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Peer Reviewer of World Journal of Gastrointestinal Surgery, Deven Juneja, DNB, FNB, EDIC, FCCP, Director, Department of Critical Care Medicine, Max Super Speciality Hospital, New Delhi 110017, India. devenjuneja@gmail.com

AIMS AND SCOPE

The primary aim of World Journal of Gastrointestinal Surgery (WJGS, World J Gastrointest Surg) is to provide scholars and readers from various fields of gastrointestinal surgery with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJGS mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal surgery and covering a wide range of topics including biliary tract surgical procedures, biliopancreatic diversion, colectomy, esophagectomy, esophagostomy, pancreas transplantation, and pancreatectomy, etc.

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ORIGINAL ARTICLE

Basic Study Peritoneal fluid indocyanine green test for diagnosis of gut leakage in anastomotic leakage rats and colorectal surgery patients

Yu Huang, Tian-Yang Li, Jie-Feng Weng, Hui Liu, Yu-Jie Xu, Shuai Zhang, Wei-Li Gu

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Yu Huang, Tian-Yang Li, Jie-Feng Weng, Hui Liu, Yu-Jie Xu, Shuai Zhang, Wei-Li Gu, Department of Surgery, Guangzhou First People's Hospital, Guangzhou 510180, Guangdong Province, China

Corresponding author: Yu Huang, MD, PhD, Doctor, Department of Surgery, Guangzhou First People's Hospital, No. 1 Panfu Road, Yuexiu District, Guangzhou 510180, Guangdong Province, China. eyhy@scut.edu.cn

Abstract

BACKGROUND

Application of indocyanine green (ICG) fluorescence has led to new developments in gastrointestinal surgery. However, little is known about the use of ICG for the diagnosis of postoperative gut leakage (GL). In addition, there is a lack of rapid and intuitive methods to definitively diagnose postoperative GL.

AIM

To investigate the effect of ICG in the diagnosis of anastomotic leakage in a surgical rat GL model and evaluate its diagnostic value in colorectal surgery patients.

METHODS

Sixteen rats were divided into two groups: GL group (n = 8) and sham group (n = 8) 8). Approximately 0.5 mL of ICG (2.5 mg/mL) was intravenously injected postoperatively. The peritoneal fluid was collected for the fluorescence test at 24 and 48 h. Six patients with rectal cancer who had undergone laparoscopic rectal cancer resection plus enterostomies were injected with 10 mL of ICG (2.5 mg/mL) on postoperative day 1. Their ostomy fluids were collected 24 h after ICG injection to identify the possibility of the ICG excreting from the peripheral veins to the enterostomy stoma. Participants who had undergone colectomy or rectal cancer resection were enrolled in the diagnostic test. The peritoneal fluids from drainage were collected 24 h after ICG injection. The ICG fluorescence test was conducted using OptoMedic endoscopy along with a near-infrared fluorescent imaging system.

RESULTS

The peritoneal fluids from the GL group showed ICG-dependent green fluorescence in contrast to the sham group. Six samples of ostomy fluids showed green fluorescence, indicating the possibility of ICG excreting from the peripheral veins to the enterostomy stoma in patients. The peritoneal fluid ICG test exhibited a sensitivity of 100% and a specificity of 83.3% for the diagnosis of GL. The positive



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predictive value was 71.4%, while the negative predictive value was 100%. The likelihood ratios were 6.0 for a positive test result and 0 for a negative result.

CONCLUSION

The postoperative ICG test in a drainage tube is a valuable and simple technique for the diagnosis of GL. Hence, it should be employed in clinical settings in patients with suspected GL.

Key Words: Gut leakage; Indocyanine green; Anastomotic leakage model; Diagnostic test; Diagnostic technique

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Core Tip: This study demonstrates the effectiveness of the peritoneal fluid indocyanine green (ICG) test in detecting postoperative gut leakage (GL) using rat models of surgical GL. The ICG test is a highly useful tool for diagnosing GL in patients with colorectal surgery. Our proposed method is a simple technique that can be used for both diagnosing and ruling out GL.

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INTRODUCTION

Gut leakage (GL) is a serious yet common postoperative complication of gastrointestinal (GI) surgery. It has an incidence rate of 2.4%–27.0% and a mortality rate of up to 18% among patients with resected colorectal carcinoma[1-4]. Postoperative GL may result in reoperation, delayed discharge, and increased morbidity and mortality, as well as a risk of local recurrence^[5-7]. It also reduces the quality of life of the patient after discharge^[8].

