

## THE BIOPSY OF THE SENTINEL LYMPH NODE IN COLORECTAL CANCER. LITERATURE REVIEW

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**Keywords:** colorectal cancer, sentinel lymph node, biopsy, micrometastases

**Abstract:** Colorectal cancer (CRC) is the most common tumour of the digestive tract, both in women and men, having a high mortality rate due to local recurrence and metastases. In patients with colon cancer, the rate of survival is strongly correlated with the status of the lymph nodes. Patients with no metastatic nodules (TNM stages I and II) have a five-year survival rate of 70-80%, whereas patients in stage III have a survival rate of only 45-50%.(1) The presence of lymph node metastases indicates the use of adjuvant therapy in such patients resulting in an increase in the survival rate to 5 years with approximately 10%.(2) Despite favourable prognosis in patients with colon cancer without lymph node metastases, 20-30% of them will nevertheless relapse after undergoing curative resection.(3) These patients are likely to have lymph node metastases that were not detected by using the standard method, or as a result of inadequate lymphadenectomy, or insufficient histological examination.(4) Current guidelines recommend the examination of 12 lymph nodes (LN) for the proper staging of patients with colorectal cancer.(5) Goldstein et al. argue that proper staging requires 6-18 LN.(6) Further scrutiny of LN, through immunohistochemistry and the RT-PCR reaction, can reveal small tumour deposits that were not detected by conventional examination. Several authors report a lower survival rate when LN micrometastases were detected.(7,8,9,10) Since the examination of all LN is time-consuming and expensive, examining only sentinel lymph nodes (SLN) could be helpful. SLN have the greatest potential of developing metastases, and their evaluation is more cost-effective and more efficient. Moreover, the intraoperative nodular mapping can enable the detection of aberrant lymphatic drainage pathways, which leads to a more extensive resection.

**Cuvinte cheie:** cancer colorectal, nodul limfatic santinelă, biopsie, micrometastaze

**Rezumat:** Cancerele colorectale (CCR) sunt cele mai frecvente tumori ale tractului digestiv, atât la femei cât și la bărbați, cu mortalitate crescută, datorită recidivelor locale și a metastazelor. La bolnavii cu cancer de colon rata de supraviețuire este puternic corelată cu statusul ganglionilor limfatici, rata de supraviețuire de 5 ani la pacienții fără metastaze nodulare este de 70-80% (stadiile I și II TNM), în timp ce rata de supraviețuire la bolnavii din stadiul III este de numai 45-50%. (1) Prezența metastazelor ganglionare indică utilizarea tratamentului adjuvant la acești pacienți, ducând la o creștere a ratei de supraviețuire la 5 ani cu aproximativ 10%.(2) În ciuda prognosticului favorabil, la bolnavii cu cancer de colon fără metastaze ganglionare, 20-30% din cazuri totuși vor recidiva după rezecția curativă.(3) Este posibil ca la acești pacienți să existe metastaze ganglionare, care nu au fost detectate prin metoda standard, sau datorită unei limfadenectomii inadecvate, sau examinării histologice insuficiente.(4) Ghidurile actuale recomandă examinarea a 12 noduli limfatici (NL) pentru o stadializare adecvată a bolnavilor cu cancer colorectal.(5) Goldstein și colab. susțin că pentru stadializarea corectă sunt necesari 6-18 NL.(6) Examinarea mai aprofundată a NL, prin reacția imunohistochimică și RT-PCR pot pune în evidență depozitele mici tumorale care au fost ratate la examinarea convențională. Mai mulți autori au raportat o rată de supraviețuire mai scăzută atunci când au fost detectate micrometastaze în NL.(7,8,9,10) Deoarece examinarea tuturor NL este consumatoare de timp și scumpe, examinarea doar a nodulilor limfatici santinelă (NLS) ar putea fi de ajutor. NLS au cel mai mare potențial de a fi metastazați, evaluarea lor fiind mai rentabilă și mai eficientă, iar în plus efectuarea hărții nodulare intraoperator poate detecta căile limfatice aberante de drenaj, ceea ce conduce la o rezecție mai extinsă.

