Upstaging Benefits and Accuracy of Sentinel Lymph Node Mapping in Colorectal Adenocarcinoma Nodal Staging

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Background and Objectives: Sentinel lymph node (SLN) mapping is an additional method for improving colorectal cancer nodal staging. The purpose of the study was to define the method’s accuracy in nodal staging, its upstaging benefits and to identify the predictive factors for its failure.

Methods: Lymphatic mapping was performed using technetium-99m-phytate and patent blue in 52 consecutive colorectal adenocarcinoma patients. Enhanced pathological examination was carried out on SLNs with hematoxylin–eosin step-sectioning and immunohistochemistry.

Results: The patients studied had an average tumor size of 6.5 cm; 85% had T3/T4 tumors; and rectal tumors represented 57.7% of the group. Overall SLN mapping accuracy was 79.5%, with sensitivity of 65.2% and 34.8% false negatives. Upstaging with SLN mapping was 23.1%. Colon tumors had an SLN identification rate of 90.9% and rectal tumors had 63.3% (P = 0.023). Multivariate statistical analysis identified lower rectal tumor (P = 0.009), neoadjuvant treatment (P = 0.029) and tumor size (P = 0.036) as independent risk factors for the inability to detect SLNs.

Conclusions: Upstaging benefits of SLN mapping should be considered in colon and mid- and upper rectal tumors. The method should be avoided in patients with lower rectal tumors, large tumors and having had neoadjuvant therapy.


Key Words: lymphatic mapping; micrometastasis; colon and rectal cancer; neoadjuvant treatment

INTRODUCTION

In Brazil, colorectal adenocarcinoma is the fourth most common malignancy indicated by an estimated incidence of 25,360 cases in 2006, where there was a 76.7% increase in new cases in the last two decades for men and 69.0% for women [1]. The treatment of colorectal cancer has become a matter of public health concern and methods of nodal staging improvement are welcomed by national public authorities.

Lymph node status of colorectal adenocarcinoma patients is an important prognostic factor and defines the need for adjuvant treatment [2]. Current guidelines indicate chemotherapy in patients with metastatic nodes (TNM stage III) and not in node-negative patients (TNM stage II) [3,4]. Since 20–40% of TNM stage II patients submitted to curative surgical treatment develop recurrence of the cancer, it has been postulated that they might not have been adequately staged [2,5]. Therefore, while lymph node status in colorectal cancer is one of the most important prognostic factors, it is not a true predictor of outcome, probably because node-positive patients might have been considered node-negative.

The examination of a minimum of 8–12 lymph nodes has been established to consider a colorectal cancer patient as being true node negative (N0) [6,7]. This parameter (to be determined by oncological surgeons and pathologists) would increase the accuracy of lymph node status to achieve proper cancer staging. The number of dissected lymph nodes should be correlated with one of the fundamentals of surgical oncology, that is, regional lymphadenectomy. The greater the number of examined nodes in the surgical specimen, the more accurate cancer staging becomes, as the possibility of identifying metastatic nodes increases.

Another procedure that has been postulated as capable of increasing metastatic lymph nodes identification in colorectal cancer has been sentinel lymph node (SLN) mapping [8–28]. The aim of this method is to identify metastatic lymph nodes on a direct drainage pathway from the tumor’s lymphatic basin. Unlike SLN mapping in breast cancer and melanoma, the method in colorectal cancer does not implicate the indication or not for regional lymphadenectomy, but it is used rather as a tool for increasing staging accuracy [8–28]. Performing lymphatic mapping during standard colorectal surgery provides the retrieval of a small number of SLNs that could be representative of the entire surgical specimens’ lymph node status [8–28]. Submitting these SLNs to expensive and time-consuming pathological examination improves patients’ nodal staging [8–28]. Performing a more detailed pathological examination of the SLNs results in the detection of lymph node metastases that otherwise would go undetected.

The aims of this study were to test the accuracy of sentinel node mapping in colorectal cancer nodal staging, to determine its ability of upstaging patients that could be considered node negative if the method was not used, and to identify the predictive factors of the inability of SLN identification.

