Hypothesis: Ten percent fluorescein may be successfully used as an alternative to 1% Lymphazurin (1% iso-sulfan blue; US Surgical Corp, North Haven, Conn) in sentinel lymph node (SLN) mapping for the accurate staging of colorectal tumors.

Design: Review of prospectively gathered data.

Setting: University-affiliated regional medical center.

Patients: Sentinel lymph node mapping was performed in 120 consecutive patients with colorectal malignancies.

Interventions: The first 1 to 4 blue nodes detected within 5 minutes were designated as Lymphazurin-detected SLNs. The first 1 to 4 fluorescent nodes seen under the Wood light were designated as fluorescein-detected SLNs. Multilevel serial sections for hematoxylin-eosin and immunohistochemistry studies for cytokeratin were performed on all SLNs.

Main Outcome Measures: Successful mapping, accuracy, skip metastasis, adverse reactions, occult micrometastases detection, and cost.

Results: Mapping was successful using Lymphazurin in 99% of the patients vs 97% of the patients using fluorescein ($P=0.89$). The accuracy of predicting nodal metastases with each tracer was 95.8% vs 93.1%, respectively ($P=0.82$). The skip metastases rate was 4.2% for Lymphazurin vs 6.9% for fluorescein ($P=0.37$). The 5 patients in whom nodal disease was only identified as occult micrometastasis in the SLNs had a total of 5 SLNs, all of which were identified by both tracers. No adverse reactions occurred. The cost for Lymphazurin was $99.00, while the cost for fluorescein was $2.10.

Conclusions: With the exception of cost, there were no statistically significant differences between the 2 dyes. While easy availability and lower cost remain distinct advantages of fluorescein, Lymphazurin remains the gold standard. In patients with known hypersensitivity to Lymphazurin and when availability and cost are an issue, fluorescein may be used effectively for SLN mapping in colorectal tumors.

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Sentinel lymph node (SLN) mapping is widely accepted in the surgical management of solid tumors including breast cancer and melanoma. This technique permits the accurate staging of these malignant neoplasms while sparing those patients without nodal disease the morbidities associated with regional lymph node dissection. In the treatment of colorectal tumors, however, SLN mapping has been developed as a means for reducing the substantial amount of understaging associated with these malignant neoplasms. This understaging is evidenced by the fact that about 20% to 30% of the patients with colon cancer who undergo a presumably curative resection (American Joint Committee on Cancer stages I and II), ultimately develop and die of distant metastatic disease. With more accurate staging, the upstaged patients may benefit from adjuvant chemotherapy that has been shown to be curative in about one third of the patients with nodal metastases. The SLNs are presumed to be the first 1 to 4 lymph nodes in the lymphatic drainage pathway of a primary tumor and are theoretically the nodes with the highest likelihood of harboring metastatic disease. The pathologist may then perform focused analysis of these lymph nodes using advanced pathological techniques. These techniques include multilevel serial sectioning, immunohistochemical staining, and molecular analysis with reverse transcriptase polymerase chain reaction, all of which may lead to the identification of occult micrometastatic disease and more accurate staging of the disease. While these costly, time-consuming, and labor-intensive techniques have improved the detection of nodal
metastases in SLNs, their indiscriminate use for all lymph nodes is highly impractical. The technical feasibility and staging accuracy of the SLN mapping technique with focused pathological analysis of the SLNs in colorectal tumors have been demonstrated in several publications.15-21 It is estimated that between 10% and 20% of patients have occult micrometastases that would likely be missed by conventional histological examination, and perhaps account for the subset of patients who ultimately develop recurrent systemic metastases despite a presumed curative resection.

Traditionally, 1% Lymphazurin (1% isosulfan blue; US Surgical Corp, North Haven, Conn) has been the dye of choice used in SLN mapping for breast cancer, melanoma, and gastrointestinal tumors. However, this blue dye’s associations with reports of adverse effects, including severe anaphylaxis,22-23 and its interference with accurate pulse oximetry readings26-28 have prompted a search for an alternative tracer in SNL mapping for colorectal tumors. Ten percent fluorescein is a conventionally safer substance and is widely used to assess vascular integrity and viability of tissues, without any clearly associated adverse effects. The chemical structure of the fluorescein molecule is shown in Figure 1. It is metabolized in the liver and eliminated from the body by the kidneys. This substance is widely available and can be purchased at a small fraction of the cost of 1% Lymphazurin. Despite these positive qualities, to our knowledge, the use of fluorescein in SLN mapping for colorectal tumors has not been described previously. A prospective study was undertaken, performing SLN mapping in colorectal tumors using both 1% Lymphazurin and 10% fluorescein. A comparative statistical analysis between the 2 tracers was then performed with regards to the success of SLN mapping, predictive accuracy of nodal metastasis, rate of skip metastases, rate of occult micrometastases detection, rate of adverse reactions, and cost.