The sentinel lymph node (SLN) is defined as the first lymph node relay, being placed in the drainage area of a tumour and is considered to be very important in the practice of oncology. The concept of sentinel node is based on the theory of the sequential lymphatic dissemination of tumors.(11,12,13) In clinical practice, SLN biopsy has proved to be very effective in accurately estimating the lymphatic nodular status of malignant melanoma and breast cancer.(14) If SLN is negative in breast cancer and malignant melanoma, surgery can be reduced to a

minimum. When SLN is positive, the dissection of the lymph nodes needs to be performed. SLN detection can be performed by injecting dye or radiotracer into the area around the tumour, after the completion of a specific protocol for validating the technique.(15) Over the last two decades, more and more researchers have tried to broaden the scope of the concept of SLN, and have performed biopsy of the sentinel node in various malignancies.(16)

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In the case of CRC, the benefit offered by SLN biopsy is different from that obtained in breast cancer or melanoma. SNL biopsy is not meant to reduce the extent of the resection, but aims to identify situations that would require a more extensive dissection of the lymph nodes. Furthermore, another purpose is to determine the patients' nodular status accurately so as to be able to identify the risks of recurrence or progression of the disease.(17) The presence or absence of lymph node metastases, without distant metastases, is the most important survival and recurrence prognostic factor for patients with operated CRC.(18,19) Stage I and II CRC patients, whose staging does not indicate the presence of lymph node metastases, nevertheless relapse in 25% of the cases, a fact that can be explained either by an incorrect evaluation of the lymph nodes, or by dissemination through blood.(20,21) Thus, there is a constant concern in the scientific community with finding methods that can identify these high-risk patients so that they may benefit from chemotherapy from the outset of the disease. Many studies indicate that a more thorough evaluation of regional LN can enable the detection of nodular metastases that were not revealed by the conventional method. These methods include the multiserial IHC and RT-PCR examination. These nodular metastasis detection methods have not been applied in a multicenter clinical trial to determine their relationship to the prognosis and treatment outcome of the disease.(22) There are many obstacles that hinder the application of these methods in multicenter trials, the most important being time and high cost, as a large number of LNs have to be examined. For this reason, SLN tests attempt to identify the regional LN that are most likely to have developed metastases by their detection during surgery, as they are the first lymph nodes draining the lymph fluid. Many studies have attempted to determine the applicability of the concept of SLN in colon cancer.(23,24,25) Several studies suggest that this concept can be successfully applied in colon cancer, while other studies indicate that LN directly draining the tumour lie outside the conventional limits of regional lymphadenectomy (23,26,27). Paramo et al. found that SLN with no metastases reflect the status of non-sentinel lymph nodes in 97% of the cases.(28) Encouraging results have also been obtained by Tsioulis et al., who have performed SLN mapping during laparoscopic surgery in 14 patients with incipient CRC. SLN were detected in all 14 patients: an average of 13.5 LN and of 1.3 SLN per patient was sampled. All LN were examined with HE, and SLN underwent step sectioning and immunohistochemical analysis with cytokeratin. The condition of SLN accurately reflected the status of regional LN in 93% of the cases (29). In their review, Van der Zaag ES et al. (30) centralized the results and obtained a rate of SLN detection of 90% in CRC patients, by using the *ex vivo* method. The procedure was approximately 70% accurate. Higher accuracy was obtained in several subgroups, which included patients with four or more identified SLN versus patients with less than four identified SLN, and patients with colonic versus rectal localization, incipient carcinoma, ie pT1 / 2 vs pT3 / 4. In a recent study, Saha et al. have shown to what extent SLN biopsy can be used in routine practice.(31) They have investigated 192 patients and identified 22% patients with aberrant lymphatic drainage, i.e. drainage that is outside the standard resection margins. These patients needed a change in surgery conduct, as they manifested higher nodular positivity (62%) than those with standard resection (43%). The major drawback is the relatively low rate of accuracy in the detection of tumour metastasis. The main factors that significantly influence the detection rate are the body mass index, the expertise of the medical centre and the learning curve.(14) The high number of false negatives results can be explained by the aberrant lymphatic drainage and by the

