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Autologous blood, a novel agent for preoperative colonic localization: a safety and efficacy comparison study

Eui Joo Kim[1] · Jun-Won Chung[1] · Su Young Kim[1] · Jung Ho Kim[1] · Yoon Jae Kim[1] · Kyoung Oh Kim[1] · Kwang An Kwon[1] · Dong Kyun Park[1] · Duck Joo Choi[1] · Sung Won Park[2] · Jeong-Heum Baek[2] · Won-Suk Lee[2]

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Abstract

Background Preoperative localization is essential for minimally invasive colorectal surgery. However, conventional endoscopic tattooing agents such as India ink have safety issues. The availability of new endoscopic markers such as non-India-ink-based agent is limited. We assessed the efficacy and safety of preoperative endoscopic tattooing using autologous blood in colorectal surgery.

Methods From February 2016, all patients who required localization of a target lesion before colorectal surgery underwent endoscopic tattooing using autologous blood, and the outcomes were collected prospectively. As a comparison, we retrospectively reviewed the medical records of a further 51 consecutive patients who underwent endoscopic tattooing using India ink before February 2016. A total of 102 patients who underwent endoscopic tattooing using either India ink or autologous blood were included in this study. The primary outcomes were the visibility of the tattooing in the peritoneal cavity and related adverse events.

Results Endoscopic tattoos produced using India ink were visible in 49 (96.1%) patients, and tattoos created using autologous blood were visible in 47 (92.2%) patients. In the autologous blood group, the tattoo could not be identified in four patients due to excessive peritoneal fat, bleeding tendency, congenital anomaly, and suboptimal tattooing. Seven (13.7%) patients in the India ink group and three (5.9%) patients in the autologous blood group experienced endoscopic tattooing-related adverse events.

Conclusions Autologous blood is a feasible and safe tattooing agent for preoperative endoscopic localization of colorectal lesions within maximal interval of 5 days.

Keywords Colorectal neoplasia · Endoscopy · Tattooing · Minimally invasive surgical procedures · Laparoscopy

In patients with small or flat early colon cancer or malignant polyps resected by endoscopic mucosal resection or endoscopic submucosal dissection, identification of the culprit lesion is difficult, even during open surgery. Thus, several techniques for the preoperative localization of colorectal tumors have been developed, such as barium imaging, computed tomography combined with colonoscopy, intraoperative endoscopy, endoscopic tattooing with India ink or indocyanine green (ICG), and endoscopic metal clipping [1–4].

Although tattooing with India ink or ICG is frequently performed, its safety and efficacy are unclear [4–7]. India ink is a foreign substance that comprises diverse hazardous materials (such as ethylene glycol, phenol, shellac, and gelatin) that can cause adverse events [8–12]. In addition, the availability of recently developed endoscopic markers such as pre-sterilized and diluted India ink (Endomark™) or non-India-ink-based pure carbon particle product (Spot™), which does not contain phenols, shellacs, or ammonia is limited in Republic of Korea.

In one retrospective study, Lee et al. used patients' own blood for the colonic localization showing 92% (23/25) of

✉ Jun-Won Chung
junwonchung@daum.net

[1] Department of Internal Medicine, Gachon University Gil Medical Center, Gachon University College of Medicine, 21, Namdong-daero 774 beon-gil, Namdong-gu, Incheon 21565, Republic of Korea

[2] Department of Surgery, Gachon University Gil Medical Center, Gachon University College of Medicine, Incheon, Republic of Korea

successful detection rate by laparoscopy [13]. The current study compares success rates and complications of endoscopic tattooing method involving the use of autologous blood for preoperative colorectal tumor localization with those using India ink at a single tertiary medical center and the causes of the autologous blood tattooing failure were elucidated.

