

Assessment of Sentinel Lymph Node Biopsy in Colon Cancer and Its Impact on Staging

Emad Hokkam¹, Soliman El-Kammash¹, Amr Abdelaziz², Sherif Farrag¹, Hamada Fathy¹, Ahmed Gomaa¹

¹Department of Surgery, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

²Department of Pathology, Faculty, Suez Canal University, Ismailia, Egypt

Email address:

ehokkam@gmail.com (E. Hokkam)

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Abstract: *Background:* Missed nodal metastases during resection for colon cancer or missed occult metastases during pathological examination leads to down staging of the disease and increase the recurrence rate. The sentinel lymph node is a technique used to properly detect nodal metastases hence improving staging accuracy with subsequent proper application of adjuvant therapy. The aim of this study is to determine the feasibility of sentinel lymph node technique and its effect on staging of the disease. *Methods:* A total number of forty five patients with primary colon cancer (T1-3, any N, M0) were enrolled in the study. They were subjected to appropriate colonic resection based on the anatomic location of the tumor. A combined method of lymphatic mapping using technetium 99mTc -labeled sulfur colloid and patent blue was performed. After few minutes of injecting the tracers, the colon and its mesentery were examined for any blue-stained glands and areas of high radioactivity using a hand-held gamma probe. After colonic resection, the sentinel lymph node(s) and non-sentinel lymph nodes were sent for H&E staining. Positive sentinel lymph node(s) underwent no further analysis while negative nodes were submitted for immunohistochemical staining. *Results:* Sentinel lymph node(s) were successfully identified in 43 patients (95.6%) with a mean of 1.7 node/patient. The false negative rate is 7.1%, Sensitivity is 92.9%, specificity is 100%, negative predictive value is 88.2% and positive predictive value is 100%. Detailed focused examination using the immunohistochemical staining discovered 4 more positive patients who were supposed to be negative by the ordinary H&E staining resulting in upstaging rate of 9.3% among the whole study group and 21% among the negative-nodes patients. *Conclusion:* Sentinel lymph node mapping is a feasible technique with a relatively high identification rate. It can upstage some patients who will get benefit from further adjuvant chemotherapy resulting in reduced recurrence and better prognosis.

Keywords: Sentinel Lymph Node, Lymphatic Mapping, Colon Cancer, Upstaging

1. Introduction

Lymph node (LN) status is considered the most predictive factor for local recurrence and survival in patients with colorectal cancer. Although stage I and II should not contain metastatic LNs (according to the American Joint Committee on Cancer), yet 10-25% of patients with these stages will have local recurrences within five years [1-4]. Two factors lead to this fact; improper staging through retrieval of inadequate number of LNs and the presence of occult tumor cells (OTCs) that could not be detected by ordinary pathological examination [3-5]. A minimum number of 12 nodes have been suggested to

accurately stage the disease [6, 7]. However; Josephet al [8] found that 40 nodes has to be examined to achieve an 85% probability of true node negativity.

OTCs are not usually detected by conventional haematoxylin and eosin (H&E) staining. A variety of techniques have been employed for the detection of OTC which include serial sectioning, immunohistochemistry (IHC), step sectioning, polymerase chain reaction and reverse transcriptase polymerase chain reaction [9]. Gusterson [10] reported that up to 20% of cases being diagnosed as node-negative on routine single section examination can be demonstrated to contain OTC after serial sectioning. It is calculated that a single section through the center of a LN

measuring 1 cm in diameter samples approximately 0.06% of the node which is presumed to reflect the entire LNs [11]. The need for dissection of huge number of LNs and the infeasibility of doing detailed focused examination to this such number has directed surgeons to adopt the policy of sentinel lymph node (SLN) mapping. The accuracy rate of SLN in colorectal cancer reaches up to 90% and results in upstaging of 10-30% of patients with early stages(stage I & II) [12].

