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Sentinel lymph node study in colorectal cancer using serial sectioning and Hematoxylin–Eosin staining: importance and limitations

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Abstract

The lymph node involvement represents an important predictor for survival in colorectal cancer; consequently, the best pathologic evaluation is necessary in order to adequately assess the lymph node status. This study aims to evaluate the impact of sentinel lymph node technique in colorectal cancer in lymphatic basin staging. The study included 43 consecutive operated cases, in which the identification of sentinel lymph node was performed during surgery (*in vivo* procedure – colon cancer) or immediately after the removal of the resection specimen (*ex vivo* procedure – rectal cancer). These cases were matched with 45 control cases. The identified sentinel lymph node was separately examined using multiple sections and Hematoxylin–Eosin staining method. The detection rate, accuracy, sensitivity and false negative rate were better for colon cancer (86.36%; 84.21%; 66.66%; 23.07%) *vs.* rectal cancer (61.9%; 84.61%; 50%; 18.18%), but there are no arguments for the feasibility of the technique in every day practice. Further studies and methods are mandatory in order to improve the staging of the *pN* status in colon and rectal cancer.

Keywords: colon cancer, rectal cancer, sentinel lymph node.

Introduction

One of the most important predictors for survival in colorectal cancer is represented by the lymph node status: the lymph node involvement decreases significantly the 5-year-survival rate, from 85–95% in the absence of lymph node involvement, to 59% in the case of lymph node invasion [1]. The prognosis is worsening with the increasing number of involved lymph nodes [2].

The correct evaluation of the lymph node basin in colorectal cancer depends on the number of the identified and pathological assessed lymph nodes: a minimum of 12 lymph nodes is recommended in order to accurately assess the *pN* stage. In fact, the more lymph nodes are examined, the better the chances to classify the lymph node status [3–6].

This extended examination is a time-consuming procedure, and a series of studies have demonstrated the failure of this recommendation [3, 7, 8]. Consequently, an important number of patients will have an incorrect substaging of *pN* stage, and this correlates with a poor prognosis [9, 10].

The technique of the sentinel lymph node in colorectal cancer, although does not change the surgical procedure, may help to improve the *pN* staging, and could also reduce the time for pathological examination by limiting the extended examination over those cases in which the lymph node is positive on initial examination. Of course, this is valid only if the technique of the sentinel lymph node will reproduce the same good results as in malignant melanoma and breast cancer.

The objective of this study was to assess the value (detection rate, accuracy, sensitivity, false negative rate) of the sentinel lymph node pathological evaluation by serial sectioning and usual Hematoxylin–Eosin staining method.

Materials and Methods

Patients

This study was performed on 299 consecutive colorectal cancer diagnosed patients, out of which 262 cases with the possibility of surgically removing the specimen. The following were excluded from the study: emergency operated cases; non-resectable cases, T_4 cases and patients with distant metastasis; all cases with enlarged, invaded lymph nodes at the time of surgery; synchronous colorectal cancers; the patient's refusal of the procedure, and generally severely altered status (ASA IV); previous surgery, due to postoperative adhesions.

After applying the exclusion criteria, there were 43 cases in which the sentinel lymph node procedure was possible; in order to demonstrate a real benefit from the procedure in postoperative staging, these cases were compared with 45 cases (non-sentinel group), with similar clinical and pathological characteristics (Table 1).

Table 1 – *Characteristics of patients included in the study*

Characteristics		Sentinel procedure group	Control group	*p*
No. of cases		43	45	
Gender	Women	23	24	
	Men	20	21	*0.988*
Age [years]		64.48 (±10.63)	63.91 (±9.93)	
Tumor topography	Right colon	11	12	
	Transverse colon	2	3	
	Left colon	9	11	0.157
	Colon (total)	22	26	
	Rectum	21	19	*0.533*
Invaded lymph nodes	n	16	12	*0.28*
	%	37.2	26.67	

Methods

In this study, it was considered as a sentinel lymph node, the first blue stained pericolic or perirectal lymph node, 5–10 minutes after the peritumoral subserosal injection of 1–2 mL, of 1% Methylene Blue dye.

In colon cancer, the technique was performed during surgery (*in vivo* technique), avoiding tumor massaging; in case of a tumor located on the fixed segments of the colon before the Methylene Blue injection, that segment was mobilized. Lymph node identification was possible usually 1–6 minutes after the injection, helped by the blue stain, contrasting with the yellowish surrounding mesentery (Figure 1). In rectal cancer, the technique was performed in an identical manner, but *ex vivo*, after the rectal resection was performed [11, 12] (Figure 2).