Methods

Patients

From February 2016, all patients who required localization of a target lesion before colorectal surgery at a single tertiary medical center underwent endoscopic tattooing using autologous blood, and the outcomes were collected prospectively. A total of 51 consecutive patients who underwent autologous blood tattooing between February 2016 and June 2017 were included in this study. As a comparison, the medical records of a further consecutive 51 patients who underwent endoscopic tattooing using India ink between January 2014 and February 2016 were retrospectively reviewed. The indications for colorectal surgery included large lateral spreading tumors that could not be treated endoscopically, malignant colorectal tumors, and malignant polyps treated endoscopically that required additional colorectal resection. The exclusion criterion was a previous history of gastrointestinal surgery that altered the gastrointestinal anatomy. All surgeries were elective. All patients in the autologous blood tattooing group underwent preoperative colonoscopy before elective surgery and received mechanical bowel preparations. Endoscopic tattooing was performed 24–48 h prior to surgery in most patients, with the exception of two patients who required additional preoperational evaluation and management for reasons not related to tattooing. The surgical outcomes and adverse events of these patients were reviewed retrospectively. This study was approved by the Institutional Review Board (IRB No. GAIRB2016-367).

Tattooing procedure

Endoscopic tattooing was performed at a single tertiary medical center according to a modification of a previously reported tattooing procedure using India ink or autologous blood by five gastroenterologists with at least 5 years of therapeutic endoscopy experience each. In the India ink group, commercially available autoclave sterilized India ink was injected at two to four sites (at the discretion of the endoscopist) 1–2 cm proximal or distal to the target lesion in a circumferential manner using a conventional endoscopic needle after normal saline bleb formation to avoid perforation or intraperitoneal spillage with or without endoscopic clipping. In the autologous blood group, before preoperational colonoscopy, an intravenous catheter was inserted to obtain blood. Because the blood was not prepared to prevent coagulation, autologous venous blood was sampled after identification of the lesion by endoscopy. Immediately after blood sampling, 2–3 ml of blood were injected into one site and 2–3 other sites at the discretion of endoscopists. As a result, 6–12 ml of autologous blood were injected submucosally at three to four sites circumferentially around the target lesion using a conventional endoscopic needle without submucosal injection of normal saline. Two endoscopic clips were applied on the assumption that the autologous blood tattoo may not be visible in some patients (Fig. 1). None of the patients were administered prophylactic antibiotics prior to the tattooing procedure.

Primary endpoint assessment

As a retrospective study based on prospectively collected data, the primary outcome was visibility of tattooing in the peritoneal cavity during surgery (Fig. 2). The visibility

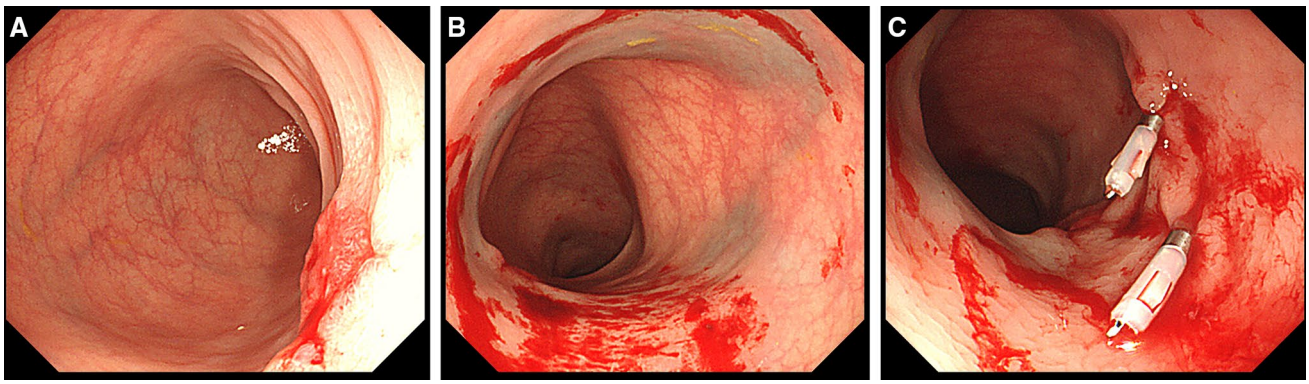

Fig. 1 Endoscopic autologous blood tattooing procedure. **A** A flat target lesion was observed at the colonoscopy. **B** Endoscopic autologous blood tattooing was performed. **C** Additional metal clips were applied

