

Peer Review File

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Reviewer A

In this study, the authors evaluated the impact of the spleen dose-volume parameters with the absolute lymphocyte count (ALC) and overall survival in patients with esophageal cancer.

The study is well written. The key finding is that the unintended increase in spleen dose can have a significant impact on ALC.

Reply: Thank you for evaluating my study in detail.

However, I have some remarks.

1. Why did the authors not adjust this analysis for any potential confounding (i.e. multivariable analysis).

Reply: The factors that affect the number of lymphocytes are assumed to be those shown in the Table 1. The most important of these is chemotherapy, and some previous reports have considered chemotherapy as a separate model, but in this study the type and amount of chemotherapy is not constant. Therefore it cannot be used as a factor in multivariate analysis. Considering that only the spleen dose is a factor directly related to the number of lymphocytes, we decided to perform univariate analysis only.

2. Which patients received more spleen dose and are these patients comparable to patients with less spleen dose? An additional baseline table stratified for high versus low spleen dose would potentially address this.

Reply: Patients with lesions in the lower esophagus and higher total doses also have higher spleen doses. Regarding spleen dose, we did not create a stratified table among them because there are multiple factors such as average dose and V5-V30.

3. More information is required regarding the spleen dose and chemotherapy. For example, some patients received radiotherapy alone. These patients are not comparable to patients who received concurrent chemoradiotherapy. Consider removing these patients or providing more information about these patients in the manuscript.

Reply: Past studies have reported that lymphocyte counts are not affected by chemotherapy. By taking the NLR in this study, it was considered that the spleen dose had a greater effect than the effect of chemotherapy. Patients with radiation alone

should be omitted if chemotherapy was affected, but patients with radiation alone were left included in the study because of the greater effect of spleen dose, although it is a consequential theory. Please see Page 14, line 249-253.

The reason for choosing irradiation alone as a treatment method is described. Please see Page 7, line 119 and Page 10, line 171. I'm sorry, I don't have any more information about reason for irradiation alone.

4. The results of this study are not inline with the conclusion. No impact of spleen dose on overall survival was demonstrated. Therefore, the conclusion of this study cannot be that reducing spleen dose using RT techniques may affect overall survival.

Reply: It is as you pointed out. The relevant part about survival has been deleted. (Please see Page 8, line 307-309).

5. More information about the blood collection is required. When were pre-treatment blood samples collected?

Reply: We apologize for the lack of information regarding blood collection. We added some information about blood collection (Please see Page 8, line 139-142). Since blood was not always collected immediately before treatment, in that case, we mainly referred to the blood collection results within one month. For example, blood collection at first visit (mainly within one months).

6. When the period from the end of treatment to blood collection was ≥ 1 week, the result obtained immediately before the end of treatment was used as a reference. In my opinion this not validate and consider removing these patients.

Reply: Due to the retrospective study, it was difficult to unify the blood sampling time. Even if the blood sampling time is determined to be immediately after treatment, there is a difference in the total treatment period, and it is not possible to make a rigorous evaluation considering the recovery of lymphocytes. Blood sampling immediately before treatment with several irradiations left was considered to be acceptable for a retrospective study, although there are uncertainties. This point was described as a limitation in the discussion section. (Please see Page 17, line 298-301)

7. Provide a flowchart regarding the patient inclusion and exclusion.

This study only excluded complications and pre-existing conditions that affect lymphocytes and did not make multiple complicated exclusions or allocations. Even if create figure, it will be very simple and have little information. How about just the description in the text?

Reviewer B

I have read your paper with great interest. The authors revealed an association between radiation dose of the spleen and lymphocytopenia. There are several concerns on this paper as follows.

Reply: Thank you for evaluating my study in detail.

1. The explanation for Figure 2 is not explicitly stated in the manuscript.

Reply: I'm sorry. It was my deficiency. We added "Figure2" in the sentence... (Please see Page 11, line 192)

2. V5 and V10 were shown to be associated with lymphopenia. Is it possible to show the threshold values of V5 and V10 that cause G3 lymphopenia, for example, by using ROC curve analysis?

Reply: We would like to thank you for your accurate opinions. Analysis using the ROC curve has also been reported in past reports. Since this retrospective study cannot exclude uncertainties such as the presence or absence of chemotherapy and the timing of blood sampling, we thought that it was necessary to collect more cases or conduct a prospective study to refer to the threshold value in the ROC curve.

3. As mentioned in Discussion, the effects of irradiation field size and chemotherapy cannot be ignored. Will the results of this study change depending on the size of the irradiation field or the presence of chemotherapy?

Reply: This study we used a ratio (NLR) to confirm the effects of chemotherapy. That is, if chemotherapy reduces lymphocytes, neutrophils should also decrease, and the ratio should remain the same. Using NLR, we concluded that the effects of spleen dose were higher than chemotherapy. Please see Page 14, line 248-250. Also, it seems that how much the spleen is irradiated is more important than the size of the irradiation field. However, when intensity-modulated radiotherapy is used, low doses area may spread to the bone marrow, and the size of the irradiation field may affect lymphocyte counts. Although the bone marrow dose could not be examined in this study, it was concluded that the effect of the spleen dose is greater than the irradiation to the bone marrow in the past reports and distribution of lymphocytes to the bone marrow.