Histological and immunohistochemical study on sentinel lymph node in colorectal cancer – values and limitations

**Abstract**
Colorectal cancer is currently one of the most common malignancies in both men and women. Surgical resection remains the essential element in the local control of the disease but the development of novel diagnostic and therapeutic tools can enhance the results of radical surgery. The indication for adjuvant treatment majorly depends on a correct pathological assessment of the surgical specimen – a correct pTNM staging. For patients diagnosed with stage III disease (characterized by the presence of lymph node metastases), adjuvant chemotherapy increases the survival rate, while in stage II disease, in most cases, the chemotherapy is contraindicated, due to increase morbidity without real benefit. This is why an accurate pN stage becomes essential. It is proven that classic pathological exam sometimes fails to identify lymph node micrometastases or isolate tumor cells, which might explain local or distant relapses in stage II patients. In our study, we evaluated a total of 39 surgical specimens of cTNM stage II patients operated for colon or rectal cancer. In the attempt to enhance the accuracy of pTNM staging we used ex vivo lymph node mapping combined with sentinel node analysis on serial sections in both classical histological and immunohistochemical (IHC) staining. We have demonstrated that the IHC staining on sentinel lymph node can improve the accuracy of pTNM staging we used

**Keywords**: colorectal cancer, ex vivo sentinel lymph node, immunohistochemistry, staging, metastases.

## Introduction

In the last 20–30 years, colorectal cancer (CRC) has become a public health problem [1–3], being the third type of cancer in men and the second most frequent type in women all over the world [4]. In 2012, world widely there were recorded 1.36 million new cases of colorectal cancer and approximately 694 000 deaths related to it [4].

The surgical treatment remains the central key for the treatment of colorectal cancer. The development of adjuvant and neoadjuvant means of diagnosis and therapy, and especially their accurate use according to the stage, localization, the patient’s age, presence or absence of metastases, genetic profile, etc, are elements that may improve the results of an adequate surgical treatment at a maximum rate [5, 6]. In order to provide an optimal management for the patients operated for colorectal neoplasias, it is necessary an accurate and complete post-operative pathological diagnosis (pTNM). From this point of view, the accurate assessment of the lymph nodes status remains the most important diagnosis indicator [7, 8]. The patients presenting metastases in the regional lymph nodes are included in stage III of the disease, according to the staging system TNM proposed by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer, having a high risk of relapse for the neoplastic disease [9]; this is why these patients should receive adjuvant chemotherapy. Patients with no lymph node metastases (stages I, II) do not receive any anti-tumor chemotherapy, this bringing more morbidity, mortality and unjustified costs. Still, the surviving rates in colon cancer published by AJCC show that survival is lower in stage IIIb compared to stage III. Thus, we ask ourselves the natural question whether these patients who did not receive any adjuvant therapy have been accurately included in stage II or we are faced with a substaging process.

The microscopic analysis of the peritumoral and regional lymphatic lymph nodes for detecting lymph node metastases is essential for the staging of colorectal cancer, establishment of the treatment protocol and prognosis, due to the fact that various studies showed that the presence of lymph node metastases decreases the patients’ five years survival rate by 30% [10]. Relatively recent data show that almost a third of the patients with colorectal cancer without any metastases in the lymphatic lymph nodes suffer relapses due to occult lymph node micrometastases that are not detected during the routine histological examination of regional lymph nodes [11, 12]. According to AJCC, the accurate staging of colorectal cancer, with an obvious impact upon prognosis, surveillance and especially on adjuvant therapy decision, involves the
examination of at least 12 lymph nodes from the lymphatic basin of tumoral drainage. Because this target is not constantly reached and sometimes impossible to attain, in the last 15 years emphasis was put on the study of the sentinel lymph nodes. The sentinel lymph nodes are the first lymph nodes receiving the lymph from the primary tumor and have the highest probability of harboring metastasis, if they exist. Therefore, the pathological study of the sentinel lymph nodes may be a good indicator of the regional lymph nodes involvement and of the tumor extension process [13].