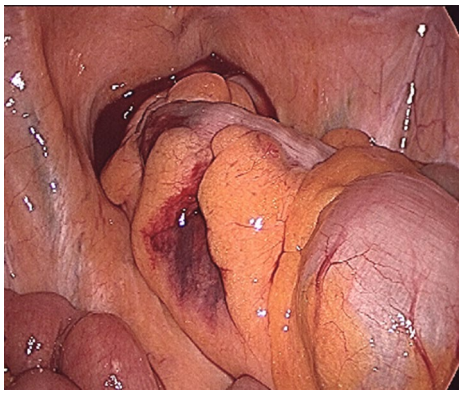

Fig. 2 Intraoperative finding of target lesion with autologous blood tattoo

of tattooing with autologous blood and the reason for the non-visibility of the tattoo were prospectively collected by the surgeon who has more than 20 years of experience in colorectal surgery with more than 100 cases per year. The visibility of tattooing with India ink was assessed by reviewing medical records retrospectively. The tattooing with India ink was considered to be invisible if there were records about the invisibility in the surgical report or if there was a need for the intraoperative colonoscopy. Other adverse events related to endoscopic tattooing, such as abscess formation, peritonitis, perforation, post-tattoo fever, post-tattoo abdominal pain, and intraperitoneal spillage of tattooing agent, were evaluated in both groups.

Statistical analysis

The primary objective of this study was to show non-inferiority of autologous blood tattooing compared with conventional dye tattooing. Based on previous studies which reported the rates of successful visualization of endoscopic tattooing ranging from 70 to 97%, success rates of 97 and 92% were assumed for the India ink group and the autologous blood group, respectively [1, 6, 13]. With settings of 90% power, 5% significance level, and non-inferiority margin of 20%, sample sizes of 40 patients in each group were required [14]. Statistical analyses were performed using SPSS for Windows version 23.0 (IBM Corporation, Armonk, NY, USA). Continuous variables are presented as means and standard deviations for normally distributed variables or medians with ranges for non-normally distributed variables. Categorical variables are given as numbers and percentages. To compare descriptive variables between groups, the χ^2 test was used for categorical variables and the *t* test or Mann–Whitney U test was used for continuous variables with a two-sided significance level of $p < 0.05$.

Results

Baseline characteristics

In the India ink group, there were 34 (66.7%) male patients with a mean age of 64 years. Fifteen patients were classified as obesity I (body mass index ≥ 25 kg/m^2 according to the World Health Organization Asia-Pacific guideline) and 4 patients were assigned to obesity II (≥ 30 kg/m^2) [15]. The most frequent indication for surgery was adenocarcinoma (94.1%) and the most frequent site of tattooing was the sigmoid colon (43.1%). In the autologous blood group, there were 36 (70.6%) male patients with a mean age of 66 years. In total, 22 patients were classified as obesity I and 4 were classified as obesity II. There were 46 (90.2%) patients with adenocarcinoma and five (9.8%) patients with large lateral spreading tumors. The most frequent site of tattooing was the sigmoid colon (33.3%). The median interval between tattooing and surgery was 1 day. There were two patients with liver cirrhosis, one of whom also had thrombocytopenia (platelet count < 100,000/μl). There were no significant differences in medical history, type of colorectal neoplasm, and tattooing site between the groups; however, the interval between tattooing and surgery differed significantly ($p < 0.001$; Table 1).

Most of the included patients (74.5%) were referred to our hospital after pathologic diagnosis by endoscopic biopsy or polypectomy. None of the patients who underwent polypectomy at our hospital had complications such as abdominal pain or fever, and patients who had undergone polypectomy at other hospitals before the referral to our institution were symptom-free at the time of initial visit for the tattooing and surgery. There were no emergent or unplanned admissions related to the polypectomy.

