. For each set of blood cultures, 10 ml of blood was drawn under sterile conditions and immediately inoculated into separate culture bottles (Organon Teknika, Durham, NC, USA) for aerobic and anaerobic cultures. Blood samples were incubated until bacterial growth was detected or for 7 days. Bacteremia was diagnosed when a single blood culture grew an isolate of organisms, unless the isolate was *Staphylococcus epidermidis* or coagulase-negative *Staphylococcus* species, which were considered contaminating skin flora. In these instances (i.e. presence of *Staphylococcus epidermidis* or coagulase-negative *Staphylococcus* species), diagnosis required isolation from two or more blood cultures [25].

Statistical analysis

Statistical analyses were performed using Dr. SPSS II for Windows Version 11.0.1J (SPSS, Chicago, IL, USA). Quantitative data are expressed as the means with standard deviations. The *t*-test was used to compare parametric data. Categorical data were compared using the Pearson χ^2 test or Fisher's exact test, as appropriate. A *P*-value of <0.05 was considered statistically significant.

Results

Demographics of study patients

Between July 2012 and September 2013, a total of 63 consecutive patients underwent combined liver and extrahepatic bile resection with cholangiojejunostomy. Of these, 13 patients were excluded due to refusing study enrollment, missing blood samples, or limited minor hepatectomy. The remaining 50 patients were enrolled in the study, including 30 men and 20 women with a mean age of 69 ± 9 years (range, 40 to 81 years); 44 patients had cholangiocarcinoma, three patients had gallbladder carcinoma, and three patients had other malignancies. Of the 50 patients, 48 (96%) had obstructive jaundice and were treated with endoscopic nasobiliary drainage ($n = 42$), percutaneous transhepatic biliary drainage ($n = 5$), or endoscopic biliary stenting ($n = 1$) [26, 27].

The types of hepatectomy included right trisectionectomy ($n = 3$), right hemihepatectomy ($n = 13$), left trisectionectomy ($n = 18$), and left hemihepatectomy ($n = 16$). All patients underwent combined en bloc caudate lobectomy. Combined vascular resection was performed in 15 (30%) patients, including resection of the portal vein only in six patients, resection of the hepatic artery only in two patients, and simultaneous resection of the portal vein and hepatic artery in seven patients [21]. The mean operative time in the 50 patients was 591 ± 104 min (range, 410 to 895 min), blood loss was 979 ± 662 ml (range, 283 to 3334 ml), and the total Pringle time was 86 ± 26 min (range, 45 to 157 min).

Incidence of portal bacteremia detected by the RT-qPCR method

Microbes (including obligate anaerobes, facultative anaerobes, and aerobes) in the portal blood were detected by the RT-qPCR method in 11 (22%) of the 50 study patients (Table 1). The incidence of microbe detection was not different among PV-1 samples (8% = 4/50), PV-2 samples (14% = 7/50), and PV-3 samples (14% = 7/50) ($P = 0.567$). Four patients (cases 1, 4, 9, and 11) had positive PV-1 or PV-2 samples but negative PV-3 samples. Only two patients (cases 2 and 7) had negative PV-1, negative PV-2, and positive PV-3 samples. No microbes were detected with the conventional culture method in any of the PV-1, PV-2, or PV-3 samples.

Obligate anaerobes, detected in all of the 11 patients, were predominant. Facultative anaerobic and aerobic microbes were detected in only three patients. The number of microorganisms detected was very small, fewer than 10 cells/ml in nine of the 11 patients (Table 1).

Comparison of PV-3 positive and PV-3 negative patients

Several pre- and intraoperative factors of patients with (PV-3 positive) and without (PV-3 negative) portal bacteremia following liver transection were compared. No significant between-group differences were found among these factors (Table 2).

Correlations between portal bacteremia following liver transection and postoperative infectious complications were also analyzed. Several types of postoperative infectious complications were observed in 25 patients. No differences in the prevalence of infectious complications between the two groups were found (Table 3).

