Sentinel Lymph Node Study: Its Significance In Colorectal Cancer.

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ABSTRACT

Background: Intraoperative SLNB using methylene blue is technic ally simple and quick (usually within 10 minutes of operating time) procedure. It allows the pathologist to focus attention on a limited number of nodes (1-5) for detailed focused analysis, which saves time and is less costly. Methods: Acolonooscopic biopsy-proven diagnosis of clinical stage I and II colon or rectal cancer were prospectively studied. A standard oncological en-bloc resection of the neoplasm and the regional LNs was then performed. The SLNs (sentinel lymph node biopsy) were dissected from the surgical specimen immediately after the completion of the operation and were sent separately to the pathology department together with the specimen. The SLNs were submitted in their entirety for microscopic examination. Results: SLNB is highly accurate because it accurately predicts the regional lymph node status in 92.85% of cases. The absence of metastases in the SLN accurately predicts the status of the non-SLNs 85.7% of the time. Conclusion: SLNB improves the staging of patients with colon cancer by upstaging 14.29% of patients, who may benefit from further adjuvant chemotherapy.

Keywords: Colorectal Cancer, Sentinel Lymph Node.

INTRODUCTION

Globally colorectal cancer is the third most common cancer in males and the second in females. The most important prognostic factor in CRC is the stage of the tumor at the time of initial diagnosis and the presence of lymph node (LN) metastases decreases the 5-year survival by approximately 20% to 30%. Surgery remains the only curative option but adjuvant chemotherapy (CT) has been shown to improve survival in patients with positive lymph nodes (LNs) (i.e. Stage III CRC). Accurate identification of LNs involved by metastases is thereby of vital importance as it facilitates decision-making with regard to adjuvant systemic therapy. The role of SLNB (sentinel lymph node biopsy) in CRC is not to decide on the extent of lymphadenectomy but to increase the histological yield of positive LNs following standard radical resection. Detection of malignant involvement of LNs outside the traditional drainage area and resection line is another potential use of SLNB. The purpose of this study was to validate the feasibility of SLNB in patients with CRC.

MATERIALS AND METHODS

All patients, from June 2014 to June 2016, with a colonoscopic biopsy-proven diagnosis of clinical stage I and II colon or rectal cancer, were prospectively studied under an Institutional Review Committee approved Protocol after obtaining informed consent. Patient characteristics, including age, sex, and tumor location, were documented. Preoperative staging evaluation was done with physical examination including digital rectal examination, laboratory studies (including blood chemistry and carcinoembryonic antigen (CEA), liver function tests), fecal occult blood test (FOBT), chest x-ray, computed tomographic scan of the abdomen and pelvis (CT Scan), and colonoscopy in all patients. Transrectal ultrasound was not routinely done.

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Inclusion Criteria
1. Colonoscopic biopsy-proven diagnosis of colonic or rectal adenocarcinoma
2. Clinical Stage I or Stage II resectable disease
3. No evidence of Distant Metastasis
4. Performance status of less than or equal to 2 on the Eastern Cooperative Oncology Group (ECOG) scale

**Exclusion Criteria**
1. Patients requiring emergency surgery (such as for malignant bowel obstruction, perforation)
2. History of Crohn disease, chronic ulcerative colitis, familial polyposis, intestinal tuberculosis, autoimmune diseases
3. Prior history of malignancy (including colorectal malignancy), irradiation or prior abdominal surgery
4. Patients found to have metastases intraoperatively

### Surgical Technique
All of the patients were approached via open surgical procedures such as anterior resection (AR), abdomino-perineal resection (APR) and segmental resection and end-to-end anastomosis or hemicolectomy. Patients were brought to the operating room, where an exploratory laparotomy was performed. The location of the primary neoplasm was confirmed together with assessment of the extent of the primary tumor and any distant metastases were excluded. None / barest minimal of the extent of the primary tumor and any distant metastases. Once the primary lesion was identified, 0.5 mL of 1% Methylene Blue was injected into the bowel wall subserosally circumferentially around the primary tumor at 4 sites (total 2 ml) by using a tuberculin syringe. Care was taken to ensure that there was no injection into the lumen of the bowel. Proctoscope was used to inject the dye on the distal aspect in rectal cancers submucosally.

The blue dye travels quickly via the lymphatics to the draining LNs, which turn pale to deep blue. Within the first 10 minutes after injection, the first to fifth nodes closest to the tumor, that were highlighted with blue dye against a background of yellow mesenteric fat were identified as the SLNs and were marked with silk sutures.