The idea beyond the use of SLN in colon cancer is different from that of breast cancer. In breast cancer SLN aimed to avoid unnecessary lymphadenectomy for node negative patients thus decreasing the operative morbidity while in colon cancer lymphadenectomy is an integral part of the operative resection usually without complications or morbidity. Moreover, one-stage abdominal surgery is usually preferred. In colon cancer; SLN aimed primarily at detecting the OTC thus optimize staging accuracy and guide the use of adjuvant chemotherapy. Another advantage for SLN in colon cancer is the ability to identify the aberrant lymphatic drainage patterns which may occur in about 8-14% of the patients, leading to change in the initial resection plan [13, 15].

Once identified, the SLN can be assessed through detailed focused pathologic examination. OTC detection becomes attainable as the number of LNs to be examined is greatly reduced [16, 17]. There are several tracers that can be used separately or in combination to localize the SLN; these include soluble blue dye (isosulfan blue, patent blue), radioactive material (human serum albumin, technetium) or fluorescent tracers [18]. Several studies [19-21] showed that a combination of isosulfan blue dye and technetium-99m sulfur colloid gives the best result in detecting SLN in colon cancer. The current study assesses the validity of SLN technique and whether it accurately predicts the status of the lymphatic basin and its value on proper staging of colon cancer patients.

2. Methods

This is a prospective cross-sectional study that conducted on patients with primary colon cancer in the department of surgery, Suez Canal University Hospital, Ismailia, Egypt in the period between 2010 and 2012. The study protocol was revised and approved from the local ethical committee of the faculty of medicine and an informed consent was obtained from each patient.

All patients of any age in both sexes with endoscopic biopsy proven as primary colon cancer (T1-3, any N, M0) were enrolled in the study. Excluded from the study are patients underwent neo-adjuvant chemoradiotherapy, patients having double lesions, patients with distant metastatic disease and patients with rectal lesions. All included patients were subjected to detailed medical history, full general examination, thoroughly abdominal examination and per rectal examination. Liver function tests, chest radiograph, CT scans of the abdomen and pelvis were performed to exclude distant metastasis.

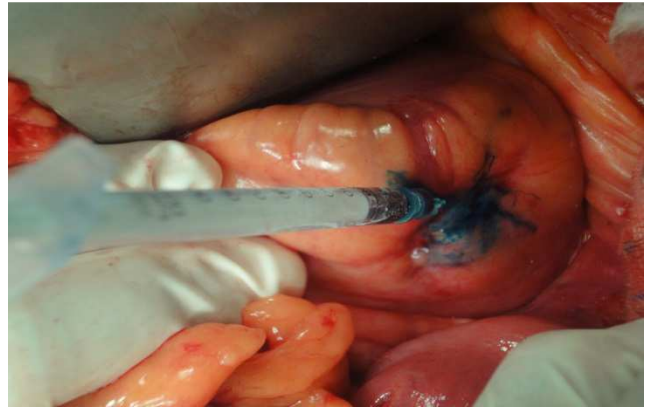


Figure 1. Subserosal injection of blue dye at site of colonic carcinoma.



Figure 2. Blue dye at transverse colon mass and stained mesenteric SLN.

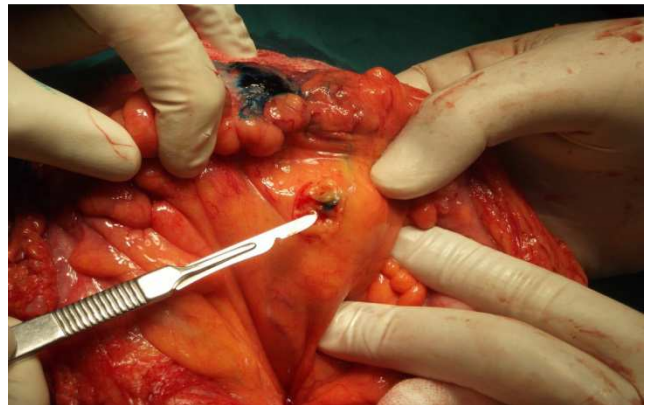


Figure 3. Dissected SLN at sigmoid mesentery with obvious blue stained afferent lymphatic channels.

