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Systemic indocyanine green administration to detect bile leakage after liver surgery: a prospective clinical trial, using historical controls

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ABSTRACT

Objective The aim of this study was to evaluate the clinical impact of intraoperative indocyanine green (ICG) assessment and subsequent interventions on the total bilirubin concentration in postoperative drainage fluid after hepatectomy. Specifically, this study was conducted to determine whether systemic ICG administration and near-infrared (NIR) imaging to detect and address bile leakage (BL) during liver surgery could improve postoperative outcomes in an intervention group compared with a historical control group.

Design This was a prospective clinical trial with historical controls that involved inverse probability of treatment weighting (IPTW) analysis to adjust for potential confounding biases resulting from nonrandomised treatment assignments.

Setting Tottori University Hospital, Japan.

Participants This study included 84 participants who were undergoing hepatectomy. Among these participants, 40 were prospectively enrolled in the intervention group. The remaining 44 participants underwent hepatectomy without ICG-based assessment or interventions and served as historical controls.

Intervention In the intervention group, 10 mg of ICG was intravenously administered before liver parenchymal dissection. After hepatic dissection, the resection plane was evaluated and treated as necessary via NIR imaging to detect and address BL.

Primary outcome measure The primary outcome measure was the total bilirubin concentration in the drainage fluid on postoperative day 3 (POD 3).

Results According to the IPTW analysis, the total bilirubin concentration in the drainage fluid on POD 3 was significantly lower in the intervention group than in the historical control group. The adjusted mean difference was −1.11 mg/dL (95% CI: −1.49 to −0.72; p<0.001). No adverse events or side effects related to ICG administration were reported in the intervention group, indicating both the efficacy and safety of this approach in reducing postoperative bilirubin levels.

Conclusions Intraoperative ICG administration and assessment effectively lower bilirubin levels in drainage fluid during hepatectomy, potentially reducing the incidence of BL.

Trial registration number jRCTs061210043.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study employed a prospective cohort design combined with historical controls, enabling structured data collection within a clinical setting.
⇒ Inverse probability of treatment weighting was applied to adjust for potential confounding factors in the absence of randomised allocation.
⇒ The use of predefined inclusion and exclusion criteria ensured consistency across both intervention and historical control groups.
⇒ The study was conducted at a single institution, which may limit the external validity and generalisability of the findings.
⇒ The nonrandomised design and use of historical controls introduce the potential for selection bias and temporal confounding.

INTRODUCTION

Bile leakage (BL) is a common complication following hepatectomy that is sometimes intractable.[1 2] Despite recent improvements in surgical techniques and perioperative management, BL can cause biliary fistulae and is sometimes associated with serious conditions such as liver failure and severe intra-abdominal infection.[3] In addition, BL has been linked to prolonged hospital stays, increased risks of secondary interventions such as percutaneous drainage or reoperation, and overall increased healthcare burdens. The frequency of BL after hepatic resection has recently been reported to range from 4.0% to 8.0%.[4–6]

In 2011, the International Study Group of Liver Surgery (ISGLS) indicated that posthepatectomy BL could be diagnosed in patients with a 'drain-to-serum total bilirubin ratio of≥3 at postoperative day 3 (POD3) after resection or later'.[1] BL severity is classified by the ISGLS as follows: Grade A: no change or little change in patients' clinical management; Grade B: requires a change in patients' clinical management (eg, additional diagnostic or interventional procedures) but is manageable without relaparotomy, or is a Grade A BL lasting for >1 week; and Grade C: relaparotomy is required.[1]

Since Grade B or Grade C BLs significantly impact a patient's postoperative clinical course, various efforts to reduce BL have been reported. Detecting and managing the collapsed bile ducts that cause BL by injecting dyes, including indocyanine green (ICG), into the bile ducts is widely known to be a valid treatment.[7–9] However, there is a risk of inducing bacteraemia and barotrauma to the small bile ducts on the liver incision plane associated with intrabile duct injections. In addition, placing a C-tube or T-tube into the bile duct for internal decompression is helpful for reducing the incidence of BL. This approach has been reported in many cases.[10 11] However, these methods are practical only for bile duct injuries connected to the common bile duct side; they are entirely ineffective for treating BL stemming from bile duct injuries that do not involve the common bile duct, such as those identified as type D injuries according to Nagano's classification.[12] Therefore, a more effective and sensitive method is needed to prevent postoperative BL completely.

ICG is a standard drug used for evaluating liver function, and in recent years, reports of navigation surgery using ICG have increased.[13] We also reported a case in which BL was avoided during hepatectomy by detecting BL through the excretion of ICG in bile juice after intravenous administration.[3 14] Between ordinary observation with the naked eye and observation with a near-infrared (NIR) camera, the number of leakage spots detected on the live section plane was significantly greater with NIR camera observation.[15 16] Our retrospective observations revealed that the total bilirubin concentration in the drainage effluent on postoperative day 3 (POD 3) was significantly lower in the group assessed via ICG administration.[16]

As noted earlier, the incidence of BL after hepatic resection remains clinically relevant and should not be overlooked; its occurrence is influenced by a complex interplay of factors, including the degree of fibrosis in the background liver, the surgeon's skill, the patient's medical history and the extent of liver resection.