METHODS

Within our group, SLN mapping has been performed in more than 250 patients with colorectal tumors. For the last 120 consecutive cases, both Lymphazurin and fluorescein were used as tracers for SLN mapping. These patients were prospectively enrolled in an institutional review board–approved study. The preoperative workup of these patients included colonoscopy, computed tomography of the abdomen and pelvis, liver function tests, and serum carcinoembryonic antigen measurements. At the time of laparotomy, 0.5 to 2 mL of 1% Lymphazurin and 0.5 to 2 mL of 10% fluorescein were injected into the subserosal layer surrounding the palpable tumor using a tuberculin syringe and a 30-gauge needle (Figure 2). The amount of tracer used (within the stated range of 0.5-2 mL) depended on the size of the tumor. Great care was taken to avoid injection into the lumen or spillage on the mesentery. Intraluminal injection may lead to absorption of the dye further down the colon with highlighting of lymph nodes that drain the area of absorption rather than those draining the primary neoplasm. Spillage of Lymphazurin or fluorescein on the mesentery can make the visualization of the lymphatics leading to SLNs difficult and the identification of the SLNs challenging. For low to midrectal lesions the injection of the Lymphazurin was performed from below via a rigid proctoscope. Lymphazurin-detected SLNs were defined as the first 1 to 4 lymph nodes to acquire a blue color within the first 5 to 10 minutes after injection. The operating room lights were then momentarily dimmed and Wood light illumination was projected on the mesentery. Fluorescein-detected SLNs were defined as the first 1 to 4 lymph nodes to become fluorescent in the dark room under Wood’s light illumination (Figure 3). It is essential to immediately mark the SLNs with suture once they are identified, as these 2 tracers can quickly travel through the lymphatics and the lymph nodes. By the time the specimen reaches the pathologist, the dyes may highlight lymph nodes that are further down the lymphatic chain and erroneously lead the pa-
thologist into treating those lymph nodes as SLNs. At this time, the true SLNs may no longer be stained at all. Following the SLN mapping, a standard surgical resection of the bowel with en-bloc regional lymphadenectomy was performed.

Once the specimen reaches the pathology department, the SLNs are immediately dissected. For each SLN, 5 to 10 microsections are obtained by serial sectioning at 20- to 40-µm intervals. One section is also stained for cytokeratin immunohistochemistry (AE-1/AE-3; Ventana Medical System, Tucson, Ariz.). A standard pathological examination is then performed on the remainder of the specimen including all other identified non-SLN lymph nodes.

The 2 tracers were statistically compared for feasibility rate, failure rate, accuracy rate, skip metastasis rates, cost, adverse reactions rate, upstaging rate by detection of micrometastatic disease, and the number of SLNs per patient. The accuracy rate is defined as the number of patients in which the histological status of the nodal basin was accurately predicted by analysis of the SLNs divided by all patients in which SLN mapping was successful. The skip metastases rate is defined as the number of patients in which the SLNs were histologically negative and metastatic disease was found in non-SLNs divided by all the patients in which the SLN mapping using that particular tracer was successful. The accuracy rate and skip metastasis rate of each tracer were calculated using only those SLNs identified by that particular dye. The rate of occult micrometastases is defined as the number of patients in which only micrometastatic disease (immunohistochemistry-positive result or <2 mm on multilevel serial sections) was identified divided by all of the patients in the series in which nodal metastatic disease was detected. A 2-tailed probability for the null hypothesis was used to determine whether there was a difference between the 2 techniques regarding any of the studied parameters. The critical α value for statistical significance was set at P<.05.

