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Reviewer A

Comment 1. The grammar should be improved by professional editing.
Reply: Thank you for your careful checking. We have checked the full text and revised all the language mistakes and format errors, please review.

Comment 2. In methods, case-control study was designed to conduct the study. However, it was not identical case-control study to investigate the relationship of exposure and outcome according to STROBE statement checklist. As the study mainly focused on the description of 14 patients with ICI-mediated myocarditis, the purpose of control group should be further explained.
Reply: In this study, we set up the control group to compare the case group with the control group, aiming to analyze differences in basic characteristics between patients with and without ICI-associated myocarditis, and finally determine risk factors of ICI-myocarditis. In order to make the article clearer, we have revised the Results and Discussion section on baseline characteristics and risk factors (Manuscript Line 116-125, 130-133, and 199-222). Please review.

Results section: In comparison with controls, myocarditis patients had a higher prevalence of liver and kidney disease, and previous use of beta-blockers and angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (ACEI or ARB) was more common (Table 1). Logistic regression including the above factors shows that liver disease (Odd ratio, OR: 20.40, 95% confidence interval, 95%CI: 3.20-130.13, P=0.001) and previous use of beta-blockers (OR: 16.46, 95%CI: 1.05-258.96, P=0.046) are independently associated with ICI-associated myocarditis (Table 2). Compared with controls, 64% of myocarditis cases had experienced other concurrent irAEs, with a higher incidence of myositis (50%) and peripheral neuritis (35.7%, Table 1). There were no statistically significant differences in characteristics between cases and controls, including prior cancer treatment, type of PD-1 inhibitors, and prevalence of other ICI-related side effects (Table 1).
Discussion section: The risk factors for ICI-associated myocarditis are not well characterized. In our study, renal disease, liver disease, and previous use of beta-blockers, ACEI, or ARB were correlated with ICI-related myocarditis, among which liver disease and previous use of beta-blockers were independent risk factors for ICI-associated myocarditis. ICI combination, diabetes, and obesity are identified as the independent risk factors for ICI cardiotoxicity in an international registry. With regard to other potential risk factors, it has been reported that abnormal creatinine and AST (suggesting renal and hepatic dysfunction) are risk factors for fulminant myocarditis. It has been reported that patients with liver cirrhosis had higher risks of ICI-associated major adverse cardiovascular events. Likewise, renal dysfunction is considered to have a major impact on the progression of fulminant myocarditis. Therefore, monitoring

renal and liver function may be critical in the early identification of ICI-related myocarditis and the effective management of fulminant myocarditis. Besides, our myocarditis cases tended to be more likely to have prior use of ACEI or ARB, which was consistent with a multicentre international registry in which the interpretation is based upon proportional analysis rather than multivariate regression analysis. Since beta-blockers, ACEI, and ARB are all cardiovascular drugs, it is suggested that cardiovascular disease may be related to ICI-associated myocarditis. In a retrospective study discussing the relationship between cardiovascular factors and ICI-associated myocarditis, history of heart failure and history of acute coronary syndrome help identify patients at higher risk of ICI-induced myocarditis. In a meta-analysis, all cases that experienced cardiovascular irAEs presented cardiovascular risk factors. However, our study did not show a difference in previous heart history between the myocarditis and control groups.

Comment 3. The diagnosis criteria of ICI-mediated myocarditis should be introduced to clarity the definition of "Probable", "Possible", "Definite" myocarditis.

Reply: Thanks for your careful review. We have supplemented diagnosis criteria of ICI-mediated myocarditis in Method and Online Supplement section (Manuscript Line 74-75; Online Supplement Line 1-42). Please review.

Manuscript: Diagnosis criteria of ICI-associated myocarditis were represented in online supplement.

Online Supplement: Diagnosis criteria of immune checkpoint inhibitor-associated myocarditis

Immune checkpoint inhibitor (ICI)-associated myocarditis was diagnosed based on Chinese expert consensus on the surveillance and management of immune checkpoint inhibitor-related myocarditis (2020 version) (1). Diagnosis criteria were as followed:

Definite Myocarditis: Any of the following:

1. Tissue pathology diagnostic of myocarditis (e.g. on biopsy or autopsy)
2. Cardiac magnetic resonance imaging (CMR) diagnostic of myocarditis, a clinical syndrome, and one of following:

a. Elevated biomarker of cardiac myonecrosis
b. Electrocardiographic (ECG) evidence of myo-pericarditis

3. New wall motion abnormality (WMA) on echocardiogram not explained by another diagnosis (e.g. acute coronary syndrome ruled out by angiography, trauma, stress induced cardiomyopathy, sepsis) and all of the following:

a. Clinical syndrome consistent with myocarditis
b. Elevated biomarker of cardiac myonecrosis
c. ECG evidence of myo-pericarditis
d. Negative angiography or other testing to exclude obstructive coronary disease