With this background, we focused on detecting intraoperative BL as a surrogate marker for the development of biliary fistulae, as it has been reported in the past that BL detected during hepatectomy is a risk factor for biliary fistulae.[17] Traditionally, the determination of the presence of BL following hepatic resection has relied solely on visual inspection with the naked eye. Leaks that cannot be confirmed visually are generally overlooked and progress to biliary fistulae after BL. Therefore, this study assessed whether monitoring for ICG leakage into the bile intraoperatively, alongside visual checks for BL during surgery, could improve the accuracy of BL detection and management. The aim of this approach was to reduce the elevated levels of total bilirubin in the drainage fluid and prevent the occurrence of biliary fistulae, which are postsurgical complications.

METHODS

This study adhered to the guidelines of the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) statement[18](online supplemental material 1), and the study protocol is available in an open-access publication.[19]

Objective

This study was a prospective, single-centre, nonrandomised trial with historical controls at the Tottori University Hospital; trial registration number jRCTs061210043 (https://jrct.niph.go.jp/en-latest-detail/jRCTs061210043). Using a prospective cohort (ICG group, I-G) and a historical control group (H group, H-G), we assessed whether the evaluation and treatment of ICG (10 mg/body, administered intravascularly) would result in improved total bilirubin levels in drainage fluid on POD 3.

Endpoints

The primary outcome of this trial was the total bilirubin concentration in the drainage fluid on POD 3. The secondary outcomes included the following: (1) the frequency of BL after hepatectomy as defined by the International Society of Clinical Oncology (ISGLS) criteria[1]; (2) the drain-to-serum total bilirubin concentration ratio on POD 3; (3) the detection and number of BL spots after hepatectomy; (4) changes in the total bilirubin concentration in the drainage fluid over time, specifically between PODs 1 and 3; (5) the amount of total bilirubin in the drainage fluid over 24 hours on POD 1; (6) the identification and classification of bacteria in the drainage fluid; (7) the duration of the postoperative hospital stay; and (8) the type, severity, and frequency of adverse events, side effects and postoperative complications as classified by the Clavien–Dindo grading system.[20] The amount of total bilirubin in the drainage fluid over 24 hours on POD 1 was estimated by the product of the total bilirubin concentration in the drain effluent on POD 1 and the total volume of effluent for that day.

Eligibility criteria in this prospective cohort (ICG group, I-G)

Eligibility was evaluated for all consecutive patients who were scheduled to undergo hepatectomy at Tottori University Hospital.

Inclusion criteria in this prospective cohort (ICG group, I-G)

The inclusion criteria for patients were as follows: (1) underwent hepatectomy as a planned surgery for primary

liver cancer, metastatic liver cancer, or benign liver lesions, whether open or laparoscopic; (2) had an Eastern Cooperative Oncology Group-Performance Status (ECOG-PS)[21] ≤1; (3) were male or female patients aged 20 years or older at the time consent for the study was obtained; (4) did not have severe comorbidities and (5) received a full explanation of the content of the study from the principal investigator, understood the purpose of the study and provided free and voluntary written consent to participate in the study.

Exclusion criteria in this prospective cohort (ICG group, I-G)

The exclusion criteria include (1) Patients who underwent emergency hepatectomy (ie, hepatectomy for trauma injury); (2) patients known to be hypersensitive to any of the components of ICG; (3) patients who had a history of hypersensitivity to iodine; (4) pregnant women or patients who may have been pregnant; (5) patients who were breastfeeding; (6) patients who had participated in other clinical trials or who had used unapproved or uninsured medicinal products within the 3 months prior to enrolment; and (7) patients who were judged by the principal investigator or coinvestigators to be inappropriate for participation in this study owing to clinical or ethical concerns (eg, unstable general condition or cognitive impairment affecting consent).

Inclusion criteria for the historical controls (historical control group, H-G)

The inclusion criteria include (1) consecutive patients who underwent hepatic resection for primary liver cancer, metastatic liver cancer or benign liver lesions, whether open or laparoscopic, before July 2020; (2) ECOG-PS≤1; (3) male and female patients aged 20 years or older at the time of surgery; (4) patients with data on the total bilirubin concentration in the drainage fluid on POD 3; and (5) patients without severe preoperative comorbidities.

The H-G was intentionally selected from a period before July 2020, when intraoperative ICG assessment had not yet been introduced at our institution; this ensured that all patients in the H-G underwent hepatectomy without exposure to ICG-based evaluation or intervention, thus representing the standard surgical practice of that time. After July 2020, ICG was progressively incorporated into routine care, making it difficult to establish a contemporaneous control group without potential contamination or variability in implementation.

