

The sentinel lymph node (SLN) significance in colorectal cancer: methods and results. General report

MARIA CRISTINA SFECLAN¹⁾, IONICĂ DANIEL VÎLCEA¹⁾, GORAN BARIŠIĆ²⁾, STELIAN ȘTEFĂNIȚĂ MOGOANTĂ¹⁾, EMIL MORARU¹⁾, MIHAI CĂLIN CIORBAGIU¹⁾, ION VASILE¹⁾, CRISTIN CONSTANTIN VERE³⁾, ALINA MARIA VÎLCEA⁴⁾, CECIL SORIN MIREA¹⁾

¹⁾Department of Surgery, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania

²⁾Department of Surgery, Faculty of Medicine, University of Belgrade, Serbia

³⁾Department of Gastroenterology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania

⁴⁾Department of Dermatology–Venereology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania

Abstract

Colorectal cancer appears to be one of the most important malignancies in the world, with a survival rate depending on the TNM stage. The presence of lymph nodes metastasis indicates the necessity of adjuvant chemotherapy but exact classification of the N stage requires at least 12 lymph nodes to be pathologically examined. The sentinel lymph node (SLN) is considered to be the closest lymph node to the tumor, bearing the highest risk of malignant cells colonization. The main advantage of the sentinel lymph node mapping in colorectal cancer is identification and separate pathological examination of the nodes carrying the highest risk of metastasis. There are still open questions regarding the best method for sentinel lymph node mapping (*in vivo* or *ex vivo*), the factors influencing it, which substance is better for identification and which are the best histological methods and markers to be used. Numerous studies have discussed the quality and applicability of the method, but the importance of the SLN in colorectal carcinoma remains an open issue.

Keywords: colorectal cancer, sentinel lymph node, mapping, histopathological, immunohistochemistry.

Introduction

Colorectal cancer is the third most commonly diagnosed malignancy worldwide, with nearly 1.4 million new cases diagnosed in 2012 [1], third in males and the second in females [2]. The survival rates are mainly dependent on the TNM stage: in early stages (I and II) the five-year survival rates are between 82% and 93%, decreasing to 59% in the presence of lymph node metastases (stage III) [3]. Patients with rectal cancer present with a rate of 30–40% lymph nodes invasion [4]. Thus, evaluation of the lymph nodes involvement has a paramount importance for the prognosis of the colorectal cancer [5].

Lymph node metastases necessitate the use of adjuvant chemotherapy, which may increase the five-year survival rate up to 10%. Despite the favorable prognosis of patients with localized colon carcinoma without regional lymph node metastasis, 20–30% of these patients will develop recurrent disease after an apparently curative resection, mainly due to the microscopic residual disease, especially in the lymph nodes, liver or peritoneum. According to the international guidelines, examination of at least 12 lymph nodes is recommended for exact staging of the colon and rectal carcinomas [6, 7]. In fact, the more lymph nodes are examined, the more accurate is the lymph node staging.

The importance of the SLN mapping

The sentinel lymph node (SLN) concept was introduced by Gould *et al.* [8], in 1960, for parotid carcinoma and by Cabanas (1977) in penile carcinoma [9]. In colon cancer, this concept was introduced in 1999 by Joosten *et al.*

[10], in order to decrease the false negative results rates, knowing the importance of the lymph node involvement for further therapy and the survival rate of these patients.

The sentinel lymph node is considered the lymph node(s) located the closest to the tumor, on the lymphatic drainage pathways, bearing the highest risk of tumoral involvement; using lymphatic mapping, 2–4 lymph nodes may be identified and closely examined, thus increasing the chance of the metastatic involvement recognition.

Standard pathological evaluation is performed on a limited surface of the lymph node (1–2 sections), a large area of the lymph node remaining unexamined, with the subsequent risk of undetected residual micrometastases. When lymph nodes are analyzed only with standard pathological techniques, such as the Hematoxylin–Eosin (HE) technique, 30% of the patients will present with local or distant metastases at five years [1, 11].

The main advantage of SLN mapping in colon cancer appear to be the identification of nodes that carry the higher risk of metastasis, which will be submitted to a detailed pathologic scrutiny, including more sections, immunohistochemistry, hence optimizing the staging accuracy. Still it is uncertain if the lymph node mapping can improve outcomes for patients with colorectal cancer, while the influence of the detected micrometastases on the survival and therapeutic decision is, yet to be discussed.