Despite these serious adverse effects of postoperative GL, no simple method for its definitive diagnosis has yet been developed. Patients with GL generally display systemic inflammatory response symptoms, such as severe abdominal pain, high fever, rapid heart and respiratory rates, peritonitis, fecal matter in the drainage tube, and increased leukocytes postoperative day (POD) 5-8[9,10]. However, it is not easy to diagnose GL based on clinical symptoms alone. Postoperative monitoring of combined changes in C-reactive protein (CRP) and procalcitonin (PCT) tests affords both good positive and negative predictive values for GL, usually diagnosed at POD 3-5[11,12]. The drainage matrix metalloprotein 9 (MMP9) levels on POD 3 can be used to predict the risk of GL[13]. However, the currently available evidence for MMP9 is inconsistent[14]. At present, the abdominal computed tomography (CT) scan is the most commonly used postoperative imaging method to diagnose or rule out GL. However, the CT scan is an expensive technology. In addition, it may lead to false-negative results, which may delay re-intervention[15]. Therefore, it is necessary to develop simpler methods for the diagnosis of GL.

Indocyanine green (ICG) fluorescence has helped advance the field of GI surgery. Intraoperative usage of ICG for assessing anastomosis perfusion has been well studied [16,17]. However, little is known about using ICG for diagnosing postoperative GL. In the present study, we design a GL rat model by conducting surgical abscission of the sigmoid colon and test the effectiveness of ICG for diagnosing postoperative GL in rat models. In addition, we investigate whether ICG can be used for the diagnosis of postoperative GL in human patients who have undergone colorectal cancer resections.

MATERIALS AND METHODS

Study design

We designed an enterostomy test to identify any possible leakage of ICG from the peripheral veins to the enterostomy stoma. In addition, we developed a diagnostic test to explore the diagnostic effect of the ICG test on GL in patients with colorectal surgery. The human study was approved by the Research Ethics Committee of the Guangzhou First People's Hospital (Approval No. K-2019-173-01). This study was registered in the Chinese Clinical Trial Registry (registration number: ChiCTR1900028537).

Diagnostic criteria for leakage

We employed the following criteria to diagnose leakage: (1) Feces or pus discharged from an abdominal drainage tube, the rectum, or rectovaginal fistula[18]; and (2) peritonitis with sepsis, or with one of the following clinical features: Leukocytosis (> 12 × 10°/L), elevated CRP (> 10 mg/L), PCT (> 0.5 ng/mL), abnormal drainage volume (> 100 mL lasting 3 d), significant increase in reduced drainage (increased > 30 mL from the previous day), or abnormal drainage color



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(turbid or brown)[18]. The ICG index test for the diagnosis of leakage involved the detection of green fluorescence emitted by the collected peritoneal fluid under near-infrared light.

Sample size

The sample size was estimated as follows: $n = Z^2 P (1-P)/d^2$ [19]. P is a pre-determined value of sensitivity ascertained based on our previous clinician experience, and d is the marginal error. Here, P = 0.95, Z = 1.96, a = 0.05, d = 0.1. Hence, the total required sample size was calculated as follows: $n = 1.96^2 \times 0.95 \times (1-0.95) / 0.1^2 = 18$.

Human patients

Six patients were enrolled in the enterostomy test. We used the following inclusion criteria for enrollment: (1) Patients that underwent a temporary or permanent enterostomy, including a colostomy or ileostomy for the first time; (2) patients should not have any postoperative intestinal obstructions and have smooth draining from the stoma; and (3) patients should be \geq 18 years of age. Patients with a history of adverse reactions or known allergies to ICG, iodine, or iodine dyes were excluded from the test.

For the diagnostic test, we assessed 21 patients for eligibility. However, we enrolled only 17. Patients aged \geq 18 years who had undergone GI reconstruction surgery, including colectomy or rectal cancer resection, were enrolled in the diagnostic test. We employed the following inclusion criteria for the diagnostic test: (1) The abdominal drainage tube, rectum, or rectovaginal fistula should contain feces or pus; (2) peritonitis, sepsis, or any of the following clinical features: leukocytosis (> 12 × 10^o/L), elevated CRP (> 10 mg/L), PCT (> 0.5 ng/mL), abnormal drainage volume (> 100 mL lasting 3 d), significant increase in reduced drainage (increased > 30 mL from the previous day), or abnormal color of the drainage (turbid or brown); and (3) patients with one of the following features [20]: Tumor location (lower rectum), distance from the anal verge (< 6 cm), clinical T stage (T3/4), intraoperative blood loss (> 50 mL), number of linear staples (> 2), operative time (> 3 h), tachycardia POD 1 (\geq 100 bpm), and postoperative fever (\geq 38°C). We used the following exclusion criteria: (1) Patients with a history of adverse reactions or known allergies to ICG, iodine, or iodine dyes; and (2) patients who underwent a temporary or permanent enterostomy. All patients provided written informed consent before participating in the study.