Exclusion criteria for the historical controls (historical control group, H-G)

The exclusion criteria include (1) patients who underwent emergency surgery; (2) patients who were administered ICG during surgery; (3) patients for whom the principal investigator or coinvestigator determined that it would be inappropriate to participate in this study and (4) patients who refused to allow the use of data collected from their medical records.

Data collection and registration

In the 28 days before surgery, we assessed and logged various preoperative metrics, such as complete blood counts; levels of enzymes and substances such as aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, alkaline phosphatase, total and direct bilirubin, total cholesterol, albumin, blood urea nitrogen, creatinine, C-reactive protein, serum sodium and serum potassium; and blood coagulation and ICG test results and radiological evaluations. Sample analysis was performed on hospital admission, 2 days before surgery, and on designated PODs (PODs 1, 2, 3, 5 and 7). The recorded intraoperative details included the surgical method (laparoscopic vs open), liver resection type (anatomical vs nonanatomical), weight of the liver portion removed, drain placement, surgery duration, volume of blood lost during surgery, blood transfusion status and quantity of BL spots visible with and without NIR imaging. Measurements of total bilirubin in the drainage fluid were conducted on PODs 1 and 3, as well as on PODs 5 and 7 if drainage remained, with simultaneous fluid cultures. Additionally, the duration of the postoperative hospital stay was noted. Evaluations of the presence of BL or biliary fistulas, the drainage effluent volume and postsurgical complications occurred daily during hospitalisation and at the first follow-up after discharge (between PODs 21 and 35). All adverse events reported during the study were documented.

The study included 40 patients in a prospective cohort (I-G) and 45 in a retrospective cohort (H-G), all of whom underwent hepatectomy. Prospective participants provided written consent between 28 and 3 days before their surgeries without any offered incentives. Medical charts and associated reports served as the source documents for this research.

Operative procedure and administration of ICG

Hepatectomy (open or laparoscopic) was performed as a routine medical practice. ICG (Daiichi Sankyo Company Limited, Tokyo, Japan) 10 mg/2 mL was administered intravascularly, intraportally or intravenously; during the initial phase of liver parenchymal dissection, approximately 1–2 hours before its completion, the ICG was administered by either the attending anaesthesiologist or the surgeon involved. After completion of hepatic dissection, the abdominal cavity was thoroughly rinsed with saline and visually observed for the presence and number of bile leaks (by naked eye observation). The liver resection plane was then irradiated with NIR using a Stryker 1588 AIM system (Stryker Corporation, Kalamazoo, MI, USA) to check for the presence or absence of ICG fluorescence on the gauze at the site of the liver dissection plane excretion into the bile, and the number of leakage spots was also recorded. Representative images of intraoperative ICG leakage visualised via NIR imaging are provided in online supplemental material 2. When in-bile ICG leakage could be confirmed during NIR camera observation or when BL could be confirmed grossly, the leaking

bile duct was sutured to ensure the cessation of leakage. After leakage cessation was achieved, the operation was completed by placing a low-pressure continuous suction drain (ϕ 6 mm CLIO DRAIN VAC, Sumitomo Bakelite Company Limited, Tokyo, Japan) near the hepatic dissection plane.

Throughout the study period, there were no major changes in the clinical team responsible for hepatectomy or postoperative care. Surgical techniques and perioperative management practices were consistent across both groups, following the usual standards of care at Tottori University Hospital.

Statistical analysis

Based on historical data from 50 non-ICG-treated patients and 22 ICG-treated patients at Tottori University Hospital, the mean concentrations of total bilirubin in the drainage fluid on POD 3 were 2.476 mg/dL (SD=2.266) in the non-ICG group and 1.377 mg/dL (SD=0.596) in the ICG group. Based on empirical evidence, this study was designed to detect the mean difference, assuming that the mean concentration of total bilirubin in the experimental drug group was 1.3 mg/dL (SD, 1.1 mg/dL), with a two-sided significance level of 5% and 80% statistical power. Under these conditions, a minimum of 40 participants were needed, and the sample size of the ICG group in this study was set to 40 patients. Therefore, the sample size calculation was based on historical pre-IPTW data, without accounting for the potential effects of IPTW adjustment.