Table 1 Types and number of microbes detected in the portal blood

	Case 1			Case 2			Case 3			Case 4		
	PV-1	PV-2	PV-3	PV-1	PV-2	PV-3	PV-1	PV-2	PV-3	PV-1	PV-2	PV-3
Obligate anaerobes												
Clostridium coccoides group	2	–	–	–	–	–	20	12	38	–	–	–
Clostridium leptum subgroup	–	–	–	–	–	81	53	–	43	–	3	–
Bacteroides frag xilis group	–	–	–	–	–	5	9	6	10	–	–	–
Bifidobacterium	–	–	–	–	–	–	–	–	–	–	–	–
Atopobium cluster	–	–	–	–	–	–	–	–	–	–	–	–
Facultative anaerobes + aerobes												
Enterobacteroaceae	–	–	–	–	–	–	–	–	–	–	–	–
Streptococcus	–	–	–	–	–	–	1	–	–	–	–	–
	Case 5			Case 6			Case 7			Case 8		
Obligate anaerobes												
Clostridium coccoides group	2	–	–	–	–	–	–	–	–	–	–	3
Clostridium leptum subgroup	–	–	1	–	1	2	–	–	2	–	1	–
Bacteroides fragilis group	–	–	–	–	–	–	–	–	–	–	–	–
Bifidobacterium	–	–	–	–	–	–	–	–	–	–	–	7
Atopobium cluster	7	–	–	–	2	–	–	–	–	–	–	–
Facultative anaerobes + aerobes												
Enterobacteroaceae	–	–	–	–	–	–	–	–	–	–	–	–
Streptococcus	–	–	–	–	–	2	–	–	–	–	–	–
	Case 9			Case 10			Case 11					
Obligate anaerobes												
Clostridium coccoides group	–	3	–	1	1	4	–	2	–			
Clostridium leptum subgroup	–	–	–	–	–	5	–	–	–			
Bacteroides fragilis group	–	–	–	–	–	–	–	–	–			
Bifidobacterium	–	–	–	–	–	–	–	–	–			
Atopobium cluster	–	8	–	5	9	3	–	–	–			
Facultative anaerobes + aerobes												
Enterobacteroaceae	–	–	–	–	–	–	–	4	–			
Streptococcus	–	–	–	–	–	–	–	–	–			

The numerical unit listed in this table is cells/ml.
PV-1 blood sample taken before surgical mobilization, *PV-2* blood sample taken before liver transection after skeletonization of the hepatoduodenal ligament, *PV-3* blood sample before the preparation of cholangiojejunal reconstruction after completion of liver transection.

Effect of total Pringle time on postoperative outcome

The effect of the total Pringle time on postoperative outcome was analyzed. The 50 patients were divided into two groups according to their Pringle time (i.e. above or below the median total Pringle time of 81 min). The average Pringle time was 65 ± 10 min in the short-time group ($n = 25$) and 107 ± 20 min in the long-time group ($n = 25$) (Table 4). Operative times were significantly longer in the long-time group, whereas blood loss was similar in the two groups. No between-group differences in PV-3 positivity were found, as three (12.0%) patients in the short-time group and four (16.0%) patients in the long-time group had positive PV-3 samples.

Postoperative outcomes, including infectious complications, liver failure, and mortality, were not different between the two groups. On day 1, the concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly different between the two groups and significantly correlated with the total Pringle time (Fig. 1). The other laboratory data exhibited no between-group differences.

Discussion

The gut is an important organ that serves as a barrier against living organisms within its lumen; this function is known as the "gut barrier function". However, it is well known that BT occurs during surgical procedures [6–11, 15–17]. We previously studied BT to the mesenteric lymph node with the same detection method used in the current study in patients who underwent hepatobiliary resection for biliary malignancy

Table 2 Comparison between patients with portal bacteremia after Pringle maneuver (PV-3 positive) and patients without (PV-3 negative)

	PV-3 positive ($n = 7$)	PV-3 negative ($n = 43$)	*P*
Preoperative factors			
Gender (male/female)	3 / 4	27 / 16	0.416
Age (years)	72.1 ± 7.6	68.4 ± 9.6	0.329
Diabetes mellitus (present)	1	4	0.546
Preoperative biliary drainage (present)	6	38	1.000
Preoperative portal vein embolization	6	28	0.406
ICG-R15 (%)	10.2 ± 3.0	11.2 ± 5.1	0.646
Estimated extent of liver resection (%)	48.9 ± 13.1	47.3 ± 14.5	0.787
Intraoperative factors			
Operative time (min)	640 ± 117	583 ± 101	0.179
Blood loss (ml)	1444 ± 1277	903 ± 486	0.308
Combined vascular resection (present)	2	13	1.000
Total Pringle time (min)	85 ± 22	87 ± 27	0.884

ICG-R15 retention rate of indocyanine green 15 min after administration.

