Title:
WAYS TO PERFORM AN ENDOSCOPIC TATTOO. PROSPECTIVE AND RANDOMIZED STUDY IN PATIENTS WITH COLORECTAL NEOPLASM

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Ways to perform an endoscopic tattoo. Prospective and randomized study in patients with colorectal neoplasm

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ABSTRACT
Background and aims: intraoperative identification of colonic lesions previously detected via colonoscopy may be difficult. Endoscopic tattooing facilitates identification, but there is no evidence regarding which is the best tattoo technique. The goal of the study was to describe the efficacy and safety of endoscopic tattooing and to detect technical and clinical factors associated with its efficacy.

Patients and methods: a prospective and randomized study was performed. All tattoo candidate patients were included prior to surgery and randomized into four groups;
Multiple variables were registered.

**Results:** one hundred and ninety-five patients were included with an endoscopic tattoo and who subsequently underwent a surgical intervention, the mean age was 70.1 years and 67.2% were male. The laparoscopic approach was applied in 57.9% of cases. The intraoperative visibility of the endoscopic tattoo was 89.7% and 30% of rectal lesions were not visible. Excluding the rectum, the marking was visible intraoperatively in 92% of patients, without significant differences according to the surgical approach, the type of marking or any of the variables collected. The tattoo was safe in 92.3% of the cases. The adverse effect rate was 7.7% and none of the complications were clinically significant. There were no significant differences between any variables collected in relation to adverse effects.

**Conclusions:** endoscopic colon tattoo is safe and effective regardless of the technique used. We recommend the technique of two injection points and 1 ml of marking volume for its simplicity, efficiency and safety.

**Keywords:** Tattoo. Endoscopic Marker. Colorectal Neoplasia. Laparoscopy

**INTRODUCTION**

Intraoperative identification of a lesion previously detected via endoscopy is often difficult, particularly in laparoscopic surgery (1). The estimation of the location of an injury during colonoscopy may be incorrect in up to 14% of cases (1,2). The lack of precision in the identification of an injury during laparoscopic surgery can lead to unnecessary resection of an intestinal segment (3). In this sense, the endoscopic tattoo has great value and seems superior to other localization techniques, such as endoscopic clips or laparoscopic clips placed during an intraoperative endoscopy, among other techniques.

A wide variety of substances have been used for endoscopic tattooing, including Indian ink, methylene blue, indigo carmine, radiolabeled colloids and others. Several setbacks have limited their use such as a lack of permanence, infections or inflammatory reactions (4) or complex preparation in some cases. There are even reports of
perforation with the use of methylene blue (5). The appearance of a ready-to-use sterile carbon suspension (SCS) preparation (Spot® 5 ml syringes; GI Supply Camp Hill, Pa, United States) has managed to overcome these limitations by making the practice of endoscopic tattoo easier and more accessible. As a result, the indications of the endoscopic tattoo have increased. These include the marking of colonic neoplasms, areas of maximum exploration in enteroscopy, and polyps with a difficult location, partially resected or suspected of malignancy, among others (6,7).

Sterile carbon suspensions (SCSs) are not biologically inert and have been associated with significant clinical complications. This includes cases of peritonitis after a transmural injection with the resulting instillation of carbon particles to the peritoneum (8,9). Not surprisingly, in a study of 63 patients who had been tattooed with Spot®, peritoneal remnants were found during surgery in six cases (9.5 %) (9). In this sense, performing the injection with a maximum precision in the submucosa is recommended. It is advisable to insert the injection needle at an oblique angle (close to 0°) to the colon wall to avoid penetrating the serosa (10,11). Another possible complication of the use of SCS may occur if the tattoo is performed very close to the lesion, since carbon particles can spread through the submucosa at a remarkable distance. This may cause submucosal fibrosis making it difficult to perform (or even precluding the performance of) an endoscopic mucosal resection (EMR) or an endoscopic submucosal dissection (ESD) which, on the other hand, could have been indicated (8,12,13). Multiple studies have proven that endoscopic tattooing is generally a safe technique with a very low complication rate (8-13).