The sentinel lymph node was identified and separately harvested, for special pathologic evaluation: a midsection of the lymph node was initially made, followed by paraffin embedding; micro-sectioning was performed at 0.4 μm, and then 6–10 sections were stained and pathologically assessed, using only the Hematoxylin–Eosin staining method.

All other lymph nodes, identified on the resection specimens, were pathologically assessed, using the usual pathologic examination of the lymph node in colorectal cancer, both in sentinel lymph node group and in non-sentinel lymph node group.

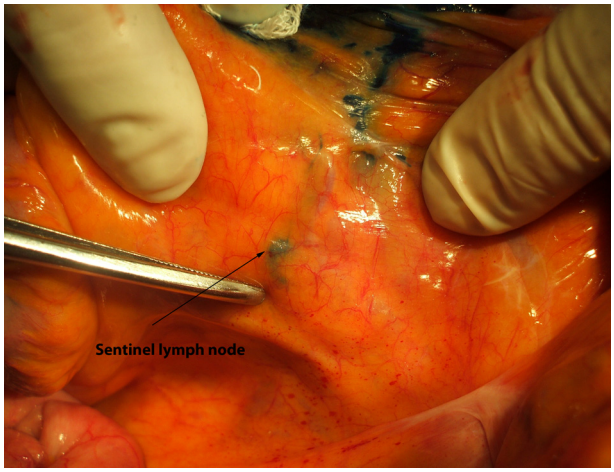

Figure 1 – *Blue stained sentinel lymph node in colon cancer (in vivo technique).*

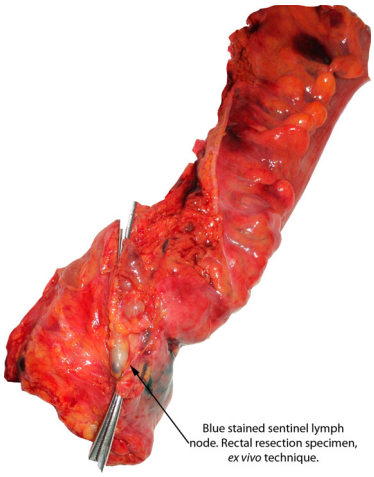

Figure 2 – *Blue stained sentinel lymph node in a rectal resection specimen (ex vivo technique).*

Statistics

The statistical results were reported as detection rate of the sentinel lymph node, accuracy and sensitivity of the test, and false negative rate; formulas, for the assessment of these parameters were as follows [13, 14]:

$$Accuracy = \frac{True\ positive + True\ negative}{Positive + Negative}$$

$$Sensitivity = \frac{True\ positive}{True\ positive + False\ negative}$$

$$FNR = \frac{False\ negative\ lymph\ nodes\ (Fn)}{False\ negative + True\ negative}$$

The staging benefit was calculated by comparison between *pN* staging in the sentinel lymph node group and *pN* staging in the non-sentinel lymph node group. The comparison between groups was performed using the *chi-square* test; the significance was assumed for $p<0.05$ (95% confidence interval). The statistics were performed using XLSTAT 2010 (Addinsoft 1995–2010) software.

Results

In this study, there were 32 cases out of 43, in which the identification of the sentinel lymph node was possible; in colon cancer, sentinel lymph nodes were identified in 19 out of 22 operated cases, while in rectal cancer, there were 13 out of 21 cases with identified sentinel lymph nodes. In colon cancer, in two cases, the identification failed, due to a large amount of blue dye that contaminated the mesentery, and made the lymph node identification impossible, while, in one case, no blue stained lymph node was identified. In rectal cancer, the identification failed in eight cases, due to blue dye spillage in two cases, and because no lymph node was identified in six cases.

In this group, in which the sentinel lymph node identification was possible, there were eight positive sentinel lymph nodes, while, in five cases, the sentinel lymph node was negative on serial sectioning, but there were other positive lymph nodes; overall, there were 13 cases staged pN^+ in this group. However, the number of

cases with lymph node involvement was 16, because in the other 11 cases in which the identification of the sentinel lymph node failed, the lymph node metastasis occurred in three cases (Table 2).

Table 2 – ***The results in the sentinel lymph node group (no. of cases)***

	No. of cases	Identified	True positive	False negative	Total pN^+	True negative
Colon	22	19	6	3	9	10
Rectum	21	13	2	2	4	9
Total	43	32	8	5	13	19

In colon cancer, there were nine positive lymph nodes, six cases with positive sentinel lymph nodes and three cases with positive lymph nodes, other than sentinel lymph node (false negative cases); in the failed identification group, there were no positive lymph nodes detected.

In rectal cancer, there were two cases with positive sentinel lymph nodes, two cases with nodal involvement other than sentinel node (false negative cases), and three cases with positive lymph nodes in the failed identification group.

