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Research Article

The role of sentinel lymph node analysis in patients with colorectal cancer undergoing surgery

[1]Bogdan Micu, [1,2]Carmen Micu, [3]Alexandra Gherman, [3]Mădălina Sava, [1]Tudor R. Pop [1]Nicolae Constantea

[1] Vth Surgical Departament, "Iuliu Hatieganu" University of Medicine and Pharmacy, Muncipal Clinical Hospital, Cluj-Napoca, Romania; [2] Department of Anatomy and Embryology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [3] "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [4] IInd Surgical Departament, "Iuliu Hatieganu" University of Medicine and Pharmacy, Emergency County Clinic Hospital, Cluj-Napoca, Romania.

Abstract. Aim: our aim was to identify and analyse sentinel lymph nodes in patients undergoing curative surgery for colorectal cancer, using two distinct methods, in vivo and ex vivo. Material and methods: we conducted a prospective study consisting of a total of 22 patients diagnosed with stage I-III colorectal cancer, hospitalized and undergoing radical surgery. Two different techniques were employed to identify sentinel lymph nodes, in vivo and ex vivo, using 1% methylene blue vital stain. Sentinel lymph nodes and resections were examined morphopathologically using the standard method. N0 was the case of patients with negative sentinel lymph nodes or other types of lymph nodes, performed together with immunohistochemistry in order to identify micrometastasis for both sentinel lymph nodes and other lymph node biopsies. Resuts: Mean age in the study group was 70 years, patients aged between 59 and 84 years. Of these, 12 (54.5%) were men and 10 (45.5%) were women. In terms of topography, the tumor was localized in the cecum in 3 (13.6%) cases, in the ascending colon in 3 patients (13.6%), in the transverse colon in 3 (13.6%) cases, in the left colic flexure in one case, in the descending colon in one case, in the sigmoid colon in 8 (36.6%) patients and in the rectum in 3 (13.6%) patients. The ex vivo technique was characterized by 88.8% accuracy, 83.3% sensitivity and a false negative rate of 16.7%. The in vivo technique was characterized by 75% accuracy, 75% sensitivity and a false negative rate of 25%. Conclusion: The two techniques, ex vivo and in vivo, cand be used with comparables results.

Key Words: colorectal cancer, ex vivo, in vivo, sentinel lymph nodes.

Corresponding Authors: A. Gherman, email: allexandragherman@gmail.com

Introduction

Treatment for colorectal cancer first consists in the en bloc surgical removal of the tumor-bearing segment of the bowel, together with the adjacent lymph drainage area, as far as the cancer has spread. Daily clinical practice has shown that half of bowel resections contain less than 12 lymph nodes, thus resulting in understaging colorectal cancer (Mitchell et al 2009). Cancer recurrence rate is over 30% in patients with stage I and II colorectal cancer undergoing curative resections (Figueredo et al 2008). Hematoxylin-eosin multiserial examination, immunohistochemistry and polymerase chain reaction are techniques required for an accurate staging (Iddings et al 2006). Ideally, all regional lymph nodes should be examined using these techniques, which are expensive and time consuming and cannot be introduced into everyday practice.

The concept of sentinel lymph node could provide a solution (Micu et al 2014). This procedure would allow the anatomical pathologist a more accurate and thorough examination of a smaller but highly representative number of lymph nodes, identifying lymph node metastasis. Therefore, sentinel lymph node analysis would redefine the staging and identification of patients who would benefit from adjuvant chemotherapy. Sentinel lymph node identification processes are not standardized and the methods, materials, techniques and patient selection criteria differ between institutions and surgeons (van der Zaag et al 2012).

Based on data available in the literature, our aim was to identify and analyse sentinel lymph nodes in patients undergoing curative surgery for colorectal cancer, using two distinct methods, in vivo and ex vivo. Moreover, our intention was to identify and analyse to what extent these methods can be useful for improving cancer staging, identifying anomalous drainage pathways, identifying micrometastasis and optimizing the prognosis in patients undergoing colorectal cancer surgery at the Fifth Surgical Clinic of the Municipal Clinical Hospital in Cluj-Napoca.

