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ORIGINAL ARTICLE

Sterile carbon particle suspension vs India ink for endoscopic tattooing of colonic lesions: a randomized controlled trial

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Abstract

Background Different markers have been used preoperatively to mark colonic lesions, especially India ink. In recent years, another kind of marker has been developed: sterile carbon particle suspension (SCPS). No comparison between these two markers has yet been made. The aim of the present study was to compare the pyrogenic, inflammatory and intraperitoneal effect of these two markers.

Methods From September 2015 to December 2018, adult patients who were candidates for elective laparoscopic colon resection were randomized to the SCPS or conventional India ink injection group using computer-based randomization. The primary endpoint of the study was the presence of intraoperative adhesions related to the endoscopic tattoo. Secondary endpoints were differences in white blood cell, C-reactive protein, and fibrinogen levels as well as, abdominal pain and body temperature at baseline (before endoscopic tattooing) and 6 and 24 h after colonoscopy. Finally, the visibility of the tattoo during the minimally invasive intervention was assessed.

Results Ninety-four patients were included in the study, 47 for each arm. There were 45/94 females (47.9%) and 49/94 males (52.1%), with a median age of 67.85 ± 9.22 years. No differences were found between groups in WBC, fibrinogen levels, body temperature or VAS scores, but we documented significantly higher CRP values at 6 and 24 h after endoscopic tattooing with India ink injection. There were significantly fewer adhesions in the SCPS Endoscopic Marker group. All the endoscopic tattoos were clearly visible.

Conclusions SCPS is an effective method for tattooing colonic lesions and has a better safety profile than traditional India ink in terms of post-procedure inflammatory response and intraoperative bowel adhesions.

Clinical trial registration clinicaltrials.gov (ID: NCT03637933).

Keywords Colon · Cancer · Tattooing · Endoscopy · Sterile carbon particle suspension · India ink

Introduction

Preoperative endoscopic tattooing is commonly used to correctly identify the location of a colonic lesion [1]. The need to mark a lesion has increased with the advent of minimally invasive surgery, especially in case of flat, small or previously excised lesions [2]. Different substances have been used to mark colonic lesions, especially India ink. Recently, another kind of marker has been developed: sterile carbon particle suspension (SCPS). No comparison between these two markers has yet been published.

The aim of our study was to compare the pyrogenic, inflammatory and intraperitoneal effect of India ink and SCPS.

Materials and methods

Study design

This study was a single-centre, interventional randomized controlled trial conducted at Federico II University,

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Naples and designed to compare the effect of two types of endoscopic markers, India ink and SCPS. This trial was registered on clinicaltrials.gov (ID: NCT03637933).

Participants

After approval of the study by the Ethics Committee of Federico II University, all consecutive patients ≥ 18 years of age with a histologically confirmed colonic malignancy who were scheduled to have elective, segmental, minimally invasive colectomy were recruited. A written informed consent was obtained from all the study participants before enrollment in the study.

Exclusion criteria were as follows: open surgery, inflammatory bowel disease, previous abdominal surgery or presence of severe systemic disease that contraindicate minimally invasive surgery. Moreover, patients in whom peritoneal distribution of the ink (spillage) was observed intraoperatively were excluded from the study.

Randomization

From September 2015 to December 2018, patients were randomized to the SCPS or conventional India ink injection group using computer-based randomization with sealed envelopes after written informed consent had been obtained in the outpatient clinic.

The experimental group included patients who had had preoperative endoscopic tattooing with SCPS-based ink (Black Eye®, The Standard Co., Ltd., Seoul, Korea). The control group included patients who had had preoperative endoscopic tattooing with India ink.

Patients were equally randomized (1:1 allocation) to one of the two groups. The endoscopists and surgeons were blinded to the marker allocation.

Study interventions

All the tattooing was performed by an expert endoscopist (at least 500 colonoscopies per year). The needle was inserted obliquely at 45° to confine the injection to the submucosa. The tattoo was placed 1 or 2 cm distal to the lesion and tattooing was performed in at least two of the four quadrants of the bowel. After 0.5–1 mL saline was injected to raise a submucosal bleb, a similar quantity of India ink or SCPS was injected.

