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Carbon Dye Staining of Sentinel Lymph Nodes Facilitates Microstaging of Colon Cancer Patients

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Abstract

Background: Carbon dye, when peritumourally injected, permanently marks the drainage site of sentinel lymph nodes (SLN). The objective of the current study was to evaluate whether the use of carbon dye facilitated the detection of small nodal tumour infiltrates in colon cancer patients.

Methods: In a prospective trial, 19 patients underwent open, oncological resections of localized colon cancer and SLN procedure according to a standardized protocol. Isosulfan blue 1% and sterile filtered carbon dye (mixed 1:1) were injected into the subserosa circumferentially around the tumour. Lymph nodes staining blue were marked as SLN. Serial sections of each SLN were stained with hematoxylin and eosin (H&E) and with the pancytokeratin marker AE1/AE3. The intranodal presence and site of carbon particles were noted and compared with the location of possible tumour infiltrates.

Results: Identification of at least one SLN was successful in 18 patients (identification rate 95%). Four patients (22%) were pN+, 11 (61%) were pN0(i–). Three patients (17%) were upstaged from pN0(i–) to pN0(i+) as isolated tumour cells were detected in their SLN: in two (11%) of the three patients, carbon dye and isolated tumour cells were found in the same nodal compartment, hence facilitating the recognition of isolated tumour cells by the pathologist.

Conclusion: The use of carbon dye in the SLN procedure for colon cancer may facilitate the detection of small nodal tumour infiltrates.

Lymph node involvement represents the most powerful prognostic factor in colon cancer patients. Accurate assessment of lymph nodes is of paramount importance for prognosis and therapy. There is some evidence that even small nodal tumour infiltrates, such as micrometastases, isolated tumour cells, or metastatic deposits at the molecular level, are associated with poorer disease-specific survival.[1,2]

Sentinel lymph node (SLN) procedure is currently under active evaluation for colon cancer patients. It strives to detect small nodal tumour infiltrates based on in-depth analysis of one (or a few) first echelon lymph nodes.[3] Typically, isosulfan blue is injected around the primary tumour and travels along the lymphatic vessels. The first lymph nodes to receive lymphatic drainage from the primary tumour are stained blue and are harvested as SLN. Multilevel sectioning and immunohistochemistry enable the detection of small nodal tumour infiltrates in the SLN. The important advantage of this procedure is that these techniques can be applied to one (or a few) SLN without

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having to analyze all resected lymph nodes. However, directing the pathologist's attention to a region of the SLN that is most likely to harbour metastatic deposits may further increase the detection rate of small nodal tumour infiltrates and lead to more accurate staging.[4]

Morton et al.[5] introduced the use of carbon dye as an adjunct to isosulfan blue and ^{99m}Tc-sulfur colloid for lymphatic mapping in melanoma patients.[4] The site of carbon particles inside a tumour-positive SLN was correlated with the location of nodal tumour infiltrates, therefore allowing ''intranodal mapping''.[5] When used for lymphatic mapping, carbon particles are transported with the lymph from the site of injection to draining lymph nodes where they are phagocyted by macrophages in the subcapsular sinus, leading to a permanent, microscopically detectable mark on the lymph node.[5] This is in contrast to the only temporary blue staining by isosulfan blue.

This is the first report on the use of carbon dye as an adjunct to isosulfan blue for SLN procedure in colon cancer patients. The objective of the current study was to evaluate whether the use of carbon dye facilitated detection of small nodal tumour infiltrates within the SLN.

MATERIALS AND METHODS

Patients

Nineteen patients (median age: 78.1-years; range: 65–92; 11 men, 8 women) underwent conventional open resections for localized colon cancer and SLN procedure according to a standardized protocol. This pilot study was part of our prospective multicenter trial investigating SLN procedure in colon cancer patients using the blue dye technique.[6] All operations were performed by the same surgeon with extensive experience in SLN procedure for colon cancer at a single, university-affiliated institution. Data were collected prospectively. Exclusion criteria were prior abdominal tumour surgery, extraperitoneal rectal cancers, other malignancies, allergies to the dye (isosulfan blue and/or India ink), pregnancy and breastfeeding. The study protocol was approved by the local ethics committee, and written consent was obtained from all patients.