RESULTS

The sample of 120 consecutive patients with colorectal tumors consisted of 61 females and 59 males ranging in age between 41 and 90 years (median age, 72 years). The anatomical distribution of the tumors was as follows: appendix, 1 (0.8%); cecum, 20 (16.7%); ascending colon, 36 (30.0%); hepatic flexure, 4 (3.3%); transverse colon, 10 (8.3%); descending colon, 5 (4.2%); sigmoid colon, 26 (21.7%); rectosigmoid, 4 (3.3%); and rectum, 14 (11.7%). The T-stage distribution of these patients was Tis, 20 (16.7%); T1, 18 (15.0%); T2, 15 (12.5%); T3, 64 (53.3%); and T4, 3 (2.5%). Of these 120 patients, in which both modalities for SLN mapping were attempted, 119 (99%) underwent successful mapping using 1% Lymphazurin and 116 (97%) underwent successful SLN mapping using 10% fluorescein (P=.89). The distribution of the number removed per patient was as follows: 0 SLN, 1 patient; 1 SLN, 25 patients; 2 SLNs, 28 patients; 3 SLNs, 45 patients; 4 SLNs, 19 patients; and more than 4 SLNs, 2 patients. One patient underwent unsuccessful SLN mapping with both methods (we could not detect any SLNs in that particular case). A mean of 13.3 total lymph nodes per patient (total of 1,590) and a mean of 2.5 SLNs per patient (total of 301) were identified. In those patients who underwent successful SLN mapping with either of the 2 tracers, there was a mean of 2.5 (297 SLNs per 119 patients) Lymphazurin-detected SLNs per patient and a mean of 2.3 (270 SLNs per 116 patients) fluorescein-detected SLNs per patient. Of the total Lymphazurin-detected SLNs, 21% (62/297) were positive for metastases, while 19% (52/270) of the fluorescein-detected SLNs were positive for metastases. The accuracy rate for correctly predicting the status of the nodal basin did not differ significantly by technique and was 95.8% (114/119) for 1% Lymphazurin vs 93.1% (108/116) for 10% fluorescein (P=.82). The skip metastases rate (false-negative results) for Lymphazurin-detected SLNs only was 4.2% or 5 of the 119 patients who had successful Lymphazurin mapping, whereas the rate of skip metastases if fluorescein would have been the only mapping modality was 6.9% or 8 of the 116 patients who underwent successful fluorescein mapping. The difference did not differ significantly (P=.37). Of the 301 SLNs, 31 (10.3%) were identified only by 1% Lymphazurin and only 4 SLNs (1.3%) were detected solely by 10% fluorescein. Of those 31 SLNs detected only by Lymphazurin, 32.3% (10/31) were histologically positive for metastases while none (0%) of the 4 SLNs detected by fluorescein only were histologically positive for nodal metastases (P=.17). The remaining 266 SLNs (88.4%) were detected concurrently by both Lymphazurin and fluorescein and had a histological positivity rate of 19.9% (53/266).

The diagnosis of invasive carcinoma was established in 100 of the total 120 patients. The remaining 20 patients had in situ disease. Nodal metastases were detected in 43 (43%) of these 100 patients. Nodal metastases were identified solely in the SLN in 13 (30.2%) of these 43 patients, and only as occult micrometastatic disease in 5 (11.6%) of these 43 patients. The 13 patients in whom metastatic disease was only identified in the SLN had 18 SLNs, all of which were identified by 1% Lymphazurin and 15 of which were identified by 10% fluorescein. The 5 patients in whom nodal disease was only identified as occult micrometastases in the SLNs had 5 SLNs, all of which were identified by both tracers. There were no adverse reactions observed with the use of either dye. The pharmacy cost for 1% Lymphazurin was $99.00 per vial vs $2.10 per vial for 10% fluorescein. The Table gives a comparative analysis for these parameters.

COMMENT

Lymphazurin (isosulfan blue or 2,5-disulfonated patent blue—triphenylmethane family) was the first dye to be approved by the Food and Drug Administration for the localization of lymphatic channels. Although various similar blue dyes have been used as early as the 1930s without formally being studied or approved, isosulfan blue has remained the gold standard for SLN mapping owing to the simplicity of its use and availability in the United States. Lymphazurin has the quality of lymphatic tropism because it weakly binds to serum proteins. With the advent of SLN mapping for the staging of solid tumors, the intraoperative use of these dyes has been increasing significantly over the past 2 decades. Along with the emergence of isosulfan blue as the dye of choice in SLN mapping, the frequency of reported problems with its use has increased. Several authors have reported erroneous pulse oximetry readings (ie, desaturations) as-
reported reactions include erythema, bronchospasms, and oxygen saturation readings and unchanged vital signs of oxyhemoglobin, hence, producing a falsely reduced measurement at this wavelength results in decreased measurements of the pulse oximeter.