A standard oncological en-bloc resection of the neoplasm and the regional LNs was then performed. The SLNs were dissected from the surgical specimen immediately after the completion of the operation and were sent separately to the pathology department together with the specimen. The SLNs were submitted in their entirety for microscopic examination.

### Pathological Analysis
The surgical specimen was dissected manually to identify other LNs contained in the mesenteric fat. No chemical clearance method was used. After resection of the specimen, the tagged LNs were excised and separately processed to further examination as SLNs. Thereafter, as many non-sentinel lymph nodes (NSLN)S as could be identified, were dissected from the specimen (aiming at a minimum of 12 lymph nodes as recommended by the UICC/AJCC).

#### Analysis of SLNs
All the SLNs were bisected and embedded in paraffin. Single section was routinely performed. Slices were stained by H&E staining. If the result was negative, all SLNs paraffin blocks were sectioned at 20 micron intervals to five slices 4 microns thick and stained by H&E in the second step of pathological evaluation.

#### Analysis of non-SLNs
All non-SLNs were bisected and embedded in paraffin. Single section was routinely performed and stained by H&E staining.

#### Analysis of Specimen
Standard processing of the tumor included reporting the tumor size and grade, T stage, and surgical margin status. The number of nodes, and the number of positive nodes were recorded. Tumor deposits within LNs were classified and staged according to the revised guidelines set by the AJCC and UICC. Tumor status in SLN was compared with the status in all other harvested regional nodes for each patient.

### RESULTS

#### Table 1: Characteristics of Patients and Interventions

<table>
<thead>
<tr>
<th>General Parameter</th>
<th>Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. of Patients</td>
<td>17 (100%)</td>
</tr>
<tr>
<td>Male [No. (%)]</td>
<td>10 (58.8%)</td>
</tr>
<tr>
<td>Female [No. (%)]</td>
<td>7 (41.2%)</td>
</tr>
<tr>
<td>Age (yr) [mean (range)]</td>
<td>53.1 (36 – 86)</td>
</tr>
<tr>
<td>Tumor Distribution</td>
<td></td>
</tr>
<tr>
<td>Colon [No. (%)]</td>
<td>8 (47.1%)</td>
</tr>
<tr>
<td>Right Colon [No. (%)]</td>
<td>4 (23.5%)</td>
</tr>
<tr>
<td>Descending Colon [No. (%)]</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>Sigmoid Colon [No. (%)]</td>
<td>3 (17.7%)</td>
</tr>
<tr>
<td>Rectum</td>
<td>9 (52.9%)</td>
</tr>
<tr>
<td>Operative Procedure</td>
<td></td>
</tr>
<tr>
<td>Right Hemicolectomy [No. (%)]</td>
<td>4 (23.5%)</td>
</tr>
<tr>
<td>Left Hemicolectomy [No. (%)]</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>Segmental Colectomy [No. (%)]</td>
<td>3 (17.6%)</td>
</tr>
<tr>
<td>Abdominoperineal Resection [No. (%)]</td>
<td>8 (47.1%)</td>
</tr>
<tr>
<td>Resection &amp; Hartman’s [No. (%)]</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>T-Stage</td>
<td></td>
</tr>
<tr>
<td>T1 [No. (%)]</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>T2 [No. (%)]</td>
<td>4 (23.5%)</td>
</tr>
<tr>
<td>T3 [No. (%)]</td>
<td>6 (35.3%)</td>
</tr>
<tr>
<td>T4 [No. (%)]</td>
<td>7 (41.2%)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
</tr>
<tr>
<td>G1[No. (%)]</td>
<td>5 (29.4%)</td>
</tr>
<tr>
<td>G2[No. (%)]</td>
<td>11 (64.7%)</td>
</tr>
<tr>
<td>G3[No. (%)]</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>G4[No. (%)]</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Nodes</td>
<td></td>
</tr>
<tr>
<td>Sentinel Nodes[No. (%)]</td>
<td>28 (21.2%)</td>
</tr>
<tr>
<td>Positive[No. (%)]</td>
<td>9 (32.1%)</td>
</tr>
<tr>
<td>Non-Sentinel Nodes[No. (%)]</td>
<td>104 (78.8%)</td>
</tr>
<tr>
<td>Positive[No. (%)]</td>
<td>31 (29.8%)</td>
</tr>
</tbody>
</table>

The number of sentinel nodes was compared with the status in all other harvested regional nodes for each patient.
The average number of nodes and SLNs was more in colon cancers (9.12 per case and 2 per case, respectively) in comparison to rectal cancers (6.55 per case and 1.33 per case, respectively) but average positive nodes and positive sentinel nodes were more in rectal cancers (3.11 per case and 0.77 per case, respectively) as compared to colon cancer cases (1.5 per case and 0.25 per case, respectively).

