Dear editor,

Thank you for providing us with the opportunity to revise our manuscript for publication. We have carefully considered all of the comments made by reviewers and have taken them into account while preparing the attached revised version of our manuscript. We appreciate the insightful feedback and suggestions for improvement and have made every effort to address all the issues raised.

As requested, we have made revisions to our article and have highlighted these changes in yellow for your convenience. We believe that the revisions we have made have significantly improved the quality and clarity of our paper. We are hopeful that our manuscript is now suitable for publication and we look forward to your positive response soon.

Thank you for your time and consideration. Here are the formal responses to the editor and the reviewers:

Reviewer #1: By collecting clinical data of patients receiving immune checkpoint inhibitors for HCC in the international database, this study analyzed the relationship between NLR, PLR and clinically significant immune-related adverse events in ICI treatment of HCC, as well as the relationship between antibiotics, steroid exposure, the choice of ICI combined treatment regimen and IrAEs. It was concluded that lower baseline NLR and PLR could predict IrAEs in ICI treatment of HCC. Statistical methods were properly used in the study, and the conclusion has certain guiding value for clinical practice. However, the discussion content is slightly superficial, and the manuscript may be fuller if the following contents could be added: 1. There were few content about possible mechanisms by which baseline NLR could predict the occurrence of IrAEs in ICI treatment of HCC, and the possible relationship between baseline inflammation and IrAEs was not clearly explained; 2. It was mentioned in the paper that the possible mechanism by which baseline PLR could predict the occurrence of IrAEs was the hypercortisolemic state in chronic inflammatory diseases, and steroid exposure was associated with the occurrence of IrAEs, although 74% of patients were exposed to steroids for the treatment of IrAEs, what do you think about the relationship between PLR level and cortisol level in patients receiving ICI treatment for liver cancer? 3. It is mentioned in the article that "there is no significant difference in NLR and PLR levels between steroid exposed and non-exposed patients". Are the NLR and PLR levels here at baseline or after exposure?

Author Response: Thank you for bringing up these interesting points, see below for our response to each point mentioned above:

1. We added a paragraph to further explain the relationship between baseline inflammation and IrAEs in discussion – "Additionally, Lymphocytes are an integral components in the immune response against tumors, and an elevated presence of tumor-infiltrating lymphocytes within the neoplastic tissue holds prognostic value, correlating with improved survival outcomes(47). Patients with higher NLR and PLR have a state of relative lymphopenia, signifying an impaired immune response. This likely accounts for both a reduced incidence of IrAEs as well as poorer prognostic outcomes on ICI."

2. Paragraph added to further explain the relationship of PLR – "The mechanistic rationale for the predictive nature of PLR is less well understood, though may be partly explained by the hypercortisolemic state of chronic inflammatory disease (48). Hypercortisolemia results in elevated platelet counts, heightened platelet activation, and concurrently lowers lymphocyte counts(49-51). Thrombocytosis has been demonstrated to exert pro-tumor effects, facilitating tumor progression and metastasis through the production of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) production. These factors subsequently recruit neutrophils and monocytes, a phenomenon that ultimately culminates in adverse prognostic outcomes(40, 52). "Because we didn't check serum cortisol levels, and only baseline PLR was drawn, we are unable to comment directly on effects of endogenous and exogenous steroids on PLR in our cohort.

3. As described in the methods section, NLR and PLR were collected at baseline for all patients. Patients were considered exposed to corticosteroid if >10 mg of prednisone (or equivalent) was administered for >24 hours within 30 days prior to or concomitantly until permanent cessation of immunotherapy.

Reviewer #1:
Scientific Quality: Grade B (Very good)
Language Quality: Grade A (Priority publishing)
Conclusion: Minor revision
Specific Comments to Authors: Human neutrophils and platelets produce a host of cytokines and growth factors relevant to tumor growth and progression. Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have been reported as predictive factors in several cancer types. Elevated NLR and PLR were also found to be associated with poor response to transarterial chemoembolization (TACE) and sorafenib treatment in HCC. As for immunotherapy in HCC, the same predictive effect has also been reported. In a subcohort of 242 patients in the CheckMate 040 trial (J Hepatol,2020,73:1460–9), patients with NLR in the low tertile showed better OS than those with medium or high tertile. A similar result was observed in PLR. Patients with

complete response or partial response (CR/PR) had lower PLR than those with progressive disease. In another cohort of 194 HCC patients treated with nivolumab (Liver Int,2021,41:2189–99), those with baseline NLR≥3 presented poorer progression-free survival (PFS) and OS. Moreover, a dynamic increase of NLR at 4 weeks was associated with an increased risk of death. Interestingly, in this study, NLR increased at 4 weeks also had a role in predicting hyperprogressive disease, which may guide treatment plan in an early phase. In a cohort of 362 HCC patients treated with mono or combination immunotherapy (Cancers,2021,14:186), patients with higher NLR and PLR at baseline were reported to have a higher incidence of portal vein thrombosis (PVT), higher Eastern Cooperative Oncology Group (ECOG) performance status, and more advanced Barcelona Clinic Liver Cancer (BCLC) stage. Significantly shorter OS and PFS were observed in patients with NLR≥5 and PLR≥300. Therefore, I don't believe Dharmapuri's article tell us new information. However, it is a qualified multicenter clinical study. Please cite the above published paper in the manuscript and compare your results with them.

