

# Immunohistochemical Evaluation of Sentinel Lymph Nodes in Colon Cancer

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**Background:** Lymph node status is the most important predictive factor in the treatment of colic cancer. As sentinel lymph node (SLN) biopsy might upstage stage II colon cancer, it could have therapeutic consequences in the future.

**Aim:** To investigate and evaluate nodal microstaging and ultrastaging using cytokeratin immunohistochemistry.

**Material and methods:** In 20 consecutive patients operated on First Surgery Clinic of the County Hospital Mures for colon cancer, subserosal injection with Patent Blue dye was used for SLN detection. In searching for occult micrometastases, each SLN was examined. In tumor-negative SLNs at routine hematoxylin-eosin (H&E) examination (pN0) we performed cytokeratin(CK) immunohistochemistry (IHC).

**Results:** The procedure was successful in 19 out of 20 patients (95%). The SLN was negative in 12 patients detected by H&E and IHC, in 10 patients the non-SLN was also negative, leading to a negative predictive value of 89% and an accuracy of 93%. In 6 patients with SLN negative by HE was positive by IHC, leading to a 33% value of upstaging.

**Conclusions:** The SLN concept in colon carcinoma using Patent Blue V is feasible and accurate. It leads to upstaging of nodal status in 6 cases (33%) when IHC techniques are involved. The clinical value of the method will be evaluated by postoperative chemotherapy efficiency.

**Keywords:** sentinel lymph node, colon, carcinoma

## Introduction

Colorectal carcinoma is the most common gastrointestinal malignancy. Lymph node status as the most important predictor of outcome indicates the use of adjuvant chemotherapy. The reported 5-year survival rate is 70–80% for patients with node negative disease (st. I–II), but only 45–50% for those with node positive tumors (st.III) [1]. Adjuvant chemotherapy significantly improves the 5-year survival in patients with node positive disease. Despite the favorable prognosis of patients with localized colon cancer without regional lymph node metastasis, 20–30% of these patients will develop recurrent disease, after apparently curative resection. It is therefore necessary to perform a more detailed histological examination of negative lymph nodes by histological examination with haematoxylin-eosine staining (HE) and immunohistochemistry with cytokeratin (CK). Understaging may be the result of inadequate numbers of examined lymph nodes, missing some metastases [1,2,3]. For adequate staging and treatment of patients with colon cancer, meticulous examination of at least 12 nodes harvested by pathological analysis is mandatory [4].

Sentinel node technique was described by Cabanas in 1977 in penile cancer, and Morton Giuliano introduced the method for melanoma and breast cancer. In colon cancer the sentinel lymph node is defined as the first tumor draining lymph node, with the highest potential to harbor metastatic disease [5,6,7]. This allows a targeted examination of a smaller number of nodes that can be examined

with multiple sections and immunohistochemistry for the accurate detection of metastases and micrometastases and to provide a better staging of colon cancer.

We used methylene blue dye to identify sentinel lymph nodes and examined them with haematoxylin-eosine staining and immunohistochemical technique with cytokeratin. In tumor-negative SLN's at routine hematoxylin-eosin (H&E) examination (pN0) we performed CK8/CK18 immunohistochemistry (IHC).

## Material and methods

Only patients with histological proven primary colon carcinoma were included in the study. Patients with distant metastases or gross lymph node involvement as shown by preoperative examinations or palpation during surgery were excluded.

Sentinel lymph node mapping was carried out through an open procedure by injection of 1–3 ml Blue Dye with a tuberculin syringe and 29 gauge needle subserosally in 4 quadrants around the tumor. The subserosal injection was carried out prior to vascular ligation. Within 5 to 10 minutes after the blue dye injection, the SLN's could be identified by following blue stained lymphatic vessels leading to the blue stained sentinel node [8–12]. These nodes were tagged with a long suture. Sentinel nodes were defined as the first four bluestaining nodes seen within the regional basin. After marking of the SLN's, routine resection was performed.

Entire abstract:  
Source: Kelder p. 54  
Match value: ~ 72%

Some values (89%, 93%, 33%) are the same as those reported by Kelder in a different sample (30 participants with 1 unsuccessful case).

Entire Introduction:  
Source: Kelder p. 55.  
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Kelder, p. 55.  
The word "disease" replaced the original "CRC" (colorectal carcinoma).

Entire Methods section:  
Source: Kelder pp. 56-57.  
Match value: ~ 92%

Kelder, p. 55.  
The word "mandatory" replaced the original: "warranted according to the Dutch National Cancer Centre Guidelines and international guidelines".

Kelder, p. 55.  
The word "cancer" replaced the original "carcinoma".

The red highlighting reflects a mistake. The original source refers to "Morton et al." and "Giuliano et al.". They are included in the reference list as separate entries (no 22 & 23, p. 16). In this paper, the two research groups were reported as a single person, but are inserted as separate entries in the reference list.