In the absence of an international consensus that guides the practice of endoscopic tattooing, there is great variability in the clinical practice (13). Although it is recommended to perform the tattoo about 3 cm distal to the distal part of the lesion, with a marking in line with the lesion and one or two more marking points on the opposite side of the lumen (8,14). In this sense, a study performed in 19 patients with a colonic lesion that was marked prior to surgery with Indian ink at a single point showed that the tattoo was only visible in 13 patients (68.4 %) intraoperatively and in 14 (73.6 %) in the surgical specimen (15). The authors concluded that at least two marking points should be performed when tattooing surgical colon lesions. With
regard to this technique, the injection of 0.5-1 ml (more usually 1 ml) of SCS is recommended after the creation of a submucosal cushion with physiological serum. This technique has been described in two different ways, the most conventional one consisting in the creation of a submucosal cushion with physiological serum and without removing the needle from the submucosa, replacing the serum syringe by the SCS syringe. After injecting the marker, the physiological serum syringe is again placed and the marker remaining in the injection catheter is dragged by injecting additional serum (16). In the second technique, called double injection, the physiological serum is first injected with a needle and then the SCS marker is injected with a different needle (17). The first technique is easier and more efficient than the second one.

In general, the evidence shows that the accuracy of the endoscopic tattoo to locate lesions varies between 70 and 100 %, with a false positive rate during surgery and the inability to detect the tattoo between 1.6-7 % and 1.6-15 %, respectively. Most cases of non-detection of the tattoo are explained by a superficial injection or by an injection in the mesenteric or retroperitoneal side of the intestine. The incidence of the intraperitoneal injection of the tattooing substance is between 2.4 % and 13 % (18).

Given the absence of a specific recommendation, the objective of this study was to determine the most effective way to perform an endoscopic tattoo with SCS injection, using the conventional technique of endoscopic injection, in colorectal neoplasms prior to surgery. In particular, the study aimed to evaluate the proportion of visible tattoos during surgery and in the surgical specimen, comparing the conventional injection in two versus three points and, independently, with a marking injection volume of 1 ml versus 1.5 ml. Two secondary objectives were to identify clinical factors associated with the non-location of the endoscopic tattoo and to assess the rate of complications related to the tattoo.

**MATERIAL AND METHODS**

The study was performed at the Hospital of Sant Joan Despí Moisès Broggi, Barcelona, covering an area of 400,000 inhabitants. The Endoscopy Service of the hospital is composed of 12 endoscopists with extensive experience and performs an annual average of colonoscopies between 7,000 and 8,000. All colonoscopies were performed
with CO₂ and under sedation by the Anesthesiology Department, composed of several doctors that rotate through our unit according to their organization chart. The nursing team has an exclusive dedication and extensive experience in endoscopy.

The General Surgery Department of our center performs more than 200 colorectal cancer surgeries annually. In the last five years, approximately 215 patients were intervened every year, 55 of whom had rectal cancer and 160 non-rectal colon cancer; 55 and 65-70 %, respectively, underwent laparoscopic surgery.

**Design**

A prospective and randomized study was performed. All patients with a scheduled colonoscopy were randomized to one of the four of the following arms: a) two injection points, a volume of 1 ml; b) two injection points, a volume of 1.5 ml; c) three injection points, a volume of 1 ml; and d) three injection points, a volume of 1.5 ml. In all the groups, the marking was performed with the conventional technique described above. Randomization was performed prior to the tattooing procedure, regardless of the endoscopist and the auxiliary nurse.

Given the impossibility to determine prior to colonoscopy which patients would have a neoplasm that required tattooing, all patients undergoing a colonoscopy were informed of the study. Only those patients who accepted to be part of the study and signed the specific informed consent were included. Before the endoscopic diagnosis of a colorectal neoplasm, according to the randomization list, marking was performed with the conventional technique with two or three injection points and with a volume of 1 or 1.5 ml.

High-definition Pentax® equipment (EPK-i 7010 processor model and F38-i10L endoscope model) was used. The injection needles were Boston® Interject 23 g x 240 cm, USA, and the marking was performed with 5 ml syringes Spot® (GI Supply Camp Hill, Pa, United States). The surgeons collected data on the visibility of tattoos during surgery and in the surgical piece. They also registered the complications of the tattoo and the different clinical factors that could influence the visibility of the tattoo.

**Patient selection**
All patients of a legal age, who gave their consent and were diagnosed in the endoscopy room with a colorectal neoplasm (Fig. 1), were endoscopically tattooed and subsequently intervened on a scheduled basis.

**Sample size**
The sample size was determined to estimate the percentage of visible tattoos during the surgical procedure, as no information was available in the literature about the differences to be expected among groups. Therefore, a minimum of 46 subjects were needed per group to estimate an expected proportion of 86.1%, with a 95% confidence and a precision of 0.1. This figure was obtained from our previous series.

**Statistical analysis**
All the relevant variables of the patients (age, sex, body mass index), the endoscopist, the assistant nurse, the specific tattoo technique performed, the location of the endoscopic and surgical lesion, the time elapsed between the tattoo and surgery, the surgeon who performed the intervention, the visibility of the intraoperative tattoo and the surgical specimen, the type of surgical approach and the complications of the tattoo were recorded.