In Table 3, the results of the sentinel lymph node group are presented.

Table 3 – ***The quality of the sentinel lymph node procedure***

	Detection rate	Accuracy	Sensitivity	FNR
Colon	86.36	84.21	66.66	23.07
Rectum	61.9	84.61	50	18.18
Total	74.41	84.37	61.53	20.83

In the 13 positive identified sentinel lymph nodes, micrometastases were diagnosed in three cases (9.37%), in which the initial examination (usual two sectioning) was considered negative: two cases of colon and one case of rectal cancer (Figure 3).

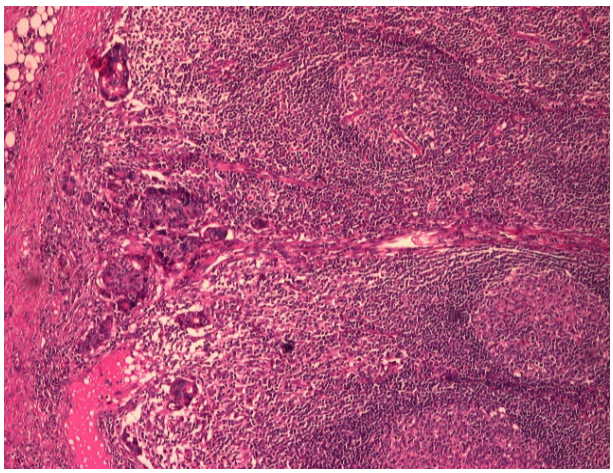

Figure 3 – ***Micrometastasis in a sentinel lymph node (HE stain, ×40).***

In four cases, metastases were identified only in the sentinel lymph nodes, the other examined lymph nodes being negative (three cases of colon cancer and one case of rectal cancer). In five cases, the sentinel lymph nodes were negative, but the examination of the other mesenteric lymph nodes identified metastasis (false-negative sentinel lymph nodes). The upstaging benefit in the sentinel lymph node group is presented in Table 4.

Table 4 – ***The upstaging due to the sentinel lymph node technique***

	Sentinel group			Control group			*p*
	No. of cases	*pN^+*	*%*	*No. of cases*	*pN^+*	*%*	
Colon	22	9	40.9	26	7	26.92	*0.3*
Rectum	21	7	33.33	19	5	26.31	*0.7*
Total	43	16	37.2	45	12	26.67	0.28

Discussion

The importance of the lymph node involvement in colorectal cancer, as well as the importance of the number of identified, pathologically assessed lymph nodes and the number of involved lymph nodes was emphasized in many articles [1, 2, 15–17].

Nowadays, recommendations include a minimum of 12 lymph nodes identified and pathologically assessed for each colorectal resection specimen, in order to ensure a better degree of certainty of pN_0 staging. In fact, the more lymph nodes examined, the better postoperative staging is, which finally influences the patient's prognosis [9, 10, 18, 19]. In every day practice, this recommendation is often eluded, as demonstrated by other authors' studies [3, 7, 8]. This is partially explained by the difficulties in the identification of the small, often invaded, mesentery or mesorectal lymph nodes, and the large volume of work for pathologists, in order to accomplish the current recommendations [20, 21].

The technique used by the pathologists is also very important: in every day practice, the enlarged lymph nodes are manually dissected, half-sectioned and examined using two sections, which means that only 0.4% of the lymph node surface is covered [22]. The special techniques (fat-clearing technique), that allow an increase in the number of identified and examined lymph nodes, are too costly and also increase the work-load for every pathologist; therefore, they are not useful in every day practice [6, 7, 23].

In order to increase the quality of the staging, and limit the number of the lymph nodes necessary to be identified and pathologically assessed, the sentinel lymph node technique was described by Morton in malignant melanoma and, subsequently, in breast cancer, with confirmed success [11].

The importance of the sentinel lymph node study in colorectal cancer is represented by the possibility of limiting the number of cases in which the minimum of 12 lymph nodes must be assessed to those cases in which the sentinel lymph node (the most probable lymph node to carry metastases) is positive. Obviously, this may be done only if the value of the technique is demonstrated in large trials; the value of the technique must be quantified in the probability of the detection rate, accuracy, sensitivity and specificity, which must be as high as possible, and also, in the number of false-negative or false-positive rate, which must be the lowest possible. The study must also demonstrate an important rate of upstaging through a better identification and selection of cases, with respect to the current recommendations.