existence of skip metastases. These occur when the lymph vessels are blocked by a tumour. In the study conducted by Retter et al., 63% of the tumours were false negatives, the lymphatic and venous invasion being present.(32) Conventional histopathological examinations used in the detection of lymph node metastases are insufficient on account of the low number of nodules sampled and evaluated. Only a small part of the node is examined, and, because of the low volume, the pathologist may not recognize cancer cells. Alternatively, owing to the existence of aberrant lymph drainage, the positive nodules may be missing from the resected piece.(31) The extension of the pathological examination through the multiserial examination with hematoxylin-eosin (HE) and the use of immunohistochemistry (IHC) techniques and molecular methods applying the polymerase chain reaction (RT-PCR) lead to an improved identification of micrometastases and isolated tumor cells, but the examination of all resected lymph nodes is time-consuming and expensive.(33) To reduce the number of examined lymph nodes, it has been suggested that only SLN should be examined. Following the multiserial and IHC examination and using anti-pankeratin antibodies, Van der Zaag et al. obtained an average increase in staging to 18.5%.(30) Although many authors have addressed this issue, the optimum method of examining SLN has not been found yet. Bembenek et al. conducted a prospective multicenter study in order to assess the clinical value of the nodal status and to identify the factors that influence these results. At least one SLN was detected in 268 out of 315 patients (85%). The detection rate was directly proportional to the expertise of the center, nodular invasion, BMI and learning curve. False negative results were obtained in 46% of the patients. In patients with a BMI equal to or less than 24, the detection rate is higher, i.e. 88%. In 21% of the patients whose routine examination indicates pN0, micrometastases or isolated tumour cells were detected. The contribution of SLN biopsy (SLNB) as compared to standard biopsy is still unspecified. Technical issues must be solved before drawing conclusions. In a quarter of the stage II patients, BNLS showed micrometastases and isolated tumour cells in lymph nodes.(14) Current records on SLNB in colon cancer are conflicting. Several studies have reported a high predictive value of SLNB for nodal status.(34,35) Others have hypothesized a better staging by detecting occult metastases and increased efficiency of nodule sampling (36,37), and reported a significant percentage of aberrant lymphatic drainage outside the resection margin.(38) Read T.E. et al. concluded that mapping and the routine processing of SLN do not improve the accuracy of staging in patients with colon cancer.(39) In a study conducted by Fleig et al., SLN was detected in 98% of the patients, while non-sentinel lymph nodes developed metastases. The false negative rate was of 38%.(40) Merrie et al. also came to the conclusion that there is no consistency between positive non-sentinel lymph nodes and SLN.(41) Van der Zaag used a standard method, making sections at intervals of 500 microns through all lymph nodes sampled from 58 patients with N0. Subsequently, LNs were examined with three types of the antibodies. The examination revealed isolated tumour cells in 33% of the cases, which originally indicated pN0 in the standard examination (12% MM, 21% CTI).(17) Other researchers injected methylene blue *ex vivo* submucosally, subserosally, but also intra-arterially, to improve SLN sampling. After removal, SLNs were examined using the standard method, and the negative ones were subjected to the multiserial examination with HE and IHC.  $3 \pm 1$  SLN were sampled on average. The SLN detection rate was of 78%. An accuracy of 75% in detecting the metastases was registered. Positive LNs were found in 43% of the cases. Skip metastases were found in 4 of

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the cases. A change in stage, i.e. an upstaging from N0 to N1mi, was found in 1 case out of 23 cases (4%) in SLN, following a thorough evaluation of the nodules.(42) Another study conducted by 25 surgeons from 13 institutions aimed to determine whether the conventional pathological examination of SLN accurately predicted nodal status in patients with resectable colon cancer. SLN were identified after 10 minutes from injecting isosulfan blue dye 1%. SLN were identified in 79 patients, followed by the multiserial examination of nodules by a single pathologist. The histological examination indicated that 7 patients had benign tumours. Out of the 72 cancers, 48 had negative SLN and 24 had metastatic nodules. SLNs were found in 66 cases amounting to 92%, with an average of 2.1 nodes per patient. SLN was negative in 14 out of 24 N + cases, i.e. 58%. The multiserial examination indicated the presence of SLN metastases in one case; as a result, the false negative rate is of 54% in the SLN examination. This study showed that in colon cancer patients with nodular metastases, the SLN examination by the multiserial method failed to establish the nodal status in 54% of the cases. The SLN biopsy, followed by the multiserial examination used alone, does not improve the staging of resectable colon cancer.(19) The most important prognostic factor in CRC with healing potential is the status of regional lymph nodes. Thus, patients with regional LN + have a high risk of relapse, so they will benefit from adjuvant therapy.(19) The results concerning CRC staging obtained by AJCC / UICC mirror the fact that the TNM staging is currently the most powerful prognostic parameter. The indication of adjuvant therapy for patients with CRC is guided mainly by the presence of metastases in the regional lymph nodes. Defining the nodular status is influenced by many factors, such as the extension of lymphadenectomy and the thoroughness with which the pathologist evaluates the sampled LN. Moreover, there are inconsistencies between the various editions of the TNM AJCC / UICC staging of CRC tumours, such as the minimum number of sampled LN. Methods commonly used, including methylene blue injection and compression with acetone, are designed to sample as many LN found in the adipose tissue. The ratio of lymph nodes, corresponding to the number of positive LN out of the total number of sampled LN, is a better predictor than the absolute number of LN that have developed metastases. Extracapsular invasion is considered to be an additional prognostic factor. The multiserial examination with hematoxylin-eosin staining and the use of immunohistochemical techniques and of the polymerase chain reaction of the sampled lymph nodes have led to an increased accuracy of the histopathological diagnosis, but the clinical value of this progress has not been defined yet.(46) In a recent study, Resch A. and Langner C. have analyzed the parameters that may affect the clinical significance of nodular status, focusing on changing the definition of LN, lymph node metastases, tumor deposits in the various editions of the AJCC / UICC, the TNM staging system regarding the minimum number of evaluated LN, the nodular ratio, the extracapsular invasion, the SLN biopsy and the benefits offered by recent techniques, such as IHC and molecular analysis.(46) Adjuvant chemotherapy reduced the number of tumor relapse in stage III patients after the introduction of the TNM AJCC / UICC staging system. Adjuvant chemotherapy and total mesorectal excision reduced the incidence of local relapse.(43,44,45) The clinical relevance of the sampled SLN for everyday practice has not been established yet, since there is no standardized method of evaluating the resected SLN, which has entailed a number of controversies. The extension of lymphadenectomy, the pathologist's expertise, the application of techniques that increase the efficiency of LN detection, the permanent changes