In the present study, we tried to assess some histological and immunohistochemical features of the sentinel lymph nodes in the patients operated for colorectal cancer, in order to establish a prognosis value for the analysis of these lymph nodes, as well as the applicability of the concept in a third degree center for colorectal oncological surgery.

Materials and Methods

In our study, we included 39 patients with colorectal cancer operated in the IIth General Surgery Clinic of Emergency County Hospital of Craiova, Romania, between 2013 and 2015, of which 21 with colon cancer and 18 with rectal cancer. From this study, there were excluded the patients that were operated for emergency complications, patients that had a history of colic resections, patients with chronic inflammations of the colon (rectocolitis, Crohn’s disease), patients that presented synchro nous neoplasias or metastases (clinically and/or imagistically detected). Also, there were excluded the cases of unresectable cancers or stage IV cases (clinically and imagistically confirmed), as well as the patients that presented evident adenopathies or adenopathic blocks during surgery (macroscopic stage III), or received resections that did not comply with the criteria of oncological radicality. An “informed consent” was obtained for every patient.

Immediately after surgery, all the resection pieces were washed, there were removed the pedicular ligatures, followed by peritumoral, serous injection of 1% Methylene Blue, 1–2 mL, in four opposed quadrants, by using a syringe for insulin administration in diabetic patients. Then, there was performed a light massage for 3–5 minutes upon the peritumoral area where there was injected the dye for its diffusion within the lymphatic ways towards the regional lymph nodes. The waiting interval for the dye diffusion lasted for 10 minutes. By this method of “ex vivo lymph node mapping”, we wanted to highlight the sentinel lymph nodes. After identifying the sentinel lymph nodes, there was performed their dissection from the resection piece. The harvested lymph nodes were immediately introduced in a 10% formalin solution and sent to a histopathological (HP) examination. After the sentinel lymph node harvesting, the dissection of all macroscopically identifiable lymph nodes was continued, grouped by node stations (epicolic, paracolic, intermediary, central). Those nodes were sent separately for the HP examination. For every case, we took macroscopic images of the dissected pieces.

After the formalin fixing of pieces, there followed the paraffin inclusion, by using the classical histopathological technique. Of the pieces included in paraffin, there were performed microtome sections, with a thickness of 4 μm, subsequently stained with Hematoxylin–Eosin (HE) and the Green Light trichrome.

The immunohistochemical study was performed strictly on the sentinel lymph nodes included in paraffin. Thus, there were performed seriate histological sections with a thickness of 3 μm that were collected on poly-L-Lysine blades and kept in a thermostatic at 37°C for 24 hours, in order to increase the biological material adherence. After paraffin removal and hydration of the histological sections, the biological material was incubated in a solution of 1% oxygenated water (hydrogen peroxide) for 30 minutes. The sections were then washed in tap water for 5 minutes, after which they were boiled in a sodium citrate solution pH 6 for 20 minutes, for the antigen demasking. After boiling, they were washed in a phosphate-buffered saline (PBS), followed by the blocking of the non-specific sites by placing the biological material in 2% skimmed milk for 30 minutes. Then, the sections were incubated with the primary antibodies for 14 hours, at 4°C, and the next day, there was applied the secondary biotinylated antibody for 30 minutes, at room temperature. Next, there was performed a washing of the pieces in 1% PBS (three baths of 5 minutes each), followed by the appliance of Streptavidin-HRP (Hors eradish Peroxidase) for 30 minutes at room temperature, followed by another wash in 1% PBS (three baths × 5 minutes). The signal was detected with 3,3′-Diaminobenzidine (DAB) under a microscopic control, the reaction being stopped by a PBS washing, when the interest structures had a maximum of color. The contrasting was performed with Mayer’s Hematoxylin for 1–3 minutes, followed by dehydration in ethanol, xylene clarifying and blade fixing by using DPX (Fluka).