The average number of LNs as well as SLNs was more in pT4 patients in comparison to pT2 or pT3 patients. The average number of positive nodes as well as positive sentinel nodes increased with increasing T-stage.

The average number of LNs and SLNs were more in grade 1 patients than in grade 2 patients. Grade 2 patients had more positive LNs and SLNs in comparison to grade 1 patients.
The average numbers of LN s as well as SLNs were more in patients with lymphovascular invasion. The average number of positive SLNs were also higher in patients with lymphovascular invasion.

Table 7: Showing Nodal and SLN Detection and Positivity per case in relation to Tumor Size in CRC patients.

<table>
<thead>
<tr>
<th>Tumor Size (cms)</th>
<th>No. of Cases</th>
<th>Total Nodes (Nodes per case)</th>
<th>Positive Nodes (Positive Nodes per case)</th>
<th>No. of SLNs (SLNs per case)</th>
<th>No. of Positive SLNs (Positive SLNs per case)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 3.0</td>
<td>1</td>
<td>5 (5)</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3.1 – 6.0</td>
<td>13</td>
<td>92 (7.08)</td>
<td>22 (1.69)</td>
<td>23 (1.77)</td>
<td>7 (0.54)</td>
</tr>
<tr>
<td>6.1 – 9.0</td>
<td>1</td>
<td>14 (14)</td>
<td>3 (3)</td>
<td>24 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>9.1 – 12.0</td>
<td>1</td>
<td>6 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>12.1 – 15.0</td>
<td>1</td>
<td>15 (15)</td>
<td>15 (15)</td>
<td>2 (2)</td>
<td>2 (2)</td>
</tr>
</tbody>
</table>

The average number of positive LNs as well as positive SLNs were more with tumors of maximum size in this study.

Table 8: Showing the Nodal Positivity in Colorectal cancer cases in this study

<table>
<thead>
<tr>
<th>Nodes</th>
<th>No. of Nodes</th>
<th>No. of Positive Nodes</th>
<th>Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sentinel Nodes</td>
<td>28</td>
<td>9</td>
<td>32.1%</td>
</tr>
<tr>
<td>(9 of 28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Sentinel Nodes</td>
<td>104</td>
<td>31</td>
<td>29.8%</td>
</tr>
<tr>
<td>(31 of 104)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nodal Positivity of SLNs vs NSLNs
SLNs were more often positive as compared to non-sentinel nodes.

Table 9: Showing the Distribution of Positive and Negative SLNs in CRC cases in this study

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no of Patients</td>
<td>17</td>
</tr>
<tr>
<td>Total patients with SLN identified</td>
<td>14</td>
</tr>
<tr>
<td>Total patients with negative SLN (no evidence of metastases)</td>
<td>7</td>
</tr>
<tr>
<td>Total patients with negative SLN and positive non-SLN</td>
<td>1</td>
</tr>
<tr>
<td>Total patients with positive SLNL (evidence of metastases)</td>
<td>7</td>
</tr>
<tr>
<td>Total patients with positive SLN on MLS (micrometastases only)</td>
<td>1</td>
</tr>
</tbody>
</table>

Sensitivity of SLNB to predict Metastases and False Negative Rate
Overall, 8 of 14 patients (50%) were found to have metastases (macrometastases or micrometastases) in SLNs or non-SLNs, and thus, were considered as node positive (pN1 or pN2). In 7 of these patients, the SLNs were positive for metastases resulting in a sensitivity (i.e. the probability of detecting tumor in an SLN when nodal metastasis is present) of 87.5% (7 of 8), whereas in the remaining 1 patient the SLN was not positive for macrometastases but metastases were identified in non-SLNs, resulting in a false-negative rate (i.e. the probability of not finding tumor in a SLN when nodal metastasis is present) of 12.5% (1 of 8).

Identification of micrometastases by Multi-level sectioning of SLN
Out of 14 patients in whom SLNs were identified, macrometastases were identified in 6 of 14 patients with SLNs, one patient had no macrometastases in SLNs but macrometastases was present in Non-SLNs. The remaining 7 patients classified as node negative (N0) by routine H&E staining underwent multilevel sections of the SLN. One of these 7 patients revealed micrometastases (14.29%).