Once the patient is under anesthesia, an exploratory laparotomy is performed, and the tumor is identified. The colon adjacent to the tumor is mobilized carefully, trying to minimize manipulation of the mesentery. Then; lymphatic mapping is performed using 1 millicuries (mCi) of technetium 99mTc -labeled sulfur colloid followed by 1-2 ml of isosulfan blue dye (Patent Blue, 2.5% Guerbet laboratories, France). Both materials are injected sequentially into the subserosa at four quadrants of the tumor (Figure 1).

After few minutes (5-10 min), the colon and the surrounding mesentery are examined using a hand-held gamma tracer (Navigator GPS; USSC, Norwalk, CT, USA).

Areas of high radioactivity are marked with sutures. The mesentery is also examined for blue-stained LNs (Figure 2 & 3). The first stained LNs are also marked with sutures. An appropriate colonic resection based on the anatomic location of the primary tumor is then performed. After the resection, any suture-marked LNs are removed from the specimen and labeled individually for pathological examination.

The primary tumor and its draining lymphatic basin containing the non-SLNs are processed histologically in the standard fashion with H&E staining. Each SLN is cut into sections and stained with H&E. SLNs with tumor cells detected by routine H&E staining underwent no further analysis while SLNs that are determined to be negative by conventional H&E staining are submitted for IHC staining for cytokeratin 20 antibody (CK20).

Data from history, operative findings and pathological assessment of SLNs and non-SLNs (after H&E staining and IHC staining) were analyzed by statistical package for social science SPSS 20 (SPSS Inc., Chicago, IL, USA). Quantitative data expressed as mean and standard deviation while qualitative data expressed as number and percentage of the total.

3. Results

From 48 patients met the preoperative inclusion criteria, three were excluded intraoperatively. Two of them were found to have T4 disease and the third had double colonic lesions. Analysis of the sociodemographic characteristics of the remaining 45 patients has revealed that twenty seven patients (60%) were males and eighteen (40%) were females. The age ranged from 30 to 67 years with a mean of 50.6 years.

The tumor was located in the right colon in 11 patients, transverse colon in 8 patients, left colon in 16 patients and sigmoid colon in 10 patients. The distal colonic cancers (left and sigmoid) represent 58% of included patients (Table 1). All patients showed one type of pathology which was colonic adenocarcinoma. As regards T stage, the highest percentage was for T2 (66.7%). T4 lesions were excluded from the study (Table 2). Thirty six patients (80%) has grade II tumors and nine patients (20%) has grade I.

The median number of LNs retrieved per patient during routine lymphadenectomy was 16.4 (ranged from 8 to 32). Among the studied 45 patients, SLNs were successfully retrieved in 43 patients (95.6%) with a mean of 1.7 SLNs per patient (ranged from 0 to 4). Among these forty three patients; the total number of patients with positive non-SLNs detected during conventional H&E staining is eighteen patients (41.9%) while twenty five patients (58.1%) have negative nodes. The two patients in whom SLNs could not be detected show positive non-SLNs during their conventional H&E staining.

Routine histopathological examination of the SLNs for the 43 patients revealed that 16 patients (37.2%) were positive for metastasis and 27 patients (62.8%) were negative. Among the sixteen patients with positive SLNs, 10 patients have

positive non-SLNs in their conventional H&E staining and 6 patients were negative. Among the twenty seven patients with negative SLNs, 8 patients have positive non-SLNs in their conventional H&E staining and 19 patients were negative. Table 3 shows the positivity and negativity of SLNs and Non-SLNs during their routine Histopathological analysis.

IHC examination for CK 20 was applied to all the 27 patients in whom SLNs were negative by conventional H&E staining. The result revealed that 10 patients were positive for metastasis and 17 patients were negative. Among the ten patients with positive SLNs, 6 patients have positive non-SLNs in their conventional H&E staining and 4 patients were negative. All the 17 patients with negative SLNs (by IHC examination) have also negative non-SLNs (by conventional H&E staining). Table 4 shows the positivity and negativity of SLNs during their IHC examination.

Table 1. Tumor site distribution.

	Frequency	Percent
Right colon	11	24.4
transverse colon	8	17.8
Left colon	16	35.6
sigmoid colon	10	22.2
Total	45	100.0

Table 2. Tumor T stage distribution.