Animals

Sixteen male Sprague–Dawley rats aged 7 wk and weighing 220 ± 20 g were purchased from Guangdong Medical Laboratory Animal Center (Guangdong, China). All rats were housed in smooth-bottomed plastic cages in a pathogen-free animal room with controlled temperature ($22 \pm 2^{\circ}$ C), humidity ($50\% \pm 10\%$), and light (12 h light-dark cycles), with free access to rodent chow and water. To acclimate the animals to the laboratory environment, an acclimation period of 1 wk was allowed before the initiation of the experiment. This animal study was approved by the Institutional Animal Care and Use Committee of the Second Affiliated Hospital of South China University of Technology (Protocol No. 2022079).

Surgical anastomotic leakage model

Eight rats were first anesthetized with inhaled isoflurane and then a laparotomy was performed in their inferior middle abdomen. Their colon was exposed approximately 6-8 cm from the anus and incised with scissors. The disassociated ends of the colon were then anastomosed with non-absorbable monofilament sutures at two diagonal points (GL group, n = 8). The abdomen was closed using 5-0 braided silk sutures in layers. The remaining eight rats did not undergo colon surgery for leakage and were used as controls (sham group, n = 8).

ICG administration in rats

ICG was obtained from the Department of Pharmacy of Guangzhou First People's Hospital. The ICG solution was diluted to 2.5 mg/mL, according to the instruction manual. Approximately 1.25 mg (0.5 mL) of diluted ICG was intravenously injected into the penile vein of the rats.

ICG administration in human patients

ICG was administered immediately post-surgery to human patients. On POD 1, 25 mg (10 mL) of diluted ICG was intravenously injected into six patients who had undergone enterostomy with a stoma. Next, 17 patients diagnosed with or suspected to have GL were intravenously injected with 25 mg (10 mL) of diluted ICG at the following times: POD 1 for 4 cases, POD 2 for 8 cases, POD 4 for 1 case, and POD 6 for 4 cases.

Peritoneal fluid collection

The rats were anesthetized with inhaled isoflurane at 24 and 48 h after post-surgical ICG injection. Their abdominal cavity was opened, and their intestines at the surgical points were exposed. After photographing the site, the abdominal cavity was washed with 5 mL of saline solution containing 2% v/v of fetal bovine serum albumin. The wash solution containing leaked stool, ingested food, bile, and digestive juices was collected and preserved at 4°C until fluorescence detection.

In human patients, the ostomy fluid or drainage liquid was collected from the enterostomy stoma or abdominal drainage tubes on the second day after ICG administration. This drainage liquid was also preserved at 4°C until the fluorescence test.

Fluorescence test

This test was performed using a fluorescence laparoscope equipped with a near-infrared fluorescence imaging system



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Figure 1 Indocyanine green test diagnosed leakage in rat gut leakage model. A: Gut anastomotic leakage model in rat. The sigmoid colon was cut, disassociated, and anastomosed with two stitches (blue triangles); B: Indocyanine green (ICG) test results of peritoneal fluids between gut leakage (GL) group and sham group at 24 and 48 h. Data show green fluorescence in the GL model; C: Gray values indicate ICG test results in each group at 24 and 48 h. Data show increased gray values in the GL model compared with that in the sham group. Data are presented as a bar graph with mean ± SD and were compared using the student's *t* test, ^a*P* < 0.0001.

(OptoMedic Technologies Inc., Guangzhou, China). The images were collected in three modes: white light, fluorescence, and gray. The fluorescence intensities were measured by ImageJ software with the gray-mode pictures.

Statistical analysis

The data were presented as the mean \pm SD or the actual number of cases. A two-sided *P* value of < 0.05 was considered statistically significant. Intergroup comparisons of continuous variables were conducted using a two-sided Student's t test. Statistical analysis was performed using SPSS 26.0, and the figures were generated using GraphPad Prism.