The analyzed data were expressed as means ± 1 standard deviation (SD) for quantitative variables and in absolute numbers and percentages for qualitative variables. Statistical significance was set at a p value less than 0.05. Data analysis was performed with the statistical program IBM SPSS Statistics, version 17.0 (SPPS Inc., Chicago, IL). To compare the categorical variables between the four groups, the Chi-squared test or Fisher’s exact test was applied if the application conditions were not met. In the analysis of quantitative variables, an analysis of variance (ANOVA) was used, after checking the normality of their distributions.

**RESULTS**
Between January 2016 and August 2018, 314 patients were included in the study. Of the total eligible cases (314) that were registered by surgery, 195 were referred for the study. There were no demographic or clinical differences between referred and non-
referred patients. Of the 195 patients who completed all phases of the study, 131 (67.2 %) were male and 64 (32.8 %) were female. The mean age was 70.1 years (SD 10.8) and the average body mass index (BMI) was 27.5 (SD 4.5). The majority were outpatients (174, 89.2 %) and the indications of colonoscopy were the presence of fecal occult blood in 69 cases (35.4 %) and symptoms suggestive of colorectal cancer (altered bowel habits, presence of blood in the stool and/or weight loss) in 66 cases (33.8 %).

Patients were randomized into four groups, 42 (21.5 %) in group 1 (two points of 1 ml), 38 (19.5 %) in group 2 (two points 1.5 ml), 42 (21.5 %) in group 3 (three points of 1 ml) and 48 (24.6 %) in group 4 (three points of 1.5 ml) (Fig. 2). There were 25 (12.8 %) patients who underwent an endoscopic tattoo without a concrete record of the technique used, due to a temporary problem in the data collection computer system (Table 1).

Of the 195 patients, the surgical approach was by laparoscopy in 113 (57.9 %) and by open surgery in 82 (42.1 %). The average number of days between colonoscopy and surgery was 34.8 (SD 27.7) and there were no significant differences for the different study groups.

All lesions referred for surgery were tattooed, including those that by location (rectum and right/blind colon) are not usually tattooed in the clinical practice. Regarding the intraoperative visibility of the endoscopic marking, this was positive in 175 cases (89.7 %) and the tattoo was also visible in the surgical specimen in all of them. In the 20 cases in which the tattoo was not visible, neither intraoperatively nor in the surgical specimen, the most frequent location was the rectum (6/20: 30 %), which was significantly different (p < 0.001) with respect to the other locations (Table 2). After excluding the rectum (13 cases) in order to avoid this confounding factor, the overall visibility of the intraoperative endoscopic marking was 92 % with no significant differences between the laparoscopic approach (94.5 %) and the open surgery (88.5 %).

There were no significant differences in the visibility of the endoscopic marking according to the tattoo technique performed or when analyzing the rest of the variables collected from both the patient, the location of the lesion or the personnel...
involved in the process. There were no differences among the four groups according to the marking technique (Table 3). Interestingly, the group with the worst visibility rate was the group where the form of marking was not recorded, although this difference does not reach statistical significance.

To evaluate the relevance of the location of the lesions, the colon was divided into the following sections: right colon, hepatic flexure, transverse colon, splenic flexure, descending colon, sigma and rectum. The location that accumulated the most lesions was the sigma, which amounted to 97 (49.7 %) of the total. The endoscopic location was correct in 144 cases (73.8 %). In the 51 (26.2%) non-successful cases, the affected segment was the closest to the one suggested by the endoscopy in 80 % of the cases. Thus, the endoscopic approach regarding the exact location of the lesion was reliable in 94.4 % of the cases, considering that the colon had been divided into specific segments.

In 180 cases (92.3 %), there were no complications related to the tattoo technique. In the 15 cases (7.7 %) in which a complication was detected, the most frequent was the presence of marking in the peritoneum (in 14 cases, 7.2 %) (Fig. 3). In another case (0.5 %), a wide extent of the marking was detected in the submucosa. None of the complications were clinically relevant. Complications were not statistically related to any of the other clinical variables collected.

**DISCUSSION**

Colorectal cancer (CRC) is one of the most common malignancies in Western countries. In Spain, it is the second cause of cancer in males, behind prostate cancer, and the second in females after breast cancer. However, when both sexes are considered globally, the most frequent type of cancer is CRC, with almost 40,000 new cases per year and with mortality close to 50 % (19).