Accuracy
The accuracy to predict the nodal status by SLNB was 92.85% (13 of 14).

Negative Predictive Value
The negative predictive value for the prediction of the absence of metastases was 87.5% (7 of 8)

Aberrant Lymphatic Drainage
Aberrant lymphatic drainage could not be identified in any of our 17 patients.

Complications
There were no complications specifically related to the SLN technique in any of the 17 cases.

DISCUSSION
The prognostic significance of LN metastases in solid tumors is well known. LN metastases decrease the overall survival in CRC by about 30%. CRC patients with LN metastases are usually treated with adjuvant chemotherapy, with a reported reduction in cancer-related mortality by approximately 33%. Patients with AJCC stage I/II disease often are not treated outside a protocol study with adjuvant chemotherapy because of a lack of strong supporting data in the published literature. Unfortunately, 20% to 30% of these patients will experience recurrence of their disease and die of metastatic disease within 5 years of diagnosis, despite undergoing curative surgery. A likely explanation of this high mortality rate is that conventional methods may falsely underestimate micrometastatic nodal involvement in some patients, who may then not receive effective adjuvant chemotherapy and, consequently, may suffer from increased mortality. A total of 17 patients, from June 2007 to June 2009 with a colonoscopic biopsy-proven diagnosis of
clinical stage I and II colon or rectal cancers were studied. The in vivo dye injection technique used in this study was similar to Saha et al and Weise et al (but 1% Methylene blue was used in place of 1% isosulfan blue used by Saha et al and Weise et al). Care was taken to ensure that there was no injection into the lumen of the bowel. Within the first 10 minutes after injection, the first to fifth nodes closest to the tumor, that were highlighted with blue dye against a background of yellow mesenteric fat were identified as the SLNs and were marked with silk sutures. The SLNs were sent separately to the pathology department together with the specimens. Studies have used isosulfan blue (1% Lymphazurin), 1% methylene blue, carbon dye (India Ink), 10% fluorescein, radiolabeled colloids (99mTc-sulfur colloid in the United States, 99mTc-nanocolloidal and 99mTc-antimony sulfide in Europe and Australia) either alone or in combination. In this study methylene blue was used as it is cheaper, does not cause hypersensitivity reactions and blue discoloration of urine and stool are temporary. Some studies have recommended that blue dye and radio-tracer mapping be combined as this approach may yield a higher SLN identification rate (0 – 9.5%) than blue dye alone. Radio-tracer mapping is very expensive (prohibitive so in most developing countries), cumbersome (requiring time-consuming preoperative preparation and increased operating time), has no significant SLN detection advantage as single agent over blue dye and may pose radiation risk. Besides, the proximity to the primary site could lead to a “shine-through” effect, reducing the sensitivity of the radioactivity of the SLNs.

In this study the surgical specimen was dissected manually to identify other lymph nodes contained in the mesenteric fat. Hermanek et al determined that the average number of nodes present in a standard colon resection after use of a fat-clearance technique was 47. Without the aid of a fat-clearance technique, the authors found 31 nodes on average. Although this number is almost one third less, it is still significantly greater than the mean of 7.7 nodes per patient in this study. The number of lymph nodes found by the clearance techniques ranges from 34 to 68 per surgical specimen. The higher number of lymph nodes discovered affords a higher possibility of detection of metastases.

Herrera-Ornelas et al studied metastases in small nodes from colon cancer and concluded that the use of clearance techniques in surgical specimens from colon cancer enhances pathologic staging by increasing detection of metastases in small lymph nodes. Lymph node metastases from colon cancer occur most frequently in small lymph nodes (≤ 5 mm). Lymph nodes 10 mm in diameter and larger can be found with nests of tumor cells lodged in the subcapsular area, but small lymph nodes were always found almost completely replaced with tumor. In this study, the SLN detection rate was more in colonic tumors (87.5%) in comparison to rectal tumors (77.7%), but this association was not statistically significant (p > 0.05). SLNB sensitivity for metastasis was more in rectal tumors (100%) in comparison to colonic tumors (50%) but again this was not statistically significant (p > 0.05). This can be due to more advanced cancers in the rectal cancer group (one pT2 tumor, three pT3 and five pT4 tumors) compared to colon cancer group (three pT2 tumors, three pT3 tumors and two pT4 tumors).

