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Identification of Sentinel Lymph Nodes in Colon Cancer Depends on the Amount of Dye Injected Relative to Tumor Size

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Abstract. Recent studies have shown that the sentinel lymph node (SLN) procedure might improve staging in colon cancer. However, low SLN identification and high false negative rates have also been reported. In a two-institution study, the SLN procedure with isosulfan blue 1% was performed according to a standardized protocol in 31 patients with open resections for colon cancer. Data were collected prospectively. The database was analyzed retrospectively to determine factors contributing to a low identification rate. The SLN identification rate was 87% and the false negative rate was 50%. Successful SLN identification was significantly associated with application of higher volumes of dye relative to the tumor diameter ($p = 0.04$) and more frequent tumor localization in the sigmoid colon ($p = 0.04$) as compared to missing SLN identification. The tumor diameter was not significantly different in the two groups. Sentinel lymph node identification in colon cancer depends on the amount of dye injected relative to the tumor size. Application of only 1 ml of dye—the amount generally recommended in the literature—is not sufficient in large tumors.

Lymph node status is still considered the most important prognostic factor in colon cancer [1], indicating that its accurate assessment is of paramount importance for prognosis and therapy. If lymph node metastases are found (AJCC/UICC stage III [2]), adjuvant chemotherapy is indicated. Patients without lymph node metastases (stage I or II) usually do not qualify for adjuvant therapy outside of formal clinical trials. Nevertheless a subset of this population suffers from recurrences over the years [3]. Although the importance of lymph node *micro*metastases (recently defined as 0.2–2 mm in diameter [4, 5]) is still debated [1], patients with micrometastases not found during routine histopathological work-up (occult micrometastases) are suspected to be part of a subgroup with higher risk that might benefit from adjuvant chemotherapy. Several studies using immunohistochemical staining (IHC) for the retrospective analysis of lymph nodes considered free of disease by conventional histopathology have found micrometastases in 25%–76% of the patients [6–11]. So far, only Greenson et al. have demonstrated a significantly shorter survival in these patients [6]. Similarly, Liefers and co-workers re-examined the resected lymph nodes of patients initially considered to have stage II colon cancer with a carcinoembryonic antigen (CEA)–specific reverse transcriptase-polymerase chain reaction (rt-PCR) [12]. They found a significantly lower observed as well as adjusted 5-year survival rate in patients with rt-PCR–positive lymph nodes.

A prospective randomized trial answering the question whether chemotherapy improves survival in these patients has not yet been done. Disregarding possible preferential lymph drainage patterns of a given tumor, identification of micrometastases requires serial or step-sections of *all* removed lymph nodes and the application of IHC (microstaging) or rt-PCR (ultrastaging). The costs of these procedures would clearly exceed the resources of routine histopathology. The eventual possibility to target only sentinel lymph nodes (SLN) of a given colon cancer [13, 14]—i.e., the first nodes draining the lymph fluid of the tumor and therefore those most likely to harbor metastases—while still getting the same staging information would help keep costs within reason.

The initial goal of our standardized protocol was to investigate the feasibility of the SLN procedure in colon cancer and to validate it in our institutions. Considering the low identification rate and the high false negative rate in the present investigation—two problems that have been mentioned, but not analyzed in other studies [15–18]—we reviewed our database retrospectively searching for factors contributing to these phenomena. Our findings may help improve the identification rate in the SLN procedure for colon cancer.

Patients and Methods

We conducted a two-institution feasibility and validation study of the sentinel lymph node (SLN) procedure during open surgery for all stages of colon cancer. All data were collected prospectively. Because we found a low SLN identification rate and a high false