in the various editions of the AJCC / UICC staging system, the history of neoadjuvant therapies, the absolute number of sampled LN, the presence of extracapsular invasion, SLN, the number of histological sections examined by the conventional method, the use of IHC and RT-PCR to identify micrometastases and isolated tumour cells are all parameters that can affect the clinical significance of nodular status in CRC.(46) Many studies have aroused the suspicion that a routine examination is insufficient for an accurate assessment of the nodular status, which has led to the introduction of new techniques, such as the SLN biopsy, multiserial examination, the application of IHC and molecular analysis.(47,48) As the indication of adjuvant therapy for patients with CRC is applied in the presence of regional lymph node metastasis, it is essential to identify these nodes.(42) Manual dissection and standard examination are the common methods of evaluating lymph nodes, and this approach leads to underestimation of the disease and patient substaging. In 30% of the patients classified as stages I and II according to the TNM system established by AJCC / UICC, whose standard examination indicated negative lymph nodes, relapses occur or the disease evolves, probably because undetected occult metastases.(49,50,51) A major limitation of the histopathological examination is that only a small part of the node is examined, many areas of the SLN remaining uninspected.(52) Identifying minimum SLN disease by molecular techniques as additional tools in the processing of nodes in patients with cancer may help to identify patients who are at risk of relapse and who would benefit from adjuvant therapy.(53) Molecular analysis enables the examination of the entire node, which leads to improved staging and a better selection of the patients.(54) The following characteristics could be relevant to the markers: there is no expression of the marker used in immune system cells; there is a relatively high and constant expression in tumour tissue, regardless of the tumour stage; and there is weak or no downregulation in the tumour compared to normal tissue. Ohlsson L et al. have analyzed seven biomarkers. Among these, CEA (carcinoembryonic antigen), followed by CK20 (cytokeratin 20) and MUC2 (mucin 2), met these criteria (56). The significance of the polymerase chain reaction for the detection of K20 mRNA has been investigated especially in the SLN biopsy.(57,58,59) These studies have proved the superiority of molecular analysis in comparison to conventional examinations (HE standard examination, multiserial examination and IHC). All these techniques enable the examination of the entire node, thus overcoming the problem of the standard approach, which leads to improved staging and a better selection of patients requiring adjuvant therapy. Yet the problems remain, since none of the studied markers have enough specificity to minimize false positives or negatives.(60) The problems arose as early as 1996, when Gunn et al found the expression of K19 genes in 34 out of 40 LN in patients who underwent small intestine resection for benign diseases.(61) Furthermore, biomarkers K20, ACE, GCC mRNA were detected in LN of patients with benign diseases, showing that there are specificity problems that need to be addressed.(62) A major disadvantage of molecular analyzes is the lack of standardization of tests, which hinders comparison of different studies and their incorporation into routine practice.(55)

### Conclusions:

Since the studies have used different methods and since only a small number of patients have been investigated, it is necessary to initiate a multicentre study that would provide a sufficient number of patients, an accurate SLN detection technique and a standard histopathological examination. The

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main objective is to establish the clinical value of SLN biopsy in CRC. Currently, the role of SLN is unclear.

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