The surgical approach in CCR is mainly performed laparoscopically. Thus, it is crucial that the location of the lesion is accurate and therefore, endoscopic tattooing of these lesions is strongly recommended. There are some recommendations on how to perform an endoscopic tattoo but there is no firm evidence on the best way to do it. It seems likely that a tattoo in more points or with a greater volume would be more
visible during surgery.

In this study, the visibility rates of the endoscopic tattoo, both in open and laparoscopic surgery, were very high and close to 90%. Regarding the comparison of the visibility according to the injection points and the volume used in the marking, there were no significant differences or any specific characteristics of our patients or their lesions. Thus, suggesting that a specific mark should be made. It is striking that the group of 25 patients in which the specific tattoo technique was not reflected had a lower percentage of visibility than the others. Perhaps this point is explained in part because some tattoos were performed outside the study protocol. It is important to note that the tattoo on the rectum seems unnecessary because of the ease of locating lesions at this level, due to its proximity to the anal margin. In fact, it may be counterproductive according to the opinion of our colleagues in surgery, as it was difficult for them to see the tissue suitable to perform the anastomosis on several occasions. This was due to the fact that the tone of the mucosa after the tattoo generated the doubt of whether there was necrotic tissue in some areas.

Regarding the safety of the technique and according to our data in line with the literature, endoscopic marking is a very safe technique and there are no clinical repercussions in case of complications. Our study also showed that the endoscopic approach in the definitive location of lesions is very reliable, with an efficacy around 90%. These data are similar to other recent studies performed in Spain (20).

Despite being a prospective randomized study, it has some limitations. The study was performed in a single center. Other purely technical limitations derive from the difficulty to ensure the exact volume in ml injected at the level of the submucosa, as similar volumes have been applied in the different groups. Another limitation may derive from the intrinsic differences in the ability and ways of working of each endoscopist, nurse and even surgeons. Finally, the number of patients included may not be sufficient to detect significant differences between the different groups. However, it is possible to determine a trend that can be confirmed in subsequent studies.

In conclusion, endoscopic marking is a safe and effective technique, regardless of the marking technique. With the data obtained in this study, we recommend using the
technique of endoscopic tattoo of two injection points and 1 ml of volume, due to its simplicity, efficacy and safety. Although the study was not designed to answer this issue, we also believe that lesions clearly located in the rectum and right colon do not require tattooing. On the other hand, the endoscopic approach in the definitive location of the lesions is very reliable, which is an important fact to take into account during surgery. Finally, more studies are needed to support our data and recommendations.

REFERENCES
9. Park JW, Sohn DK, Hong CW, et al. The usefulness of preoperative colonoscopic tattooing using a saline test injection method with prepackaged sterile India ink for


Table 1. Groups according to the tattoo technique.

<table>
<thead>
<tr>
<th>Total; n</th>
<th>195</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (2 points, 1 ml)</td>
<td>42 (21.5 %)</td>
</tr>
<tr>
<td>Group 2 (2 points, 1.5 ml)</td>
<td>38 (19.5 %)</td>
</tr>
<tr>
<td>Group 3 (3 points, 1 ml)</td>
<td>42 (21.5 %)</td>
</tr>
<tr>
<td>Group 4 (3 points, 1.5 ml)</td>
<td>48 (24.6 %)</td>
</tr>
<tr>
<td>No data</td>
<td>25 (12.8 %)</td>
</tr>
</tbody>
</table>
Table 2. Tattoos not seen in surgery and location.

<table>
<thead>
<tr>
<th>Tattoos not seen in surgery/total cases</th>
<th>20/195 (10.3 %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right colon</td>
<td>4/27 (14.8 %)</td>
</tr>
<tr>
<td>Splenic flexure</td>
<td>1/16 (6.3 %)</td>
</tr>
<tr>
<td>Sigmoid colon</td>
<td>9/97 (9.3 %)</td>
</tr>
<tr>
<td>Rectum</td>
<td>6/13 (46.2 %); p &lt; 0.001</td>
</tr>
</tbody>
</table>
Table 3. Tattoos seen during the surgical intervention according to the tattoo technique.

<table>
<thead>
<tr>
<th>Tattoos seen during the surgical intervention</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>91.7 %</td>
</tr>
<tr>
<td>Group 2</td>
<td>93.3 %</td>
</tr>
<tr>
<td>Group 3</td>
<td>91.9 %</td>
</tr>
<tr>
<td>Group 4</td>
<td>94.9 %</td>
</tr>
<tr>
<td>No group</td>
<td>83.7 %</td>
</tr>
</tbody>
</table>
Fig. 1. Colorectal cancer.
Fig. 2. Endoscopic tattoo.
Fig. 3. Marking in the peritoneum.