Materials and methods

To achieve our objectives, we conducted a prospective study consisting of a total of 22 patients hospitalized at the Surgical Clinic of the Municipal Clinical Hospital in Cluj-Napoca between September 2012 and March 2014. The study included patients

diagnosed with stage I-III colorectal cancer, hospitalized and undergoing radical surgery. All patients included in the study signed the informed consent form and the study was approved by the Ethics Committee of the Municipal Clinical Hospital in Cluj-Napoca. The diagnosis of colorectal cancer had been established preoperatively by clinical exams, laboratory tests and paraclinical investigations (chest x-ray, abdominal ultrasound, lower gastrointestinal endoscopy with biopsy). Patients with the following manifestations were excluded from the study: distant metastasis detected preoperatively or intraoperatively, synchronous primary tumors, large tumors with peritoneal serous invasion or the invasion of adjacent organs (T4a, T4b), history of gastrointestinal surgery, patients undergoing emergency surgery, patients undergoing palliative surgery, those with neoadjuvant chemotherapy, patients with rectal tumors located in the medial and lower part of the rectum, obese patients with BMI>30, patients preoperatively tested positive as allergic to methylene blue, patients with incomplete data and those who did not sign the informed consent form.

Open surgeries were performed by three teams. All markings were performed by a single surgeon, who was also the main surgeon for the in vivo method.

Two different techniques were employed to identify sentinel lymph nodes, in vivo and ex vivo, using 1% methylene blue vital stain.

Sentinel lymph node identification for the in vivo technique was performed as described by Saha et al (2000). Ex vivo identification of sentinel lymph nodes was done in accordance with the technique described by Wong et al (2001).

Sentinel lymph nodes and resections were examined morphopathologically using the standard method. N0 was the case of patients with negative sentinel lymph nodes or other types of lymph nodes, performed together with immunohistochemistry in order to identify micrometastasis for both sentinel lymph nodes and other lymph node biopsies.

For the immunohistochemical examination, 4-micron sections were cut from each paraffin block where sentinel lymph nodes and other types of lymph node biopsies were embedded, further being dewaxed and rehydrated. The sections were then incubated with anti-cytokeratin 20 antibodies (CK20; K20.8 clone, 1: 400 dilution, DAKO, Carpinteria, CA) for 30 minutes. The Streptavidin-biotin complex method was used for detection of antigen-antibody reaction. The presence of staining of cell nuclei of any intensity was considered positive and assessed by the anatomical pathologist (Iddings et al 2006).

The aim was to study the identification rate, defined as the number of successful procedures of the total number of procedures performed; accuracy, defined as the number of correct predictions of sentinel lymph nodes in relation to the number of patients with sentinel lymph nodes identified; sensitivity, defined as the number of patients with positive sentinel lymph nodes for the hematoxylin-eosin examination in relation to the number of patients with positive lymph nodes; false negative rate, defined as the number of cases with negative sentinel lymph nodes, but with other positive lymph nodes; overstaging, defined as the number of patients with micrometastasis in sentinel lymph nodes in relation to the number of patients staged N0 during routine histopathological examination. Moreover, our intention was to compare the two methods, in vivo and ex vivo, in sentinel lymph node identification in patients with colorectal cancer undergoing surgery (Saha et al 2000; Viehl et al 2012; van der Zaag et al 2012; Viehl et al 2013).

Results

After applying inclusion and exclusion criteria, a total number of 22 patients resulted and entered the study. The in vivo technique for the identification of sentinel lymph nodes was performed in 11 patients and the ex vivo technique in the other 11 patients. Mean age in the study group was 70 years, patients aged between 59 and 84 years. Of these, 12 (54.5%) were men and 10 (45.5%) were women. In terms of topography, the tumor was localized in the cecum in 3 (13.6%) cases, in the ascending colon in 3 patients (13.6%), in the transverse colon in 3 (13.6%) cases, in the left colic flexure in one case, in the descending colon in one case, in the sigmoid colon in 8 (36.6%) patients and in the rectum in 3 (13.6%) patients.