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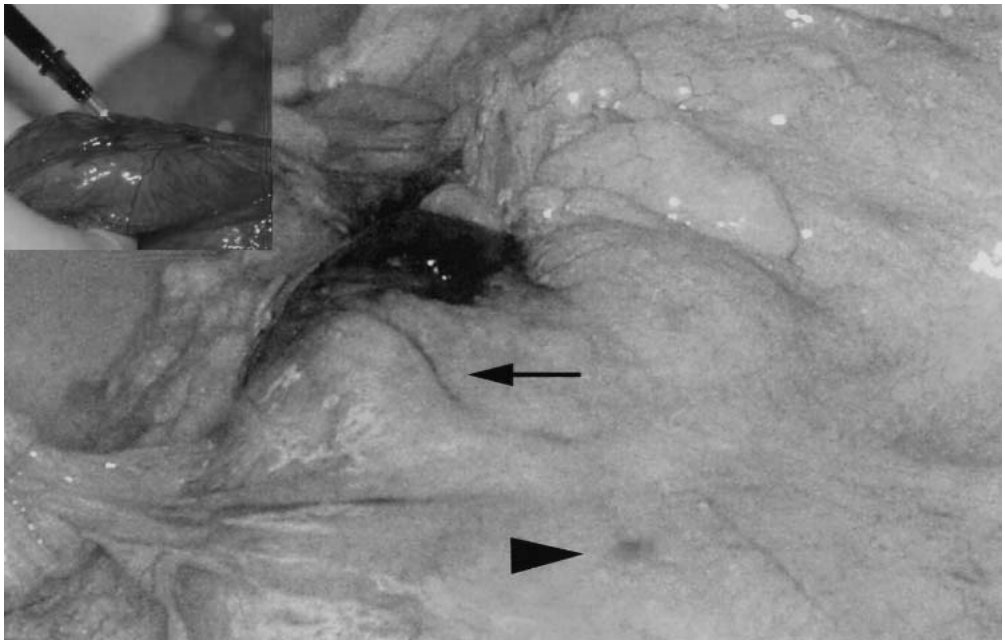

Fig. 1. Isosulfanblue 1% is injected subserosally around the tumor with a tuberculin syringe (inlet). The blue-stained lymphatic vessels (arrow) lead to a blue-stained lymph node (= SLN, arrowhead), which is marked with a long suture.

negative rate, we analyzed our database retrospectively and tested the following question: Does non-identification, or a false negative SLN result, depend on patient characteristics, on tumor characteristics, on the surgeon performing the SLN procedure, or the amount of dye injected?

Patients

Patients were consecutively enrolled in the study, from April 2000 to December 2001. Patient characteristics, peri- and postoperative data were documented continuously. Inclusion criteria were open resection for stage I–IV colon cancer and possibility of transabdominal injection of the dye. Exclusion criteria were prior intraabdominal tumor surgery, cancers below the peritoneal fold necessitating rectoscopic dye injection, other malignancies, allergies to the dye (isosulfan blue), pregnancy, and breast-feeding. The study protocol was approved by the local ethics committees, and written consent was obtained from every patient.

Technique

The technique of the SLN procedure was standardized in the protocol and followed the recommendations described in detail by Saha et al. [14]. Briefly, after careful mobilization of the affected colon segment to get access to the tumor, isosulfan blue vital dye 1% (Lymphazurin 1% [Ben Venue Labs Inc., Bedford OH, USA], or Isosulfan Blue USZ 1% [University Hospital of Zurich, Switzerland]) was injected into the subserosa circumferentially around the tumor with a tuberculin syringe with 29-gauge needle. A minimum of 1 ml of dye was required by the protocol, leaving use of greater amounts for larger tumors to the discretion of the respective surgeon. The first blue-stained lymph nodes (= SLN) shining through the peritoneum were marked with a long suture (Fig. 1). The procedure was completed by a resection of the whole affected intestinal segment according to standard procedures in oncological surgery (en bloc resection which includes the marked SLN). Data considering technical details, such as the amount of dye applied, were collected immediately after surgery. Performing the SLN procedure has been restricted to three selected surgeons at the two institutions (F.H., C.T.V., M.Z.), two of whom have extensive experience in the SLN procedure for breast cancer [19].

Histopathologic Examination

All marked SLN were processed as standardized in the protocol: Five serial sections of each SLN were obtained at three different representative levels and were mounted on separate slides. The first section of each level was stained with hematoxylin and eosin (H&E). When no metastatic tumor was identified by H&E, typically the fourth section of each level was immunostained with the pancytokeratin markers CK22 (Medite, Nunningen, Switzerland) or Lu-5 (BMA Biomedicals, Augst, Switzerland). Manual dissection of the fixed surgical specimens was performed to identify the remaining lymph nodes (non-SLN). The mesentery was sliced at 0.3–0.5 cm intervals to allow thorough visual and digital inspection. Done carefully this gross dissection method should reveal all lymph nodes 0.3 cm in size and larger. The non-SLN were bivalved and examined in H&E. If no macrometastases were found in the SLN or in the non-SLN, all non-SLN were examined in the same way as the SLN with serial sections and IHC.