Micrometastases refer to deposits of single tumor cells or very small clusters of neoplastic cells with a diameter less than 2 mm. The level of micrometastatic nodal disease that warrants postoperative adjuvant therapy is yet to be determined; the mere presence of the neoplastic cells

outside the site of the primary tumor may warrant systemic treatment [12].

☐ Sentinel lymph node mapping – available techniques

Ex vivo or *in vivo* methods?

The mapping can be performed *in vivo* or *ex vivo* with various substances: blue dyes, fluorescent or radioactive tracers. Most commonly used is the blue dye, both for *in vivo* and *ex vivo* technique. Results from various studies showed that *in vivo* mapping has the same accuracy as the *ex vivo*, but the last one lack the possibility of detection of the aberrant lymphatic drainage [13].

Injecting with a tuberculin syringe 1–2 mL of blue dye into the subserosa, around the tumor, performs the *in vivo* analysis. After 5–10 minutes, the first blue-colored lymph node is removed and sent separately to the pathologist.

The *ex vivo* mapping is usually performed about 30 minutes after resection, when 0.5–2 mL of blue dye is injected into the subserosa and circumferentially around the tumor. After injection, the sites are massaged for five minutes to push the tracer into the lymphatic vessels. The first blue stained lymph node(s) is defined as the SLN (Figure 1).

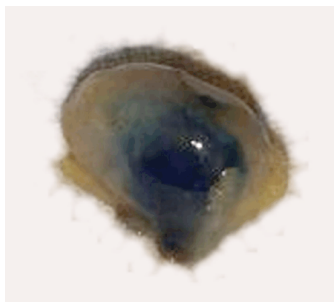


Figure 1 – Blue stained sentinel lymph node in colon cancer (*ex vivo* technique).

A multicenter prospective Swiss study regarding sentinel lymph node procedure in colon cancer was published, showing a good accuracy and identification rates of 89.1% and 83.9% respectively. The SLN was obtained by intraoperative injecting 1% Isosulfan Blue around the tumor followed by colon resection with adequate lymphadenectomy. The SLNs were stained with HE and immunostained with the pan-cytokeratin marker AE1/AE3 if HE was negative [14].

A Spanish study used the *ex vivo* technique with Methylene Blue to identify the SLN, then the lymph nodes were multiple sliced and analyzed using immunohistochemical and HE staining methods. The identification rate of SLN in 125 patients was 98%, a false-negative results were around 5%, while the up-staging was achieved in 14% [15].

A study including 32 patients with SLN, both in colon and rectal carcinoma, showed an identification rate of 74.4% (86.3% of the patients with colon cancer and 68.9% of the patients with rectal cancer), an accuracy of 84.3% and sensitivity of 61.5%. The mapping of the SLN was made by injecting 1–2 mL of Methylene Blue dye peritumorally and subserosally *in vivo* in colon cancer and *ex vivo* in rectal cancer. As a pathological method, they used serial sectioning and HE staining [16].

In rectal cancer, the SLN is usually identified through

the *ex vivo* technique because the mobilization of the middle and inferior rectum it is needed, thus the lymphatic drainage is greatly influenced. A study on 58 patients with rectal cancer was performed, the sentinel lymph nodes being identified using the *ex vivo* technique with Isosulfan Blue; 88% of the patients received neoadjuvant therapy. The sentinel lymph node detection rate was 85%, with an average sentinel lymph node harvest of 2.2 nodes per subject; 26% of the subjects had SLN metastasis on routine HE examination. The accuracy of sentinel lymph node mapping was 71%, the sensitivity was 53%, the negative predictive value was 79%, and the false negative rate was 47%. The conclusion of the study was that *ex vivo* sentinel lymph node mapping is feasible after proctectomy for rectal cancer but did not improve the staging [17].

