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THE SIGNIFICANCE OF THE SENTINEL LYMPH NODE IN COLORECTAL CANCER AND ITS ISOLATION WITH RADIOACTIVE COLLOID-A PILOT STUDY

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Abstract

Introduction. One of the most important factors for prognosis in patients with colorectal cancer (CRC), especially the 5-year survival is the status of regional lymph nodes (RLN). Threfore, today's recommendations for systematic lymphadenectomy in CRC operations are very important. For correct staging and accepted by all international recommendations, at least 12 LN must be analyzed microscopically. The sentinel lymph node (SLN) is the first lymph node that drains lymph from the tumor and thus represents a LN that has the greatest chance to be the bearer of metastatic disease. Tests to locate the SLN in CRC have started recently and so far there is no consensus on the method for its localization or its significance. The main aim of this study was to improve the tracing of SLN by using radioactive colloid. The secondary aims were to investigate the accuracy, sensitivity and rate of method's identification. Especially important for the analysis is the significance of SLN and its correlation with other RLN.

Methods. The study is performed at the University Clinic for Digestive Surgery from January 2013 and is still ongoing. A day before the surgery endoscopically around the tumor is injected radioactive colloid with Technetium 99 (Sentiscint Tc 99m Medi-radiofarma Ltd) in the amount of 4 ml, which corresponds to 4 mCi (mill curie). Immediately after that, at the Institute of Pathophysiology using a Gamma camera (Mediso DHV nucline spirit), the distribution of the colloid is monitored, which as expected is mostly accumulated in the first LN, that is the genuine sentinel lymph node, thus making lymphatic mapping that is important for identifying possible aberrant drainage. On the day of surgery at 8:00 am, a rerecording with the Gamma camera is made that shows the late distribution of contrast. All patients are operated with standard surgical technique by making resection with systematic lymphadenectomy. Promptly after removing the preparation a Gamma detector probe (Europrobe) is used to determine the radioactivity of the lymph pool and it finds the right SLN which has the highest radioactivity and it is separately sent for complete pathohistological analysis. At the Institute of Pathology all lymph nodes are first treated standardly with HE and then with immunohistochemical method.

Results. So far the study includes 10 patients, 6 men and 4 women, mean age 63 years (59-77). Until now the identification rate is 100%, which means that SLN has been found in all procedures. Only in 2 patients two sentinel lymph nodes have been revealed while the in the remaining only 1, average 1.2. At PH analysis, an average of 14.2 lymph nodes have been isolated (6-25). Only in one patient false negative 1 SLN has been revealed. The number of patients with real negative SLN is 2, which means the SLN is negative and also all the other lymph nodes. The total number of patients with real positive SLN is 7, which means SLN is positive and also some of the other lymph nodes. Therefore the accuracy of the procedure is 90%. The sensitivity of the procedure in our study that is still ongoing, is 87.5%. Up to now there have been no micrometastases detected in these 10 patients with immunohistochemical methods and because of it the up staging for now is 0%. In 2 patients the SLN is the only positive lymph node of all examined LN. In none of the patients aberrant lymphatic drainage has been discovered.

Conclusions. From the results obtained so far in this study, it can be concluded that the identification of the SLN with this method is possible; the accuracy and sensitivity are high and we expect them to be even higher, which is our motive to continue with the study and to analyze minimum 30 patients. We think this would be the highest number of discovered SLN by a surgeon and an institution and we believe it to be sufficient validation of the method.

Key words: sentinel, colorectal cancer, lymph node, radioactive, colloid

Introduction

One of the most important factors of prognosis in patients with colorectal cancer (CRC), especially related to the 5-year survival rate is the status of regionally-mph nodes (RLN). In patients with stage I and stage II of the disease with no metastasis in the LN a 5-year

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survival rate of 70%-80% has been documented, while in patients with stage III and IV with lymph node (LN) metastasis a survival rate of only 40%-50% has been documented [1]. This is the main reason for the current systematic lymphadenectomy in the surgical treatment of CRC. According to the present recommendations patients with stage III and IV of the disease, besides the surgical treatment, also receive adjuvant radiotherapy as well as chemotherapy which increase the 5-year survival rate by additional 10% [2)]. This means that the accurate staging of the disease not only is an important prognostic factor, but it is also important on the type of treatment the patient receives. For an accurate staging, according to the international recommendations, at least 12 LN have to be microscopically analyzed [3]. The standard analysis done by pathologists includes hematoxylin-eosin (HE) staining which is an inexpensive method. However, in 10% to 20% of the lymph nodes that were negative on HE, if immunohistochemistry is applied micrometastases or individual tumor cells will be detected. This leads to the conclusion that by using expensive immunohistochemistry, metastasis will be detected in 20% of the patients that would otherwise remain undetected if standard techniques have been used. This will contribute to the up-staging of these patients from stage II to stage III of the disease which will affect their medical treatment.