Of the 22 patients, one was classified as stage T1, 5 were stage T2 and the other 16 were stage T3. The tumor was poorly differentiated in one case, moderately differentiated in 17 cases and well differentiated in 4 cases. Five patients were classified as stage I according to TNM staging, 6 patients as stage IIA, one patient as stage IIIA, 9 patients stage IIIB and one patient stage IIIC. There were 72.3% patients with more than 12 resected lymph nodes in the study group. The main clinical and pathological characteristics of patients in the study group, subdivided according to the applied technique, can be seen in the table 1.

Identification of sentinel lymph nodes was performed in 8 of 11 cases (72.7%) using the in vivo technique and in 9 of 11 cases (81.8%) for the ex vivo technique. Difficulties in identifying sentinel lymph nodes when using the in vivo technique was due to an excessive injection of stain which caused the staining of the entire colonic segment, making it impossible to identify sentinel lymph nodes or to harvest blue-coloured tissue that proved not to be a lymph node for the histopathological examination. In the two ex vivo cases, one case indicated excessive staining of the surgical specimen by injecting too much stain, while the other case showed the staining of the entire postoperative specimen by applying too much pressure when injecting the dye. Failure was due to technical errors in all situations where sentinel lymph nodes were not identified.

No statistically significant differences have been found between the in vivo and the ex vivo techniques in terms of identification rate, accuracy, sensitivity, false negative rate and overstaging. The ex vivo technique was characterized by 88.8% accuracy, 83.3% sensitivity and a false negative rate of 16.7%. The in vivo technique was characterized by 75% accuracy, 75% sensitivity and a false negative rate of 25%.

In the present study, in patients where the hematoxylin-eosin examination of lymph nodes did not identify any invasion (N0), immunohistochemical examination was performed to highlight possible micrometastasis, using cytokeratin, in both sentinel lymph nodes and the other lymph node biopsies. As part of the in vivo technique, one case of micrometastasis in sentinel lymph nodes was identified in a number of 4 N0 cases, while for the ex vivo technique, one case of micrometastasis in sentinel lymph nodes was identified out of 3 N0 cases. In the two cases, the other resected lymph nodes were negative. The result

is the overstaging phenomenon, 25% in in vivo and 33.3% in ex vivo, which increased sensitivity in both cases.

Table 1. The main clinical and pathological characteristics of the study group

Clinical and pathological characteristics	Ex vivo (no./%)	In vivo (no./%)
Men	7(63.6)	5 (45.5)
Women	4 (36.4)	6 (54.5)
Ascending colon	3 (27.3)	3 (27.3)
Transverse colon	1 (9.1)	2 (18.2)
Descending colon	7 (63.3)	3 (27.3)
Rectum	-	3 (27.3)
T1	-	1 (9,.1)
T2	2 (18.2)	3 (27.3)
T3	9 (81.8)	7 (63.6)
N0	5 (45.5)	6 (54.5)
N1a	2 (18.2)	1 (9.1)
N1b	2 (18.2)	3 (27.3)
N2a	1 (9.1)	1 (9.1)
N2b	1 (9.1)	-
I	2 (18.2)	3 (27.3)
IIA	3 (27.3)	3 (27.3)
IIB	-	-
IIIA	-	1 (9.1)
IIIB	5 (45.5)	4 (36.3)
IIIC	1 (9.1)	-
G1	2 (18.2)	2 (18.2)
G2	8 (72.7)	9 (81,8)
G3	1 (9.1)	-
G4	-	-
No. of excised lymph nodes		
<12	2 (18.2)	3 (27.3)
>12	9 (81.2)	8 (72.7)

Table 2. The main characteristics of sentinel lymph node analysis in the study groups

Variable	*In vivo*	*Ex vivo*
No. of sentinel lymph nodes	1.63±0.6	1.71 ±0.9
Identification rate	8/11 (72.7%)	9/11(81.8%)
Accuracy	6/8 (75%)	8/9(88.8%)
Sensitivity	3/4 (75%)	5/6 (83.3%)
False negative rate	1/4 (25%)	1/6 (16.7%)
Overstaging	1/4(25%)	1/3 (33.3%)

During the in vivo technique, there was one case of sentinel lymph node outside the lymphatic drainage area of the corresponding colic artery, marked as aberrant lymphatic drainage. It is a tumor in the ascending colon which, after dye injection, indicated a blue staining in the left colic artery, which required a wider right hemicolectomy, elevating the anomalous sentinel lymph node.