A blood sample was taken from all patients in both groups and body temperature was measured at the following time points; pre-tattooing (time T0) and 6 and 24 h post-colonoscopy (times T1 and T2). The blood collected was used to analyse C-reactive protein (CRP), white blood cell count (WBC) and fibrinogen levels. Abdominal pain was also measured using a visual analogue scale (VAS) score before tattooing and 6 and 24 h after colonoscopy (time T0, T1 and T2).

Laparoscopic colectomy was performed between 3 and 30 days after endoscopic tattooing by an expert surgeon (at least 50 laparoscopic colectomies). During the procedures, the surgeon evaluated the visibility of the tattoo and the presence of tattooing-related adhesions, according to the Zühlke classification [3] (Table 1).

Outcome measures

The primary endpoint of the study was the presence of intraoperative adhesions related to the endoscopic tumour marking system.

Secondary endpoints were variations in WBC, CRP, and fibrinogen levels, abdominal pain and body temperature before endoscopic tattooing and at 6 and 24 h after colonoscopy.

Finally, the visibility of the tattoo during the minimally invasive intervention was assessed.

Sample size calculation

Since there are no high-level methodological level studies, randomized trials or meta-analyses about adhesions (from grade 1 to grade 4) after endoscopic tumour marking, to evaluate the sample size, we had to use the incidence recorded in our centre (34%).

To design a study with a 70% or greater minimal predefined reduction of adhesions (grade 1) with the SCPS marker compared with conventional India ink injection, at least

Table 1 Classification of adhesions according to Zühlke et al

Grade 0	No adhesions or insignificant adhesions
Grade 1	Adhesions that are filmy and easy to separate by blunt dissection
Grade 2	Adhesions where blunt dissection is possible, but some sharp dissection necessary beginning vascularization
Grade 3	Lysis of adhesions possible by sharp dissection only, clear vascularization
Grade 4	Lysis of adhesions possible by sharp dissection only, organs strongly attached with severe adhesions, damage of organs hardly preventable

47 participants in each study arm were needed to achieve greater than 80% power with a 5% α error.

Statistical analysis and data extraction

Statistical analysis was performed using the SPSS 20 system (SPSS Inc., Chicago, IL, USA). Continuous data were expressed as mean ± standard deviation (SD), and categorical variables were expressed as % changes. The Chi-square test was used to analyse categorical data, and the Mann–Whitney test was used to analyse continuous variables. All results are presented as two-tailed values with statistical significance defined as p values < 0.05. For each patient, data regarding age, sex, body mass index (BMI), American Society of Anaesthesiologists (ASA) score, tumour localization, WBC, CRP, fibrinogen, body temperature, VAS score and adhesion stage were extracted by a blinded researcher.

Results

Figure 1 shows the enrollment flow diagram according to the Consolidated Standards of Reporting Trials (CONSORT) statement. One hundred and two patients, who were scheduled for laparoscopic resection for colon cancer after endoscopic lesion tagging, were eligible for randomization and were subsequently randomized. Eight patients were excluded for the following reasons: two patients refused to participate in the study, in five patients, spillage of the endoscopic marker or ink was found intraperitoneally, and one patient needed emergency open surgery for intestinal obstruction. Therefore, 94 patients were included in the study. Forty-seven patients were randomized to the SCPS group and 47 patients were randomized to the conventional India ink injection group.

There were 45/94 females (47.9%) and 49/94 males (52.1%), with a median age of 67.85 ± 9.22 years. The mean BMI was 25.73 ± 4.93 kg/m^2 and the mean ASA score was 2.45 ± 0.66. The majority of tumours were located in the sigmoid colon ($n = 41$), while hepatic flexure was the least common location ($n = 6$). When the two groups were compared, they were homogeneous with respect to gender ($p = 0.836$), age ($p = 0.525$), BMI ($p = 0.530$) and ASA score ($p = 0.744$) (Table 2).