Injection of Lymphazurin to the subserosal layer of the bowel has also been reported in the literature. Other reactions with pulse oxymetry when isosulfan blue dye is injected intradermally with isosulfan blue and underwent SLN dissection, Leong et al reported 3 cases of hyper-sensitivity of variable severity. The overall incidence of reactions to Lymphazurin is estimated to be around 1% to 1.5%. Despite the excellent efficacy of 1% Lymphazurin as a lymphatic mapping agent, the aforementioned problems and its high cost have justified a search for an alternative tracer. The results in our series have shown fluorescein 10% to be a suitable substitute for 1% Lymphazurin, with a comparable efficacy in terms of success rate, accuracy rate for predicting the histological status of the nodal basin, skip metastasis rate, and occult micrometastasis detection rate. The results are consistent with the feasibility rates (82%-99%), accuracy rates (83%-98%), and skip metastases rates (6%-14%) reported in the larger series in the literature. Of the 6 cases in which Lymphazurin mapping either failed (1 case in which no nodes were identified) or was inaccurate (5 cases of skip metastases), all 6 cases had identical results with fluorescein (1 failure and 5 skip metastases). Fluorescein did not add to the success rate of SLN identification or to the accuracy of the staging in any one case. This emphasizes fluorescein’s role as a substitute tracer, rather than an adjunct to increase success rate and accuracy, as is seen with technetium Tc sulfur colloid and Lymphazurin.

While Lymphazurin remains the gold standard in SLN mapping for colorectal tumors, fluorescein does have some clear advantages. Despite its widespread use in operating rooms around the world to assess the vascular integrity of tissues, fluorescein is not commonly associated with any adverse reactions. Its availability and affordability in Third World countries also render it clearly advantageous from an economic standpoint. In working with a population with a notable prevalence of obesity, we have observed that fluorescein with Wood light allows better visualization of fluorescent lymphatics leading to the SLNs within a fatty mesentery, in comparison with Lymphazurin. In our experience, however, the use of fluorescein is more technically challenging as inadvertent spillage on the mesentery will obscure the lymphatics leading to the SLNs and is more detrimental to the mapping process than is spillage of Lymphazurin. Unlike Lymphazurin, fluorescein also necessitates the use of additional equipment (Wood light).

To our knowledge, this is the first report in the literature comparing the use of 10% fluorescein to 1% Lymphazurin in SLN mapping for colorectal tumors. Lymphazurin and fluorescein are both efficacious tracers for lymphatic mapping in colorectal cancer with success rates greater than 97% and accuracy rates greater than 93%. The lack of reported adverse effects, lower cost, and easy availability are distinct advantages of fluorescein over Lymphazurin. Lymphazurin remains the gold standard, however, owing to its efficacy and the technical simplicity of its use. Hence, in patients with known hypersensitivity to Lymphazurin, and when availability and cost are prohibitive, fluorescein 10% can be an effective substitute for 1% Lymphazurin for lymphatic mapping in colorectal tumors.

Table. Comparative Analysis of Various Parameters Between 1% Lymphazurin and 10% Fluorescein in Sentinel Lymph Node (SLN) Mapping for Colorectal Cancer

<table>
<thead>
<tr>
<th>Variable</th>
<th>1% Lymphazurin (n = 120)</th>
<th>10% Fluorescein (n = 120)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Success rate</td>
<td>99 (119/120)</td>
<td>97 (116/120)</td>
<td>.89</td>
</tr>
<tr>
<td>Failure rate</td>
<td>1 (1/120)</td>
<td>3 (4/120)</td>
<td>.27</td>
</tr>
<tr>
<td>Accuracy rate</td>
<td>95.8 (114/119)</td>
<td>93.1 (108/116)</td>
<td>.82</td>
</tr>
<tr>
<td>Skip metastasis rate</td>
<td>4.2 (5/119)</td>
<td>6.9 (8/116)</td>
<td>.37</td>
</tr>
<tr>
<td>SLNs per patient</td>
<td>2.5 (297/119)</td>
<td>2.3 (270/116)</td>
<td>.21</td>
</tr>
<tr>
<td>Occult micrometastases†</td>
<td>5 Patients</td>
<td>5 Patients</td>
<td>.96</td>
</tr>
<tr>
<td>Adverse reactions</td>
<td>0</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Cost, $</td>
<td>99.00</td>
<td>2.10</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not applicable.

*Data are given as the percentage (number/total number) of subjects unless otherwise indicated. The critical p value for statistical significance was set at P<.05.

†Occult micrometastases refers to those patients who were upstaged owing only to the detection of micrometastases in multilevel serial sections (<2 mm) or by immunohistochemical studies.

REFERENCES


4. Cost, $ 99.00 2.10 NA

5. SLNs per patient 2.5 (297/119) 2.3 (270/116) .21

6. Accuracy rate 95.8 (114/119) 93.1 (108/116) .82

7. Failure rate 1 (1/120) 3 (4/120) .27

8. Success rate 99 (119/120) 97 (116/120) .89

9. Skip metastasis rate 4.2 (5/119) 6.9 (8/116) .37

10. Occult micrometastases† 5 Patients 5 Patients .96

11. Adverse reactions 0 0 NA

12. Cost, $ 99.00 2.10 NA

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