For the immunohistochemical (IHC) study, we used the following antibodies: cytokeratin 7 (CK7) (monoclonal mouse anti-human, clone OV-TL-12/30, 1:50, Dako); cytokeratin 19 (CK19) (monoclonal mouse anti-human, clone RCK 108, 1:100, Dako); cytokeratin 20 (CK20) (monoclonal mouse anti-human, clone KS 20.8, 1:100, Dako); p53 (clone DO7, Ms/Hu/monoclonal, 1:50, Dako); Ki67 (clone MIB-1, Ms/Hu/monoclonal, 1:50, Dako); D2-40 (Ms/Hu/monoclonal, clone D2-40, 1:50, Dako); matrix metalloproteinase 2 (MMP-2) (clone 8B2, 1:58, Novus); matrix metalloproteinase 9 (MMP-9) (Rb/Hu/monoclonal, clone ab 38898, 1:300, Abcam); MMP-8 (clone 100608, RD System); MMP-13 (clone VIII A2, 1:50, Novus) and MMP-14 (clone MT1 – MM, Novus).

Results

In our study we used Methylene Blue as stainer for lymphatic vessels and lymph nodes, because it does not alter the cellular and tissular structures and it enters relatively fast through the lymphatic vessels and lymph nodes, the ex vivo lymph node mapping had the objective of visualizing the lymph drainage ways from the tumor towards the regional lymph nodes, thus leading to the identification of the sentinel lymph node, considered as the first node where the metastasized neoplastic cells might be present. The dye dissemination through the lymph vessels allowed us the highlighting of one or more lymph nodes that captured the dye, an
extremely important fact, as the closest lymph node to the tumor did not capture the dye in all the cases. Our study showed that the lymph dissemination of the tumor cells most often takes place directly, towards the lymph nodes localized in the proximity of the tumor (Figure 1), but it may also take place through alternative ways that go round the closest nodes, sometimes in the areas with low probability (Figure 2) for metastasis. We consider that the identification of the sentinel lymph node has a strong practical importance, due to the fact that the pathological examination should focus on it, in order to detect any possible lymph node metastases.

Of the 39 surgical specimens subjected to ex vivo lymph node mapping procedure in our study, the sentinel node identification succeeded only in 32 cases: in 18 cases of colon cancer and in 14 cases of rectal cancer. In seven cases, we failed to identify at least one colored lymph node. Of the 32 success procedures, in three colon cancer cases, there were identified two nodes that simultaneously captured the dye; in two cases, the lymph nodes were situated at less than 2 cm one from another in the same area of the lymphatic drainage basin, and in a third case (a sigmoid tumor), the dye was captured into an intermediary node but also into an epicolic node, situated at above 12 cm from the tumor site.

The palpating examination of the resection pieces from the 39 patients included in the study allowed us to identify a total number of 418 lymph nodes with a ratio of 10.7 lymph nodes/surgical resection piece, the number of the extracted nodes from each piece varying between four and 46. The sizes of the harvested nodes varied between 0.3 and 1.8 cm. All the lymph nodes were introduced in 10% formalin solution and sent to the pathology laboratory. The pathological anatomy service was blinded for the sentinel lymph node in order to perform a standard pathological analysis – examination of 2–4 sections per lymph node in classical staining.

From the 18 cases of colon cancer, where there were identified sentinel lymph nodes by using the ex vivo lymph node mapping, the pathological examination identified the presence of metastases in the sentinel node (Methylene Blue-stained nodes) in seven cases. The pathological analysis on the other nodes allowed the identification of three more cases of lymph node metastases, where the sentinel nodes were negative instead. There should be noted that in all three cases with two sentinel lymph nodes, in all of the six nodes there were identified metastases. All the patients with lymph node metastases were included in stage III. In eight colon cancer, by using the standard pathological analysis, there were not identified any lymph node metastases, neither in the sentinel nodes nor in the other examined lymph nodes. These patients were included in stage II (Table 1).