Table 3 shows no significant differences in the study population with respect to variations of WBC count, and CRP, fibrinogen levels, or in the VAS score and body temperature at all the time points (T0, T1, T2). However, the India ink injection group had significantly higher CRP levels at T1 (2.57 ± 0.67 vs 4.13 ± 1.47, $p = 0.001$) and T2 (2.22 ± 0.87 vs 3.38 ± 1.47, $p = 0.001$) after the endoscopic tattooing procedure.

With respect to adhesions, no patient in either groups developed grade 3 or 4 adhesions (Zuhlke classification). There were significantly fewer stage I and stage II adhesions in the SCPS group than in the India ink group ($p = 0.009$) (Table 4).

No difference was found between the groups with respect to endoscopic tattoo visibility. The tattoos were clearly visible in all cases.

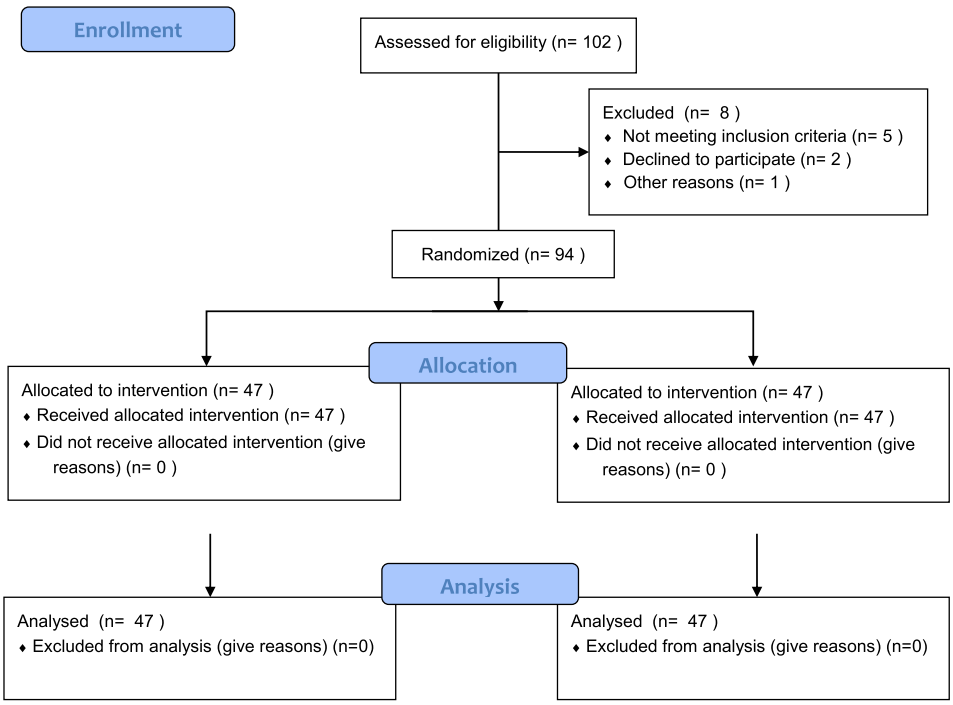

Fig. 1 CONSORT flow diagram of the study

Table 2 Demographic characteristics of patients

Characteristics	All patients ($n=94$)	SCPS ($n=47$)	India ink injection ($n=47$)	p value
Male (n, %)	49/94 (52.1%)	25/47 (53.2%)	24/47 (51.1%)	0.836
Age, years (mean ± SD)	67.85 ± 9.22	67.40 ± 9.12	68.31 ± 9.39	0.525
BM,Ikg/m^2 (mean ± SD)	25.73 ± 4.93	25.44 ± 5.36	26.03 ± 4.51	0.530
ASA score (mean ± SD)	2.45 ± 0.66	2.37 ± 0.70	2.53 ± 0.63	0.744
Tumour localization (mean ± SD)	4.38 ± 1.9	4.36 ± 1.9	4.40 ± 1.8	0.893
Right colon	15 (16%)	8 (17%)	7 (14.9%)	–
Hepatic flexure	6 (6.4%)	3 (6.4%)	3 (6.4%)	–
Transverse colon	7 (7.4%)	3 (6.4%)	4 (8.5%)	–
Splenic flexure	7 (7.4%)	3 (6.4%)	4 (8.5%)	–
Left colon	18 (19.1%)	10 (21.2%)	8 (17%)	–
Sigma	41 (43.6%)	20 (42.5%)	21 (%)	