For the primary statistical analysis, we applied inverse probability of treatment weighting (IPTW)[22] to assess the reduction in the total bilirubin concentration in the drainage fluid on POD 3 between the I-G and the H-G. IPTW analysis effectively balances the distributions of potential confounding factors between the experimental and historical control groups and can adjust the resultant bias in estimating the outcome measure.[22] The baseline characteristics of the I-G and the H-G were summarised and compared prior to IPTW adjustment. Continuous variables were compared via Student's t test and categorical variables were compared via the χ^2 test. For the calculation of propensity scores, a logistic regression approach was used, with the treatment assignment (either intervention or control) serving as the dependent variable, while independent variables included demographic and clinical characteristics such as age, sex, body mass index, extent of fibrosis in the liver background tissue (categorised as Inuyama class F0–2 or F3–4),[23] preoperative blood test results (including total bilirubin, albumin levels, platelet counts and prothrombin time as a percentage) and the weights of the resected liver specimens. The full specifications of the logistic regression model used for propensity score estimation are provided in online supplemental table 1. IPTW analysis was conducted via these propensity

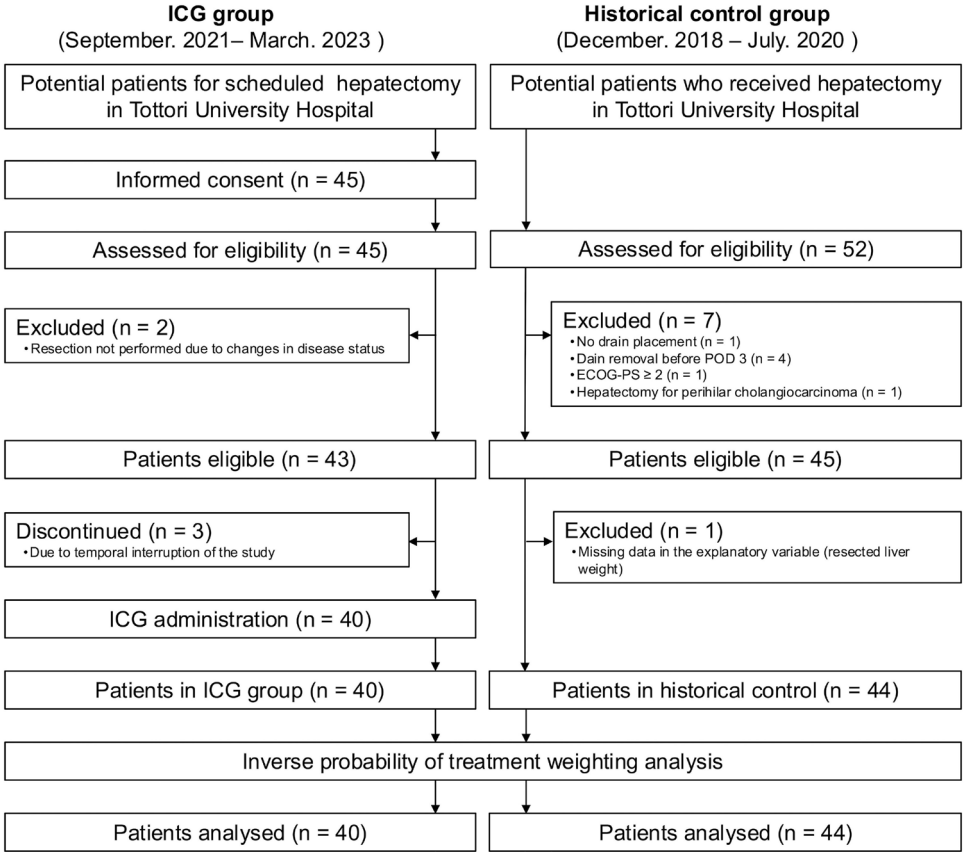

Figure 1 Study flow diagram. ECOG-PS, Eastern Cooperative Oncology Group Performance Status; ICG, indocyanine green; POD, postoperative day.

scores, and stabilised weights were applied to reduce variance. Covariate balance between groups after weighting was assessed via standardised mean differences. These results are presented in online supplemental table 2, which confirms that all covariates were well balanced after IPTW adjustment (all standardised mean differences<0.25, with most being <0.1).

All comparisons of outcomes between groups were performed using weighted data. While the actual number of patients in each group remained unchanged (n=40 and n=44), the weighted analysis represents a pseudopopulation in which baseline covariates are balanced. The mean differences were estimated via IPTW based on these propensity scores. Furthermore, an IPTW-based statistical test was conducted to test the null hypothesis that the mean difference was 0; therefore, the efficacy of ICG treatment was evaluated with a two-sided 5% significance level.

For the analyses of secondary endpoints, we first performed IPTW analysis to compare the incidence of postoperative BL after hepatectomy. Moreover, we summarised the outcome data related to the presence and number of BL spots on the hepatic dissection plane after hepatectomy in terms of the means and SDs. We performed a paired t-test to compare the mean values of changes in the number of BL spots after hepatectomy with a two-sided 5% significance level. We also summarised descriptive statistical values for changes in the total bilirubin concentration in drainage fluid between PODs 1 and 3, estimated total bilirubin in drainage fluid on POD 1, the presence/absence and type of bacteria in the drainage fluid based on effluent culture, the length of postoperative hospital stay, adverse events, side effects and postoperative complications (according to the Clavien–Dindo classification[20]) throughout the observation period.