[15] or esophagectomy for esophageal cancer [16, 17]; we found that: (1) BT to the mesenteric lymph node frequently occurred in these types of major surgeries; (2) obligate anaerobes were predominantly detected; and (3) intraoperative BT was closely associated with postoperative infectious complications. In addition, we also confirmed no significant correlation between BT to the lymph node and total Pringle time in the previous study [15]. These observations clearly indicate that BT to the lymph node frequently occurs, irrespective of Pringle maneuver, in major surgery. However, BT to the portal circulation was not investigated.

Table 3 Postoperative infectious complications according to presence (PV-3 positive) or absence (PV-3 negative) of portal bacteremia after Pringle maneuver

	PV-3 positive ($n = 7$)	PV-3 negative ($n = 43$)	*P*
Postoperative infectious complications	3 (42.9%)	22 (51.2%)	1.000
Intra-abdominal abscess	3 (42.9%)	16 (37.2%)	1.000
Wound infection	0	3 (7.0%)	1.000
Pneumonia	0	2 (4.7%)	1.000
Bacteremia[a]	1 (14.3%)	4 (9.3%)	0.546
Post-hepatectomy liver failure (Grade B or C)[b]	1 (14.3%)	3 (7.0%)	0.464
Postoperative hospital days (days)	38.0 ± 23.2	29.3 ± 15.7	0.211
Mortality	1 (14.3%)	1 (2.3%)	0.263

[a] Detected by conventional culture method
[b] According to International Study Group of Liver Surgery (ISGLS)

Table 4 Comparison of intra- and postoperative variables according to total Pringle time

	Total Pringle time		*P*
	Short-time group Less than 81 min (≤81 min, $n = 25$)	Long-time group (>81 min, $n = 25$)	
Intraoperative variables			
Pringle time (min)	65 ± 10	107 ± 20	<0.001
Operative time (min)	548 ± 75	633 ± 113	0.003
Blood loss (ml)	948 ± 653	1010 ± 683	0.747
PV-3 positive[a]	3 (12.0%)	4 (16.0%)	1.000
Postoperative status			
Infectious complications (presence)	11 (44.0%)	14 (56.0%)	0.396
Liver failure (Grade B or C)[b]	1 (4.0%)	3 (12.0%)	0.609
Postoperative hospital day (days)	27.5 ± 16.0	33.6 ± 17.6	0.210
Mortality	1 (4.0%)	1 (4.0%)	1.000
Laboratory data on day 1			
Procalcitonin (ng/ml)	7.4 ± 5.8	5.1 ± 3.4	0.103
WBC (/μl)	9076 ± 2912	8172 ± 2394	0.236
CRP (mg/dl)	3.5 ± 1.5	2.9 ± 1.4	0.179
Total bilirubin (mg/dl)	2.7 ± 1.2	2.4 ± 1.2	0.404
PT-INR	1.6 ± 0.2	1.6 ± 0.3	0.361
AST (IU/l)	374 ± 175	661 ± 387	0.002
ALT (IU/l)	282 ± 132	553 ± 403	0.003

[a] See Table 2.
[b] According to International Study Group of Liver Surgery (ISGLS)
ALT alanine aminotransferase, *AST* aspartate aminotransferase, *CRP* C-reactive protein, *PT-INR* prothrombin time-international normalized ratio, *WBC* white blood cell count

Although the intermittent Pringle maneuver is widely used in everyday clinical settings, this procedure may itself promote BT by inducing mesenteric venous stasis with increasing pressure in the microvascular network of the intestine. Evidence of Pringle maneuver-induced BT to the mesenteric lymph node has been shown in both experimental [6, 8, 9] and clinical studies [10, 11, 15–17]. To our knowledge, however, BT to the portal circulation following the Pringle maneuver has been described only in experimental studies [6, 8, 9].