Table I. Sex and age distribution

Patient sex	Number of patients	Average age
Male	8	62.25
Female	12	60.91
Total	20	61.45

The tumor and all lymph nodes were examined according to standard guidelines. If the SLN's were negative after routine hematoxylin-eosin (H&E) staining, they were sectioned at 150 µm intervals and examined at 3 levels with H&E as well as immunohistochemistry on cytokeratins (CK8/CK18). Metastases between 0.2 mm and 2 mm were referred to as micrometastases. Metastases smaller than 0.2 mm were described as isolated tumor cell [13].

A total of 20 patients were included in the study, 8 men and 12 women, with ages between 49 and 79 years old (the average age of 61.45) (Table I).

## Results

The procedure was successful in 19 out of 20 patients (95%), but failed in one patient. The SLN was negative in 12 patients detected by H&E and IHC, in 10 patients the non-SLN was also negative, leading to a negative predictive value of 89% and an accuracy of 93% (Table II).

A total number of 275 lymph nodes from the samples were examined, with an average of 13.75 lymph nodes/sample (between 3 and 32 sampled lymph nodes). A number of 41 were marked as the SLN (an average of 2.05). The presence of metastases was detected in 6 patients, SLN was negative; the other lymph nodes from the surgical sample were also negative.

Without taking into account the SLN examination (41 marked lymph nodes), there was no spread to the lymph nodes in 14 patients (70%) staged as pN0. Out of the 6 patients (30%) with positive SLN, 4 (20%) were staged as pN1 and 2 (10%) as pN2. In 6 patients of the other 13 patients where the SLN was negative by HE, it was evidenced as positive by IHC, leading to a 33% value of upstaging. (Table III).

## Discussions

Unlike the validated SLN concept in breast cancer and melanoma mandating lymphatic dissection, the main reason for SLN mapping in colon cancer is to focus pathologic IHC evaluation of SLN after in vivo mapping with patent blue. In colon cancer patients examination of the SLN's, will increase the accuracy of nodal staging, resulting

Table III. Analysis of the examined lymph nodes

The analysis of the examined lymph nodes	SLN(%)	nonSLN(%)
Total lymph nodes = 275	41 (14.9)	234 (85.1)
Negative lymph nodes at HE = 185	9	176
Positive lymph nodes at HE = 90	32 (11.6)	58 (21.1)
Positive lymph nodes at HE + IHC = 108	37	61

Table II. Examination of SLN

	No.	%
Total number of patients	20	100
Total number of patients with SLN detected	19	95
Total number of patients with positive SLN at HE	6	30
Total number of patients with negative SLN at HE	13	75
Total number of patients with negative SLN at HE, and positive at IHC	6	30
Total number of patients with positive SLN and the other lymph nodes positive	6	30

in a higher percentage of node-positive patients, who may benefit from adjuvant chemotherapy [14,15,16].

Upstaging by H&E conventional examination is difficult to measure. It might be explained by the focused examination of blue stained nodes, because these blue nodes can be very small nodes and would otherwise not have been detected. The IHC in our study was performed on cytokeratins. Most studies performed sectioning with intervals of 500 µm or immunohistochemistry on 1-4 levels in total. Increasing the number of slices for immunohistochemistry probably improves the detection rate of micrometastases smaller than 2 mm. A variety of results on this subject have been described [19,20,21] (Table IV).

We found an upstaging by immunohistochemical staining in 33% of patients. We should wait for the results after follow up in a large group of patients before assessing the real impact. If future results confirm the importance of microstaging and ultrastaging in CRC, the sentinel node concept can help the pathologist to focus the examination on one or two sentinel nodes in H&E negative cases. The detection of micrometastases might then select a subgroup of patients who could benefit from adjuvant treatment.

## Conclusions

The sentinel node concept in colon carcinoma using Blue Dye is feasible and accurate. It leads to an upstaging of nodal status in 33 % of patients when IHC techniques are combined and may detect aberrant lymphatic drainage. This procedure can be performed in a multi-center study under adequate supervision during the learning curve and may have diagnostic and therapeutic consequences in the future.

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The word "assessing" replaced the original "we can estimate". The word "assessing" is misspelled.

The abbreviation "CRC" was lifted straight from the original. In the original source, it stands for "colorectal carcinoma". The authors of this paper did not assign an abbreviation to colorectal carcinoma and has replaced CRC with colon cancer in all other instances where CRC appeared, except here.

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Entire Discussion section:  
Source: Kelder pp. 60-62.  
Match value: ~ 91%

The word "mandating" replaces the original "which affect the need for".

The phrase "colon cancer" replaces the original "CRC" (colorectal carcinoma)

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