<table>
<thead>
<tr>
<th>Segment/ Staining</th>
<th>Cases (No.)</th>
<th>Identified SLN (cases)</th>
<th>Metastasis in SLN</th>
<th>Metastasis in other nodes</th>
<th>Absence of metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon/ HE</td>
<td>21</td>
<td>18</td>
<td>7</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Rectum/ HE</td>
<td>18</td>
<td>14</td>
<td>6</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>32</td>
<td>13</td>
<td>6</td>
<td>13</td>
</tr>
</tbody>
</table>

SLN: Sentinel lymph node; HE: Hematoxylin–Eosin.

Of the 14 patients with rectal cancer with sentinel lymph nodes highlighted by ex vivo lymph node mapping, nine presented lymph node metastases – six in the sentinel nodes, three in other lymph nodes, being included in stage III, while five of them did not present any lymph node metastases and therefore they were included in stage II. We should note that of the seven patients where there could not be obtained a sentinel lymph node, five had lymph node metastases and two were included in N0.

Regarding the main statistical indicators like success rate of the procedure, accuracy, specificity and false negative results rate, our results are in the same range with the data reported in the literature (Table 2).
After this first stage of standard pathological diagnosis that took place in the Laboratory of Pathological Anatomy within the Emergency County Hospital of Craiova, the sentinel lymph nodes were subjected to the IHC study on serial sections, performed in the Research Center for Microscopic Morphology and Immunology, University of Medicine and Pharmacy of Craiova. The examiner in this study was also blinded for the results of the classical pathological examination.

For the cases of colic cancer, this study allowed the identification of micrometastases in other four sentinel lymph nodes declared negative during the standard HP examination, of which two patients were staged N1 based on metastases in other nodes and two were staged N0. In patients with rectal cancer, the examination of serial sections allowed the identification of micrometastases in other two sentinel lymph nodes, one of them in a patient previously staged N0.

Of the 39 patients included in the study, 24 were identified in the Laboratory of Pathological Anatomy with lymph node metastases, by using the classical protocol (19 of the group where there was obtained the sentinel node and five of those where the technique failed), and 19 were found with lymph node metastases by using the serial section technique and IHC staining on the sentinel lymph node. Of these 19 cases, three patients were previously staged N0 after the standard analysis. In conclusion, the serial sectioning of the entire sentinel lymph node associated with immunohistochemistry and the study of sections by classical histological techniques, allowed the inclusion of three patients with tumor lesions in stage III, thus practically correcting a downstaging of previously staged N0.

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Table 2 – Statistical indicators after standard pathological exam

<table>
<thead>
<tr>
<th>Segment/Parameter</th>
<th>Success of the procedure</th>
<th>Accuracy</th>
<th>Sensitivity</th>
<th>False negative rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>85.71%</td>
<td>83.30%</td>
<td>70%</td>
<td>27.20%</td>
</tr>
<tr>
<td>Rectum</td>
<td>77.70%</td>
<td>78.50%</td>
<td>66.60%</td>
<td>33%</td>
</tr>
</tbody>
</table>

The pathological aspects of lymph nodes with metastases were extremely variable, from one case to another and obviously from one lymph node to another. In the sentinel lymph nodes identified by Methylen Blue mapping, the node metastases were most frequently present in the subcapsular sinus or in the cortical area, as deposits or islets of neoplastic cells (Figure 3) or forming tumoral glands of various shapes and sizes (Figure 4). The complete examination of lymph nodes on serial sections allowed the identification of a higher number of micrometastases, which were not identified during the standard pathological examination (Figures 5 and 6). Some micrometastases, distributed in the ganglionic parenchyma, were only suspected after the HP examination and confirmed by immunohistochemical techniques (Figures 7 and 8). In some patients, lymph node metastases had reduced sizes of approx. 100–150 μm, structurated as tumoral glands (more or less differentiated) or coordinated by tumoral cells, while some ganglions presented larger metastases occupying great part of the node or even the whole node volume (Figure 9).