Preparation of the Dye Mix

The carbon dye India ink (Royal Talens B.V., Apeldoorn, The Netherlands) was diluted 1:3 in NaCl 0.9%, passed through a 5 μm filter, sterilized at 121°C for 20 minutes, and mixed 1:1 with sterile isosulfan blue 1% (University Hospital Zurich, Switzerland).

SLN Procedure

After careful mobilization of the tumour-bearing colon segment, the dye mix was injected into the subserosa circumferentially around the tumour using a tuberculin syringe with a 29-gauge needle. The amount of dye injected depended on the respective tumour size;[6] on average, 4.7 ml of the dye mix were applied. Lymph nodes staining blue were marked as SLN. The procedure was completed by a resection of the affected intestinal segment according to standard procedures in surgical oncology.

Histological Examination

All marked SLN were processed separately as standardized in the protocol: Five serial sections of each SLN were obtained at 3 different representative levels. The first section of each level was stained with hematoxylin and eosin (H&E). When no metastatic tumour was detectable in H&E, the fourth section of each level was immunostained with the pancytokeratin marker AE1/AE3 (DakoCytomation, Glostrup, Denmark). The intranodal presence and site of carbon particles were noted and compared with the location of possible tumour infiltrates.

Statistical Analysis

Nominal scaled variables were analyzed with the χ^2-test. A P-value <0.05 was considered to be significant. All P-values were two-sided.

RESULTS

Identification of at least one blue SLN was successful in 18 of 19 patients (identification rate 18/19 = 95%). Of those, 4 patients (4/18 = 22%) showed macrometastases, and 14 (14/18 = 78%) had no lymph node involvement as evidenced by H&E. Eleven patients (11/18 = 61%) remained node negative after immunohistochemical staining (pN0[i−]). However, in 3 patients (3/18 = 17%) isolated tumour cells were detected after immunostaining with a pancytokeratin marker. These patients were upstaged from pN0(i−) to pN0(i+) (Table 1).[7]

Table 1.
Carbon dye facilitates the detection of isolated tumour cells in colon cancer patients

Identification rate	18/19	95%
pN1/pN2	4/18	22%
pN0(i+) = upstaging	3/18	17%
Carbon dye and isolated tumour cells in same nodal region	2/18	
pN0(i–)	11/18	61%

Carbon dye containing SLN showed a clear polarization, with carbon dye being phagocyted by macrophages in the subcapsular sinus on one side of the lymph node. Therefore, carbon dye permanently marked the drainage site of the individual SLN (Fig. 1A). In 2 (2/18 = 11%) of the 3 upstaged patients, carbon dye and isolated tumour cells were found in the same nodal region, hence facilitating the recognition of small nodal tumour infiltrates by the pathologist (''intranodal mapping''; Fig. 1B). The third patient had no carbon dye in the SLN.

In 18 patients, a total of 424 lymph nodes (median: 22; range: 15–41) were analyzed. Of those, 80 were SLN (median: 3.5; range: 1–13) and 344 were non-SLN (median: 19; range: 8–32). SLN were significantly more likely to contain carbon particles than non-SLN: 40 of 80 SLN (50%) showed carbon particles as compared with 81 of 344 non-SLN (24%; $P < 0.0001$). SLN were also significantly more likely to harbour nodal tumour infiltrates than non-SLN: Eleven of 80 SLN (14%) were found to have nodal tumour involvement as compared with 11 of 344 non-SLN (3%; $P = 0.0006$). A total of 16 SLN were analyzed in the 3 patients upstaged to pN0(i+). Four SLN harboured isolated tumour cells; in 3 SLN (75%), these isolated tumour cells were located in close proximity to phagocyted carbon dye, which greatly facilitated their detection by the pathologist.

DISCUSSION

The present investigation, the first one in the literature, provides suggestive evidence that the use of carbon dye as an adjunct to isosulfan blue for SLN procedure in colon cancer is useful in detecting small nodal tumour infiltrates. In fact, by directing the pathologist's attention to the region of interest that is most likely to harbour tumour infiltrates, carbon dye may facilitate detection of small nodal tumour infiltrates.