PATIENTS AND METHODS

A total of 52 consecutive colorectal adenocarcinoma patients were prospectively submitted to SLN mapping during their standard curative surgery. The patients studied had an average tumor size of 6.5 cm; 85% had T3/T4 tumors; and rectal tumors represented 57.7% of the group. The examination of a minimum of 8–12 lymph nodes has been established to consider a colorectal cancer patient as being true node negative (N0) [6,7]. This parameter (to be determined by oncological surgeons and pathologists) would increase the accuracy of lymph node status to achieve proper cancer staging. The number of dissected lymph nodes should be correlated with one of the fundamentals of surgical oncology, that is, regional lymphadenectomy. The greater the number of examined nodes in the surgical specimen, the more accurate cancer staging becomes, as the possibility of identifying metastatic nodes increases.

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surgery, from January 2004 to July 2005. All surgical procedures and pathological examinations were performed at a single institution, the Aristides Maltez Hospital, Salvador, Brazil. Local and national ethics committees approved the trial and written informed consent was obtained from all patients. Patients included in the study had no distant metastasis identified in preoperative or intraoperative evaluation and were submitted to curative treatment for resectable primary colorectal cancer.

In matters of tumor site of location for lymphatic mapping, colon and sigmoid colon cancers were analyzed together as they share mesentery and lymphatic chain characteristics. Patients with colon cancers presented with tumor in the cecum, ascendancy, transverse, descendant and sigmoid colon. Rectal cancers were defined as tumors originating at or below the peritoneal reflection. The rectum was divided into three anatomic regions. The upper rectum was the proximal 1/3 starting at the peritoneal reflection. The lower rectum was considered the lower 1/3 including the anal canal. The mid-rectum was the intermediate 1/3 rectal segment between the upper and lower rectum.

Indication of preoperative chemoradiotherapy (neoadjuvant treatment) occurred when a computed tomography scan suggested positive pelvic lymph nodes or T4 rectal lesions, and when pelvic rectal examination identified fixed tumors. Neoadjuvant treatment consisted of a 5.040 cGy radiotherapy dose delivered in 180-cGy fractions. Radiotherapy was combined with a daily regimen of 5 fluorouracil (375 mg/m²) and leucovorin (30 mg/m²), delivered in a 5-day cycle, one on the first and other on the third week of radiotherapy. Patients submitted to neoadjuvant treatment, even ones with T4 tumors, were included in the study.

All patients were treated by the first author who performed standard surgical procedures with classical regional lymphadenectomy and followed this study protocol using lymphatic mapping as an additional lymph node staging tool. Patients with rectal cancer were also enrolled in a second ongoing trial, in which retroperitoneal and lateral pelvic lymphadenectomy were added to the standard total mesorectal excision [29]. All surgical specimens were accessed by a single pathologist, the third author. Data obtained at the Aristides Maltez Hospital was audited at the A.C. Camargo Cancer Hospital by the second author and submitted to statistical analysis by the fourth author at the Cancer Hospital of Barretos.

After anesthetic induction, a solution containing 37 MBq of technetium-99m-phytate, diluted in 2.0 ml of saline, was injected around the tumor using a tuberculin syringe, followed by injection of 2.0 ml of a 2.5% solution of patent blue dye. In patients with colon, sigmoid colon and upper rectal tumors, the radiotracers and the dye were injected subserosally in a circumferential manner around the tumor. In mid- and lower rectal tumors, injection was performed through a transanal approach into the submucosal and muscular layer. A handheld gamma probe device (Evroprobe™, CA-Te-detector) was used for lymphatic mapping during the surgical procedure and after specimen retrieval for SLN identification.

Radioactive nodes were defined if their counts were at least three times greater than the background counts, away from the site of the technetium-99m-phosphate injection. Blue nodes were identified by visual assessment. All identified radioactive or blue nodes were considered to be SLNs. Once identified and isolated, SLNs were sent separately for pathological inspection.