Statistical Analysis

Descriptive statistics: Median values with ranges in brackets are given. Statistical tests: For nominal scaled variables we used a Fisher exact test or a maximum likelihood χ^2 test; for ordinal scaled variables, the Mann Whitney U-test (2×1 sides exact p for small n); and for interval scaled variables, the Student's t-test after variance homogeneity was confirmed by the Levene test. All tests were two-tailed and are valid even for small samples. A p value < 0.05 was considered to be significant. The tests were performed on STATISTICA version 5.5 for Windows (StatSoft, Inc., Tulsa, OK,

Table 1. TNM classification and stage.[a]

	pT1	pT2	pT3	pT4
pN0	2	3	9	2
pN1	0	0	9	1
pN2	0	0	4	1
Stage I		5		
Stage II		11		
Stage III		13		
Stage IV		2		

[a]TNM classification [21] and stage, according to the American Joint Committee on Cancer/International Union Against Cancer (AJCC/UICC [2]), for all patients.

USA). Sensitivity, specificity, negative and positive predictive values, false negative and false positive rate have been calculated as described by Walter [20].

Results

Patients and Tumors

Thirty-one patients (23 men and 8 women) with primary colon cancer have been enrolled in this study. Median age was 74.5 years (range: 45.4–85.9 years) and median body mass index (BMI) 25.3 kg/m^2 (range: 19.5–38.3). Of the 31 patients, 13 had had previous intra-abdominal surgery for nonmalignant disease. None had undergone previous radiation therapy of the abdomen. Five patients had a positive family history for colorectal cancer but none met the criteria for hereditary non-polyposis colorectal cancer (HNPCC) or familial adenomatous polyposis (FAP).

Eleven tumors were localized in the right hemicolon, six in the left hemicolon, and 14 in the sigmoid colon. TNM classification [21] and stage according to the American Joint Committee on Cancer/International Union against Cancer (AJCC/UICC [2]) are shown in Table 1. Two patients presented with liver metastases in the preoperative radiological staging (abdominal ultrasound and/or computerized tomography scan). No additional liver metastases were found by intraoperative palpation and ultrasound. Median maximal diameter of the fixed tumor as described in the histopathological report was 4.2 cm (range: 2.0–7.2 cm). The preoperative carcinoembryonic antigen (CEA) titer was 2.0 μg/l (range: 0.6–33.6 μg/l).

SLN Procedure

A median of 2.0 ml (range: 1.0–5.0 ml) isosulfan blue vital dye 1% (Lymphazurin 1% in 20 patients; Isosulfanblue USZ 1% in 11 patients) was injected subserosally around the respective tumor. In four patients no SLN could be identified (no identification group, NID). In 27 patients at least one SLN was identified after median 5.0 minutes (range: 3.0-15.0 minutes) (identification group, ID), resulting in an identification rate of 87.1%. In 21 of these patients all harvested SLN were without evidence of metastases, whereas six patients showed metastases in their SLN (true positive group, TP). In all, 15 patients without metastases in the SLN had no further evidence of metastatic deposits in the non-SLN (SLN true negative, TN). Six patients with negative SLN presented metastases in at least one non-SLN; therefore the SLN must be rated false negative (false negative group, FN). For two patients with SLN metastases the SLN was the only site of nodal metastases (possible upstaging 7.4%). The breakdown of these patient categories is shown in Figure 2.

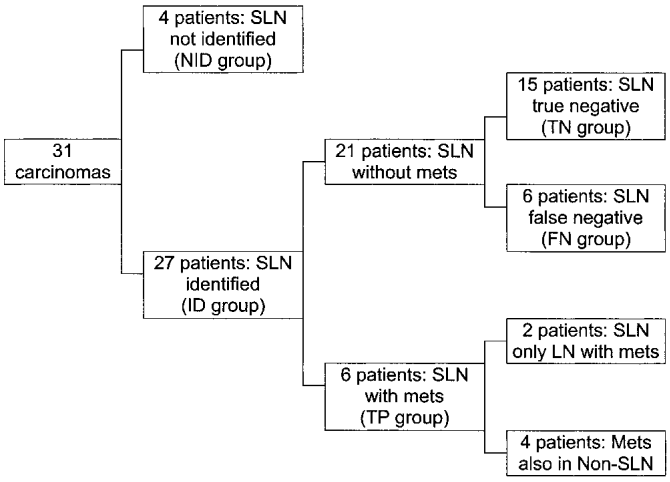

Fig. 2. Breakdown of patient categories (mets: metastases; SLN: sentinel lymph node).