SCPS sterile carbon particle suspension, *BMI* body mass index, *ASA* American Society of Anesthesiologists

Table 3 Inflammatory response to endoscopic tumour marking system

Parameters	All patients ($n=94$)	SCPS ($n=47$)	India ink injection ($n=47$)	p value
Baseline				
WBC (mean ± SD)	5.54 ± 0.82	5.57 ± 0.83	5.51 ± 0.83	0.548
CRP (mean ± SD)	1.09 ± 0.41	1.07 ± 0.49	1.11 ± 0.32	0.576
Fibrinogen (mean ± SD)	272.31 ± 52.01	273.65 ± 55.02	270.97 ± 49.37	0.553
Body temperature (mean ± SD)	36.40 ± 0.18	36.40 ± 0.12	36.40 ± 0.22	0.767
VAS score (mean ± SD)	0.17 ± 0.38	0.13 ± 0.34	0.21 ± 0.41	0.275
At 6 h from tattooing				
WBC (mean ± SD)	6.68 ± 1.07	6.79 ± 1.28	6.56 ± 0.80	0.883
CRP (mean ± SD)	3.35 ± 1.38	2.57 ± 0.67	4.13 ± 1.47	0.001
Fibrinogen (mean ± SD)	311.17 ± 55.70	319.79 ± 57.46	302.56 ± 53.11	0.081
Body temperature (mean ± SD)	36.56 ± 0.20	36.57 ± 0.21	36.56 ± 0.19	0.942
VAS score (mean ± SD)	0.79 ± 0.97	0.94 ± 1.07	0.64 ± 0.85	0.182
At 24 h from tattooing				
WBC (mean ± SD)	6.20 ± 1.17	6.31 ± 1.49	6.09 ± 0.74	0.149
CRP (mean ± SD)	2.80 ± 1.34	2.22 ± 0.87	3.38 ± 1.47	0.001
Fibrinogen (mean ± SD)	300.06 ± 53.55	309.11 ± 58.44	291.00 ± 47.07	0.137
Body temperature (mean ± SD)	36.53 ± 0.18	36.51 ± 0.18	36.55 ± 0.17	0.304
VAS score (mean ± SD)	0.22 ± 0.49	0.23 ± 0.52	0.21 ± 0.46	0.960

SCPS sterile carbon particle suspension, *WBC* white blood cells, *CRP* c-reactive protein, *VAS* visual analogue scale

Discussion

Accurate tumour localization is essential for colorectal resection in colorectal cancer, especially for early cancer or previously resected polyps [2]. The need to correctly localize a lesion has increased with the advent of minimally invasive approaches, in which the inability of the surgeon to palpate the colon during the intervention makes localization of the tumour more difficult, increasing the possibility of resection of a wrong segment of the colon [4].

For this reason, endoscopic application of a tattoo adjacent to the tumour can improve tumour identification and can permit a correct minimally invasive colorectal resection. Thus, colonoscopic tattooing is considered to be the gold standard for tumour localization prior to minimally invasive surgery [5–7]. The most commonly used agent is India ink, that creates a long-lasting tattoo on the colonic wall [8, 9]. The use of endoscopic India ink marker for the tattooing of a colonic lesion was first described by Ponsky and King in 1975 [10]. They marked ten lesions with a non-sterile India ink preparation, without adverse effects. India ink has been

Table 4 Adhesions in the two study groups

Characteristics	All patients ($n=94$)	SCPS ($n=47$)	India ink injection ($n=47$)	p value
Adhesion score (mean ± SD)	0.33 ± 0.59	0.17 ± 0.43	0.49 ± 0.69	0.009
Adhesion stage 0	69 (73.4%)	40 (85.1%)	29 (61.7%)	–
Adhesion stage 1	19 (20.2%)	6 (12.8%)	13 (27.6%)	–
Adhesion stage 2	6 (6.4%)	1 (2.1%)	5 (10.7%)	–
Adhesion stage 3	0	0	0	–
Adhesion stage 4	0	0	0	–

SCPS sterile carbon particle suspension

used by endoscopists for the past 30 years to mark colonic lesions [11, 12].