Discussions

In our study, sentinel lymph node identification rate using the two methods together was 77.3%. The identification rate was 72.7% when using the in vivo technique, and 88.8% for the ex vivo technique. The data are related to data in the literature, where the identification rate ranges from 58 to 100% (van der Zaag et al 2012). The identification rate was higher in studies assessing more than 100 patients and in studies assessing colon cancer than in those assessing rectal cancer using the ex vivo technique as well. The differences in our study occurred due to the fact that we initially performed the ex vivo technique, and only after correctly acquiring the technique we moved on to perform the in vivo technique.

There were five cases of failure, without being capable of identifying sentinel lymph nodes, which occurred in the first cases, when the techniques were first applied. Therefore, it would be necessary to have at least 5 cases to practice the technique on.

There was only one case of aberrant lymphatic drainage, representing 4.5% of all cases, which falls within the data in the literature on the identification rate of aberrant lymphatic drainage, ranging between 1.6 % and 15% (Saha et al 2000).

In the literature, the sensitivity of sentinel lymph node identification varies between 33.3% and 100% and in the present study, sensitivity was 83.3% for the ex vivo technique and 75% for the in vivo technique (van der Zaag et al 2012). The sensitivity decreases and false negative rate increases in advanced cancers (T3/T4), while in T1/T2 cancers, sensitivity increases and false negative rate decreases. The explanation lies in the fact that advanced tumors either obstruct or interrupt lymphatic drainage.

In our study, one case of exclusion criteria was stage T4, but the number of advanced stage T3 tumors accounted for 72.7% of all cases. This explains the reduced sensitivity rate and the increased false negative rate.

In our study, overstaging, one of the reasons for the identification of sentinel lymph nodes, was between 33.3% for the ex vivo technique and 25% for the in vivo technique, using immunohistochemistry to identify micrometastasis in sentinel lymph nodes, while the rest of the lymph nodes remained negative. These values were correlated with data in the literature, ranging between 0 and 50% (van der Zaag et al 2012)..

Despite the major differences between the two techniques, they both have advantages and disadvantages, their results being comparable. In our study, as in other studies in the literature, there were no statistically significant differences between the two techniques regarding the identification rate, accuracy, sensitivity, false negative rate and overstaging (Viehl et al 2013).

Conclusions

The two techniques, ex vivo and in vivo, cand be used with comparables results. We increased the sensibility of identfication of sentinel lymph node.

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Authors

•Bogdan Micu, Vth Surgical Departament, "Iuliu Hatieganu" University of Medicine and Pharmacy, Muncipal Clinical Hospital, 11 Tabacarilor Street, 400139, Cluj-Napoca, Cluj, Romania, EU, email: micubogdan@yahoo.com

•Carmen Micu, Vth Surgical Departament, "Iuliu Hatieganu" University of Medicine and Pharmacy, Muncipal Clinical Hospital, 11 Tabacarilor Street, 400139, Cluj-Napoca, Cluj, Romania, EU, email: carmenmmicu@yahoo.com

•Alexandra Gherman, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania. 8 Victor Babes Street, 400012, Cluj-Napoca, Cluj, Romania, EU, e-mail: A. Gherman, email: allexandragherman@gmail.com

•Mădălina Sava, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania. 8 Victor Babes Street, 400012, Cluj-Napoca, Cluj, Romania, EU, e-mail: madalina.sava0508@gmail.com

•Tudor R. Pop, Vth Surgical Departament, "Iuliu Hatieganu" University of Medicine and Pharmacy, Muncipal Clinical Hospital, 11 Tabacarilor Street, 400139, Cluj-Napoca, Cluj, Romania, EU, email: poptudor_2003@yahoo.com

•Nicolae Constantea, Vth Surgical Departament, "Iuliu Hatieganu" University of Medicine and Pharmacy, Muncipal Clinical Hospital, 11 Tabacarilor Street, 400139, Cluj-Napoca, Cluj, Romania, EU, email: nicuconstantea@yahoo.com

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