All the statistical analyses were performed via R V. 4.2. 1 (R Foundation for Statistical Computing, Vienna, Austria).

Deviations from the registered protocol

One minor deviation from the registered protocol[19] involved the final number of patients included in the H-G. Although the protocol specified 45 patients in each group, one patient from the H-G was excluded from the final analysis because of missing data on liver resection weight, which was one of the predefined covariates in the propensity score model used for IPTW adjustment. As this variable was essential for inclusion in the IPTW analysis, the patient could not be included. As a result, the H-G comprised 44 patients in the final analysis set. No other deviations from the protocol were identified.

Patient and public involvement

The present study involved patients and members of the public. Three lay representatives were involved in the protocol design and ethical approval. The lay representatives of the trial reviewed the study protocol and patient-related trial documents, including the information sheets and consent forms.

RESULTS

Study participants

A study flowchart of this trial is shown in figure 1. For the H-G, 52 consecutive patients were assessed for eligibility. Seven of the 52 patients were excluded on the basis of the study criteria. For the I-G, 45 patients were evaluated for eligibility after providing informed consent. The first patient was enrolled in the prospective cohort (I-G) on 15 November 2021. Of these 45 patients, two were ineligible for the present analysis because liver resection was not performed due to changes in their disease status after they provided informed consent. Of the remaining 43 patients, three were excluded because ICG was not administered intraoperatively due to temporary interruption of the study. Ultimately, 45 patients were assigned to the H-G, and 40 were assigned to the I-G and administered ICG intraoperatively. The baseline patient characteristics of this study are shown in online supplemental table 3. The full analysis set included 44 of the 45 patients in H-G, except for one with a missing liver resection weight (one of the explanatory variables) in the H-G, and 40 patients in the I-G. This study included 55/29 (65%/35%) men/women in the full analysis set; their mean age was 72±8 years, and 66 patients (79%) underwent open hepatectomy. Hepatocellular carcinoma was the most common causative pathology for liver resection (48 patients, 57%), followed by metastatic liver cancer (22 patients, 26%). BL of all grades (Grade A or higher) occurred in 14 patients (16.7%), and clinically problematic Grade B BL occurred in four patients (4.8%).

A comparison of the preoperative characteristics of the I-G and H-G patients before IPTW analysis revealed slight but statistically significant differences in the serum albumin concentration, γ-glutamyl transpeptidase, total bilirubin level, activated partial thromboplastin time and ICG retention rate at 15 min. The operation time and blood loss volume were significantly shorter (259 vs 310 min, p=0.023) and lower (247 vs 365 mL, p=0.041) in the I-G, respectively. The serum bilirubin levels on POD 3 were significantly lower in the I-G (1.0 vs 1.3 mg/dL, p=0.024), as were the bilirubin levels in drainage fluid on POD 3 (1.4 vs 2.4 mg/dL, p<0.001). In contrast, the drain/serum bilirubin ratio on POD 3 was also significantly lower in the I-G (1.4 vs 2.0, p<0.001).

Primary outcome: Total bilirubin concentration in the drainage fluid on POD 3

The total bilirubin concentration in the drainage fluid on POD 3 was significantly lower in the I-G (1.4 vs 2.4 mg/dL, p<0.001), and the incidence of BL (above Grade A) was substantially lower in the I-G (5.0 vs 27.3%, p=0.015, online supplemental table 4). Significant differences in the serum total bilirubin level and background liver fibrosis were found before IPTW adjustment, but no

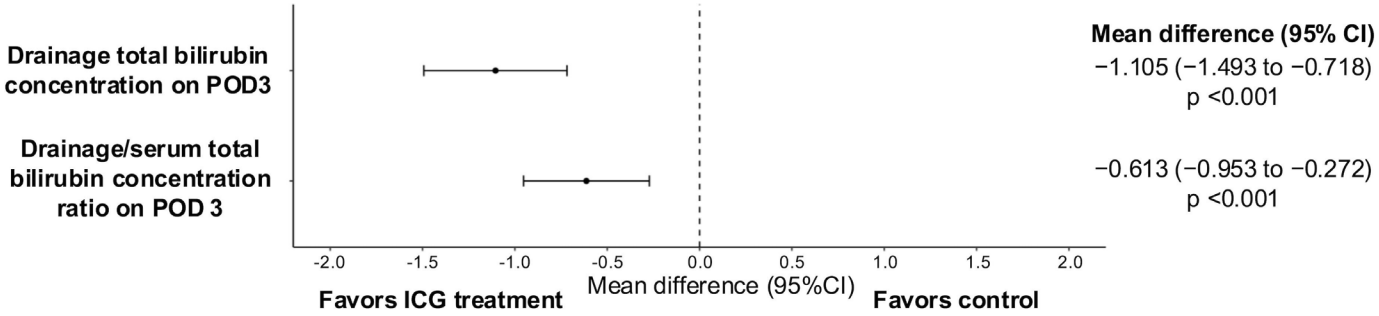

Figure 2 Forest plot showing the IPTW-adjusted parameters and 95% CIs for the mean difference in the total drainage bilirubin concentration and the serum-to-drain total bilirubin ratio on POD 3. CI, confidence interval; ICG, indocyanine green; IPTW, inverse probability of treatment weighting; POD, postoperative day.

significant differences were observed after adjustment (online supplemental table 4).

