Validation of Lymphatic Mapping in Colorectal Cancer: In Vivo, Ex Vivo, and Laparoscopic Techniques

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Background: The use of lymphatic mapping (LM) is being investigated to improve the staging of colorectal cancer (CRC) and thereby identify patients who might benefit from adjuvant chemotherapy. This study evaluated in vivo, laparoscopic, and ex vivo approaches for the ultrastaging of CRC.

Methods: Seventy-five CRC patients were enrolled in a study of LM with peritumoral injection of isosulfan blue dye. LM was undertaken during open colon resection (OCR) in 64 patients, during laparoscopic colon resection (LCR) in 9 patients, and after specimen removal (ex vivo) in 2 patients. Ex vivo LM was also undertaken in 6 patients after unsuccessful in vivo LM. All nodes were examined by hematoxylin and eosin (H&E) staining; in addition, sentinel lymph nodes (SNs) were multisectioned and examined by immunohistochemical staining with cytokeratin (CK-IHC).

Results: At least one SN was identified in 72 patients (96%). In vivo LM identified SNs in 56 of 64 (88%) patients undergoing OCR and in 9 of 9 (100%) patients undergoing LCR. Ex vivo LM was undertaken as the initial mapping procedure in 2 cases of intraperitoneal colon cancer and after in vivo LM had failed in 6 cases of extraperitoneal rectal carcinoma; an SN was identified in 7 of the 8 cases. Focused examination of the SN correctly predicted nodal status in 53 of 56 OCR cases, 9 of 9 LCR cases, and 6 of 7 ex vivo cases. Multiple sections and CK-IHC identified occult micrometastases in 13 patients (17%), representing 10 OCR, 1 LCR, and 2 ex vivo cases.

Conclusions: LM of drainage from a primary CRC can be accurately performed in vivo during OCR or LCR. Ex vivo LM can be applied when in vivo techniques are unsuccessful and may be useful for rectal tumors. During LCR, colonoscopic injection can be used to mark the primary tumor and define the lymphatic drainage so that adequate resection margins are obtained. These LM techniques improve staging accuracy in CRC.

Key Words: Colorectal carcinoma—Sentinel node—Lymphatic mapping—Staging—Laparoscopic colon resection—Ex vivo.
nodes most likely to contain metastases would be advantageous. Morton and colleagues\(^4\) popularized the sentinel lymph node (SN) concept in melanoma. Giuliano and co-workers\(^5\) later applied the concept in breast cancer. Because the first (“sentinel”) lymph nodes to receive the lymphatic drainage from the primary tumor are the most likely to contain metastasis, examination of these SNs could be used to determine which patients should not be subjected to the morbidity associated with complete lymphadenectomy. More recently, Bilchik et al.\(^6\) and Saha et al.\(^7\) have applied the lymphatic mapping (LM) technique to identify SNs in patients with CRC. However, unlike in melanoma and breast cancer, LM in CRC is not used to limit the extent of lymph node dissection, but rather to improve staging by a focused ultrastaging examination of the SNs.

In our early experience using LM in CRC (unpublished data, 1999), we successfully mapped the SN in more than 90% of cases, and most SNs were identified during open colon resection (OCR). One limitation of the technique, however, involved failures of LM for rectal tumors below the peritoneal reflection. Unpublished data from investigators at the University of Hawaii (JH Wong, May, 1999) indicate that LM of the drainage from a CRC can be performed ex vivo, i.e., after the tumor has been removed. We hypothesized that ex vivo LM might be useful when in vivo LM failed to identify an SN, especially in patients with rectal tumors, and that the approach could be applied during laparoscopic colon resection (LCR). This study expanded our initial experience with in vivo LM, and it evaluated the potential of ex vivo and laparoscopic LM to improve staging of CRC.

**METHODS**

Between August 1996 and February 2000, 75 patients with clinically localized CRC were enrolled in a study of LM undertaken in vivo during OCR or laparoscopic colon resection (LCR), or ex vivo after the tumor had been resected. Informed consent was obtained prior to surgery.

**In Vivo Techniques**

**Laparotomy and Routine Abdominal Exploration**

The tumor was localized. After resectability had been determined and before mobilization of the colon, .5–1 cc of isosulfan blue dye (Lymphazurin, Ben Venue Laboratories, Inc., Bedford, OH) was carefully injected subserosally around the periphery of the tumor using a tuberculin syringe (Fig. 1). The dye traveled from the injection site along the lymphatics to the SN(s) typically within 30–60 seconds. Occasionally gentle dissection of the mesentery was performed to trace the lymphatic path to the SN. Each blue-stained node was marked with sutures, and the colectomy performed in the standard

**FIG. 1.** A tuberculin syringe is used to inject isosulfan blue dye subserosally around the periphery of the tumor. The blue dye immediately flows in the lymphatic channels toward the SN(s).