SLN procedure is now established for melanoma[8] and for breast cancer patients.[9] SLN technique enables proving absence of regional lymph node involvement through a less invasive surgical procedure compared with formal lymph node dissection. Furthermore, a more in-depth analysis of a small and selected number of lymph nodes facilitates the identification of micrometastases.[10] Among other malignancies, the SLN concept was subsequently applied to colon tumours.[11,12] Unlike in melanoma and breast cancer, standard lymph node dissection in colon cancer surgery causes no added morbidity. The rationale for applying the procedure in colon cancer patients is to improve staging by detecting small nodal tumour infiltrates.[3] SLN procedure in colon cancer has therefore been referred to as ''upstaging procedure''. Application of time-consuming and expensive techniques, such as multilevel sectioning, immunohistochemistry (microstaging) or reverse-transcriptase polymerase chain reaction (ultrastaging), can be restricted to a few SLN.

Detection of small nodal tumour infiltrates does not necessarily indicate any prognostic significance. This certainly is a matter of ongoing debate, as several retrospective studies utilizing different techniques have described conflicting results.[13] However, accurate microstaging might be very helpful in future prospective trials evaluating the prognostic significance of small tumour infiltrates in colon cancer patients. Even with focused analysis of only a few SLN, detection of single, dispersed, isolated tumour cells still might be a challenge given the number of sections at different levels to be analyzed compared with the minute size of some nodal tumour infiltrates. Hence, supporting the pathologist by marking the region inside a lymph node most likely to harbour tumour deposits further increases chances of detecting small nodal infiltrates. Furthermore, this region might selectively undergo additional microsections in search for small nodal tumour infiltrates. We do not suggest that the use of carbon dye can replace immunohistochemistry. However, it may – based on our limited experience – be an additional analytical tool that helps correctly staging colon cancer patients.

Our current data provide evidence that SLN in colon cancer patients were significantly more likely to contain carbon dye as well as nodal tumour infiltrates. Isolated tumour cells were frequently located in the same nodal region as phagocyted carbon particles. Therefore, the concept of SLN mapping in colon cancer patients can be extended to an additional *intranodal* mapping. By marking the region of the SLN that is most likely to harbour tumour infiltrates, carbon dye may facilitate the detection of small nodal tumour infiltrates. However, the interesting and promising findings of the present pilot study need to be confirmed by investigations with larger sample sizes.

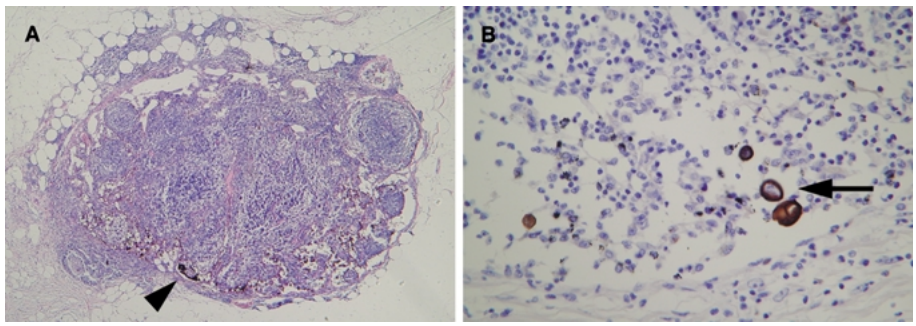

Figure 1. Carbon-dye staining of sentinel lymph nodes (SLN) allows intranodal mapping. Carbon dye injected around the tumour is transported with the lymph to the SLN where it is phagocyted by macrophages in the subcapsular sinus (*arrowhead*), leading to a permanent mark of the drainage site of the SLN (**A**. Hematoxylin and eosin staining). Focused examination of this region of interest reveals 3 isolated tumour cells (*arrow*) located within the area of phagocyted carbon particles (**B**. immunohistochemical staining with the pancytokeratin marker AE1/AE3, original magnification 400×).

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