The Sentinel Lymph Node (SLN) is the first node through which the tumor cell is being drained and therefore having the highest probability to be the carrier of metastatic disease. The researches about how to identify the SLN in CRC have only recently started and presently there is no consensus neither on how to best identify the SLN nor on its importance. However, the logic that should be applied should follow the same logic as in breast cancer and melanoma, that SLN in CRC represents a reflection of the status of the rest of the LN in a patient. This means that the accurate identification of SLN and its analysis using immunohistochemistry, instead of all nodes, will significantly reduce the cost of the PH analysis while at the same time providing more accurate results. So far the studies on detecting SLN have been using sub-serous injection of blue dye *in vivo* and the first 4 LN that turn blue are defined as SLN (4-10). In our opinion this method does not give enough precise findings so the main goal of our research is to improve the detection of SLN by using radioactive colloid. Another objective of this research is to assess the accuracy, sensitivity and the rate of identification that this method offers. Pertinent to this study is also to analyze the importance of SLN and its correlation with the rest of the regional LN.

Material and method

The study is performed at the University Clinic for Digestive Surgery from January 2013 and is still ongoing. All surgeries have been performed by one surgeon. The study includes patients with diagnosed CRC (through endoscopy and PH). Patients with stage IV of the disease and with distant metastasis confirmed through the pre-operative staging have been excluded from the research. All patients have signed consent forms. One day before surgery endoscopicallyradioactive colloid is injected around the tumor with Technetium 99m (Sentiscint Tc 99m Mediradiofarma Ltd) in the amount of 4 ml, which corresponds to 4 mCi (mill curie). Immediately after that, at the Institute of Pathophysiology using a Gamma camera (Mediso DHV nucline spirit), the distribution of the colloid is monitored, which as expected is mostly accumulated in the first LN, that is the genuine sentinel lymph node, thus making lymphatic mapping that is important for identifying possible aberrant drainage. On the day of surgery, a rerecording with the Gamma camera is made that shows the late distribution of contrast. All patients are operated with standard surgical technique by making resection with systematic lymphadenectomy. Promptly after removing the specimen a Gamma detector probe (Europrobe, France) is used to determine the radioactivity of the lymph pool and it finds the right SLN which has the highest radioactivity and it is separately sent for complete pathohistological analysis. At the Institute of Pathology all lymph nodes are first treated standardly with HE and then with immunohistochemical method.

Definitions

All of the micrometastases detected in the SLN are 0.2-2 mm in size. All individual tumor cells are less than 0.2 mm in size. When micrometastases or individual tumor cells are detected in LN that have been treated with immunohistochemistry, which have been earlier negative using HE, it becomes a case of "up-staging". False negative SLN are those that have been categorized as negative while some of the RLN have been evaluated as positive (G). Rate of identification is the number of procedures that have detected the SLN (B)/ the number of all undertaken procedures (A) x 100%. "Accuracy of procedure" is defined as the total number of patients with positive SLN (C) + number of patients with

a- number of procedures done
b- number of procedures where SLN was detected
c- number of patients with positive SLN
d- number of patients with false-negative SLN
e- number of patients with real negative SLN
f- number of patients with positive LN detected by immunohistochemical analysis

Accuracy of the method = c+e/b x 100%
Sensitivity of the method = c/(c+e) x 100%
Upstaging = f/(e+f) x 100%

true negative SLN (D)/ number of patients with detected positive SLN x 100%. Sensitivity is the number of patients with positive SLN (C)/ total number of patients with positive LN (C+E) x 100%. Upstaging represents the number of patients with positive LN detected with IHH (F)/ number of patients with negative LN confirmed through HE (F+E) x 100.

Results

So far the study includes 10 patients, 6 men and 4 women, mean age 63 years (59-77). Tumor characteristics are presented in Table 1. Five of the patients that were part of this study and have been treated with endoscopic injections were excluded from further participation due to the stage of the disease and blocks of positive lymph nodes.

Table 1. Tumor characteristics

Tumor location	
Right colon	1
Left colon	6
Rectum	3
T-stage	
1 1	
2 2	
3 6	
4 1	
Total number of isolated nodes - medium	14,2
Isolated SLN - medium	1,2

Until now the identification rate is 100%, which means that SLN has been found in all procedures. Only in 2 patients two sentinel lymph nodes have been revealed while in the remaining only 1, average 1.2. At PH analysis, an average of 14.2 lymph nodes has been isolated (6-25). Only in one patient false negative SLN has been revealed. The number of patients with real negative SLN is 2, which means that SLN is negative and also all the other lymph nodes. The total number of patients with real positive SLN is 7, which means SLN is positive and also some of the other lymph nodes. Therefore, the accuracy of the procedure is 90%. The sensitivity of the procedure in our study that is still ongoing, is 87.5%. Up to now there have been no micrometastases detected in these 10 patients with immunohistochemical methods and because of it the up staging for now is 0%. In 2 patients the SLN is the only positive lymph node of all examined LN. In none of the patients aberrant lymphatic drainage has been discovered.