All the lymph nodes identified in the surgical specimens were submitted to routine pathological analysis that consisted of examination of one lymph node section stained by hematoxylin–eosin. In the SLN, if these first section slides were negative for metastases, special techniques of enhanced pathologic analysis including step-sectioning and immunohistochemistry were used. Upstaging was only considered if enhanced pathological examination detected SLN metastases, with step-sectioning hematoxylin–eosin staining or immunohistochemical examination, and if all other non-sentinel nodes were negative. If routine pathological analysis identified metastasis in an SLN, this lymph node was not considered in the upstaging count.

Step-sectioning of SLNs consisted of cutting formalin-fixed and paraffin-embedded tissues at 4 µm, creating three distinct levels with two sections in each level. One section of each level was stained with hematoxylin–eosin and the other used for immunohistochemistry. The first level consisted of the two sides of the sectioned lymph node in its longitudinal axis, examined in routine pathological examination. The next four µm of the node were discarded, and a second level was obtained; again four µm were discarded, and a third level of sections was obtained.

Immunohistochemical examination was performed using the streptavidin–biotin–peroxidase method (LSAB-Dako, Carpinteria, CA). A primary monoclonal antibody against cytokeratin (clone AE1/AE3) was used at a dilution of 1:100. Before the application of the monoclonal antibody, the sections were pretreated in citrate buffer at pH 6 in a 96°C steam bath for 35 min.

SLNs were considered to be positive when unequivocal micrometastases or macrometastases were identified by histological or immunohistochemical examination. The parameter for micrometa-stases identification established by Saha et al. [20] was used, defining micrometastases as a tumor focus within a single node that measures less than 0.2 cm in greatest dimension or nodal tumor that was only detectable by immunohistochemistry. Nodal metastases not meeting these criteria were considered macrometastases [20]. Micrometastases were used in nodal upstaging [20].

The accuracy of lymphatic mapping in colorectal cancer lymph node staging was evaluated using the following statistical measures described by Saha et al. [20]. The identification rate was calculated as the number of lymphatic mapping procedures where at least one SLN was identified, divided by the total number of procedures in which the method was attempted. True positives (TP) were defined as the cases where SLNs had metastatic cells whether or not metastatic cells were found in other nodes. True negatives (TN) were the cases where both SLNs and non-sentinel lymph nodes did not have metastatic cells. False negatives (FN) were defined as cases where the SLNs were negative whereas the non-sentinel nodes were positive. The equations of the variables are demonstrated as follows: sensitivity was calculated using the formula TP/(TP + FN), false negative as FN/(FN + TP), negative predictor value as TN/(FN + TN) and accuracy as (TP + TN)/(TP + FP + FN + TN).

Comparison of the category variables was performed with Fischer’s exact test and the continuous variables were compared using the Mann–Whitney test. Logistic regression was used to identify the predictive factors for the inability of SLN identification. The model was constructed using the stepwise forward selection method. The final model was adjusted by tumor size (continuous variable) and neoadjuvant therapy. In all statistical tests the alpha error was set at 5%.

**RESULTS**

SLN mapping was performed in 52 consecutive colorectal adenocarcinoma patients. The group consisted of 57.7% (30/52) rectal cancer patients and 42.3% (22/52) colon cancer patients. The mean patient age was 55.9 years (SD = 14.9) and median was of 56.0 years (range from 22 to 79 years). Female gender was predominant with 63.5%. The total number of lymph nodes examined by the routine pathological method was 987, with a mean of 19 nodes retrieved per patient (987/52). SLNs totaled 137, with an average of 3.5 nodes per patient (137/39).

In terms of TNM staging, the group was formed of 31 stage III patients (60%), 19 stage II patients (36%) and 2 stage I patients (4%). No patient had a T1 tumor; 15% of the patients had T2 tumors, 54% T3
tumors and 31% T4 tumors. The average tumor size was 6.5 cm (SD = 4.8), with a median of 5.0 cm (range from 2.0 to 32.5 cm). There was a statistical significant difference (Mann–Whitney test: P = 0.017) between the mean tumor size of colon tumors (8.3 cm; SD = 6.4 cm) when compared to the mean tumor size of rectal tumors (5.2 cm; SD = 2.7 cm).