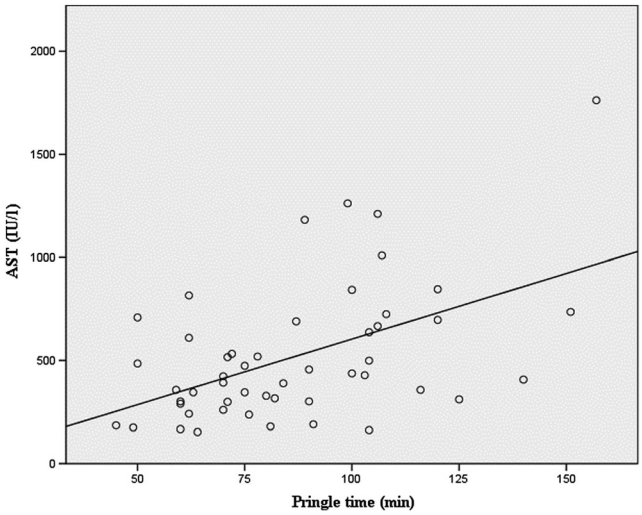

Fig. 1 Correlation between the concentrations of aspartate aminotransferase on day 1 and the total Pringle time

Erenogluet al. [8] showed that more than 90% of rabbits had positive mesenteric lymph node and portal blood cultures after 30 min of portal clamping. In contrast, Ferri et al. [10] who studied BT in 14 patients who underwent the Pringle maneuver during hepatectomy, reported that positive cultures by the conventional method were observed in the mesenteric lymph nodes of six patients (43% = 6/14) but in the portal blood of none of the patients (0% = 0/14). The current study showed that the incidence of microbe detection in the portal blood was low, even though the RT-qPCR method is very sensitive. Furthermore, the incidence of detection was similar before and after the Pringle maneuver, even under difficult clinical settings. These observations strongly indicate that the Pringle maneuver is unlikely to induce BT to the portal circulation in humans. Although speculative, the intestinal barrier function of the portal circulation may be different from that of the lymphatic channel.

Another important finding is that portal bacteremia following the Pringle maneuver (PV-3 positive) is not associated with the incidence of postoperative infectious complications. This finding is in strong contrast with findings of our previous studies [15–17] showing that the occurrence of RT-qPCR-positive mesenteric lymph nodes following resection is closely associated with the occurrence of infectious complications. This difference may be explained by the following two reasons. First, portal bacteremia following the Pringle maneuver in the present study must not have resulted from a true BT, as the incidence of microbe detection was the same in the PV-2 and PV-3 samples; the detection of microbes may have occurred by accident for unknown reasons. In contrast, the occurrence of RT-qPCR-positive mesenteric lymph nodes following resection noted in previous studies resulted from a true BT. Second, the numbers of microbes detected in the portal blood were very small (fewer than 10 cells/ml in most cases) (Table 1). These numbers were approximately 1000-fold lower than the numbers of microbes detected in mesenteric lymph nodes in our previous studies [15–17]. This observation may explain the large difference in the flow volume between the portal and lymphatic flows. Nevertheless, we speculate that the low numbers of microbes detected in the current study are explained by the fact that the detected microbes were trapped in the reticuloendothelial system of the liver and would therefore have had no impact on postoperative infection.

Positivity of the PV-1 samples in some cases implies that a small amount of microbes is present in the portal blood. In this regard, Sato et al. reported interesting observations [28]. They analyzed peripheral blood samples, with the same detection method used in the current study, in 50 control volunteers and 50 patients with type 2 diabetes, and showed that gut bacteria was detected in two (4%) of the control subjects and 14 (28%) of the diabetic patients. Taking these observations into consideration, BT to the circulation could rarely occur even in healthy people and often in some disease/condition. A small amount of microbes may be trapped in the reticuloendotherial system of the liver and has no impact on clinical course.

The conventional culture method has been shown to underestimate biodiversity [29, 30] because certain groups of bacteria such as obligate anaerobes are difficult to culture or uncultivable due to the difficulty in maintaining optimal culture conditions. In contrast, the RT-qPCR method targeting bacterial rRNA can more accurately detect the bacteria that are truly present in samples. The comprehensive sequencing analysis of $>$13,000 16S rRNA gene clones revealed that the human intestinal microbiome is dominated by obligate anaerobes that account for more than 99% of the identified phylogenetic types [31]. Our finding that obligate anaerobes were predominant in the portal blood is therefore expected. The results of previous studies showing that facultative and aerobic microorganisms were predominantly detected by the conventional culture method [6–11] should be interpreted carefully.

