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Answering Reviewers

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 31014

Title: Prognostic significance of preoperative and postoperative CK19 and CEA messenger RNA levels in the peripheral blood of patients with gastric cardiac cancer

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Dear Editors and Reviewers:

Thank you for your letter and for your comments regarding our manuscript titled "Prognostic significance of preoperative and postoperative CK19 and CEA messenger RNA levels in the peripheral blood of patients with gastric cardiac cancer" (ESPS Manuscript NO: 31014). Your comments are valuable and helpful in our revision and improvement of our paper, and in providing guidance to our research. We have studied your comments carefully and accomplished the corresponding revisions, which we hope will meet with your approval. These changes will not influence the content and framework of the paper. The revised sections are marked in red in the paper. The main revisions and responses to the reviewers' comments are as follows.

Responds to the reviewer's comments:

Abstract 1. In the background add some more information about cytokeratin-19 (CK-19) and carcinoembryonic antigen (CEA) mRNA

Response：Thanks you for the reviewer's suggestion. We have added some more information about cytokeratin 19 (CK19) and carcinoembryonic antigen (CEA) mRNA in the abstract section. Please refer to the **abstract**.

2. key word : prognostic , clinicopathological need to be looked up Introduction However, the detection and clinical significance of CEA and CK19 mRNA in the peripheral blood of GCC patients have been few reported in the previous data. On the other hand, with the decrease of tumor burden after surgery, the level of tumor marker expression will change. Some studies supported preoperative CEA levels as predictors for prognosis in GC[11, 12], other studies reported the prognostic value of postoperative CEA levels[13]. The prognostic significance of preoperative and postoperative CK19 in GCC has been few reported.-----Rephrase the sentences.

Response：Thanks very much for the reviewer's suggestion. We have looked up all the key words carefully. "Prognostic" should be "prognosis" and "clinicopathological" should be "clinicopathological factor". In the Introduction section, we have rephrase the sentences according to the reviewer's suggestion. Please refer to the **Key words and Introduction** sections.

3. overall English must be improved e.g.in method And all of the patients written informed consent.

Response: We highly acknowledge the reviewer's comments and suggestions, which have been valuable in the improvement of the quality of our manuscript. According to the reviewer's comments, we have revises the language of our manuscript from a professional English language editing company as you suggested and provided a language editing certificate in the attachments, which we hope will meet your approval.

4. follow the instruction for references.

Response：Thanks for the reviewer's suggestion. As the reviewer has suggested, we

have update the format of all the references according to the Format for references guidelines of the Journal. Please refer to all **references**.

5. what was sample collection method , sample volume , sample preparation must mention

Response：Thanks for the reviewer's question. In our study, **3 to 5** ml peripheral blood samples were obtained through a catheter inserted into a peripheral vessel and collected into EDTA tubes from each GCC patient before and after surgical resection. Sample processing was performed within two hours after blood collection. Karyocytes were isolated from the blood samples using a lymphocyte separation medium according to the manufacturer's instructions (Solarbio, Beijing, China). Briefly, blood samples were subjected to Ficoll-sodium diatrizoate density gradient centrifugation. Then, after discarding the plasma layer, the samples were mixed with a wash buffer and centrifuged at 200 × g for 10 minutes. The pelleted cells were resuspended in red blood cell lysis buffer (Solarbio) and centrifuged another two times. The remaining cells, which are karyocytes in PB, were washed with PBS, and the total cellular RNA isolation and cDNA synthesis were performed as previously described. Please refer to the **"Total RNA isolation and complementary DNA (cDNA) synthesis"** section**.**

We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval. Looking forward to hearing from you.

Sincerely yours,

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