After IPTW analysis, compared with that in the H-G, the mean difference in the total bilirubin concentration in the drainage fluid on POD 3 in the I-G was −1.105 mg/dL (95% CI: −1.493 to −0.718 mg/dL, p<0.001; figure 2).

To further account for potential residual confounding due to baseline imbalances, we conducted a sensitivity analysis via a multivariate linear regression model adjusting for the same covariates used in the IPTW analysis. This analysis yielded a consistent result, with an adjusted mean difference of −0.74 mg/dL (95% CI: −1.07 to −0.41, p<0.001), supporting the robustness of the primary outcome.

These findings suggest that intraoperative ICG administration was independently associated with a significant reduction in the total bilirubin concentration in postoperative drainage fluid, even after adjusting for potentially confounding clinical and operative variables.

Secondary outcomes

A summary of the secondary endpoints is as follows:

- The incidence of postoperative BL (Grade A or higher) was significantly lower in the I-G than in the H-G.
- The drain-to-serum total bilirubin ratio on POD 3 was also significantly reduced in the I-G.
- More BL points were detected by NIR imaging than by visual inspection in the I-G.
- Patients in the I-G had a significantly shorter hospital stay and fewer Clavien–Dindo grade ≥II complications.
- No drug-related side effects were observed.

Incidence of postoperative BL after hepatectomy (according to the ISGLS definition)

The incidence of postoperative BL (Grade A or higher) after hepatectomy following IPTW was significantly lower in the I-G than in the H-G at −0.239 (95% CI: −0.387 to −0.091, p=0.002). The ratio of the BL incidence rate in the I-G to that in the H-G was 0.152 (95% CI: 0.036 to 0.646, p=0.011), which was significantly lower than that in the I-G (table 1).

The difference in Grade B or higher BL incidence was −0.064 (95% CI: −0.122 to −0.007) between the I-G and the H-G, with the I-G demonstrating a significantly lower incidence (p=0.028). Furthermore, the incidence of BL (above Grade A) was 5.0% (95% CI: 0.61% to 16.92%) in the I-G and 27.3% (95% CI: 14.96% to 42.79%) in the H-G. The incidence of clinically significant bile leakage (above Grade B) was 0.0% (95% CI: 0.00% to 8.81%) in the I-G and 9.1% (95% CI: 2.53% to 21.67%) in the H-G.

Drainage/serum total bilirubin concentration ratio on POD 3

The drainage/serum total bilirubin concentration ratio on POD 3 following IPTW analysis was significantly lower in the I-G at −0.613 (95% CI: −0.953 to −0.272) than in the H-G, suggesting that bilirubin levels in drainage fluid were lower in the I-G independent of serum bilirubin levels (p<0.001, figure 2).

Presence and number of bile leaks after hepatectomy

The presence and number of bile leaks after hepatectomy in I-G were compared. Conventional naked-eye observation alone revealed at least one leak in 4 of 40 patients. Conversely, ICG leakage confirmed via an NIR

Table 1 Risk ratio of and risk difference in BL incidence for the ICG group relative to the historical control group after adjustment by IPTW

	Risk ratio	(95% CI)	P value	Risk difference	(95% CI)	P
Incidence of BL (above Grade A)	0.152	(0.036 to 0.646)	0.011	−0.239	(−0.387 to 0.091)	0.002
Incidence of BL (above Grade B)	Incalculable*	–	–	−0.064	(−0.122 to 0.007)	0.028

*Not applicable because there were no patients with Grade B or higher disease in the ICG group.
BL, bile leakage; ICG, indocyanine green; IPTW, inverse probability of treatment weighting.

camera revealed ≥1 leakage in 19 of 40 patients. The number of ICG leakage sites detected via an NIR camera averaged 1.15±1.58 per patient compared with 0.10±0.30 per patient, as determined by naked-eye observation alone. The difference between the number of leakage sites detected by the NIR camera and the number of sites detected by the naked eye was 1.05 (95% CI: 1.52 to 0.58), and the number of leakage sites detected with the NIR camera was significantly greater (p<0.001).

Changes in the total bilirubin concentration in the drainage fluid over time

On POD 1, the drainage fluid total bilirubin level was 1.40±4.23 mg/dL for the I-G and 1.36±2.2 mg/dL for the H-G (p=0.950). However, on POD 3, total bilirubin levels in the drainage fluid were significantly lower in the I-G (1.35±0.68 mg/dL) than in the H-G (2.40±1.36 mg/dL), indicating a significant reduction (p<0.001).