	Frequency	Percent
T1	4	8.9
T2	30	66.7
T3	11	24.4
Total	45	100.0

Table 3. The positivity and negativity of SLNs and Non-SLNs during their routine Histopathological analysis.

		SLNs		Total
		+	-	
Non-SLNs	+	10	8	18
(Harvested during routine lymphadenectomy)	-	6	19	25
	Total	16	27	43

Table 4. The positivity and negativity of SLNs during their immunohistochemical examination.

		SLNs		Total
		+	-	
Non-SLNs	+	6	0	6
(Harvested during routine lymphadenectomy)	-	4	17	21
	Total	10	17	27

Analyzing data in table 3 & table 4 revealed that the false negative result was found in two patients (false negative rate is 7.1%) while no patient has false positive result (false positive by definition is zero). Sensitivity is 92.9%, specificity is 100%, negative predictive value is 88.2% and positive predictive value is 100%. The IHC examination of the SLNs detected 4 positive patients who were previously

assumed to have negative nodes by routine histopathological examination with upstaging rate of 9.3% in the whole study sample and 21% in the negative nodes patients.

4. Discussion

Colorectal cancer is the third most common cancer and the second leading cause of cancer deaths when both sexes are combined [22]. Recurrence of the tumour in its early stage and its associated morbidity and mortality despite the potentially curative operations denotes inadequate surgical excision or pathological understaging. The accuracy of staging depends mainly on number of LNs examined and the technique used to detect the OTC. Understaging is most likely to occur after harvesting insufficient number of LNs or the OTC have not been identified by the conventional H&E staining [5, 7, 23]. SLN mapping for colorectal cancer has been prompted in an effort to overcome the problem through selecting nodes that are at greatest risk for harboring metastases. Once it is identified, SLN can undergo a detailed pathologic analysis to identify the OTCs [13, 17]. In the current study, total number of dissected LNs ranged from 8 to 32. In most cases; this number was sufficient to face the minimum recommended number for good and standardized staging requirement (12 LNs) [6, 7].

The number of identified SLNs is a matter of variation among literature. Although some authors [13, 24, 25] define sentinel nodes as the first one to four blue nodes that appear within 5 to 10 minutes of dye injection, others have regarded all blue-staining nodes as sentinel nodes [19, 26]. Consequently, there is a large variation in examined sentinel nodes (0 to 21) in the literature. In the present study; the identified SLNs ranged from 0 to 4 and it was looked for within the first 5 to 10 minutes after injection of both dye and radio labelled colloid with a mean of 1.7 LNs. In this study; the identification rate of SLN was 95.6% which emphasize on the inflated success with the usage of the combined blue dye method and radio colloid method. This is consistent with the results showed by Trocha et al, 2003 [20] (identification rate: 96%) and Saha et al, 2004 [19] who got a result of 100% identification rate in a series included 57 patients.

The false negative rate in our series was 7.1%. This represents 2 patients who did not show evidence of SLN metastasis either by routine histopathology or IHC while showed metastasis to non-SLNs during conventional lymphadenectomy. This false negative rate is comparable to what has been reported by Bilchik and Trocha, 2003 [27] (false negative rate: 8%), while it is much better than what were reported by Saha et al, 2004 [19] and Patten et al 2004 [28] (16% and 17% respectively). These false negative results could be due to excessive infiltration of the LNs that lead to directional flow changes of lymph, leading to skip phenomenon whereby the SLN is bypassed and the tracer is taken up by non-SLNs [6, 29].

In our series, 4 patients proved to have higher stage after application of SLN technique and IHC, and this represent 9.3% upstaging rate. This upstaging rate is more than what

has been reported by Saha et al, 2004 [19] (5%) and being lower than that of Patten et al 2004 [28] and Bilchik and Trocha, 2003 [27] (20% and 31% respectively). This variation in upstaging rates may be due to different mapping tracers and different methods used to detect the OTC.

In conclusion; SLN technique is feasible and reliable in colon cancer patients. It can upstage the disease resulting in more accurate determination of patients need postoperative adjuvant therapy in such way that surely affect prognosis of the disease.

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