The lymph node stroma in the metastases nodes appeared more or less changed, according to the metastases sizes. Where metastases were reduced, the node stroma was less modified, while in large metastases the lymph node stroma was intensely modified, both regarding the stromal conjunctive tissue and the lymphatic and blood vessels. Most often, the lymph node stroma had a desmoplastic aspect, with various fibroblasts and collagen fibers situated between the tumoral glands (Figure 10).

For the immunohistochemical characterization of the lymph node metastases, we used various antibodies. Cytokeratins are structural elements that characterize the epithelial cells. Based on that feature, anti-cytokeratin IHC stainings are the most often used in the detection of colorectal carcinoma metastases. In our study, the reaction to anti-cytokeratin 7 was absent (Figure 11), but very intense to anti-cytokeratins 19 (Figure 12) and 20 (Figure 13). The tumor cells were constantly and intensely positive to anti-Ki67 (Figure 14), while only about 45% of the lymph node metastases cells were positive to anti-p53 (Figure 15).

The use of D2-40 antibody allowed us to observe deep changes of the lymphatic vessels in the lymph nodes with metastases. In the lymph nodes with large metastases or with a desmoplastic reaction, the lymphatic vessels had a lower number or they were even absent. In some sentinel lymphatic lymph nodes, identified by lymph node mapping, the lymphatic vessels had large sizes, some with tumor cells nests in their lumen (Figure 16). The change of the intraganglionary lymphatic vessel network by the tumor metastases could explain the absence of staining during lymph node mapping of some lymph nodes located in the immediate proximity of the tumor.

Starting from the observation that the structure of the lymph node with tumor metastases is modified, we evaluated the reaction of matrix metalloproteinases (MMPs) in these lymph nodes. MMP-9 had an intense reaction mainly in the tumor cells, but also in some stromal cells (Figure 17), while MMP-2 presented a reduced reaction in the tumor cells and more intense in stromal cells (Figure 18). MMP-13 had a poor reaction in the tumor cells and a totally absent reaction in the stromal cells (Figure 19), while MMP-14 was negative both in tumor cells and in stromal ones (Figure 20).
Figure 3 – Micrometastasis in the subcapsular sinus in the sentinel lymph node. Goldner–Szekely (GS) trichromic staining, ×200.

Figure 4 – Metastasis present in the cortical lymph node. GS trichromic staining, ×100.

Figure 5 – Isolated metastasis in a sentinel lymph node, present in the subcapsular sinus, identified by the examination of serial sections of the entire lymph node. HE staining, ×40.

Figure 6 – Adenocarcinoma gland with necrotic debris in the lumen. Detail from previous figure. HE staining, ×200.

Figure 7 – Microscopic image of the parenchyma of a sentinel lymph node where we can observe the presence of abnormal cells, suspect of being carcinoma cells. HE staining, ×200.

Figure 8 – Isolated tumor cells, positive to anti-p53 antibody. Anti-p53 antibody immunostaining, ×200.
Figure 9 – Overall image of a lymph node almost entirely metastasized. GS trichromic staining, ×40.

Figure 10 – Lymph node metastasis with a desmoplastic reaction of the tumor stroma. GS trichromic staining, ×200.

Figure 11 – Lymph node metastasis with negative reaction to anti-CK7 antibody. Anti-CK7 antibody immunostaining, ×200.

Figure 12 – Lymph node metastasis with intense reaction to CK19. Anti-CK19 antibody immunostaining, ×200.

Figure 13 – Intense reaction of tumoral metastases to CK20. Anti-CK20 antibody immunostaining, ×200.

Figure 14 – Lymph node metastases with an intense positive reaction to anti-Ki67 antibody. Anti-Ki67 antibody immunostaining, ×200.
Figure 15 – Tumoral cells in a lymph node metastasis with inconstant reaction to anti-p53 antibody. Anti-p53 antibody immunostaining, ×200.

Figure 16 – Dilated lymph vessels, with nests of tumoral cells in the lumen. Anti-D2-40 antibody immunostaining, ×400.

