

April 17, 2023

Dear Dr. Peter Schemmer  
Editor-in-Chief & Co-Editor  
*World Journal of Gastrointestinal Surgery*

Title: Histological study of the structural layers around the esophagus in the lower mediastinum  
Manuscript ID: 84494

Thank you for giving us an opportunity to submit our revised manuscript. We are grateful for your insightful comments and the reviewers' valuable suggestions on our manuscript. We have incorporated changes that reflect the suggestions you have graciously provided. We also hope that our edits and the responses below satisfactorily address all the issues and concerns you and the reviewers have noted. Our response to the comments is as follows:

Reviewer #1:  
Specific Comments to Authors: Nil

**Response:** We appreciate you taking the time to read and evaluate our manuscript.

Reviewer #2:  
Specific Comments to Authors: Thank you for the opportunity to review the manuscript titled, Histological study of the structural layers around the esophagus in the lower mediastinum. There are no important problems with the methodology, but the findings of the study are underwhelming and of limited clinical significance.

**Response:** We appreciate you providing important comments. We are thankful for the time and energy you expended. We agree that our report has certain limitations because this is a histological study without cancer tissues. However, we believe that the results of our study are very meaningful. Recently, the number of operations for esophagogastric junction adenocarcinoma is increasing, however, there is no consensus about the membrane and layer structure of the lower mediastinum. Also, the boundaries of lower mediastinal lymph nodes are unclear, and esophageal surgeons often complain that lymph node classification is obscure. Our study revealed the fascias in the lower mediastinum, which we believe will resolve these issues. We also revealed that tissues

on the dorsal side of the esophagus were thicker on the caudal side than the cranial side, which we consider is associated with the aortic invasion of the cancer. It has been already reported that aortic invasion is more common in middle thoracic esophageal cancer than lower thoracic esophageal cancer, however, to the best of our knowledge, we believe this is the first report to mention histological factors of aortic invasion. Furthermore, clinical studies such as the therapeutic value of mediastinal lymph node dissection can be accurately performed based on our study. So, we believe that our study has significant potential for further development. Thank you.

Reviewer #3:

Unfortunately, the incidence and mortality from cancer of the esophagus and cardia continues to grow in the world. The most common localization is the esophageal-gastric junction. In the initial and local stages of the disease, the main method of treatment is a surgical method, including regional lymph node dissection. However, this localization, characterized by a complex relationship of anatomical structures of the mediastinum, is clearly insufficiently studied. In particular, the localization of the lymphatic structures of the mediastinum, as well as the peculiarities of the blood supply to the esophagus, have not been sufficiently studied, which can affect the effectiveness of operations performed, especially endoscopic ones. In this regard, the manuscript presented by the authors is of undoubted interest, and the results obtained may be of practical importance in planning and performing surgical interventions for diseases of the lower third of the esophagus and cancer of the esophagogastric junction. In addition, the authors' assumptions are interesting that the intermediate area on the dorsal side of the esophagus may be a barrier preventing the invasion of tumors of the abdominal esophagus and esophageal-gastric junction into the aorta. The manuscript is well structured, the information is logically and consistently presented in good English. The text is well illustrated with pictures. The tables below supplement the information in the manuscript. There are several issues that may require clarification of the authors (at the discretion of the authors).

1. Duration of fixation of corpses in 10% formalin?

**Response:** Thank you very much for providing important comments. We are thankful for the time and energy you expended. We fixed cadavers in 10% formalin for a week. We added a description of this duration to the “1. Specimen preparation and histological examination” section of materials and methods part.

2. Unfortunately, when stained with antibodies to podoplanin, marker expression is often observed not only in lymphatic endothelial cells, but also in other stromal cells. In cancer, expression can also be observed in tumor cells. This fact is well documented in the scientific literature. Perhaps it makes sense to explain in which case the positive staining of the marker was regarded as expression in the endothelial cells of the lymphatic vessels.

**Response:** We agree that marker expression is observed not only in lymphatic endothelial cells. In our pilot study, it was found that stromal cells, esophageal mucosa and vessel walls could also be stained by podoplanin antibodies, depending on the duration of chemical reaction and concentration of the reagent. To resolve this problem, we diluted the primary antibody to 1000 times. Also, after the incubation with secondary antibody, we used 3,3-diaminobenzidine (DAB) to detect the immunocomplexes. During this process, we made several microscopic observations to confirm that the lymphatic vessels around the lymph nodes and the endothelial cells of the thoracic duct were stained and stop the DAB reaction before any other cells got stained. We used these methods to ensure that only lymphatic tissues were stained. We added this explanation to the materials and methods part (2. Immunohistochemical examination). Thank you for pointing this out.

3. Figure 1. Perhaps it will be more clear if you label the appropriate structures of the mediastinum, as you did in Figures 2 and 3.

**Response:** Thank you for your suggestion. I will label the structures of the mediastinum in Figure1. Thank you for your cooperation.

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