Reviewer 1

Paper is relevant and adds to the literature. The use of cytokines is an active area of research and this group appears to be at the forefront of this investigation. No major concerns.

Minor issues:

1) word space

⇒ We corrected the manuscript.

2) would consider adding more insight into why each cytokine was chosen and may represent ie not just a fishing exercise

⇒ Cytokines which we analyzed in this study were known to be associated with hepatocellular carcinoma. Especially, IL-6 is the well-characterized pro-tumorigenic cytokine and previous studies reported that IL-6 was associated with treatment response. We edited sentence in Materials and methods. (page 6)

3) limitations: add comment on the statistical probability of picking up these changes ie some would be positive by chance based on the statistical methodology

⇒ According to your suggestion, we added comment in discussion. (page 11)

Minor changes to strengthen the paper, but would recommend publication even without the changes.

Reviewer 2

This paper is well written and adds to the literature. Although a small number of patients limits the significance of this study, the results are clear and seems to promote further studies.

Detailed comments:

- Check the abbreviation for radiotherapy in Abstract.

⇒ It was a mistake. We corrected the abbreviation for radiotherapy in Abstract. (page 3)

- Insert the relevant references in the Introduction section.

⇒ Following your comment, we added more references in the introduction. (page 5)

- Although the 51 patients were accrued for a prospective study, the study was not aimed to evaluate the association between cytokines and RT outcomes according to the informed consent. Please explain about this aspect.

⇒ Initially, this study was planned to investigate VEGF levels in the patient receiving RT for HCC. And we performed the additional analysis for serum cytokine levels.

- In the results, there was no association between the change of serum cytokines and survival or treatment failures. How did you analyze the association?

⇒ The increase or decrease of cytokine levels after RT was not consistent between previous studies and it is not clear how the changes of serum cytokine levels affect treatment outcome. RT effect on immune system was induced by various mechanisms and this might be different between patients. The small number of patients could be one of reason for no statistically significance.

- In the Materials and Methods section, mRECIST was used for tumor response evaluation. By the way, there is no data about the relationship between cytokine levels and response. Delete "the sentence about mRECIST", or please describe the results with the timing of response evaluation.

⇒ We analyzed tumor response and treatment failure was defined as a progression disease in mRECIST criteria. However, we did not analyze the relationship between cytokine levels and response such as CR and PR. Following your comment, we deleted sentence about mRECST. (page 6)

- What does "no cases of RILD" in the results mean? For any grade or only for severe RILD? Describe the range of mean liver dose.

⇒ It meant severe RILD and we added the range of mean liver dose. (page 8)

- There was no correlation between cytokine levels and the interval between the date of last pre-RT treatment and RT. Please, describe the interval (median, range) between pre-RT treatment and RT instead of "data not shown".

⇒ We describe the median time interval between pre-RT treatment and RT. (page 9)

- In Figure 1, numbering is wrong.

⇒ It was a mistake. We corrected the legend of Figure 1. (page 22)

Reviewer 3

The author detected the serum levels of cytokines IL-1, IL-6, IL-8, IL-10, IL-12, and TNF-α in 51 hepatocellular carcinoma patients before and after radiotherapy, and claimed that baseline serum level of IL-6 is a useful biomarker for predicting radiotherapy outcomes. This manuscript is not well-designed and lack of novelty.

Major issues:

(1) The author classified patterns of failure into three categories: infield failure, outfield-intrahepatic failure, and extrahepatic failure, what is it based on? An explanation should be given for each category.

⇒ In HCC patients treated with RT, treatment failure outside the RT field is more common than local failure. Therefore, we classified treatment failure into infield, outfield-intrahepatic, and extrahepatic failure. And we demonstrated that clinical factors related to recurrence after RT was different between each treatment failure in previous reports.

We added the explanation for each category. (page 5)

(2) IL-6 level was not changed significantly after radiotherapy, which means radiotherapy has not much effects on IL-6, is it suitable to take it as the biomarker for predicting radiotherapy?

⇒ We suggested that baseline IL-6 level was more suitable to predict to RT outcome than change of serum level. The change of serum cytokine level after RT was not consistent between studies and the effect of change on treatment outcome was not definite. Further large study will be needed to investigate whether the change of serum cytokine levels by RT affect treatment outcome.

(3) The author claimed that the cut-off value for IL-6 was set to 9.735 pg/mL based on a receiver operating characteristic (ROC) curve, ROC curve should be provided. And the number of patients for each group should be given.

⇒ Following your comment, we edited Figure 2. We added ROC curve and describe the number of patients of each subgroup.

(4) The exact time for blood collecting from patients after radiotherapy should be provided. Generally, the serum levels of cytokines are fluctuating, it is more reasonable to detect the cytokines at different times.

⇒ Blood samplings were performed at the last day of RT schedule (page 6) and median duration of RT was 34 days (range, 25-56 days). (page 7)

(5) Total number of patients is 51, and it was further subdivided into different groups, too small numbers of patients are not suitable for statistic analysis.

⇒ The small number of patients was a limitation of our study. The number of patients of subgroup was also small, but graph of infield FFS between subgroups showed a large difference. To overcome this limitation and confirm our results, further large scale studies are needed.

(6) In figure 1, the unit at Y axis should be provided. The symbols used in figure were not consistent with that in figure legends.

⇒ The unit of cytokine level was pg/mL and we added the unit in Y axis.

The discordance of symbols between figure and figure legends was our mistake. We corrected the figure legends. (page 22)

(7) In figure 2, the patient numbers for each group should be given.

⇒ We added the number of patients for each group in Figure 2. (page 23)

(8) English writing should be improved, some sentences are not understandable.

⇒ We did an English proofreading before submission. If it is necessary, we will proceed further proofreading process.