RESULTS

ICG test diagnosed leakage in rat

To simulate clinical postoperative GL in the colon, we established a GL rat model by surgically incising the sigmoid colon with an incomplete colon anastomosis sutured by only two stitches (Figure 1A). The incomplete anastomosis caused a state of intestinal discontinuity for at least 2 d postoperative. During this period, the stool comprising ingested food, bile, and digestive juices leaked into the abdominal cavity. The sham group showed no fluorescence, while the GL group showed green fluorescence in the collected peritoneal fluids at 24 and 48 h (Figure 1B). The semiquantitative analysis of gray values in the images showed that the GL group exhibited increased fluorescence intensity at 24 and 48 h compared to that shown by the sham group (Figure 1C).

ICG detected in ostomy fluids in enterostomy stoma patients

To test the status of ICG from the peripheral veins to the enterostomy port in patients, six patients (four women and two men), with an average age of 66.3 ± 10.6 years, who had undergone laparoscopic rectal cancer resection along with enterostomies, were selected for ICG injection on POD 1 (Table 1). The ostomy fluid at the stoma was collected on POD 2 and used to detect the ICG-dependent fluorescence (Figure 2). Five samples of stoma fluids from the aforementioned six cases showed strong green fluorescence, while one exhibited weak green fluorescence (Figure 2).

Diagnostic test

In the diagnostic test (Figure 3), 21 patients were assessed for eligibility. Of these, four patients were excluded. Three of these excluded patients did not meet the inclusion criteria, while one patient refused to participate. Finally, 17 were enrolled for the ICG test analysis. The baseline demographic and clinical characteristics of the participants are listed in Table 2. In the ICG test, seven of the 17 patients showed green fluorescence, while the other did not (Figure 4A). When



Table 1 Characteristics of six patients with enterostomy stomas		
Patients	<i>n</i> = 6	
Age (yr), mean ± SD	66.3 ± 10.6	
Gender (male/female)	2/4	
Diagnose	6 (100%)	
Rectal cancer		
Surgery approach	6 (100%)	
Laparoscopic rectal cancer resection plus ileostomy		

Table 2 Patient	characteris	tics in the <i>i</i>	diagnostic test
	characteria		ulagnostic test

Characteristic	Value (<i>n</i> = 17) (percentage)
Age (yr), mean ± SD	58.6 ± 11.4
Gender (male/female)	10/7
Diagnosis, n (%)	
Ascending colon cancer	3 (17.6)
Descending colon cancer	2 (11.8)
Sigmoid colon cancer	4 (23.5)
Rectal cancer	8 (47.1)
Surgery, n (%)	
Laparoscopic right hemicolectomy	3 (17.6)
Laparoscopic left hemicolectomy	2 (11.8)
Laparoscopic sigmoidectomy	4 (23.5)
Laparoscopic rectal cancer resection	8 (47.1)
Enrollment criteria	
Feces or pus discharged from drainage tube, <i>n</i> (%)	2 (11.8)
Peritonitis with leukocytosis, n (%)	5 (29.4)
Peritonitis with procalcitonin, n (%)	5 (29.4)
Peritonitis with C-reactive protein, <i>n</i> (%)	3 (17.6)
Abnormal drainage volume (> 100 mL lasting 3 d), n (%)	2 (11.8)
Postoperative fever (> 38°C), n (%)	7 (41.2)
Intraoperative blood loss > 50 mL, n (%)	3 (17.6)
Operative time > 3 h, n (%)	6 (35.3)
Tachycardia POD 1 (\geq 100 bpm), <i>n</i> (%)	2 (11.8)
Clinical T stage (T3/T4)	7/3

tested with a reference standard, five of the seven patients who tested positive in the index test were diagnosed as GL positive, while all the other 10 who tested negative were diagnosed as GL negative (Table 3). The ICG test exhibited 100% sensitivity and 83.3% specificity for the diagnosis of GL. The positive predictive value was 71.4% and the negative predictive value was 100%. The likelihood ratios were 6.0 for a positive test result and 0 for a negative result. We ranked the fluorescence intensity based on the gray values of the 17 drainage collections and generated the receiver operating curve (ROC) to determine the predictive values of ICG for identifying postoperative GL. The ROC analysis showed the area under the curve as 0.933 (95% CI: 0.813–1.000; Figure 4B).

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Table 3 Contingency table evaluating the accuracy of indocyanine green measurement for the diagnosis of postoperative gut leakage

100 test	Postoperative gut leakage	Total		
ico test	Positive	Negative	- 10(8)	
Positive	5	2	7	
Negative	0	10	10	
Total	5	12	17	

ICG: Indocyanine green.



Figure 2 Indocyanine green detected in ostomy fluids in patients with enterostomy stomas. Indocyanine green test results of ostomy liquids from six patients using fluorescence and gray modes. The collection can be seen in green in the fluorescence mode.