Author Response: The reviewer is discussing several studies, all of which investigate the correlation between NLR (Neutrophil-to-Lymphocyte Ratio) and PLR (Platelet-to-Lymphocyte Ratio) with clinical outcomes, such as overall survival and progression-free survival. However, our study stands out as it is designed to assess the relationship between these biomarkers and the occurrence of immune related adverse events. It's worth noting that the relationship the reviewer mentions has already been examined within the same cohort utilized in our study and has been previously published elsewhere. We have appropriately cited this study in the discussion section of our manuscript (Page 9, Paragraph 2, Line 5; References 39, 40). Consequently, we do not believe it would be meaningful to cite the aforementioned studies and compare their results with ours, given the differences in research objectives.

Reviewer #2:
Scientific Quality: Grade A (Excellent)
Language Quality: Grade A (Priority publishing)
Conclusion: Accept (High priority)
Specific Comments to Authors: Congratulatioms! excellent work and huge effort to collect data from center on 3 continents. The work is signaling an importamt issue in forecast of imune related adverse effects by simple laboratory data. Work of high practical importance!

Author Response: Thank you!

Reviewer #3:
Scientific Quality: Grade D (Fair)
Language Quality: Grade B (Minor language polishing)
Conclusion: Major revision

Specific Comments to Authors: Strength: It's an interesting subject. Data from multicenter and the general sample is relatively large. Weakness: A retrospective study, the study design was not sufficent, and the results of statistical analyses were not precision. There were no correlative study of the therapeutic effects with the immune-related adverse events (IrAEs). The immune-related adverse events (IrAEs) may be a part of the therapy response, and mechanism had better be discussed. If a patient with HCC has poor respose to the immune treatment, what is the immune-related adverse events?

Author response: we have discussed in detail in paragraph 4 & 5 of the manuscript the relationship between NLR, PLR and IrAEs. We have also made several edits to further explain this relationship – "An elevated NLR in a chronic inflammatory state such as cancer, has been shown to be associated with an increased concentration of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) in the peripheral blood (45) as well as tumor-infiltrating neutrophils(43). The role of PMN-MDSCs in immune tolerance by means of upregulated expression of arginase-1, increased production of reactive oxygen species, Nitrous Oxide, prostaglandin E2 among others is well described in literature(46). It may therefore be reasonable to hypothesize that the suppression of the anti-tumor T lymphocyte responses in the tumor microenvironment heralded by PMN-MDSCs likely contributes to the poor clinical outcomes and a lower incidence of IrAEs. Additionally, Lymphocytes are an integral components in the immune response against tumors, and an elevated presence of tumor-infiltrating lymphocytes within the neoplastic tissue holds prognostic value, correlating with improved survival outcomes(47). Patients with higher NLR and PLR have a state of relative lymphopenia, signifying an impaired immune response. This likely accounts for both a reduced incidence of IrAEs as well as poorer prognostic outcomes on ICI.

The mechanistic rationale for the predictive nature of PLR is less well understood, though may be partly explained by the hypercortisolemic state of chronic inflammatory disease (48). Hypercortisolemia results in elevated platelet counts, heightened platelet activation, and concurrently lowers lymphocyte counts(49-51). Thrombocytosis has been demonstrated to exert pro-tumor effects, facilitating tumor progression and metastasis through the production of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) production. These factors subsequently recruit

neutrophils and monocytes, a phenomenon that ultimately culminates in adverse prognostic outcomes(40, 52). Beyond their role in hemostasis, platelets modulate the immune response via both pro and anti-inflammatory mechanisms. Platelets have been shown to express functional CD154, a molecule critical for primary and memory T cell responses(53). In addition to acting as antigen presenting cells(54), platelets also augment CD8 T cell responses via CD154 mediated secondary signaling(55). Their anti-inflammatory effect is exerted via platelet-derived cytokine TGF β which inhibits FoxP3(-) T-cells, among other mechanisms (56). The vast role of platelets in immune modulation remains underrepresented in literature."

Some specific concerns: The title should be rephrased to be better consistent to the central findings of this study (objectively and accurately).

Author response: Title edited to – "Baseline Neutrophil-Lymphocyte Ratio & Platelet-Lymphocyte Ratio Appear Predictive of Immune Treatment Related Toxicity in Hepatocellular Carcinoma" which we think is more precise and informative.