In the ID group 582 lymph nodes (median 21 per patient [range: 5–40]) were processed histopathologically. Of those 68 (median 2 [range:1–8]) were SLN, 514 (median: 16 [range: 1–38]) were non-SLN. Seven of 68 SLN (10.3%) in six patients were positive in H&E, one SLN (1.5%) was positive in IHC only (as a non-SLN in this patient was positive in H&E, this was not rated as upstaging). In all, 21 of 514 non-SLN (4.1%) were positive in H&E, but no additional non-SLN turned positive in IHC.

Comparison of Patients with and without SLN Identification

The ID and the NID groups did not differ significantly in age, sex, BMI, incidence of previous intra-abdominal surgery, or positive family history. The localization of the tumor ($p = 0.04$) and the quotient amount of dye applied over maximal tumor diameter ($p = 0.04$) were significantly different between the two groups (Table 2). No statistical difference was found for the surgeon performing the SLN procedure, the type of dye applied, the total number of lymph nodes harvested, or the TNM stage. The identification rate did not change during the time of the study (87.5% for the first 16 patients, 86.7% for the following 15 patients).

Comparison of Patients with True Positive and False Negative SLN

A similar analysis comparing the TP with the FN group revealed no significant difference (Table 3). However, larger tumors showed a tendency to be associated with false negative SLN ($p = 0.09$). We found slightly more SLN in the TP group than in the FN group (median: 2.0 and 1.5, respectively; non-significant difference). All the positive non-SLN in the FN group were macrometastases (found in H&E), not micrometastases. The false negative results were evenly distributed throughout the protocol time (three of the first 16 patients, three of the following 15 patients).

Comparison of Final Nodal Status and SLN Status

In Table 4 the SLN status (positive or negative) is compared to the final nodal status (pN0, pN1, or pN2), and the different rates reflecting the accuracy of the SLN procedure are calculated.

Table 2. Comparison of patients with and without SLN identification.[a]

	All	ID	NID	*p*
n	31 (100.0%)	27 (87.1%)	4 (12.9%)	
Localization				
• Right colon	12	10	2	
• Left colon	5	3	2	
• Sigmoid colon	14	14	0	0.04
Maximal tumor diameter (cm)	4.2 (2.0–7.2)	4.0 (2.0–7.2)	6.3 (2.1–7.0)	0.38
Amount of dye (ml)	2.0 (1.0–5.0)	2.0 (1.3–5.0)	1.0 (1.0–2.0)	0.05
ml of dye/cm tumor diameter	0.48 (0.14–1.5)	0.55 (0.21–1.5)	0.31 (0.14–0.48)	0.04

[a]Comparison of all patients and patients with (ID) and without (NID) identification of at least one SLN. Median values are given with ranges in brackets; *p* values when comparing ID to NID group.

Table 3. Comparison of patients with true positive and false negative SLN.[a]

	TP	FN	*p*
n	6	6	
Localization			
• Right colon	2	4	
• Left colon	0	1	
• Sigmoid colon	4	1	0.14
Maximal tumor diameter (cm)	3.4 (3.0–4.0)	4.5 (2.5–7.0)	0.09
Amount of dye (ml)	2.0 (1.3–4.0)	3.0 (2.0–5.0)	0.22
ml of dye/cm tumor diameter	0.63 (0.37–1.3)	0.67 (0.29–1.1)	0.89

[a]Comparison of patients with true positive SLN (TP) and patients with false negative SLN (FN). Median values are given with ranges in brackets; *p* values when comparing TP to FN group.

Table 4. Comparison of final nodal status and SLN status.[a]

SLN	pN0	pN1/pN2	Total	
Negative	15	6		21
Positive	0	6		6
Total	15	12		27
Sensitivity			6/12	50.0%
Specificity			15/15	100.0%
False negative rate			6/12	50.0%
False positive rate			0/15	0.0%
Negative predictive value			15/21	71.4%
Positive predictive value			6/6	100.0%

[a]Comparison of final nodal status (pN) and SLN status (negative: all SLN without metastases; positive: nodal metastases in at least one of the SLN) in cases with successful SLN identification and the calculated corresponding rates. Specificity is 100% per definition since a false positive result is not possible.