In this study, the average number of nodes and SLNs were more in colon cancers (9.12 per case and 2 per case, respectively) in comparison to rectal cancers (6.55 per case and 1.33 per case, respectively) but average positive nodes and positive sentinel nodes were more in rectal cancers (3.11 per case and 0.77 per case, respectively) as compared to colon cancer cases (1.5 per case and 0.25 per case, respectively). This can be due to more advanced cancers in the rectal cancer group (one pT2 tumor, three pT3 and five pT4 tumors) compared to colon cancer group (three pT2 tumors, three pT3 tumors and two pT4 tumors).

Quadros et al (2008), using combined technique in 52 patients reported low rectal location (P=0.009) was an independent risk factor for inability to detect SLNs in multivariate analysis, using H&E, SS, and IHC. Shen et al (2009) reviewed clinicopathologic factors of 434 consecutive cases of CRC treated by surgical resection correlated with number of lymph nodes recovered and found that tumor location was associated with number of lymph nodes harvested. More lymph nodes were present in resection specimens of cecum/ascending colon and descending colon cancers than in those of transverse colon, sigmoid colon, and rectal cancers in multivariate regression analysis. In this study, the SLN detection rate was more in colon cancers (87.5%) in comparison to rectal tumors (77.7%) but this association was not statistically significant (p > 0.05). The average number of nodes and SLNs were more in colon cancers (9.12 per case and 2 per case, respectively) in comparison to rectal cancers (6.55 per case and 1.33 per case, respectively) but average positive nodes and positive sentinel nodes were more in rectal cancers (3.11 per case and 0.77 per case, respectively) as compared to colon cancer cases (1.5 per case and 0.25 per case, respectively). Kitagawa (2002) studied 56 CRC patients with Tc-99m labeled colloid and H&E staining and had SLN DR and accuracy of 100% for patients with T1 or T2 primary tumors were as overall SLN DR was 91%. All the 4 false negative cases were advanced cases with T3 primary tumors. Broderick-Villa et al (2002) studied SLNB in 50 CRC patients using blue dye and H&E with CK-IHC if negative by IHC, and found that tumor stage was not associated with higher false negative rate. In this study, the SLN Detection rate as well as SLNB sensitivity was
highest in pT4 tumors as compared to pT3 and pT2 tumors, though, not statistically significant (p > 0.05). There were no cases with pT1 tumors. The average number of LNs as well as SLNs were more in pT4 patients in comparison to pT2 or pT3 patients. The average number of positive nodes as well as positive sentinel nodes increased with increasing T-stage. Fang et al retrospectively studied 1127 CRC patients undergoing resection and found by univariate as well as multivariate logistic regression analysis that the lowering tumor histological differentiation was a statistically significant risk factor for lymph node metastasis. In this study, grade 2 tumors were most common (64.7%), with only one grade 3 tumor and no grade 4 tumor. SLN sensitivity was more in grade 2 patients as compared to grade 1 patients. This was however, not statistically significant (p > 0.05). The average number of LNs and SLNs were more in grade 1 patients than in grade 2 patients.

Bembenek et al (2007) studied 315 colon cancer patients using blue dye injection followed by SS and IHC if LNs were negative on routine H&E. LVI was positively associated with SLNB detection rate. In this study, SLN detection rate and SLNB sensitivity increased with the presence of lymphovascular invasion. This was however, not statistically significant (p > 0.05). The average numbers of LNs as well as SLNs were more in patients with lymphovascular invasion. Wolmark et al studied 670 colon cancer patients and 236 rectal cancer patients in NSABP prospective trials and found that there was no correlation between the longest diameter of the primary tumor and the status of regional lymph nodes for either colon or rectal cancer. Quadros et al (2008), using combined technique in 52 patients reported tumor size (P=0.036) was an independent risk factor for inability to detect SLNs in multivariate analysis, using H&E, SS, and IHC. In this study, the mean tumor size 5.79 (± 2.63) X 3.04 (± 1.08) cms and the range was 2.5 to 13 cms. Maximum SLNB sensitivity was seen in tumors with maximum size (100% and 12.1 – 15.0 cms, respectively). This was not statistically significant (p > 0.05). SLN detection rate however, was unrelated to tumor size.

In this study, a total of 132 nodes were harvested (7.7 per patient): 104 non-SLNs (6.1 per patient) versus 28 SLNs (1.6 per patient). The average number of SLN retrieved is the range of 70% to 100%. In this study, the SLNB improves the staging of patients with colon cancer by upstaging 14.29% of patients, who may benefit from further adjuvant chemotherapy. A selected group of patients staged as node negative by SLN biopsy, likely to be cured by the surgical intervention alone are spared from the toxic effects of chemotherapy.

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