

## **A CLEARING TECHNIQUE TO INCREASE DETECTABILITY OF LYMPH NODES IN RADICAL SURGERY FOR COLON AND RECTAL CANCER**

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### **ABSTRACT**

A method is proposed to detect the smallest lymph nodes located in adipose-lymph-vascular tissues of the meso of the intraoperatively collected specimen. The method combines traditional dissection with modified fat-clearing.

Immediately after surgery, the meso of the fresh specimen was dissected into layers, and the lymph nodes detected by palpation and inspection were removed. They were then grouped according to specificity of lymph dissemination and staging of tumors. Thus detected, the lymph nodes were investigated using routine histological methods. Fatty lymph vessel tissue was separated from the organ and was further processed using a fat clearing technique, by dipping it into 10% formalin solution with 0.01% methylene blue, alcohol and xylol. The mass obtained was translucent, which allowed detecting even minute bluish solid lymph nodes. The latter were dissected, fixed and placed into formalin to be later histologically investigated.

After the experimental stage, the method was introduced into routine clinical practice. So far, we found that in 15 cases the technique of processing the specimens helped to detect 5 to 11 additional lymph nodes, otherwise undetectable.

**Key words:** lymph nodes detection, clearing techniques

### **INTRODUCTION:**

Lymph dissection of regional lymph nodes is standard practice in surgical operations for colorectal carcinoma but more often than not the number of the lymph nodes found for investigation is rather small, thus casting doubt on both diagnostic and therapeutic value of this part of radical surgery. The differences observed in the number, size and location of the lymph

nodes in the specimen could be attributed to anatomical differences, biological relationships and the tumor itself. As well as to surgery techniques and methods of detection.

### **MATERIALS AND METHODS:**

Immediately after operation, all the material removed (colon and its meso) was examined by the so-called post-operative lymph dissection in a specially equipped laboratory. The material was washed with water; the colon was resected along the antemesenteric wall and placed on a well-lit transparent surface. Lymph nodes were detected by palpation and plotted on a specially designed "lymph map", taking into account the location, texture and size of the nodes. Then the material was laid with the meso frontwards, and sliced into longitudinal lamellae 1-2cm thick. Three or four such lamellae were formed on the spreadsheet of mesorectum, and 2 or three – on the mesocolon. To that moment, routine manual lymph node processing was applied. Then, each group of lymph nodes were removed from the "lymph map", and sent for histological investigation placed in glass bottles with 10% formalin solution with labels indicating the group and number of lymph nodes.

The rest of the material collected was processed using a fat-clearing technique, applied as follows:

The adipose lymph vessel tissue was separated from the colon wall and was put into 10% formalin solution for 24 hours, dyed with methylene blue. Then it was consecutively placed into several solutions until complete fat removal was obtained and the material had become translucent. It should be noted that care was taken to have the solution bathe all surfaces of the material. The solutions were used in the following order: 95% alcohol, 100% acetone, 100% xylol. The material was left in each solution for 24 hours. After

soaking in xylol, the material was thoroughly washed with water and was processed in the lab for postoperative lymph dissection on a well-lit glass surface, on which through combining palpation and trans-illumination, even minute lymph nodes could be detected, usually bluish in color and harder than the adjacent tissue layers. Thus detected, the lymph nodes were sent for routine histological investigation.

#### **RESULTS:**

Our first results from applying the methods described above showed that in 15 patients,  $8 \pm 3$  lymph nodes were detected, which was impossible to achieve by routine investigation.

#### **CONCLUSION:**

In view of the importance of detecting as many lymph nodes as possible for clinical practice, and requirements of good practices today to investigate at least 12 lymph nodes in colorectal cancer for the purposes of correct and reliable staging, the fat-clearing method may prove a reliable approach in clinical practice. What is more, fat-clearing could be used to improve approaches to lymphatics in clinical practice and be useful in research work concerning lymphogenic metastases. Fat clearing is indispensable in cases when routine investigations do not allow the detection of 12 lymph nodes necessary.

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