Discussion

The sentinel lymph node (SLN) procedure in melanoma and breast cancer patients was designed to determine the histological lymph node status by means of a surgical intervention less invasive than a formal lymphadenectomy. The latter is performed only in SLN positive cases, thus avoiding more extensive surgery in SLN negative ones. Furthermore a more in depth-analysis of a small number of SLN allowed the identification of micrometastases in some patients [22], which raised questions about their importance.

The SLN concept was subsequently adapted to colonic tumors [13, 14]. Other than in breast cancer the standard lymphadenectomy in colon cancer surgery has no added morbidity. The rationale for applying the SLN procedure is therefore not less surgery but improved accuracy of the nodal staging on less material while still doing a standard resection. Patients with a SLN as the only site of lymph node metastasis are *possibly upstaged* through the procedure, as conventional pathological dissection of the mesentery might have missed this lymph node [14]. Focusing the pathologist's attention on the tagged SLN leads to the detection of otherwise occult metastases, therefore upstaging these patients to stage III (pN+). Second, more in-depth examination of the SLN by serial sections and IHC (microstaging) may reveal micrometastases that are regularly missed in routine H&E, resulting in an *upstaging* to stage III [14].

The first study on the SLN procedure in colorectal cancer showed a SLN identification rate of only 70% and a high false negative rate of 60% [13]. The poor identification rate was partly explained by the injection technique at the beginning of the study. Saha and co-workers [14] presented very promising results in a series of 86 patients where at least one SLN was identified in 99% of the patients. In 18% of the patients, the SLN was the only positive LN (possible upstaging); in 8% of the cases micrometastases in SLN were found with serial sections and immunohistochemical staining (upstaging). This subgroup of patients was assigned to stage III thanks to the focused histopathological work-up of the SLN. The false negative rate (when calculated with the formula FN/[FN + TP] [20]) was 9%. These results were confirmed in an update with a total of 131 patients [23]. Likewise, Wood and associates [24] found a good identification rate of 94% and an acceptable false negative rate of 12% while upstaging 20% of the patients.

Some further studies failed to confirm these promising results [15–18, 25], although the injection technique described by Saha et al. [14] was used. Intraoperative identification rates ranging from 50%–90% have been reported [15, 16]. Similarly, the identification rate of 87% in the present study is unsatisfactory. As the reasons for low identification rates are not analyzed in the current literature, we compared our SLN identification group (ID) with our no SLN identification group (NID). Advanced (pT3 and pT4) and bulky tumors have been associated with a low identification and a high false negative rate [24, 25]. In our study, neither the TNM-stage nor the tumor diameter itself correlated significantly with poor identification. When calculating the ratio between the amount of dye injected and the maximal tumor diameter—thereby analyzing the quantity of dye relative to the tumor size—we found significantly better identification rates with higher volumes of dye in larger tumors. The amount of dye injected was therefore found to be an important predictor for successful identification of the SLN, suggesting that the low identification rates in earlier reports were related, at least in part, to insufficient amounts of injected dye.

The difficulty of SLN identification in large colon cancers has

been previously described [14, 17, 18, 25], but this is the first report establishing a significant correlation between the amount of dye relative to the tumor diameter and the SLN identification rate. Too little volumes of dye do not allow a completely circumferential injection around the tumor [18]. Attempts to improve the identification rate by additional use of a radiocolloid tracer yielded identification rates not higher than 90% [17, 26]. The SLN experience in large tumors is still limited in general because malignant melanomas or breast cancers are usually small tumors: e.g., Giuliano mentions only 10.3% of breast cancers over 5 cm [27], whereas 32.2% of colon cancers in our series were larger than 5 cm. In some SLN protocols for breast cancer, larger tumors (e.g. > 3 cm) are even excluded [19]. Saha et al. propose the injection of slightly more than 1 ml of the dye in tumors over 5 cm [14]. When dealing with this specific problem in SLN procedure for colon cancer, we suggest injecting clearly more than 1 ml of dye. In the present study the SLN identification was always successful when at least 0.5 ml of dye per centimeter of tumor diameter was applied (Table 2), i.e., 2.5 ml of isosulfan blue in a tumor 5 cm in diameter. Until more data covering this topic are available, we suggest this as the minimum amount of dye to be injected for the SLN procedure in colon cancer.