Probable Myocarditis: Any of the scenarios below that are not explained by another diagnosis (e.g. acute coronary syndrome, trauma, stress induced cardiomyopathy)

1. CMR with findings diagnostic of myocarditis without any of the following (when

screening CMR is being performed routinely as in the context of trial procedure)
a. Clinical syndrome consistent with myocarditis
b. Elevated biomarker of cardiac myonecrosis
c. ECG evidence of myo-pericarditis
2. Non-specific CMR findings suggestive of myocarditis with any 1 or more of the following:
a. Clinical syndrome consistent with myocarditis
b. Elevated biomarker of cardiac myonecrosis
c. ECG evidence of myo-pericarditis
3. New WMA on echocardiogram with a clinical syndrome consistent with myocarditis and either:
a. Elevated biomarker of cardiac myonecrosis
b. ECG evidence of myo-pericarditis
4. A scenario meeting criteria for Possible Myocarditis (see below) with 18F-Fluorodeoxyglucose (FDG) Positron Emission Tomography (PET) imaging showing patchy cardiac FDG uptake without another explanation
Possible Myocarditis: Any of the scenarios below that are not explained by another diagnosis (e.g. acute coronary syndrome, trauma, stress induced cardiomyopathy)
1. Non-specific CMR findings suggestive of myocarditis with none of the following:
a. Clinical syndrome consistent with myocarditis
b. Elevated biomarker of cardiac myonecrosis
c. ECG evidence of myo-pericarditis
2. New WMA on echocardiogram and 1 of the following:
a. Clinical syndrome consistent with myocarditis
b. ECG evidence of myo-pericarditis
3. New elevated biomarker (beyond baseline) and 1 of the following:
a. Clinical syndrome consistent with myocarditis
b. ECG evidence of myo-pericarditis
Subclinical myocardial injury
1. Only cardiac injury biomarkers were elevated (excluding other diseases)
2. Without clinical symptoms, electrocardiogram, echocardiogram, or CMR changes

Comment 4. The results of logistic regression mentioned in 3.1 needs more statistical illustration and explanation.

Reply: Thanks for your helpful suggestion. We have re-analyzed our data using logistic regression and the results showed that liver disease and previous use of beta-blockers were independent risk factors of ICI-associated myocarditis. According to logistic regression results, we have added a table of logistic regression (Table 2) and revised the Result and Discussion section (Manuscript Line 122-125 and 199-222), please review.

Result section: Logistic regression including the above factors shows that liver disease (Odd ratio, OR: 20.40, 95% confidence interval, 95%CI: 3.20-130.13, P=0.001) and previous use of beta-blockers (OR: 16.46, 95%CI: 1.05-258.96, P=0.046) are independently associated with ICI-associated myocarditis (Table 2).

Discussion section: The risk factors for ICI-associated myocarditis are not well characterized. In our study, renal disease, liver disease, and previous use of beta-blockers, ACEI, or ARB were correlated with ICI-related myocarditis, among which liver disease and previous use of beta-blockers were independent risk factors for ICI-associated myocarditis. ICI combination, diabetes, and obesity are identified as the independent risk factors for ICI cardiotoxicity in an international registry. With regard to other potential risk factors, it has been reported that abnormal creatinine and AST (suggesting renal and hepatic dysfunction) are risk factors for fulminant myocarditis. It has been reported that patients with liver cirrhosis had higher risks of ICI-associated major adverse cardiovascular events. Likewise, renal dysfunction is considered to have a major impact on the progression of fulminant myocarditis. Therefore, monitoring renal and liver function may be critical in the early identification of ICI-related myocarditis and the effective management of fulminant myocarditis. Besides, our myocarditis cases tended to be more likely to have prior use of ACEI or ARB, which was consistent with a multicentre international registry in which the interpretation is based upon proportional analysis rather than multivariate regression analysis. Since beta-blockers, ACEI, and ARB are all cardiovascular drugs, it is suggested that cardiovascular disease may be related to ICI-associated myocarditis. In a retrospective study discussing the relationship between cardiovascular factors and ICI-associated myocarditis, history of heart failure and history of acute coronary syndrome help identify patients at higher risk of ICI-induced myocarditis. In a meta-analysis, all cases that experienced cardiovascular irAEs presented cardiovascular risk factors. However, our study did not show a difference in previous heart history between the myocarditis and control groups.

Comment 5. Treatments of myocarditis has been elaborately described. Were they based on guideline? If did, please add references. If not, please identified the modified management strategy and clinical outcome.