The identification (detection) rate of the sentinel lymph node varies between 85–97% of cases in the literature, depending on the substance used (Methylene Blue 1%, Isosulfan Blue), but especially on the tumor's topography and surgical team experience with the procedure. Thus, the best detection rate was achieved in colon carcinoma by Kelder W *et al.* and Bilchik AJ *et al.* (96%, and 97% respectively), while Bembenek AE *et al.* achieved an 85% detection rate for the same topography [4, 24, 25]. In the current study, the overall detection rate was 74.41%, with 86.36% detection rate for colon cancer. Although a good detection rate, the main differences may be due to the substance used in our study, but probably at least another two parameters accounted for this result: the experience (a small number of cases in our study), and the fact that we considered as sentinel lymph node only the first blue dyed lymph node, while in the others authors' opinions, the number of sentinel nodes varies from 1 to 4, for each specimen.

For rectal topography, the detection rate is significantly lower (61.9%) than for colon cancer, in all studies but the study of Baton O *et al.*, which demonstrated a 97% detection rate for *ex vivo* technique in rectal carcinoma (this result was not reproduced in other studies). Consequently, the applicability of the sentinel lymph node technique in rectal cancer requires further studies and cautions [11, 12]. The importance of sentinel lymph node detection in rectal cancer will significantly increase in case of extended radical dissection validity in rectal cancer, knowing that the risk of lateral lymph node involvement is significantly higher in cases with perirectal lymph node invasion.

The accuracy of the sentinel lymph node technique is a very important parameter, defining the diagnostic efficiency of the method; the accuracy is influenced by many parameters, in our study the accuracy being relatively good, regardless of the tumor's topography: 84.21% for colon cancer, and 84.61% for rectal cancer. The accuracy may increase with the number of lymph node sections examined, and with the technique of staining used: immunohistochemistry or molecular studies will increase the accuracy, as demonstrated in many studies, but will also increase the cost and the workload for pathologists [4, 24, 25].

Even though the accuracy was good for colon and rectal cancer, the sensitivity of the method was very low (66.66% for colon and 50% for rectal cancer), and the false-negative rate was high (23.07% for colon cancer and 18.18% for rectal cancer). The sensitivity of the method varies in the literature between 54% in the study of the Bembenek AE *et al.*, and 88.2–89% in the study of Bilchik AJ *et al.*, and Kelder W *et al.* [4, 24, 25].

The smallest false-negative rate was achieved by Bilchick AJ *et al.* (7.4%), but other authors reported a significantly higher rate of false-negative results (46% for colon cancer in the study of Bembenek AE *et al.* and 43% in rectal cancer in the study of Baton O *et al.*) [4, 12, 24].

Thus, with such high risk of failure (lower detection rate, low sensitivity and high false-negativity rate), the technique of sentinel lymph node in rectal cancer is obviously not feasible; in colon cancer, the method may be improved by increasing the number of the examined lymph nodes, and using specific immunohistochemical staining methods. However, doing so, it will not represent a relief for pathologist, but probably will increase the quality of the *pN* staging.

In this matter, our study has shown an increase in the detection of the positive lymph nodes (37.2% N^+ in sentinel lymph node group *vs.* 26.67% in the control group), but statistical significance was not reached. Moreover, the quality of the upstaging was not determined by the examination technique itself (micrometastases were detected in only two cases – 9.37% upstaging rate), but probably by the increased number of the identified and examined lymph nodes in the studied group *vs.* comparison group (the blue staining of the lymph node in the study group made it easy to identify them, and probably an increasing awareness and close collaboration between the surgeon and pathologist) [21].

In literature, there are better results in upstaging the *pN* category, using the sentinel lymph node technique, varying from 15% for rectal cancer (Baton O *et al.*) to 18–23.6% in colon cancer (Kelder W *et al.*, Bembenek AE *et al.*, and Bilchik AJ *et al.*) [12, 21, 24, 25].

Conclusions

The number of involved lymph nodes represents an important prognostic factor in colorectal cancer, and is largely dependent on the number of the identified and pathologically assessed lymph nodes.

Close collaboration between surgeon and pathologist is mandatory in order to ensure a good staging in colorectal cancer.

The pathologic examination technique is very important in order to avoid a false pN_0 staging; examining a small number of lymph nodes, and analyzing only 1–2 sections for each lymph node increase the risk of missing nodal invasion, especially micrometastatic disease.

Even though the sentinel lymph node technique allows close examination of the most probable lymph node to carry metastases, it appears that this technique is not feasible for colorectal cancer, due to its low sensitivity and high rate of false-negative results.

The quality of the technique will probably increase with the examination of multiple sections for each lymph node, and using specific immunohistochemical and molecular reactions; still, this will increase the cost and workload for clinicians and pathologists, and requires further studies for confirmation of the validity.

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