Our results demonstrated that the total Pringle time did not affect any of the surgical outcomes. The concentrations of AST and ALT on day 1 were closely associated with clamping time, which is consistent with results of previous studies [5]. The total Pringle time in the current study was relatively long, with a mean time of 86 min, indicating that all of the hepatectomies, which were performed for hilar malignancies, were difficult and complicated. Ishizaki et al. [4] studied patients in whom the cumulative clamping time during hepatectomy was 90 min or longer and reported that such prolonged intermittent clamping is safe and useful when the hepatectomy is difficult; the authors also indicated that such clamping can be used for cumulative periods exceeding 120 min without intraoperative blood loss or complications. The current study confirmed the validity of their study [4].

In conclusion, the intermittent Pringle maneuver is unlikely to induce BT to the portal circulation and is safe, even for difficult, complicated hepatobiliary resections requiring long clamping times.

Conflict of interest None declared.

References

1. Pringle JH. Notes on the arrest of hepatic hemorrhage due to trauma. Ann Surg. 1908;48:541–9.
2. Petrowsky H, McCormack L, Trujillo M, Selzner M, Jochum W, Clavien PA. A prospective, randomized, controlled trial comparing intermittent portal triad clamping versus ischemic preconditioning with continuous clamping for major liver resection. Ann Surg. 2006;244:921–8.
3. Torzilli G, Procopio F, Donadon M, Del Fabbro D, Cimino M, Montorsi M. Safety of intermittent Pringle maneuver cumulative time exceeding 120 minutes in liver resection: a further step in favor of the "radical but conservative" policy. Ann Surg. 2012;255:270–80.
4. Ishizaki Y, Yoshimoto J, Miwa K, Sugo H, Kawasaki S. Safety of prolonged intermittent Pringle maneuver during hepatic resection. Arch Surg. 2006;141:649–53.
5. Sugiyama Y, Ishizaki Y, Imamura H, Sugo H, Yoshimoto J, Kawasaki S. Effects of intermittent Pringle's manoeuvre on cirrhotic compared to normal liver. Br J Surg. 2010;97:1062–9.
6. Garcia-Tsao G, Albillos A, Barden GB, West AB. Bacterial translocation in acute and chronic portal hypertension. Hepatology. 1993;17:1081–5.
7. Dello SA, Reisinger KW, van Dam RM, Bemelmans MH, van Kuppevelt TH, van den Broek MA, et al. Total intermittent Pringle maneuver during liver resection can induce intestinal epithelial cell damage and endotoxemia. PLoS One. 2012;7:e30539.
8. Erenoglu B, Gokturk HS, Kucukkartallar T, Sahin M, Tekin A, Tatkan Y, et al. Mechanical intestinal cleaning and antibiotic prophylaxis for preventing bacterial translocation during the Pringle maneuver in rabbits. Surg Today. 2011;41:824–8.
9. Ypsilantis P, Lambropoulou M, Grapsa A, Tentes I, Tsigalou C, Panopoulou M, et al. Pringle maneuver deteriorates gut barrier dysfunction induced by extended-liver radiofrequency ablation. Dig Dis Sci. 2011;56:1548–56.
10. Ferri M, Gabrieal S, Gavelli A, Franconeri P, Huguet C. Bacterial translocation during portal clamping for liver resection: A clinical study. Arch Surg. 1997;132:162–5.
11. Yeh DC, Wu CC, Ho WM, Cheng SB, Lu IY, Liu TJ, et al. Bacterial translocation after cirrhotic liver resection: A clinical investigation of 181 patients. J Surg Res. 2003;111:209–14.
12. Harmsen HJ, Gibson GR, Elfferich P, Raangs GC, Wildeboer-Veloo AC, Arqaiz A, et al. Comparison of viable cell counts and fluorescence in situ hybridization using specific rRNA-based probes for the quantification of human fecal bacteria. FEMS Microbiol Lett. 2000;183:125–9.
13. Matsuda K, Tsuji H, Asahara T, Kado Y, Nomoto K. Sensitive quantitative detection of commensal bacteria by rRNA-targeted reverse transcription - PCR. Appl Environ Microbiol. 2007;73:32–9.