Reply: Treatment of ICI-associated myocarditis is based on Chinese expert consensus on the surveillance and management of immune checkpoint inhibitor-related myocarditis (2020 version) (1). The detailed treatment of ICI-myocarditis has been added in Online Supplement (Line 44-78), please review.

Online Supplement: Treatment of immune checkpoint inhibitor-associated myocarditis
Treatment strategy for immune checkpoint inhibitor (ICI)-associated myocarditis was based on the Chinese expert consensus on the surveillance and management of immune checkpoint inhibitor-related myocarditis (2020 version) (1). Treatment details are as follows:

1. General treatment

- Discontinue ICI immediately
- Consult with a cardiovascular physician or critical care physician if necessary
- Attention should be paid to concurrent immunotoxicity of other organs, and multidisciplinary diagnosis and treatment teams should be formed if necessary.
- Electrocardiographic (ECG), blood pressure, and oxygenation monitoring should

be performed in patients with arrhythmia and unstable hemodynamics. Patients with heart failure should be managed according to heart failure guidelines.

2. Administration of steroids

- For unstable subclinical myocardial damage (progressive elevation of cardiac troponin), oral prednisone (1-2 mg/kg/day) is recommended, and the dose should be reduced after 5 to 7 days. The first dose reduction is 25% to 40%, and then the dose is reduced once a week. The reduction process should not be shorter than 4 weeks.
- For mild myocarditis, intravenous methylprednisolone (1-2 mg/kg/day) or equivalent (4 mg methylprednisolone = 5 mg prednisone) oral prednisone 5-7 days depending on the situation is recommended. After the improvement of the condition, the dose could be reduced once every 1 to 2 weeks. The reduction process should not be shorter than 4-6 weeks.
- For severe and critical myocarditis, intravenous methylprednisolone (1 g/day) is recommended for 3 to 5 days. After the improvement of the condition, the dose of methylprednisolone could be changed to 1 to 2 mg/kg/day. After the recovery of conduction block and cardiac function, the dose could be reduced once every 1 to 2 weeks. The reduction process should not be shorter than 6-8 weeks.
- Steroids should be the first-line and critical treatment for ICI-associated myocarditis
- If myocarditis aggravates in the process of steroids reduction, up-dose steroids or combination of mycophenolate mofetil (MMF), tacrolimus, infliximab, and other agents are should be selected as appropriate.

3. Further interventions

- If no improvement within 24–48 hours on steroids, further interventions should be considered.
- The dose of intravenous immunoglobulin is 2 g/kg (20-40 g/d for the first 2 days, and then 10-20 g/d for 5 to 7 days).
- Plasmapheresis and lymphocyte clearance suppress humoral and cellular immunity by removing cytokines, immune complexes, and activated lymphocytes from plasma.
- When available, critical myocarditis is recommended for referral to the cardiovascular ward or intensive care unit with respiratory and circulatory support.

1. Society of Integrative Cardio-Oncology China Anti-Cancer Association, The Cardio-Oncology Group of the Chinese Society of Cardiovascular Diseases of Chinese Medical Association, Chinese College of Cardiovascular Physicians Specialized Committee on Cardio-Oncology Chinese Medical Docotor Association, et al. Chinese expert consensus on the surveillance and management of immune checkpoint inhibitor-related myocarditis (2020 version) [J]. CHINESE JOURNAL OF CLINICAL ONCOLOGY 2020; 47: 1027-38

Comment 6. The correlations of previous cardiac- and non-cardiac comorbidities and risk factors with the occurrence of myocarditis need more analysis and explanations.

Reply: Thanks for your constructive suggestion. We have collected and compared data

on cardiac and non-cardiac comorbidities, basic clinical characteristics, tumor status, and tumor therapy strategies in case and control groups to identify risk factors for ICI-associated myocarditis before (Manuscript Line 115-122, 131-133, 202-205, and 206-216). In order to make our study more convincing and comprehensive, we have added the results of logistic regression and supplemented the discussion of risk factors according to your advice (Manuscript Line 122-125, 199-202, 205-206, and 216-222), please review.

Results section: Baseline characteristics of patients are described in table 1. The mean age of ICI-associated myocarditis cases (n = 14) was 60 ± 14 years, with 71.4% being male. In comparison with controls, myocarditis patients had a higher prevalence of liver and kidney disease, and previous use of beta-blockers and angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (ACEI or ARB) was more common (Table 1). Logistic regression including the above factors shows that liver disease (Odd ratio, OR: 20.40, 95% confidence interval, 95%CI: 3.20-130.13, P=0.001) and previous use of beta-blockers (OR: 16.46, 95%CI: 1.05-258.96, P=0.046) are independently associated with ICI-associated myocarditis (Table 2). There were no statistically significant differences in characteristics between cases and controls, including prior cancer treatment, type of PD-1 inhibitors, and prevalence of other ICI-related side effects (Table 1).