Although the use of India ink is considered safe and feasible, several complications were described during the past decades. The complications reported are peritonitis, covered perforation, and inflammatory pseudotumour [13–15]. However, the incidence of these complications still remains below 1% [16–19].

It is important to underline that many of these complications seem not to be related to India ink per se, but to compounds used to stabilize the suspension a promote a smooth flow. These include ethylene glycol, ammonium hydroxide, sodium tetraborate, propylene glycol, surfactant and gelatines [20].

Thus, to prevent these complications, a technique involving sterilization or dilution of the India ink before injection was developed, even though the techniques remain cumbersome. In recent years, a marker using SCPS was developed. It is a specially formulated, biocompatible agent designed for endoscopically marking lesions in the gastrointestinal tract. This kind of marker is a suspension containing highly purified, sterile, pre-packaged, very fine carbon particles.

To the best of our knowledge, this is the first study which compared the well-established endoscopic India ink tattooing method with the new SCPS marker.

There are few studies in the literature which analysed tissue responses to tattoo agents and their clinical implications. One of the earliest reports, by Lane et al. [20], demonstrated that India ink injections cause a pronounced acute inflammatory reaction with vasculitis and consequent foci of mucosal and submucosal necrosis, and later (after > 6 weeks) can cause an extensive and dense fibrosis, due to fibroblastic proliferation, of the submucosa as well as the muscularis propria.

Similarly, Hammond and colleagues [21] showed that, in comparison with other tattoo agents, India ink produces a much more pronounced and significant inflammatory reaction with microhemorrhage and thrombosis. In biopsy specimens taken from tattoo marks at post-polypectomy surveillance colonoscopy, mild chronic inflammation was seen in 6/56 (10.7%) patients and hyperplastic changes in 1/56 (1.7%) patients [16].

In the present series, patients in the SCPS group had significantly less grade 1 and 2 adhesions, according to the Zühlke score than patients in the India ink group. This score is based on tenacity and some morphologic features of adhesions, without covering the extent of adhesions. The recently introduced Peritoneal Adhesion Index integrates tenacity and extent of adhesion in a single score, but it is more complex to calculate [22]. According to these findings and to its self-explanatory nature, the Zühlke score is still the most frequently used by the majority of surgeons and gynaecologists as shown by the recent evidence-based guidelines of the Word Society of Emergency Surgeons [23]. The higher incidence of adhesions in the SCCP group is of clinical relevance, since it could result in an increase of operating time and possibly of intraoperative complications. Moreover, a case of adhesion ileus caused by tattoo-marking has been reported by Yano and colleagues [24]: in a patient who had a laparoscopic-assisted partial transverse colectomy for early colon cancer, the small intestine strongly adhered to India ink stuck on the mesentery.

The different components of India ink solution, associated with its lack of its sterility and with the inoculation of intestinal flora into an injection site were suspected to play a role in the development of complications. This does not seem to be true of SCPS. Askin et al. [25] described that, for 113 patients who had endoscopic tattooing with SCPS and surgery within 69 days after injection, there was no inflammation or fibrosis identified in the resection specimen.

Our results clearly confirm what has been previously reported in the literature: in a homogeneous population with colon cancer, an endoscopic tattoo with India ink determines a higher inflammatory tissue response attested by a rise in CRP levels 6 and 24 h after the procedure. This could explain the different amount of bowel adhesions in the two groups that we found during surgery.

Considering that we excluded from the study all the cases of spillage, eliminating a possible source of bias, it is evident

that the differences in terms of inflammatory reaction can derive only from the marker used.

Furthermore, the presence of significantly more tenacious adhesions in the group treated with India ink, could be linked to the presence of microscopic phenomenon of necrosis and ischemia which have already been described in connection with India ink tattooing [15, 26].

Conclusions

Our results show that SCPS is an effective colonic tattoo-marker with a better safety profile than traditional India ink in terms of post-procedure inflammatory response and intra-operative bowel adhesions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by Ethics Committee of Federico II University.

Informed consent A written informed consent was obtained from all the study participants before enrollment in the study.

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