14. Matsuda K, Tsuji H, Asahara T, Matsumoto K, Takada T, Nomoto K. Establishment of an analytical system for the human fecal microbiota, based on reverse transcription – quantitative PCR targeting of multicopy rRNA molecules. Appl Environ Microbiol. 2009;75:1961–9.
15. Mizuno T, Yokoyama Y, Nishio H, Ebata T, Sugawara G, Asahara T, et al. Intraoperative bacterial translocation detected by bacterium-specific ribosomal RNA-targeted reverse-transcriptase polymerase chain reaction for the meseneric lymph node strongly predicts postoperative infectious complications after major hepatectomy for biliary malignancies. Ann Surg. 2010;252:1013–9.
16. Nishigaki E, Abe T, Yokoyama Y, Fukaya M, Asahara T, Nomoto K, et al. The detection of intraoperative bacterial translocation in the mesenteric lymph nodes is useful in predicting patients at high risk for postoperative infectious complications after esophagectomy. Ann Surg. 2014;259:477–84.
17. Yokoyama Y, Nishigaki E, Abe T, Fukaya M, Asahara T, Nomoto K, et al. Randomized clinical trial of the effect of perioperative synbiotics versus no synbiotics on bacterial translocation after oesophagectomy. Br J Surg. 2014;101:189–99.
18. Kanazawa H, Nagino M, Kamiya S, Komatsu S, Mayumi T, Takagi K, et al. Synbiotics reduce postoperative infectious complications: a randomized controlled trial in biliary cancer patients undergoing hepatectomy. Langenbecks Arch Surg. 2005;390:104–13.
19. Sugawara G, Nagino M, Nishio H, Ebata T, Takagi K, Asahara T, et al. Perioperative synbiotic treatment to prevent postoperative infectious complications in biliary cancer surgery: A randomized controlled trial. Ann Surg. 2006;244:706–14.
20. Kamiya S, Nagino M, Kanazawa H, Komatsu S, Mayumi T, Takagi K, et al. The value of bile replacement during external biliary drainage: an analysis of intestinal permeability, integrity, and microflora. Ann Surg. 2004;239:510–7.
21. Nagino M, Nimura Y, Nishio H, Ebata T, Igami T, Matsushita M, et al. Hepatectomy with simultaneous resection of the portal vein and hepatic artery for advanced perihilar cholangiocarcinoma: An audit of 50 consecutive cases. Ann Surg. 2010;252:115–23.
22. Nagino M, Nishio H, Ebata T, Yokoyama Y, Igami T, Nimura Y. Intrahepatic cholangiojejunostomy following hepatobiliary resection. Br J Surg. 2007;94:70–7.
23. Sakaguchi S, Saito M, Tsuji H, Asahara T, Takata O, Fujimura J, et al. Bacterial rRNA-targeted reverse transcription-PCR used to identify pathogens responsible for fever with neutropenia. J Clin Microbiol. 2010;48:1624–8.
24. Wada M, Lkhagvadorj E, Bian L, Wang C, Chiba Y, Nagata S, et al. Quantitative reverse transcription-PCR assay for the rapid detection of methicillin-resistant Staphylococcus aureus. J Appl Microbiol. 2010;108:779–88.
25. Shigeta H, Nagino M, Kamiya J, Uesaka K, Sano T, Yamamoto H, et al. Bacteremia after hepatectomy: an analysis of a single-center, 10-year experience with 407 patients. Langenbecks Arch Surg. 2002;387:117–24.
26. Kawashima H, Itoh A, Ohno E, Itoh Y, Ebata T, Nagino M, et al. Preoperative endoscopic nasobiliary drainage in 164 consecutive patients with suspected perihilar cholangiocarcinoma: a retrospective study of efficacy and risk factors related to complications. Ann Surg. 2013;257:121–7.
27. Nagino M. Prihilar cholangiocarcinoma: a surgeon's viewpoint on current topics. J Gastroenterol. 2012;47:1165–76.
28. Sato J, Kanazawa A, Ikeda F, Yoshihara T, Goto H, Abe H, et al. Gut dysbiosis and detection of live gut bacteria in blood of Japanese patients with type 2 diabetes. Diabetes Care. 2014;37:2342–50.
29. Wilson KH, Blitchington RB. Human colonic biota studied by ribosomal DNA sequence analysis. Appl Environ Microbiol. 1996;62:2273–8.
30. Franks AH, Harmsen HJ, Raangs GC, Jansen GJ, Schut F, Welling GW. Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. Appl Environ Microbiol. 1998;64:3336–45.
31. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science. 2005;308:1635–8.