Tumors located in the sigmoid colon had a significantly better SLN identification rate in this study than tumors in other locations. This could be due to the better mobility of the sigmoid colon compared to the right or left hemicolon, allowing better exposure with less mobilization leading to disruption of lymphatic vessels. Nevertheless the surgeon cannot influence this factor.

It is unlikely that our low identification rate is related to a learning curve as the cases without SLN identification occurred evenly in the first and second halves of the study. Moreover the learning curve in the SLN procedure for colon cancer has been considered much shorter than for other SLN procedures [14, 28]. In a recent paper, Paramo et al., found that an almost 100% identification rate was achieved after only five cases per surgeon in a multisurgeon setting [29].

As others groups have done [16, 17, 25], we are also dealing with a high false negative rate between 30% and 50%. When comparing the false negative group (FN) with the true positive group (TP) we could not find any significant difference regarding patient or tumor characteristics or factors related to the SLN procedure. In large tumors a trend to false negative results was observed ($p = 0.09$), indicating that a large tumor size could contribute, at least in part, to the false negative results. However, the calculated ratio between the amount of dye injected and the maximal tumor diameter was not significantly different in the two groups. Several reasons for false negative SLN are discussed in the literature. Lymph nodes extensively infiltrated by metastases could alter or block the lymphatic flow pattern [13], as described by Grinnell, who meticulously investigated the lymphatic system of the colon in 1965 [30]. Blue stain could be diverted to nodes that are not to be considered as true SLN. Failure to inject circumferentially around the tumor or an inadequate volume of dye are other possible explanations [17].

In our feasibility and validation study of the sentinel lymph node (SLN) procedure in colon cancer, we found that the SLN was identifiable in 87%. The amount of dye injected relative to the tumor size was found to be an important predictor for successful identification of the SLN. Further research concerning the false negative results must be conducted if the promising SLN technique should contribute to the clarification of the question whether micrometastases in colon cancer patients really matter.

Résumé. Récemment démontrée, il semble que l'étude des ganglions lymphatiques sentinelles (SLN) pourrait améliorer le staging des cancers coliques. Cependant, le taux d'identification du SLN reste peu élevé et on a également rapporté des taux élevés de faux négatifs. Dans cette étude bi-institutionnelle, on a étudié le SLN par injection de bleu isosulphane 1% selon un protocole standardisé chez 31 patients opérés d'un cancer colique par voie traditionnelle (chirurgie ouverte). Les données ont été recueillies de façon prospective. La banque de données a été analysée rétrospectivement afin de déterminer les facteurs d'une identification limitée. Le taux d'identification du SLN a été de 87% et le taux de faux négatifs, de 50%. L'identification du SLN a été positivement corrélée avec des volumes plus importants de colorant par rapport au diamètre tumoral ($p = 0.04$) et plus fréquemment dans le côlon sigmoïde ($p = 0.04$). Le diamètre tumoral ne différait pas entre les deux groupes. L'identification du SLN dans le cancer colique dépend de la quantité de colorant injecté par rapport à la taille tumorale. L'injection de seulement 1 ml de colorant, la quantité généralement recommandée dans la littérature, n'est pas suffisante en cas de tumeur volumineuse.

Resumen. Estudios recientes han demostrado que el procedimiento de identificación del ganglio linfático centinela (GLC) puede mejorar la estadificación del cáncer de colon. Sin embargo, se han informado bajas tasas de identificación del GLC y falsos negativos. Se recogió información en forma retrospectiva para realizar un estudio bi-institucional de identificación del GLC con azul isulfano al 1% mediante un protocolo estandarizado en 31 pacientes sometidos a resección abierta de cáncer de colon. La base de datos fue analizada retrospectivamente para determinar factores que pudieran contribuir una baja tasa de identificación. La tasa de identificación del GLC fue 87% y la tasa de falsos negativos 50%. Se encontró asociación significativa entre una exitosa identificación del GLC y la aplicación de un mayor volumen del colorante en relación al diámetro del tumor ($p = 0.04$) y la ubicación del tumor en el colon sigmoide ($p = 0.04$). El diámetro del tumor no fue significativamente diferente en estos dos grupos. La identificación del GLC en el cáncer de colon depende de la cantidad de colorante que se inyecte en relación al tamaño del tumor. La aplicación de apenas 1 ml de colorante -la cantidad que aparece generalmente recomendada en la literatura- no es suficiente cuando se trata de tumores grandes

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