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

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WAYS TO PERFORM AN ENDOSECOPIC TATTOO. PROSPECTIVE AND RANDOMIZED STUDY IN PATIENTS WITH COLORECTAL NEOPLASM
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ABSTRACT
Background and study aims
Intraoperative identification of colonic lesions previously detected at colonoscopy may be difficult. Endoscopic tattooing facilitates this 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
Prospective and randomized study. All tattoo candidate patients prior to surgery were included and randomized into 4 groups (tattoo at 2 or 3 injection points and with a volume of 1 or 1.5 ml of labeling). Multiple variables were registered.
Results
195 patients were included in which endoscopic tattoo and subsequent surgical intervention was performed, with a mean age of 70.1 years, 67.2% being men. The laparoscopic approach was applied in 57.9% of cases. The intraoperative visibility of the endoscopic tattoo was 89.7%. 30% of rectal lesions were not visible. Excluding the rectum, the marking was visible intraoperatively in 92% of the 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%. No 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 2 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 at endoscopy is often difficult, particularly in laparoscopic surgery. The estimation of the location of an injury during colonoscopy may be incorrect in up to 14% of cases. The lack of precision in the identification of an injury during laparoscopic surgery can lead to unnecessary resection of an intestinal segment. 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, to mention some of these 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: lack of permanence, infections or inflammatory reactions, or...
complex preparation in some cases. There are reports of even perforation with the use of methylene blue. 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 and, among others, include the marking of colonic neoplasms, area of maximum exploration in enteroscopy, polyps of difficult location partially resected or suspected of malignancy.

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. Not surprisingly, in a study of 63 patients who had been tattooed with Spot, in 6 of them (9.5%) peritoneal remnants were found during surgery. In this sense it is recommended to perform the injection with maximum precision in the submucosa. It is advisable to insert the injection needle at an oblique angle (close to 0°) to the colon wall to avoid penetrating the serosa. Another possible complication of the use of SCS may occur if the tattoo is done 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.

Multiple studies have proven that endoscopic tattooing is generally a safe technique with a very low complication rate. In the absence of an international consensus that guides the practice of endoscopic tattooing, there is great variability in clinical practice, 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 1 or 2 more marking points on the opposite side of the lumen. In this sense, a study conducted on 19 patients with a colonic lesion, marked prior to surgery with Indian ink at a single point, showed that the tattoo was visible only in 13 patients (68.4%) intraoperatively and in 14 (73.6%) in the surgical piece; authors concluded that at least 2 marking points should be performed when tattooing surgical...
colon lesions. As for the 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 creating 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 placed again and the marker remaining in the injection catheter is dragged by injecting additional serum\textsuperscript{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 \textsuperscript{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%, showing 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%\textsuperscript{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 the surgical act and in the surgical piece, comparing the conventional injection in two versus three points and, independently, with a marking injection volume of 1 ml vs 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 carried out at the Hospital of Sant Joan Despí Moisès Broggi, Barcelona, covering an area of 400,000 inhabitants.

The Endoscopy Service of the hospital, composed of 12 endoscopists with extensive experience, performs an annual average of colonoscopies that ranges between 7000 and 8000. All colonoscopies are 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 5 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**

Prospective and randomized study. All patients with a scheduled colonoscopy were randomized to one of the four following arms: (1) two injection points, a volume of 1 ml; (2) two injection points, a volume of 1.5 ml; (3) three injection points, a volume of 1 ml; (4) three injection points, a volume of 1.5 ml. In all the groups the marking was carried out with the conventional technique described above. Randomization was done prior to the tattooing procedure, regardless of the endoscopist and the auxiliary nurse.

Given the impossibility of determining prior to colonoscopy which patients would have a neoplasm that required tattooing, all patients undergoing a colonoscopy would 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, the marking with conventional technique with 2 or 3 injection points and with a volume of 1 or 1.5 ml was performed.

High-definition Pentax® equipment (EPK-i 7010 processor model and F38-i10L endoscope model) were used. The injection needles were Boston® Interject 23 g x 240 cm, USA, and the marking was done with 5 ml syringes Spot; (GI Suppy Camp Hill, Pa,
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 legal age who, having previously given their consent, were diagnosed in the Endoscopy room of a colorectal neoplasm (see Figure 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, with a 95% confidence and a precision of 0.1, an expected proportion of 86.1%. 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 the surgery, the surgeon who performed the intervention, the visibility of the intraoperative tattoo and the surgical piece, 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 p value less than 0.05. The 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-square test, or Fisher’s exact test was applied if the application conditions were not met. In the analysis of quantitative variables, after checking the normality of their
distributions, an analysis of variance (ANOVA) was used.