Amount of total bilirubin in the drainage fluid over 24 h on POD 1

The estimated amount of total bilirubin in the drainage fluid on POD 1 was 1.65±3.3 mg in the I-G and 1.78±3.7 mg in the H-G. The total bilirubin in the drainage fluid was greater in the H-G, but this difference was not statistically significant (p=0.859).

Presence and types of bacteria in the drainage fluid

The drainage fluid culture results on PODs 1 and 3 were negative for all patients in the H-G and I-G.

Length of postoperative hospital stay

The mean postoperative hospital stay was 6.1±1.0 days in the I-G compared with 17.6±30.6 days in the H-G, with a significantly shorter hospital stay in the I-G (p=0.020).

Adverse events, side effects, and postoperative complications (Clavien–Dindo classification)

The H-G demonstrated 19 (43.2%) Grade II or higher postoperative complications. In contrast, eight (20%) Grade II or higher complications occurred in the I-G. Moreover, the I-G exhibited no adverse events or side effects associated with the study drug administration.

DISCUSSION

The primary endpoint of this study was the total bilirubin concentration in the drainage fluid on POD 3. Notably, this parameter functions as a surrogate marker rather than a direct clinical outcome. While it does not in itself represent a clinically adverse event, elevated bilirubin levels in the drainage fluid have been associated with postoperative BL and biliary fistula formation in previous studies. Therefore, monitoring this surrogate provides a practical and quantifiable indicator for assessing the effectiveness of intraoperative BL management strategies.

Our findings revealed that a significant reduction in the drainage bilirubin concentration in the I-G corresponded with a markedly lower incidence of BL, shorter postoperative hospital stays, and fewer Clavien–Dindo grade ≥II complications. Although causality cannot be definitively established owing to the study design, these associations suggest that bilirubin reduction may reflect clinically meaningful improvements, such as a reduced need for reintervention, a lower risk of infection and faster recovery. Nevertheless, further randomised controlled trials are needed to validate whether this surrogate endpoint reliably predicts improved long-term outcomes and to confirm the direct clinical benefits of ICG-guided interventions. The results indicated that all bile leaks were Grade A or above and that there were significantly fewer clinically problematic Grade B bile leaks in the I-G. Compared with conventional naked-eye observation, intraoperative ICG administration performed during hepatic resection to evaluate and address leakage from the dissection plane after completing hepatic dissection was more helpful in reducing both the total bilirubin level in the drainage fluid and the drainage fluid-to-serum total bilirubin concentration ratio and decreasing the incidence of BL.

The methods of injecting dye solution, fat emulsion, contrast agent or air through the cystic duct to check for leakage from the hepatic detachment section have long been known to help detect BL.[24–26] The injection of substances into the hilar bile duct is undoubtedly beneficial. However, the following problems must be considered: (1) cannulation into the bile duct is labourious; (2) injection of substances may induce barotrauma to the unruptured cut end of the bile duct; (3) cholangitis may occur as a result of bacterial translocation into the bile duct wall due to injection pressure and (4) BL originating from bile ducts that lack a connection to the hilar bile duct, such as those classified as Nagano's type D[12] injury, are undetectable.

ICG is a standard drug used to evaluate liver function and has been widely used for many years. Recently, there has been an increase in the number of reports on navigation-guided surgery using the fluorescent property of ICG in blood, which binds to albumin and fluoresces in the NIR region.[13] More than 95% of the ICG administered into the bloodstream is secreted into the bile juice after absorption by hepatocytes.[13 27] The presence of ICG in bile has been shown to be similar to that of ICG in blood, and Matsumura *et al*[15] reported that the fluorescence intensity of ICG in bile peaked between 30 min and 4 hours after ICG administration into the blood. Based on these previous reports and our experience in detecting and managing ICG leakage in liver section planes after intravenous ICG administration,[3 14] we decided to conduct this study.[19] We reported in a previous retrospective study that ICG leak detection is more sensitive than naked-eye confirmation of BL,[16] and we found similarly sensitive leak detection in the prospective group (I-G). Furthermore, the systemic administration of ICG to evaluate the hepatic section plane and address leakage significantly reduced the bilirubin levels in the drainage fluid and the frequency of BL in the I-G compared with those in the H-G, possibly because of (1) the greater sensitivity

of ICG than that of the naked eye in detecting BL and (2) the ability to detect and treat Nagano's type D BL.[12]