SLNs were isolated in 39 patients, with an identification rate of 75% (Table I). Colon cancer had an identification rate of 90.9% and rectal cancer had an identification rate of 63.3% (P = 0.023). Total accuracy of SLN mapping for colorectal staging was 79.5%. Sensitivity was 65.2%, and negative predictive value was 66.7%, while false negatives were 34.8%.

Fifteen patients had metastases in their SLNs. Immunohistochemistry was responsible for the detection of all six cases of SLN micrometastases, and the hematoxylin–eosin step-sectioning process detected SLN macrometastases in 9 patients. In 9 patients (23.1%), the SLNs were the only metastatic nodes of the entire surgical specimen leading to a TNM upstaging from stage II to III. The total upstaging rate in colon cancer patients was 20.0% and for rectal cancer patients 26.3%. All the SLNs responsible for upstaging were within the first five identified nodes next to the colorectal cancer.

The identification rate for each surgical procedure is shown in Table II. Abdominoperineal rectal resection was the most performed procedure, being responsible for 25.0% of the surgeries, followed by anterior rectal resection (23.1%) and right coloectomy (23.1%). SLN Identification rate in abdominoperineal rectal resection was low, as only 6 patients out of 13 had SLNs identified, which resulted in an identification rate of 46.1%. The identification rate was lowest (33.3%) when abdominoperineal rectal resection was combined with colpectomy. The SLN identification rate was better in anterior rectal resection with 91.7% and right coloectomy with 83.3%.

Univariate analysis did not demonstrate statistical significance between the inability of SLN identification according to mean values of age, time between the end of radiotherapy and surgery, preoperative carcinoembryogenic antigen levels and tumor size (Table III). SLN identification ability was related to the tumor site (Table IV). A statistically significant difference occurred when the SLN identification rate of patients with colon cancer (90.9%) was compared with that for rectal cancer patients (63.3%) (P = 0.023). The lower identification rate obtained in rectal cancer patients made it necessary to access, in a separate manner, patients with mid- and upper rectal tumors and to compare them to lower rectal tumor patients.

The poor identification rate in rectal cancer patients was due to the results obtained in lower rectal tumors. The lowest SLN identification rate was obtained in patients with lower rectal tumors with 44.4%; P value was <0.001 when lower rectal tumor identification rate was compared to the identification rates of tumors of other sites (91.2%) (Table IV). Of the 30 rectal cancer patients, 11 did not have detected SLNs and 10 of those patients (90.9%) had lower rectal tumors being submitted to abdominoperineal rectal resection (9 patients) or total pelvic exenteration (1 patient). Patients with lower rectal tumors were responsible for 60% of the lymphatic mappings performed in rectal cancer patients and had an identification rate of 44.4% (Table V). Patients with mid- and upper rectal tumors had a 91.7% identification rate, similar to that obtained in patients with tumors in other sites. Only one patient in the mid- and upper rectal tumor category had no SLNs identified. There was statistical significance in the difference in identification rate between lower rectal tumors and mid- and upper rectal tumors (P = 0.018).

In the univariate statistical analysis (Table IV), the inability to identify SLNs was also associated with the type of surgery performed. The lowest identification rate of the abdominoperineal rectal resection procedure (43.8%) was significantly different (P = 0.001) compared to the identification rates of other procedures (88.9%). Statistical significance (P = 0.023) was also obtained in the ability to identify SLNs in colectomy–sigmoidectomy (90.9%) when compared to the other surgical procedures (63.3%).

Another factor that showed statistical significance in SLN identification was preoperative chemoradiotherapy (Table IV). Patients submitted to neoadjuvant treatment had poorer identification rate (42.9%) when compared to patients not submitted to neoadjuvant treatment (86.8%; P = 0.003).