There are some factors which seem to influence the *in vivo* technique accuracy such as gender, tumor location, previous abdominal surgery, intraoperative visualization of the blue lymphatic vessels, nodal status, grading, age, weight, body mass index, volume of the dye injected, tumor size, number of the identified sentinel lymph nodes, number of the positive lymph nodes. The success of this mapping technique is influenced also by procedure related factors (intraoperative visualization of blue lymphatic vessels, high number of SLN identified), which are the key for a successful SLN procedure. In a recent study regarding these factors seem that a successful identification of SLN was significantly associated with the intraoperative visualization of blue lymphatic vessels ($p=0.001$) and with female gender ($p=0.024$). True positive SLN results were significantly associated with higher numbers of SLN ($p=0.026$) and with pN2 stage ($p=0.004$). A better sensitivity was observed in patients with lower body mass index (BMI) ($p=0.050$) [18].

Tracers

The mapping can be performed with various substances: blue dyes, fluorescent or radioactive tracers. Most commonly used is the blue dye, both for *in vivo* or *ex vivo* technique; regarding the substances that are used in the sentinel lymph node mapping, blue dye and radioactive tracers appear to have the same false-negative rate [19].

Another method for identifying the SLN is the staining with activated carbon nanoparticles (ACNS). These nanoparticles are capable to pass through lymphatic vessels but not in the blood capillaries. ACNS using carbon particles with 21 nm diameter added with suspending agents is a stable suspension. ACNS dyes the lymph nodes in black, after subserous injection of 1 mL in four-quadrant region around the mass (similar for the blue dye injection) and the SLNs were harvested after 10 minutes, in order to avoid surgical destruction of the lymphatic system along the vessels. In a study, the ACNS method was compared with the Methylene Blue *in vitro* (the Methylene Blue was injected after the tumor was dissected, in the main artery of the specimen and waited 10 minutes before SLN was colored); none of these patients had an insufficient number of the detected lymph nodes in the ACNS group, with only one in the Methylene Blue group, and the detection rate was significantly higher than in the unstained group. Thus, the advantage of the stained methods is the detection of a sufficient number of lymph nodes especially

due to the fact that allows the detection of smaller lymph nodes [20].

Another method of detecting SLN is using fluorescence navigation with Indocyanine Green (ICG); for visualization, a special camera is needed, used intraoperatively after the injection of about 2 mL of ICG around the tumor. 3–10 minutes after the injection of the ICG, the ICG suspension is observed in the lymph nodal compartments in real time. In the study evaluating this method, the identification rate was 96%, which demonstrates the feasibility of fluorescence navigation for SLN (provides an accurate detection). Fluorescence detection in colon cancer aims an ultra-staging and allows lymphatic aberrant drainage detection [21]. The main disadvantage of this procedure is the high cost of the materials that are needed; however, the ICG-guided method was demonstrated to conjoin the advantages of both, the dye and radiocolloid methods, and allowed real-time lymphography.

In colon cancer, conventional methods, which used blue dye or a radiocolloid, showed a detection rate between 81% and 98% with sensitivity over 90% [10, 22, 23].

In rectal cancer, in patients who undergone preoperative radiochemotherapy for locally advanced tumors, the *ex vivo* identification of the SLN with a colloid had a detection rate of 96% a sensitivity of 44% and a false-negative rate of 56% [24].

☞ Histopathological analysis

Colorectal cancer staging is performed according to the 6th edition of the *American Joint Committee on Cancer* (AJCC). After localization of at least 12 lymph nodes, as it was recommended by the UICC/AJCC Guidelines, they are fixed in formalin and sent to the pathologist. Usual examination includes the lymph nodes paraffin embedding and sectioning, with a thickness of 5 μ m. These sections are stained, usually with HE; in case of a negative lymph node, the histological identification is continued with immunohistochemical evaluation (Figure 2).

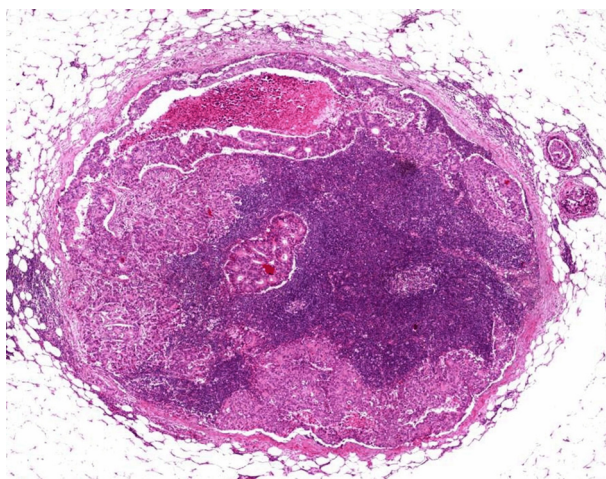


Figure 2 – Micrometastasis of sentinel lymph node in colon cancer (HE staining, $\times 40$).