We acknowledge several limitations of the present study. First, this was not a randomised controlled trial, and the I-G and H-G comprised a small number of patients. The differences in outcomes observed in this study may have been due to several unmeasured variables. Patients in the I-G were treated in the later phase of the study period, and it is undeniable that improvements in technique and skill may have affected the drainage bilirubin levels. Second, this study was conducted at a single institution. However, the patient population included a broad range of diagnoses (eg, hepatocellular carcinoma, metastatic liver cancer and intrahepatic cholangiocarcinoma) and surgical approaches (open and laparoscopic hepatectomy). Given the diversity of the cohort and the typical nature of the procedures performed, the study population may be broadly comparable with those treated at other Japanese tertiary centres. Third, although a reduction in total bilirubin levels on POD 3 was observed in the postoperative drainage fluid, there was also a significant difference between the two groups in terms of the total bilirubin levels in the blood. Although the drain-to-serum bilirubin ratio was substantially reduced, this may not be entirely due to the ICG-guided intervention. Fourth, the study's primary endpoint was not a reduction in the frequency of BL. BL generally occurs in less than 10% of patients who undergo hepatectomy.[4–6] Nevertheless, a clinical trial demonstrating a BL frequency reduction of a few percentage points would require the participation of several hundred patients in each arm. Therefore, the primary endpoint was set as the presence or absence of a reduction in bilirubin levels in drainage fluid as a surrogate marker in this study. Fifth, despite our use of IPTW to balance observed covariates between groups, the influence of unmeasured confounders, including potential improvements in surgical skills and postoperative management over the study period, cannot be entirely excluded. Such temporal factors may have partially affected the clinical outcomes. Furthermore, while liver resection weight was included as a covariate in the propensity score model because it can be reasonably estimated preoperatively based on imaging studies and surgical planning, intraoperative variables such as operation time and blood loss were not included. These variables are considered intermediate outcomes influenced by the surgical course itself, and adjusting for them could introduce bias into the estimation of treatment effects. Future multicentre, prospective, randomised trials are needed to evaluate the direct benefit of ICG administration and treatment on postoperative BL.

Furthermore, we selected the total bilirubin concentration in drainage fluid on POD 3 as a surrogate endpoint in this study based on its established correlation with the grading of BL as defined by the ISGLS.[1] Specifically, elevated bilirubin levels in drainage fluid have been associated with higher-grade BL, and thus, a reduction in this parameter reflects a corresponding improvement in the clinical severity of BL. This close relationship supported the use of drainage bilirubin concentration as a meaningful and quantifiable endpoint in evaluating the efficacy of intraoperative ICG-guided interventions.

Although several retrospective studies and case reports have suggested that intraoperative use of ICG fluorescence imaging may improve the detection of BL, the current evidence remains limited, particularly in terms of prospective clinical trials. To our knowledge, this is the first study to evaluate the impact of systemic ICG administration on postoperative BL using a prospective design with methodological rigour, including propensity score-based IPTW analysis.

Intraoperative ICG assessment and subsequent intervention significantly reduced the total bilirubin concentration in the drainage fluid on the third day after hepatectomy. This finding suggests that the use of ICG may help reduce the occurrence of postoperative biliary fistula, which is a clinically significant complication and thereby contributes to improved patient prognosis.

These findings support the routine use of ICG-guided intraoperative assessment during hepatectomy as a safe, practical and effective strategy to reduce BL; its incorporation into standard surgical protocols may improve postoperative recovery, reduce complications and enhance overall patient outcomes. By reducing the burden of postoperative BL, this approach may lead to fewer complications, shorter hospital stays and faster recovery, ultimately improving patient outcomes and patient satisfaction. Nevertheless, these findings should be interpreted with caution because of the nonrandomised design, the use of historical controls and the relatively small sample size, which may limit generalisability. Further validation in larger, multicentre trials is warranted. In particular, future studies could investigate the differential effectiveness of ICG assessment across various types of hepatectomy, including anatomical versus non-anatomical resection or major versus minor resection. Additionally, long-term follow-up studies could help clarify whether intraoperative ICG assessment contributes to a sustained reduction in the incidence of biliary fistula and associated complications. Furthermore, given the exploratory nature of this single-centre study with historical controls, the present findings should be considered hypothesis-generating, warranting validation through larger multicentre, randomised controlled trials.

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YF contributed by overseeing the manuscript's development. The manuscript underwent a critical examination and received unanimous final approval from all the contributing authors. The guarantor of the study is TaH who accepts full responsibility for the finished work and/or the conduct of the study, had access to the data and controlled the decision to publish.

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Patient consent for publication Not applicable.

Ethics approval The study spanned from September 2021 to May 2024, aligning with the principles of the 1964 Declaration of Helsinki (as updated in 2013) and Japan's Clinical Trials Act. The Tottori University Hospital Certified Review Board (CRB6200003) granted ethical approval for this protocol on 27 September 2021, with the approval number 21C002. All prospective cohort patients provided written informed consent, whereas consent from the historical control cohort was deemed unnecessary owing to the retrospective nature of the study. No significant surgical method alterations occurred following the trial's initiation.

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