Discussion

The surgical treatment is the most important step in treating patients with CRC. This includes removal of the tumor as well as of all lymph nodes that drain the tumor. These nodes are called regional. If the disease has spread to the lymph nodes they are called positive nodes, those patients are staged as III and are advised to undergo chemo- and radiotherapy. Therefore, an accurate analysis on the lymph nodes by the pathologists is of great importance not only for the prognosis but also for the treatment of patients. According to the up-to-date international guidelines it is necessary that at least 12 lymph nodes are harvested and analyzed for an appropriate staging. SLN is the first node that drains the tumor and hence it is most likely to become positive. A precise identification and PH analysis of the SLN will greatly improve the staging and consequently a more appropriate treatment can be sought. SLN presently is routinely identified and analyzed only with breast cancer and melanoma, and if found negative the lymphadenectomy is being put on hold which is of great importance for the patients. Tests to locate the SLN in CRC have started recently and so far there is no consensus on the method for its localization or its signifycance. Most of these studies have applied sub-serous injection of blue dye *in vivo* as a method of staining the lymph nodes with different sensitivity and accuracy [4,5,8,11,12] (Table 2).

Table 2. Results of multicentric studies for determination of SNL with methylene blue

Study	**No. patients**	**No. centers**	**Identification rate**		**Accuracy**	**Sensitivity Upstaging**
			(%)	**(%)**	**(%)**	**(%)**
Bilchik *et al.* [5]	40	3	100	100	100	10
Saha *et al*. [8]	131	3	99	97	92	16
Bertagnolli *et al.* [4]	72	13	92	81	42	0
Read *et al*. [11]	38	2	79	76	25	3
Kelder and Braat [12]	69	6	97	96	89	18

Although this is a method that gives results with relatively high precision, it is nevertheless based on the definition of the SLN as being the first 4 lymph nodes that have been counted. This means that it is not 1 SLN that is the subject of analysis, but a group of 4 nodes of which 1 is probably the SLN. Unlike the aforementioned method, the method applied in this study includes: peri-tumorous injection of radioactive colloid done endoscopically one day before surgery; mapping of the lymphatic drainage with a gamma camera; possible detection of aberrant lymph pathways; and standard surgical resection with lymphadenectomy after which with a gamma probe we determine the radioactivity of the lymphregion where biggest radioactivity is noted in the SLN. 1 SNL was detected in 8 out of 10 patients, whereas in the other 2 patients the same level of radio-

activity was noted in 2 lymph nodes meaning that the tumor is being drained in the 2 nodes equally. Based on the analysis done in the 10 patients so far we can conclude that the accuracy of the method is 90%, the sensitivity is 87.5% and the rate of identification is 100%. This method is most applicable in patients staged I and II, while in patients with stage III of the disease this can be used only if the lymph nodes are not blocked (part of one "package") and are not too big in size, which implies that the nodes are probably positive. In these patients there is a great possibility for lymphatic redistribution due to the blockages in the lymph nodes with metastases, since this would make the procedure imprecise and this would impact the sensitivity and the accuracy. From the 10 patients that participated in this study, 7 are stage III of whom 3 are stage IIIC which means the disease has progressed to a great extent. This explains why the accuracy and the sensitivity are not as high as in other studies. However, this study shows the highest rate of identification of 100%.
The number of lymph nodes that have been PH analyzed is 14.2 (median number) which is a reflection of a sufficient oncological resection. Only 1 patient had a false negative node while the rest of the nodes were positive making the accuracy to be 90%. Knowing that this patient was stage IVA and only 1 positive node after the SLN it can be concluded that this was probably a case of skip metastasis. In the patients that were so far subjected to immunohistochemistry no micrometastases were found, therefore the upstaging is 0%. This could also be due to how far the disease has progressed but it is too early in the process to draw conclusions. The study is in its beginning and we are of the opinion that such micrometastases will be detected.

Conclusions

From the results obtained so far in this study, it can be concluded that the identification of the SLNwith this method is possible; the accuracy and sensitivity are high and we expect them to be even higher, which is our motive to continue with the study and to analyze minimum 30 patients. We think this would be the highest number of discovered SLN with this method by one surgeon and we believe it to be sufficient for validation of the method.

Conflict of interest statement. None declared.

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