Dear Editor and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript Entitled 'Significance of Tumor-infiltrating immunocytes in Predicting the Prognosis for HBV-related HCC' (Manuscript NO: 48495). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. The main corrections in the paper and the responds to the reviewer's comments are as flowing:

Responds to the Editor's comments:

Please provide language certificate letter by professional English language editing companies (Classification of manuscript language quality evaluation is B).

For manuscripts submitted by non-native speakers of English, please provided language certificate by professional English language editing companies mentioned in **'The Revision Policies of BPG for Article'**.

Answer: Please find the below language certificate picture.

MedSci

Editing by professional editors

CERTIFICATE OF ENGLISH EDITING

Date: 17th, Jun 2019

This document certifies that the manuscript titled "Significance of tumor-infiltrating immunocytes for predicting the prognosis of hepatitis B virus related hepatocellular carcinoma" was edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English speaking editors at MedSci. Neither the research content nor the authors' intentions were altered in any way during the editing process.

Documents receiving this certification should be English-ready for publication - however, the author has the ability to accept or reject our suggestions and changes. To verify the final MedSci edited version, please visit our verification page. If you have any questions or concerns over this edited document, please contact MedSci at editing@medsci.cn

Manuscript title:	Significance of tumor-infiltrating immunocytes for predicting the prognosis of hepatitis B virus related hepatocellular carcinoma
First Author:	Qi-Feng Chen
Code:	1706-DF0D-6B6D-D0EC-917A

This certificate may be verified at www.medsciediting.com/certificate/

MedSci affiliated to BIOON Group, offers the services in paper evaluation, professional translation, language editing and publication support.

MedSci comprises a network of more than 300 editors with a biomedical background. All our editors must meet strict selection criteria. We require that each editor has completed or is attending a PhD graduate program at one of the top universities in the world. He or she must have proven written communication skills and undergo a comprehensive training period under the instruction of a managing editor. All editors are native English speakers.

We provide high quality services at the comparable price in the industry. For more information, please visit www.medsciediting.com or contact us by email at editing@medsci.cn

www.medsciediting.com | www.medsci.cn | www.medsci.cn/tw

Responds to the reviewer's comments:

Reviewer 1

The authors have investigated the proportions of immunocytes according to mRNA expression in 53 cases of HBV-related HCC using HiSeq RNASeq platform. Focus of the research is interesting and well analysed using the latest technologies. Thus, I consider this article has potential of publication. However, I have several comments.

1. The definition of "HBV-related HCC" should be clearly indicated. Only HBsAg? How deal with post-antiviral therapy cases?

Response: Thank you for your positive comments. We included HCC patients with seroprevalence of hepatitis B surface antigen (HBsAg), which is same as previous published research (*World-wide relative contribution of hepatitis B and C viruses in hepatocellular carcinoma[J]. Hepatology, 2015, 62(4):1190-1200. PMID: 26146815*). We have re-written this part according to the Reviewer's suggestion in Patient datasets and processing of MATERIALS AND METHODS, as following: '*HCC patients with seroprevalence of hepatitis B surface antigen (HBsAg) were included.*'

Please kindly understand that TCGA is a publicly-available dataset, and we could not obtain antiviral treatment for HCCs. We stated it in our limitation as following: 'anti-inflammatory drugs could not be obtained since this study was carried out on the basis of the publicly-available datasets, and this could potentially result in error or bias.'

2. The more detail of patients and materials should be described, i.e. period of collected patients, surgical case or biopsy, consecutive cases or not, and FFPE tissue or frozen tissue.

Response: We appreciate your valuable comment. As I mentioned in the response of your comment #1. The US government National Institute of Health (NIH) launched The Cancer Genome Atlas (TCGA) Project. The structure of TCGA is well organized and involves several cooperating centers responsible for collection and sample

processing (*PMID: 25691825*). Then, researches from all over the world can download the data and further analyze it for research purpose. So, the detailed information regarding to sample collection is not available, and we are afraid that this is beyond the aim of our study. Please kindly understand our decision on methods.

3. The rationale of "immunoscore" is unclear and complicated. Please explain more easily. The "prognostic risk score" in METHODS is equal to "immunoscore"? In addition, the term of "immunoscore" is used as different definition in previous study (ex, Ref 15). Please clarify it.

Response: Thank you for your positive comments. We firstly estimated 22 immunocyte type fraction using CIBERSORT method. Then, immunoscore was constructed through adopting the LASSO model, which contained 8 immunocyte fraction types.

The "prognostic risk score" in METHODS equals to "immunoscore". To avoid the confusion, the term of "prognostic risk score" has been changed to "immunoscore".

According to Ref 15, *the type, density and location of immune cells within distinct tumor regions, including tumor interior (TI) and invasive margin (IM), referred to as "Immunoscore"*. Typically, immunoscore was constructed based on CD3(+) and CD8(+) lymphocytes expressions. Here, in present study immunoscore was calculated as aforementioned method. Same methodology could be found in published studies, such as: (*Gene expression profiles for a prognostic immunoscore in gastric cancer[J]. British Journal of Surgery, 2018.*) and (*Immune cell infiltration as a biomarker for the diagnosis and prognosis of stage I–III colon cancer[J]. Cancer Immunology and Immunotherapy, 2018.*)

4. The amount of immunocytes in tumor is simply different between high-risk score cases and low-risk score cases in HE-stained slides? Please show representative pathological photographs of high- and low-risk score cases.

Response: The main aim of our study was designed to observe the profile of tumor-infiltrating immunocytes and evaluate their prognosis value for HCC using CIBERSORT, which has not been studied before. We understand that pathological photographs may better reveal the immunoscore. This is one limitation discussed in our study. Thank you for the sincere comments about this issue and further consideration should be taken to the HE-stained experiment in our future study.

5. *The quality of Figures (Figure 2-7) are too poor. Please provide the enough quality of Figures to read the characters in them.*

Response: Following your recommendation clear figures have been uploaded.

6. *I consider the information of baseline patient characteristics is quite important in this kind of study. The table regarding baseline patient characteristics should not be supplemental material. Please indicate the Stage (TMN?) and Grade (What Grade?) at footnote.*

Response: Considering your positive suggestion, we have added patient characteristics in the main text, as table 1.

Stage means TNM stage; Grade means neoplasm histologic grade documented by TCGA staff. And corresponding footnotes were also provided.

Reviewer 2

well written manuscript discussing important issue but as mentioned in the limitation of the study the small number of the study and the heterogeneity of the patients must be considered.

Response: Thank you for your positive comments.

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper.

We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions.

I look forward to hearing from you soon.

With best wishes,

Yours sincerely,