Using the stepwise forward selection method, the first multivariate analysis model only identified tumor site (lower rectum) as an independent predictive risk factor for the inability to identify SLNs (P = 0.009). Neoadjuvant treatment showed a near significant level (P = 0.081). When the first model was adjusted for tumor size (as a continuous variable) and neoadjuvant treatment, the lower rectum site still remained an independent risk factor. This new adjusted model also
identified neoadjuvant treatment and tumor size (large tumors) as other independent risk factors responsible for low SLN identification rate. Thus, this final regression model of adjusted multivariate analyses considered the lower rectal tumor site (odds ratio $= 12.4; 95\%$ confidence interval $= 1.9–81.4; P = 0.009$), neoadjuvant treatment (odds ratio $= 7.7; 95\%$ confidence interval $= 1.2–48.8; P = 0.029$) and tumor size (odds ratio $= 1.2; 95\%$ confidence interval $= 1.1–1.5; P = 0.036$) as independent risk factors for the inability of SLN identification.

**DISCUSSION**

Reviewed studies have shown poorer SLN results in patients with large tumors and advanced disease [14,15]. The Colorectal Division of the Aristides Maltez Hospital treats patients in the public health system who usually have advanced tumors. The study results demonstrate this fact, inasmuch as 85% of the patients had T3/T4 tumors, 60% being stage III, with an average tumor size of 6.5 cm. The lymphatic mapping method used in this study protocol was selected concerning the predicted study population characteristics of large advanced tumors. A broadened definition of SLN that contemplated any blue or radioactive identified node was used aiming to increase the identification and accuracy. The study results permits a retrospective reevaluation of this broaden SLN definition making it more precise and limited. If SLN definition is altered to the five first blue or radioactive nodes following the tumor lymphatic drainage channel, accuracy, identification rate and upstaging results do not change. Our further lymphatic mapping studies will apply this more restricted SLN definition.

The small-sized particle $99mTc$-phytate is currently the tracer of choice used at the Aristides Maltez Hospital for lymphatic mapping in melanoma and breast cancer. It was chosen as a tracer in this study because its features permit faster lymphatic chain migration. This characteristic was desired as the tracer’s injection and the SLN identification was performed intraoperatively. SLN mapping lacks standardization, as authors report the use of different tracers, injection methods and pathological protocols [8–28]. Patent blue or technetium-$99m$-colloid has been used in different volumes and concentrations. Only a few authors have reported using both tracers [8–28]. The delivery of these tracers has been

### TABLE IV. Univariate Statistical Analysis Concerning the Number and Percentage of Patients With Colon or Rectal Cancer According to SLN Identification and Clinical, Pathological and Treatment Related Variables