Clinical outcomes

Of the 51 patients in the India ink group, endoscopic tattooing was successfully identified in 49 (96.1%). Of the 51 patients in the autologous blood group, blood tattooing was identified in 47 (92.2%) (Table 2). Among the four patients in whom the autologous blood tattoo was not identified, in a patient who had liver cirrhosis and thrombocytopenia, it was impossible to detect the blood tattoo because multiple intra-abdominal hematomas had developed during the manipulation of the laparoscope due to a bleeding tendency. In the second patient, the tattoo was covered by excessive peritoneal fat. In the third patient, the tattoo was invisible due to a 270° malrotation of the sigmoid colon and severe peritoneal adhesion not related to the tattooing procedure; however, the blood

Table 1 Baseline characteristics

Variables	India ink ($n=51$)	Autologous blood ($n=51$)	p value
Male, *n* (%)	34 (66.7)	36 (70.6)	0.831
Age, mean (SD), years	64 (9)	66 (11)	0.461
Past medical history			
Obese I (≥ 25 kg/m^2), *n* (%)	15 (29.4)	22 (43.1)	0.353
Obese II (≥ 30 kg/m^2), *n* (%)	4 (7.8)	4 (7.8)	
DM, *n* (%)	12 (23.5)	15 (29.4)	0.654
HTN, *n* (%)	22 (43.1)	31 (60.8)	0.112
LC, *n* (%)	2 (3.9)	2 (3.9)	> 0.999
CRF, *n* (%)	0 (0)	1 (2.0)	> 0.999
Thrombocytopenia, *n* (%)	1 (2.0)	1 (2.0)	> 0.999
Type of colorectal neoplasm, *n* (%)			0.715
Adenocarcinoma	48 (94.1)	46 (90.2)	
Adenoma	3 (5.9)	5 (9.8)	
Tattoo site, *n* (%)			0.796
Ascending colon	4 (7.8)	3 (5.9)	
Hepatic flexure	3 (5.9)	5 (9.8)	
Transverse colon	5 (9.8)	4 (7.8)	
Splenic flexure	0 (0)	1 (2.0)	
Descending colon	4 (7.8)	3 (5.9)	
Sigmoid colon	22 (43.1)	17 (33.3)	
Rectosigmoid junction	10 (19.6)	11 (21.6)	
Upper rectum	3 (5.9)	7 (13.7)	
Patients treated with polypectomy before OP, *n* (%)	15 (29.4)	28 (54.9)	0.016
Time interval between polypectomy and endoscopic tattooing, median (range), days	20.5 (0–84)	27 (2–175)	0.236
Time interval between tattooing and OP, median (range), days	3.5 (1–39)	1 (0–5)	< 0.001

SD standard deviation, *DM* diabetes mellitus, *HTN* hypertension, *LC* liver cirrhosis, *CRF* chronic renal failure, *OP* operation

Table 2 Clinical outcome and complications

Variables	India ink ($n=51$)	Autologous blood ($n=51$)
Successful tattooing, *n* (%)	49 (96.1)	47 (92.2)
Complications, *n* (%)	7 (13.7)	3 (5.9)
Peritonitis	3 (5.9)	1 (2.0)
Abscess formation	1 (2.0)	0 (0)
Abdominal pain alone	1 (2.0)	0 (0)
Spillage alone	1 (2.0)	2 (3.9)
Fever alone	1(2.0)	0 (0)

tattoo was identified in the resected specimen. Suboptimal tattooing technique was suspected in the fourth patient. Of the 51 patients in the autologous blood group, an unplanned conversion to open surgery was required in one patient (Table 3).

Adverse events related to the tattooing procedure

In the India ink group, seven (13.7%) patients experienced adverse events related to the tattooing procedure or agent, including intraperitoneal spillage, perforation, peritonitis, abscess formation, post-tattooing fever, and post-tattooing abdominal pain. By contrast, three (5.9%) patients in the autologous blood group experienced adverse events. Two of these were intraperitoneal spillage of blood, and the other was minimal inflammation at the tattooing site; no patient in the autologous blood group experienced perforation, colonic abscess formation, or post-tattooing fever (Table 2).