SLN procedure is needed to improve nodal staging, in order to identify small nodes infiltration with a diameter less than 2 mm, named isolated tumor cells, which are very important for stage I and II regarding the prognosis and the five-year survival [25].

In a Swiss multicenter study of the sentinel lymph node mapping in colon cancer, including 174 patients staged I–III, an upstaging over 15% was demonstrated for the stage I and II of the disease, after pathological evaluation of the sentinel lymph nodes [14].

According to a retrospective Korean study, immunohistochemical technique is more efficient in finding isolated tumor cells than HE staining. They observed in 93 patients, with a survival rate of 86% that 14 patients died during the follow-up at 66 months, five cancer-related deaths in patients with nodal isolated tumor cells and nine in patients without it [22]. Isolated tumor cells, the term preferred by the *International Union against Cancer* (UICC) [23] are usually overlooked with HE staining.

The molecular detection of tumor cells in regional lymph nodes is associated with an increased risk of disease recurrence and poor survival in patients with node-negative colorectal cancer. Using molecular detection techniques, such as immunohistochemistry or reverse transcriptase polymerase chain reaction (RT-PCR), various studies have demonstrated occult tumor cells in regional lymph nodes in 25% to 50% of patients with node-negative colorectal cancer on routine histopathological analysis [26–28].

Two large international prospective studies showed a clear negative prognostic effect of micrometastatic nodal disease; the four-year disease-free survival (DFS) decreased from 94% to 78% in the presence of nodal micrometastasis [29].

Liefers *et al.* (1998), who used RT-PCT as a pathological method, reported that lymph node micrometastasis was detected in 54% of the examined stage II colorectal patients, and when their five-year survival rates were compared, it was 50% in the group with lymph node micrometastasis and 91% in the group without lymph node micrometastasis, a statistically significant difference ($p=0.02$) [30].

Bilchik *et al.* (2007) reported that in the patient group in which lymph node micrometastases were not detected through the use of HE staining, immunohistochemical staining and RT-PCR, the recurrence was detected in none of the cases during the follow-up period, and in the patient group in which lymph node micrometastases were detected for more than one technique, recurrences were more frequent [31].

☞ Molecular study of the SLN

Molecular staging is the newest method in the identification of the SLN. Immunohistochemical identification of the sentinel lymph node is more sensitive than HE staining but the molecular procedure is more specific, and more accurate than immunohistochemistry (IHC) in finding isolated tumor cells. However, it seems that in the literature there are not many studies regarding the molecular staging of SLN in colorectal cancer, most probably due to their high cost (both for equipment, specialists and procedures itself), and time consuming.

In a study performed in USA, the RT-PCR technique had a detection sensitivity of individual markers of 103 to 104 μ g of RNA and one to five tumor cells in 107 lymphocytes of healthy donors. In 40 patients with colon

tumors, 25% were identified micrometastases with HE staining, in 10% of the patients the micrometastases were identified with cytokeratin (CK)-IHC and of 26 remained with no evidence of micrometastasis neither by HE staining nor by IHC, 46% had positive RT-PCR results [32].

☒ Conclusions

The importance of sentinel lymph node in colorectal carcinoma remains an open discussion. The lymphatic mapping allows detection of a higher number of small lymph nodes to be identified and examined, but the best method of examination in order to achieve better staging remains unknown. Among all the techniques used for the identification of the lymph nodes, the molecular one is the most expensive, but appears to provide the most accurate up staging.

Conflict of interests

The authors declare that they have no conflict of interests.

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Corresponding author

Ionică Daniel Vilcea, Associate Professor, MD, PhD, Second Surgical Clinic, University of Medicine and Pharmacy of Craiova, 2 Petru Rareș Street, 200349 Craiova, Romania; Phone +40744–296 053, e-mail: id.vilcea@yahoo.com

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