<table>
<thead>
<tr>
<th>Variable Category</th>
<th>SLN identification</th>
<th>No n (%)</th>
<th>Yes n (%)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>8 (24.2)</td>
<td>25 (75.8)</td>
<td>1.000</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>5 (26.3)</td>
<td>14 (73.7)</td>
<td></td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td>2 (9.1)</td>
<td>20 (90.9)</td>
<td>0.023</td>
</tr>
<tr>
<td>Rectum</td>
<td></td>
<td>11 (36.7)</td>
<td>19 (63.3)</td>
<td></td>
</tr>
<tr>
<td>Mid- and upper rectum</td>
<td></td>
<td>1 (8.3)</td>
<td>11 (91.7)</td>
<td>0.253</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>12 (30.0)</td>
<td>28 (70.0)</td>
<td></td>
</tr>
<tr>
<td>Lower rectum</td>
<td></td>
<td>10 (55.6)</td>
<td>8 (44.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>3 (8.8)</td>
<td>31 (91.2)</td>
<td></td>
</tr>
<tr>
<td>Lower rectum</td>
<td></td>
<td>10 (55.6)</td>
<td>8 (44.4)</td>
<td>0.018</td>
</tr>
<tr>
<td>Histologic grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td></td>
<td>1 (7.1)</td>
<td>13 (92.9)</td>
<td>0.140</td>
</tr>
<tr>
<td>Grades 2 and 3</td>
<td></td>
<td>11 (30.6)</td>
<td>25 (69.4)</td>
<td></td>
</tr>
<tr>
<td>Lymphovascular</td>
<td>No</td>
<td>9 (22.0)</td>
<td>32 (78.0)</td>
<td>0.435</td>
</tr>
<tr>
<td>Invasive</td>
<td>Yes</td>
<td>4 (36.4)</td>
<td>7 (63.6)</td>
<td></td>
</tr>
<tr>
<td>Tumor necrosis</td>
<td>No</td>
<td>9 (22.0)</td>
<td>32 (78.0)</td>
<td>0.435</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>4 (36.4)</td>
<td>7 (63.6)</td>
<td></td>
</tr>
<tr>
<td>Ulceration</td>
<td>No</td>
<td>4 (21.1)</td>
<td>15 (78.9)</td>
<td>0.746</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>9 (27.30)</td>
<td>24 (72.7)</td>
<td></td>
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<tr>
<td>Perineural invasion</td>
<td>No</td>
<td>11 (22.4)</td>
<td>38 (77.6)</td>
<td>0.151</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2 (66.7)</td>
<td>1 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>Abdominoperineal resection</td>
<td>9 (56.2)</td>
<td>7 (43.8)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>4 (11.1)</td>
<td>32 (88.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anterior rectal resection</td>
<td>1 (8.3)</td>
<td>11 (91.7)</td>
<td>0.253</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>12 (30.0)</td>
<td>28 (70.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colectomy/sigmoidectomy</td>
<td>2 (9.1)</td>
<td>20 (90.9)</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>11 (36.7)</td>
<td>19 (63.3)</td>
<td></td>
</tr>
<tr>
<td>Preoperative chemoradiotherapy</td>
<td>No</td>
<td>5 (13.2)</td>
<td>33 (86.8)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>8 (57.1)</td>
<td>6 (42.9)</td>
<td></td>
</tr>
</tbody>
</table>

*Fisher’s exact test. SLN: sentinel lymph node.

### TABLE V. Identification Rate of Sentinel Nodes in Patients With Lower Rectal Tumors Compared With Patients With Mid- and Upper Rectal Tumors

<table>
<thead>
<tr>
<th>Primary tumor site</th>
<th>SNLM performed</th>
<th>% SLNM in rectal tumors</th>
<th>Patients with detected SLNs</th>
<th>IR per tumor site (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower rectum</td>
<td>18</td>
<td>60 (18/30)</td>
<td>8</td>
<td>44.4</td>
</tr>
<tr>
<td>Mid- and upper rectum</td>
<td>12</td>
<td>40 (12/30)</td>
<td>11</td>
<td>91.7</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
<td>19</td>
<td>$P = 0.018$</td>
</tr>
</tbody>
</table>

SLNM: sentinel lymph node mapping, SLNs: sentinel lymph nodes, IR: identification rate.
performed through preoperative colonoscopy with submucosal injection or through intraoperative subserosal injection [8–28]. There are studies that perform ex vivo lymphatic mappings in the surgical specimens [14,15]. Pathological step-sectioning examination does not always employ immunohistochemistry techniques [8–28]. The lack of standardized lymphatic mapping method and pathological examination combined with different study group characteristics results in heterogeneous SLN mapping accuracy [8–28]. As standardization has not been performed, study results should not be compared, because of the reported discrepancies. Sensitivity has been revealed to be as low as 38% [26] and as high as 100% [14], with identification rates ranging from 58% [23] to 100% [14] and false negatives from 0 [14] to 60% [8].