Discussion

Data regarding endoscopic tattooing with autologous blood for colorectal surgery are limited, and to the best of our knowledge, this study is the largest comparison study published to date that evaluates autologous blood tattooing [13]. In this retrospective study based on prospectively collected

Table 3 Summary of patients with autologous blood tattoo detection failure

No.	Sex	Age	BMI	Indication	Tattooing site	Surgical plan	Unintentional conversion to open surgery	Failure reason	Management after autologous blood tattoo detection failure
1	M	75	25	Adenocarcinoma	Sigmoid colon	Laparoscopic AR	No	Bleeding tendency	Laparoscopic AR with clipping detection
2	M	51	30	Adenocarcinoma	Upper rectum	Laparoscopic AR	No	Peritoneal fat	Laparoscopic AR with Intra OP colonoscopy
3	M	52	29	Adenocarcinoma	Sigmoid colon	Laparoscopic AR	Yes	Congenital anomaly	Conversion to open surgery
4	M	81	28	Adenocarcinoma	Splenic flexure	Transverse colectomy	No	Inadequate tattooing	Preplanned open surgery with clipping detection

No. case number, *M* male, *F* female, *BMI* body mass index, *AR* anterior resection, *OP* operation

data, we found that endoscopic tattooing with autologous blood facilitated accurate localization of colonic lesions during surgery in 92.2% of patients, which was competitive with India ink. There were no serious adverse events (such as perforation, abscess formation, or severe peritonitis causing adhesion) related to the tattooing agent. In addition, because this technique does not require a specialized tattooing agent or equipment to detect the target lesion, it is simple, practical, and can be used worldwide irrespective of the availability of tattooing agents or specialized detection equipment.

Endoscopic tattooing is standard for colorectal surgery. However, agents other than India ink or carbon-based materials (e.g., ICG, methylene blue, and indigo carmine) are not long lasting, which makes them inappropriate for localization [16–18]. In one study, tattooing using pure carbon-based tattooing agent enabled the identification of the target lesion in 97% of cases, with a low incidence of adverse events [1]. However, because recently developed carbon-based tattooing agents are expensive and their availability is limited, India ink is still the most frequently used agent [19–21].

Nevertheless, in previously published data using India ink, complications associated with India ink tattooing vary from 0.22 to 14.3% including tattooing agent spillage [21–23]. Intraperitoneal spillage of tattooing agent is not uncommon and can cause the staining of the operation field. In addition, because India ink is an aggregate of unsterilized foreign material, inadequate sterilization or inflammatory reaction to the foreign materials could lead to adverse events such as focal fat necrosis, abscess formation, or chemical peritonitis, which can be underdiagnosed clinically [8, 12, 24–26]. Relative high incidence of post-tattooing fever and focal peritonitis in the India ink group of our study can be explained by these reasons.

On the other hand, intraperitoneal spillage of autologous blood results in less staining of the operation field and milder peritonitis. In our study, two patients experienced intraperitoneal spillage of tattooing blood, of them, there was no evidence of peritonitis or inflammation. We were unable to assess the statistical superiority of autologous blood over India ink in terms of the incidence of tattooing-related adverse events due to the limited number of patients. However, the low incidence of clinically relevant adverse events in autologous blood group suggests the safety and feasibility of autologous blood as a tattooing agent (Table 2).