The abstract needs to be rewritten, making the purpose, methods, results and conclusion consistency, adequate and clear.

Author response: Abstract conclusion edited to be more precise; "Given that high baseline NLR and PLR are associated with a decreased incidence of IrAEs, lower baseline NLR & PLR may be predictive biomarkers for the appearance of IrAEs in HCC treated with ICI. This finding is in keeping with several studies in solid tumors that have shown that baseline NLR and PLR appear predictive of IrAEs."

Of 'in a subset of patients with aHCC' should be " in a subset of patients with advanced hepatocellular carcinoma (aHCC)".

Author response: Thank you for pointing out, this has been corrected.

In this study, important information were enrolled in the Table 1, however, there were absence of the details and matching analysis (e.g., propensity score matching) of the different patients, so the study design was not sound and rigorous.

Author response: Propensity score matching could not be performed, since there is no control group of patients on this study who had not received treatment. The objective of the study was to examine the relationship between inflammatory ratios such as NLR and PLR with the incidence of IrAEs in aHCC patients treated with ICI, therefore

univariable and multivariable logistic regression models were used since these were thought to be the appropriate statistical methods to determine such a relationship.

Patients with advanced hepatocellular carcinoma usually have various conditions, including underlying diseases and complications, the neutrophil-lymphocyte ratio (NLR) & platelet-lymphocyte ratio (PLR) are subjected to many confounding factors, which influence the study outcome and reliability of the results and conclusion. Other necessary materials had better be added. Of 'METHODS'. In this section there is other 'methods', please specific.

Author response: Thank you for your suggestion. We acknowledge the same on page 12 in the paragraph regarding weaknesses of the study. We have also added an additional statement to further reflect on this point – "History regarding other chronic inflammatory conditions that could affect NLR and PLR were not collected during chart review and therefore cannot be evaluated. The influence of other confounding factors such as thrombocytopenia from chronic liver disease on NLR and PLR remains a subject of investigation to be explored in a larger prospective cohort. "

Of 'A total of 361 patients had documented IrAE data and were included in the final analysis, of whom 242 (67%) were treated in the USA, 51 (14%) in Asia and 68 (19%) in Europe (Figure 1). ' These should be in the 'Methods' section in this retrospective study.

Author response: Line added to methods section: Of the 361 patients included in the final analysis, 242 (67%) were treated in the USA, 51 (14%) in Asia and 68 (19%) in Europe.

Of 'Figure 1 Study Flow Chart'. It is not a standard one. Number and reason of study sample inclusion and exclusion should be listed out. Please refer to some other articles to revise the "Study Flow Chart".

Author response: We completely agree that it is standard to indicate the number of records reviewed, included and excluded. However, we are unable to include this information in the flow chart since this information is unavailable. This is due to the extensive scope of our review by multiple reviewers across 11 medical centers spanning three continents.

Without decibel, the percentages of 19% and 10% in the Results that "NLR was ≤5 in 184 (51%) patients and >5 in 70 (19%) patients. The proportion of patients with an NLR < 5 or > 5 did not differ significantly between the IrAE groups. PLR was ≤ 300 in 217 (60%) and >300 in 37 (10%) patient" were not consistent with the figures in the Table 1. So do some other variables in the Table 1, please verify and revise.

Author response: The discrepancies in percentages between the text and table can be attributed to the differing presentation methods. In the table, the numbers are distributed between two IrAE groups, whereas in the text, they are provided as a collective total for the entire study cohort. For example: When you add up the NLR ≤5 row in the table (122(34%) +62(17%)), the total comes up to the numbers mentioned in the body of the

text (= 184 (51%)). We have verified all the numbers and minor edits made – "NLR was ≤5 in 184 (51%) patients and >5 in 70 (20%) patients. The proportion of patients with an NLR ≤ 5 or > 5 did not differ significantly between the IrAE groups. PLR was ≤ 300 in 217 (60%) and >300 in 37 (11%) patients (Table 1)"

Of "Antibiotics use was not associated with IrAE incidence (OR = 1.02; p=0.954) (Tables 3)." Tables 3 should be Table 3.

Author response: Thank you for pointing out, this has been corrected.

Of 'Table 2', 'IrAEs incidence by groups' and 'IrAE by organ system N = 167'. Note may be given to make the variables more clear.

Author response: Thank you for pointing this, a detailed note has been added.

*The incidence of immune-related adverse events (IrAEs) has been presented by patient groups categorized as those with IrAEs of grade <2 and those with IrAEs of grade ≥2, stratified by the affected organ systems.

Of 'References'. The styles should be consistent and met the requirement of the present Journal. Of 'Reference 1'. It should be replced with an updated one. Reference 9 and10 are not complete.

Author response: Reference style changed to Vancouver per journal guidelines. Ref 1, 9 and 10 have been updated as well per your recommendations.