DISCUSSION

We demonstrated the effectiveness of the peritoneal fluid ICG test in identifying postoperative GL in surgical GL rat models. We also showed the diagnostic value of the ICG test for diagnosing GL in patients undergoing colorectal surgery. Experimental models have become essential for verifying the effectiveness of ICG in diagnosing postoperative leakage. Previously reported GL models have mainly been used to study the mechanisms of intestinal wound healing[21]. Usually, in models of intestinal anastomotic sutures after segmental colonic resection, five stitches were needed to achieve a probability of 50% leakage[21,22]. A recent report also showed that a colonoscopy leakage model can be used to study the different stages of intestinal wound healing[22]. In the present study, we used only two stitches after surgically removing the sigmoid colon in rats. This technique afforded 100% leakage, with stool consisting of ingested food, bile, and digestive juices leaking into the abdominal cavity, which can be detected 24 and 48 h postoperatively. Thus, our model was suitable for testing GL as early as 24 h postoperatively.

The intravenously injected ICG was taken up by hepatocytes. It was then initially excreted into the bile and later into the bowel. If no leakage occurred, there would be no green fluorescence in the peritoneal fluid when exposed to nearinfrared light. Inversely, green peritoneal fluid is indicative of leakage, because the ICG cannot enter the enterohepatic circulation. Our animal experiment demonstrated that the ICG test can be used to detect postoperative GL because all peritoneal fluids of GL model rats showed green fluorescence. Furthermore, the ICG test of the ostomy fluids showed that this test can be used to detect postoperative GL in human patients. These results support the high sensitivity (nearly 100%) of ICG for detecting leakage.

Leakage is traditionally diagnosed based on clinical symptoms; laboratory tests such as markers of leukocytosis, CRP, and PCT; or imaging with an abdominopelvic CT scan or endoscopy[18]. Several biomarkers have been employed for diagnosing GL, such as postoperative fever, time to first defecation after operation^[20], gut microbiota^[23], MMP9^[14], cytokines IL6 and TNF α [24,25], and ischemia biomarkers such as lactic acid and pH[26]. Usually, the most effective evidence of postoperative GL is a direct clinical manifestation, such as feces or pus discharged from an abdominal drainage tube, the rectum, or rectovaginal fistula[18]. In our diagnostic data, two cases discharged feces in the abdominal drainage tube, seven had postoperative fever (\geq 38°C), four had leukocytosis, and five had elevated CRP or PCT.

The intraoperative use of ICG for assessing anastomosis perfusion has been well documented [16,17]. It has been reported that the intraoperative use of fluorescence with ICG could reduce GL rates in rectal cancer surgery [27,28]. Despite that, the postoperative application of ICG for the diagnosis of postoperative anastomotic leakage has not yet been studied. Our diagnostic test demonstrated that the ICG test conducted using drainage tubes for the diagnosis of GL had a sensitivity of 100% and a specificity of 83.3%. Hence, ICG can be used intraoperatively to assess anastomosis perfusion. In addition, it can be used postoperatively to diagnose postoperative anastomotic leakage.



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Figure 3 STARD flow diagram of the diagnostic test.



Figure 4 Diagnostic test results. A: Indocyanine green (ICG) test results of drainage fluids from 17 patients using fluorescence and gray modes; B: Receiver operating curves of predictive values of ICG for identifying postoperative gut leakage in patients.

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Our study has some limitations as well. The rat model of anastomotic leakage has not been previously reported. To verify the leakage, we used only two stitches for anastomosis in the rat model, which is not a common practice in clinical surgery for patients. Nonetheless, it was still acceptable and appropriate for this study because we needed 100% leakage. In addition, the fluorescence was detected by collecting the drainage liquid. However, this method can be applied only in patients with an abdominal drainage tube. Finally, the sample size for the diagnostic test was small. In addition, all the enrolled patients were from a single center. Therefore, it is necessary to conduct multicenter studies with a large sample size.

CONCLUSION

In conclusion, our study showed that the postoperative ICG test using the drainage tube is a valuable and simple technique for the diagnosis of GL. This simple technique is worthy of clinical promotion and application for diagnosing or ruling out GL. This method may help in proactive or early interventions in cases of postoperative GL.

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FOOTNOTES

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Country of origin: China

ORCID number: Yu Huang 0000-0002-6668-0702; Jie-Feng Weng 0000-0002-6788-6366; Shuai Zhang 0000-0003-0518-9239; Wei-Li Gu 0000-0002-0448-110X.

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