In our study, target lesion localization by autologous blood tattooing failed in four patients. In one of these patients, who had a history of liver cirrhosis and thrombocytopenia, it was impossible to discriminate the blood tattoo from multiple intraperitoneal hematomas that developed due to manipulation of the laparoscope. In the second patient, who had a BMI ≥ 30 kg/m^2, it was impossible to detect the intended tattooing site because the lesion was covered by intraperitoneal fat. In this case, endoscopic metal clipping with fluoroscopy would likely have been more effective. This case suggests that the results of this study may not be generalizable in a population with higher proportions of obese patients. In the third patient, who had malrotation of the sigmoid colon and intraperitoneal adhesion, conversion to open surgery was required because of the limited visualization of the operating field. In the fourth patient, it was impossible to detect the tattoo at the serosal surface but the tattooing blood was visible on the luminal side wall of the specimen. In this patient, we presume that injection of an inadequate amount of blood or shallow injection of blood resulted in the detection failure which was referred as "suboptimal tattoo due to poor tattooing technique" based on previous literature [27]. Although intraoperative colonoscopy might be a good candidate rescue method to detect autologous blood tattoo in these cases with intraperitoneal tattoo detection failure, intraoperative colonoscopy was performed in only

one patient in this study and we could not validate the efficacy of intraoperative colonoscopy as a rescue method.

We could not identify risk factors for failure to detect the blood tattoo because of the limited incidence of detection failure. However, because the congenitally abnormal rotation of the colon with adhesion cannot be estimated prior to surgery, the selection of patients without severe obesity and bleeding tendencies, together with the injection of an adequate amount of blood at a sufficient depth, would increase the success rate of autologous blood tattooing.

According to Botoman et al., perpendicular needle insertion to an 8 mm depth is sufficient for the full penetration of the colon wall. By contrast, the insertion of the needle at 45° to a 5 mm depth reaches only the submucosa [10]. Because it is not possible to constantly adjust the angle between the needle and colon wall during endoscopic procedures, it is challenging for endoscopists to maintain the angle and depth of needle insertion. However, based on our data, because intraperitoneal blood spillage did not cause peritonitis or adhesions, deeper penetration than tattooing using conventional agents can be feasible and might improve the visibility of the autologous blood tattoo.

This study was limited by its retrospective design and the limited number of patients, which prevented the determination of the superiority of autologous blood tattooing with regard to other localization methods. Because commercially available agents such as SPOT™ (GI Supply, Camp Hill, PA, USA) or Endomark™ (PMT corporation, Chanhassen, MN, USA) have not been approved by Ministry of Food and Drug Safety of Republic of Korea yet, we could not conduct a randomized controlled study.

Another limitation is that because most patients in the autologous blood group underwent surgery within 48 h, we could not estimate the time for which the blood tattoo remains visible. In our study, the longest interval between endoscopic blood tattooing and surgery was 5 days, and in this case the tattoo was successfully identified during surgery. Because many of the previously studied localization agents (including methylene blue, indigo carmine, toluidine blue, and ICG) other than permanent tattooing agent are absorbed and disappear within 48 h, autologous blood can be a good candidate agent for its persistence [16]. Still, because there is no prospective data that can estimate the persistency period of autologous blood yet, it is advisable to perform surgery within a relatively short period of time. Because of this indispensable requirement, preoperative colonoscopy is mandatory for the tattooing with autologous blood. Colonic localization with permanent markers at the initial colonoscopic diagnosis and additional preoperative colonoscopy with autologous blood tattooing should be considered for the cost-effectiveness analysis in the further work.

In addition, although 6–12 ml autologous blood was used for the tattooing in this study, because there is no standardization of this technique using autologous blood yet, the optimum volume and technique should be determined in further work. Further prospective randomized control studies for the efficacy and safety of tattooing with autologous blood, and other materials and techniques are required. Ideally, definite indications or guidelines for each localization method are needed.

In conclusion, preoperative endoscopic tattooing using autologous blood within maximal interval of 5 days facilitates the localization of target lesions for surgery and has an excellent safety profile, particularly in patients without a bleeding tendency.

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Compliance with ethical standards

Disclosures Eui Joo Kim, Jun-Won Chung, Su Young Kim, Jung Ho Kim, Yoon Jae Kim, Kyoung Oh Kim, Kwang An Kwon, Dong Kyun Park, Duck Joo Choi, Sung Won Park, Jeong-Heum Baek, and Won-Suk Lee have no conflicts of interest or financial ties to disclose.

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