In the present study, differences in results of SLN mapping between colon cancer and rectal cancer were a matter of SLN identification. The higher identification rate obtained in colon cancer when compared to the lower one achieved in rectal cancer patients had statistical significance. However, when the SLNs were detected, there were no significant differences between accuracy parameters in colon versus rectal cancer: accuracy rate was respectively 80.0% versus 78.9%; sensitivity was 66.7% versus 63.6%; false negative rate was 33.3% versus 36.3%; and negative predictive value was the same (66.7%).

The total lymphatic mapping identification rate of 75% obtained in this study was due to the low results obtained in patients with lower rectal tumors. The difference between the identification rate of patients with mid- and upper rectal tumors versus the rate of lower rectal tumors was statistically significant. The low identification rates of surgical procedures to which patients with lower rectal tumors were submitted to enhance the evidence that SLN mapping in low rectal tumors is cumbersome. The lower identification rates were for abdominoperineal rectal resection and its combination with colpectomy. As these two procedures were responsible for 30.8% of the surgeries (16/52) and lower rectal site tumors totaled 34.6% (18/52) of the lymphatic mappings performed, the total study identification rate was substantially decreased. If lower rectal tumors were excluded from this study, the total identification rate would be of 91.2%. The results of univariate statistical analysis showing that abdominoperineal rectal resection and lower rectal tumors had significantly poorer identification rates were confirmed by the multivariate statistical analysis, considering lower rectal tumor as an independent predictive factor for SLN identification inability.

The most recently reported SLN mapping studies enrolled only colon cancer patients or had a few number of patients with low rectal tumors [10–19,21–28]. The majority of the papers did not mention the identification rate obtained in lower rectal cancer [8–28]. They only analyzed the SLN identification rate of the whole study group, not performing a separate analysis for the different colorectal tumor sites [8–28]. In the studies reviewed, when a lower identification rate was obtained in rectal cancer patients, there was no evidence that it could be due to the lower rectal tumors [8–28]. Joosten et al. [8] are the only authors who identified a large difference in identification rates of rectal versus colon cancer patients, in a study of SLN mapping with blue dye in 50 patients. Six rectal cancer patients were included in the latter study in which an identification rate of 50% was obtained compared to a colon cancer identification rate of 76% (32/44), but the poor rectal identification result was not attributed to lower rectal tumors [8]. Kitagawa et al. [9] enrolled 21 patients with lower rectal tumors in a colorectal cancer group of 56 patients, performing lymphatic mapping with technetium-99m-colloid and obtaining a global 91% identification rate, not mentioning different identification rates according to tumor site. Saha et al. [20] published a larger SLN mapping study using isosulfan blue dye. They enrolled 500 patients, including 92 rectal cancer patients, demonstrating a 99.3% identification rate for colon cancer and 91.3% for rectal cancer [20]. Rectal cancer patients had a lower SLN identification rate with an 8% difference when compared to the colon cancer patients’ rate. The authors did not reveal the identification rate obtained in lower rectal tumors and did not consider lower rectal tumors as being responsible for the poorer identification result in the rectal cancer group [20].

Since patients with lower rectal tumors had the poorest identification rate, the possibility arose that blue or radioactive nodes were not in the total mesorectal excision specimen, but were actually in the iliac, obturator or para-aortic lymph nodes. This question was answered by our other ongoing trial [29], where 17 of 18 patients with lower rectal tumors described in this study had their retroperitoneal and lateral pelvic lymph nodes mapped by lymphoscintigraphy and blue dye [29]. Four of these 17 patients had their retroperitoneal and lateral nodes blue or radioactive, while only 1 of these 4 patients did not have SLN identification in the mesorectum. Even if the migration of the patent blue dye or technetium-99m-phytate to retroperitoneal or lateral nodes were considered in the SLN identification rate for lower rectal tumors, a non significant increase from 44.4% (8/18) to 52.9% (9/17) would occur.

Multivariate statistical analysis identified preoperative chemoradiotherapy as an independent factor for low SLN identification rate. Reviewed studies mention neoadjuvant treatment as a factor that makes difficult the identification of SLNs [8–28]. However, there has been a lack of statistical analysis data that point to chemoradiation having a significant association with low SLN identification [8–28].