Figure 17 – Lymph node metastases with an intense positive reaction of tumoral cells to anti-MMP-9 antibody. Anti-MMP-9 antibody immunostaining, ×200.

Figure 18 – Lymph node metastases with an intense positive reaction of stroma cells to anti-MMP-2 antibody. Anti-MMP-2 antibody immunostaining, ×200.

Figure 19 – Lymph node metastasis with poor reaction of tumoral cells to MMP-13. Anti-MMP-13 antibody immunostaining, ×200.

Figure 20 – Tumoral metastasis with absent reaction to MMP-14. Anti-MMP-14 antibody immunostaining, ×100.
Discussion

Examination of the regional lymph nodes on the surgical specimens in rectal and colon cancers represents a mandatory stage, essential to the stage diagnosis of the disease, in order to establish the treatment protocol, surveillance and prognostic of every case. The accuracy of pathological staging of tumor lesions is important for the improvement of long-term survival in patients with colorectal cancer, as adjuvant chemotherapy in patients with lymph node metastases may improve their survival rate. Various studies showed that adjuvant chemotherapy improves the five-year survival rate in more than a third of the patients with colorectal cancer [14, 15]. On the other side, standard chemotherapy in the patients accurately included in stage II, who underwent a surgical intervention complying with the oncologic resection criteria and without any high risk for relapse, may bring important morbidity, with a negative impact on survival, and, moreover, increasing the treatment costs.

Some studies showed that in about 30% of the patients with colorectal cancer without lymph node involvement, local relapses or distant metastases occur, thus raising a question mark upon the accuracy and precision of the pathological examination of the regional lymph nodes [16]. One of the causes of the understaging is the examination of a small number of lymph nodes from the surgical specimens. Therefore, the American Joint Committee on Cancer recommended the examination of a minimum number of 12 lymph nodes [17], and other studies recommend the examination of a number between 9 and 18 lymph nodes [18–21]. Despite these recommendations, the accordanse rates are quite low, including in the USA, on one hand due to factors related to the surgery (insufficient extent of resection in the mesenteries, altered specimen, etc.) or to the department of pathological anatomy (insufficient staff, lack of training in lymph node dissection, unavailability for fat clearing technique, etc).

In our study, the number of the harvested lymph nodes from the surgical specimens varied between 4 and 46, with a ratio of 10.7 lymph nodes/specimen, far superior to some previous data indicating a rate up to 5 examined lymph nodes/piece that were recorded in our service few years ago. Therefore, the identification of lymph nodes was performed by the surgeon, immediately after finalizing the surgery, a protocol that led to the increase of the number of the identified lymph nodes, and, at the same time, to the increase of the clinical and pathological diagnosis accuracy. The collaboration between the surgeon and the pathologist contributed to the increase of the number of the examined lymph nodes, by imposing certain standards of quality whose compliance continued even after the study was finished, although, at present, the lymph node dissection was taken over again by the department of pathology. Like other authors [22–25], we consider that the accuracy of the pathological diagnosis of lymph node metastases increases in relation to the examined lymph nodes. Still, we believe that certain low-size lymph nodes, or tumor cells localized outside the lymph nodes, escaped the process of identification. Also, in the obese patients, with a well-developed pericolic fat tissue, the palpable identification of lymph nodes was quite difficult to perform. Numerous studies showed that in colon cancer, the examination of all lymph nodes for detecting micrometastases is expensive and time consuming, thus recommending the identification and detailed examination of the sentinel lymph node [1, 26, 27], as this was supposed to be the first node containing the metastasized tumor cells.

In our study, of all the 39 cases, the method of ex vivo lymph node mapping managed to identify the sentinel lymph node only in 32 (82%) patients, in 7 (18%) cases being negative. Our data regarding the identification of sentinel lymph nodes by ex vivo lymph node mapping are similar to those in other studies [7, 10, 28]. We should notice that the identification of the sentinel lymph node is not possible in all cases, being only one of the aspects that limit this method.