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 to be included in 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 men and 64 (32.8%) women. 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 to four groups, 42 (21.5%) in group 1 (2 points of 1 ml), 38 (19.5%) in group 2 (2 points 1.5 ml), 42 (21.5%) in group 3 (3 points of 1 ml) and 48 (24.6%) in group 4 (3 points of 1.5 ml) (see Figure 2). There were 25 (12.8%) patients who underwent endoscopic tattoo without a concrete record of the technique used due to a temporary problem in the data collection computer system. (See Table 1)

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

All lesions referred to surgery were tattooed, including those that by location (rectum and right / blind colon) are not usually tattooed in clinical practice. Regarding the intraoperative visibility of the endoscopic marking, it was positive in 175 cases (89.7%), in all of them the tattoo was also visible in the surgical specimen. 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%), significantly different (p<0.001) with respect to the other locations (see 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 analyzing the rest of the variables collected from both the patient and location of the lesion or the personnel involved in the process. There were no differences among the four groups according to the marking technique (see table 3). Interestingly, the group with the worst visibility rates 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 with 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 segment actually affected was the closest to the one suggested by the endoscopy in 80% of the cases. So 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 is the presence of marking in the peritoneum (in 14 cases, 7.2%; see figure 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 men, behind prostate cancer, and the second in women 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%.

The surgical approach of CCR is mainly performed laparoscopically; it is therefore crucial for the location of the lesion to be accurate, and so endoscopic tattoo of these
lesions is strongly recommended.

There are some recommendations on how to perform an endoscopic tattoo but there is no firm evidence on which is the best way to do it. It seems likely that the tattoo in more points or with greater volume would be more visible during the surgical act.

In our 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, we have not appreciated significant differences nor have we observed any specific characteristics of our patients or their lesions, which suggests making a specific marking. It is striking that the group of 25 patients in which the specific tattoo technique was not reflected, has 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 and, even, counterproductive according to the opinion of our colleagues in Surgery since on several occasions it was difficult for them to see the tissue suitable to perform the anastomosis, since the tone of the mucosa after the tattoo generated the doubt of whether in some area was necrotic tissue.

Regarding the safety of the technique, and according to our data in line with the literature, endoscopic marking is a very safe technique and in case of complications, they have no clinical repercussions.

Our study also shows 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 conducted in our country.20

Despite being a prospective randomized study, it has some limitations. It is a study conducted in a single center. Other limitations, purely technical, derive from the difficulty of ensuring exactly the volume in ml injected at the level of the submucosa as such 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, nursing and, even surgeon. 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, for its simplicity, efficacy and safety. Although the study has not been designed to answer this issue, we also believe that those 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, this being an important fact to take into account during surgery. Finally, more studies are needed to support our data and recommendations.

REFERENCES


Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>Number (Percentage)</th>
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<tr>
<td>1</td>
<td>2 points 1 ml</td>
<td>42 (21.5%)</td>
</tr>
<tr>
<td>2</td>
<td>2 points 1.5 ml</td>
<td>38 (19.5%)</td>
</tr>
<tr>
<td>3</td>
<td>3 points 1 ml</td>
<td>42 (21.5%)</td>
</tr>
<tr>
<td>4</td>
<td>3 points 1.5 ml</td>
<td>48 (24.6%)</td>
</tr>
<tr>
<td></td>
<td>No data</td>
<td>25 (12.8%)</td>
</tr>
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</table>

Table 2.

<table>
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<tr>
<th>Description</th>
<th>Number (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tattoos not seen in surgery/total cases</td>
<td>20/195 (10.3%)</td>
</tr>
<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>
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Table 3.

<table>
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<tr>
<th>Tattoos seen during surgical intervention</th>
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<tr>
<td>Group 1</td>
<td>91.7 %</td>
</tr>
<tr>
<td>Group 2</td>
<td>93.3 %</td>
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<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>

Figure 1. Colorectal cancer.
Figure 2. Endoscopic tattoo.

Figure 3. Presence of marking in the peritoneum.