Tumor size has also been responsible for interfering in lymphatic mapping [8–28]. Authors have attributed poorer lymphatic mapping results in patients to the presence of large tumors [8–28]. As with the variable neoadjuvant treatment, there have been no studies reported showing a statistically significant association between large tumors and low SLN identification rate [8–28]. The patients included in this study had large tumors and multivariate analysis demonstrated tumor size as an independent variable for SLN identification inability. In our study, it was not possible to determine a cut-off value for tumor size that would indicate a significant difference in SLN identification. What we can affirm from our results is that as the tumor becomes larger, the SLN identification gets less effective.

In the largest multicenter study where SLN was submitted to enhanced pathological examination using immunochemistry, upstaging was 26.1% [20]. In the present study, upstaging by SLN mapping occurred in 23.1% of the patients (9/39). If lymphatic mapping was not performed, 23.1% of the patients would not have had the benefit of adjuvant therapy which has a mortality-reducing potential of up to 30% in patients with nodal metastases [30]. Saha et al. [20] demonstrated a 14.8% increase in the detection of nodal metastasis when patients submitted to conventional colorectal surgery with SLN mapping were compared to patients not undergoing lymphatic mapping (49.5% vs. 34.7%; \(P \leq 0.001\)). The percentage of patients with understaged colorectal cancer TNM stage II that have progression of their disease could be reduced significantly as nodal staging becomes more accurate with the incorporation of SLN mapping in standard colorectal surgery.

Micrometastases resulted in upstaging of 15.4% of cases (6/39), detected exclusively by immunohistochemistry. Studies have indicated a correlation between micrometastases and cancer recurrence, suggesting the need for adjuvant treatment [31–33]. Other studies showed that micrometastases were an irrelevant prognostic factor for not having a significant association with lower survival [34,35]. There is no current evidence indicating how to deal with colorectal lymph node micrometastases. As its prognostic value remains uncertain, we considered that the six patients of this study with micrometastases should be offered the choice of chemotherapy, as it was delivered.

The distinction of the present study compared to other published papers relating to sentinel lymphatic mapping is that its multivariate statistical analysis points out low rectal tumor, neoadjuvant treatment and tumor size as independent risk factors of the inability of SLN identification. This information would exclude patients with these
risk factors and indicate lymphatic mapping for patients who would benefit the most from the method. Doing so, it should increase the identification rate and accuracy of SLN mapping for proper patient nodal staging. In spite of our significant associations of identified risk factors with the inability of SLN identification, further studies should be carried out to validate our findings.

The reasons for the poorer identification rates of lower rectal tumors described in this study could be related to their anatomy and distinct lymphatic basin characteristics or to the transanal method of injecting blue dye and technetium-99m-phytate. Neoadjuvant treatment and large tumors could disrupt the lymphatic drainage pathway interfering with lymphatic mapping.

Further SLN mapping studies need to describe clearly the methods used and the characteristics of the population studied for reliable results to be obtained and for standardization to be progressively reached. Statistical analysis should be carried out so that results can be validated. Despite the lack of homogeneity of methods used, most studies agree that SLN mapping should be an auxiliary tool in standard colorectal surgery, to increase nodal staging. The great benefits that patients could gain with the incorporation of SLN mapping in clinical practice would be in matters of nodal upstaging. Patients who otherwise would be considered node negative are properly staged and receive the benefits of adjuvant therapy with lymphatic mapping.

CONCLUSIONS

SLN mapping with technetium-99m-phytate and patent blue dye should be considered an additional nodal staging method in colon and mid- and upper rectal adenocarcinoma patients, as it improves lymph node staging with acceptable accuracy and identification rates. The method led to a substantial upstaging of the patients studied to TNM stage III, offering chemotherapy benefits to patients who were not considered for adjuvant treatment. In patients with lower rectal tumors or submitted to neoadjuvant treatment or having large tumors, the performed SLN mapping method should be avoided as these characteristics were considered as independent variables associated with significantly low identification rates.

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