We believe that the lymph node identification and examination should be closely and wisely taken into consideration. It is possible that main drainage lymph node be invaded by tumoral metastases that entirely damage the intraganglionary lymph vessel network, either by their excessive development, or by the development of a desmoplastic reaction; this lymph node may be negative during the ex vivo lymph node mapping. At the same time, another lymph node, not affected by metastases, may be stained with Methylene Blue, and thus becoming a “false sentinel lymph node”. That is why we are in favor of the identification and pathological examination of all the lymph nodes from the surgical specimen.

In the last 10 years, more and more researchers observed that the classical pathological examination, based on the examination of two microscopic sections of lymph nodes, in the classical staining (HE) is insufficient and they recommended the use of other techniques as well, like immunohistochemistry on serial sections of the entire lymph node mass or molecular analyses [7, 8, 29]. In our study, the pathological examination on serial sections of the sentinel lymph node and the use of some immunohistochemical techniques highlighted the presence of lymph node micrometastases in six (15.38%) nodes that were initially declared negative on the HE staining and the standard pathological examination. The three restaged cases received chemotherapy. We showed that the classical pathological examination (only two sections of a lymph node in the HE staining) may lead to an incorrect pathological staging of the disease and thus may explain the local relapses. Other studies, as well, highlighted that the classical pathological diagnosis is not sufficient for an accurate evaluation of the development stage of colorectal cancer, as they cannot identify micrometastases unless the sectioning crosses these lesions [30–32].

Immunohistochemical assessment of nodal metastasis revealed that they are interns positive for anti-CK19 and CK20 and negative to anti-CK7. Our data are similar to those obtained by other authors [33–35] that sustain that the majority of adenocarcinomas of the colon (around 80%) are CK7(-)/CK20(+), while only 16% are CK7(+)/CK20(-) and about 4% are CK7(+)/CK20(+). Cytokeratin 19 expression in lymph node metastases of colorectal cancer was less investigated. Relatively recent studies, using new techniques for exposing CK19 (one-step nucleic acid amplification) showed that CK19 is present in the colorectal metastatic cancer cells in a huge percentage [36, 37].
The use of anti-Ki67 and anti-p53 allowed us to identify isolated tumoral cells in the sentinel lymph nodes, improving the pathological characterization and therefore the accuracy of pTNM, while the use of anti-MMP opens new perspectives in immunohistochemical characterization of tumor behavior.

We believe that the best solution for an accurate diagnosis of staging colorectal cancer is a good collaboration between the surgeon and the pathologist for examining the surgical specimens, in order to identify and examine all lymph nodes. The pathological examination of the negative lymph nodes during the standard examination should be augmented by a serial section and immunohistochemical examination of the sentinel lymph node, by IHC and by polymerase chain reaction (PCR) examination for microsatellite instability (MSI) or mismatch repair (MMR) proteins in the patients that comply with the Bethesda criteria. All these contribute to the personalization of diagnosis, a fact that leads to an adequate treatment, having the best perspectives.

Conclusions

The data obtained show that the analysis of the sentinel lymph node alone by classical pathological and immunohistochemical methods on serial sections cannot replace an extensive analysis of all the lymph nodes from the tumor lymphatic drainage basin. Instead, it may represent a complementary diagnosis method that significantly reduces the risk for understaging (approx. 10% in our study), even in the cases where there are examined over 12 lymph nodes. We consider that the sentinel lymph node examination on serial sections becomes necessary every time a patient is declared N0, without reaching a number of at least 12 examined lymph nodes. The introduction of new identification methods of possible metastasis sites, both in the intra-operative clinical practice, as well as in the laboratory of histopathology, become absolutely necessary, from this point of view. Our present research studies target the identification of high precision and specificity tracers, possibly unconditioned by the lymphatic drainage, which should allow an accurate identification and evaluation of the risk for relapse.

Conflict of interests

The authors declare that they have no conflict of interests.

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