Discussion section: The risk factors for ICI-associated myocarditis are not well characterized. In our study, renal disease, liver disease, and previous use of beta-blockers, ACEI, or ARB were correlated with ICI-related myocarditis, among which liver disease and previous use of beta-blockers were independent risk factors for ICI-associated myocarditis. ICI combination, diabetes, and obesity are identified as the independent risk factors for ICI cardiotoxicity in an international registry. With regard to other potential risk factors, it has been reported that abnormal creatinine and AST (suggesting renal and hepatic dysfunction) are risk factors for fulminant myocarditis. It has been reported that patients with liver cirrhosis had higher risks of ICI-associated major adverse cardiovascular events. Likewise, renal dysfunction is considered to have a major impact on the progression of fulminant myocarditis. Therefore, monitoring renal and liver function may be critical in the early identification of ICI-related myocarditis and the effective management of fulminant myocarditis. Besides, our myocarditis cases tended to be more likely to have prior use of ACEI or ARB, which was consistent with a multicentre international registry in which the interpretation is based upon proportional analysis rather than multivariate regression analysis. Since beta-blockers, ACEI, and ARB are all cardiovascular drugs, it is suggested that cardiovascular disease may be related to ICI-associated myocarditis. In a retrospective study discussing the relationship between cardiovascular factors and ICI-associated myocarditis, history of heart failure and history of acute coronary syndrome help identify patients at higher risk of ICI-induced myocarditis. In a meta-analysis, all cases that experienced cardiovascular irAEs presented cardiovascular risk factors. However, our study did not show a difference in previous heart history between the myocarditis and control groups.

Comment 7. It was very meaningful to conduct endomyocardial biopsy to reveal the mechanism of ICI-mediated myocarditis and provided enlightenment for treatment. Please improve Discussion from this aspect.

Reply: Thanks very much for your valuable advice. It helped us learn a lot. Based on your comments, we reviewed some literature and supplemented a discussion of endomyocardial biopsy as a useful tool to reveal the mechanism of ICI-associated myocarditis and guide therapy in Discussion section of the manuscript (Manuscript Line 285-302). Please review.

Discussion section: EMB emerges as a useful tool to reveal the mechanism of ICI-mediated myocarditis. In a study reporting two patients with fatal ICI-associated myocarditis, immunofluorescence studies, and next-generation sequencing were performed on afflicted tissues to identify cell types in the infiltrates found in the myocardium, skeletal muscle, and tumor. Immunohistochemistry showed that infiltrating cells in the myocardium and skeletal muscle were positive for T-cell marker CD3 or macrophage marker CD68. T-cell infiltrates contained an abundance of CD4+ and CD8+ T cells. Notably, immune infiltrate was limited to the myocardium and skeletal muscle. No other tissues were affected, including adjacent smooth muscle. Clonal T-cell populations infiltrating the myocardium were identical to those present in skeletal muscle and tumor, which suggested that patients with ICI-associated myocarditis were having an overactivated and potentially fatal T-cell-driven immune response. Another case report showed that there were multiple subsets of immune cells involved in inflammation, including CD4+, CD8+, and CD68+ cells. Immunohistochemical analysis of EMB specimens allows identification of the subtype of pathogenic T-cells and revelation of underlying etiology in ICI-associated myocarditis, potentially guiding appropriate therapy (immunosuppression and antiviral treatment). By using monoclonal and polyclonal antibodies to characterize inflammatory infiltrate on EMB tissue sections, markers of infection-negative immune-related myocarditis could be identified, in which case immunosuppression should be considered.

1. Palaskas NL, Segura A, Lelenwa L, et al. Immune checkpoint inhibitor myocarditis: elucidating the spectrum of disease through endomyocardial biopsy. Eur J Heart Fail 2021;23:1725-35.

2. Johnson DB, Balko JM, Compton ML, et al. Fulminant Myocarditis with Combination Immune Checkpoint Blockade. N Engl J Med 2016;375:1749-55.

3. Balanescu DV, Donisan T, Palaskas N, et al. Immunomodulatory treatment of immune checkpoint inhibitor-induced myocarditis: Pathway toward precision-based therapy. Cardiovasc Pathol 2020;47:107211.

4. Rose NR. Myocarditis: infection versus autoimmunity. J Clin Immunol 2009;29:730-7.

5. Caforio AL, Pankuweit S, Arbustini E, et al. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial

Diseases. Eur Heart J 2013;34:2636-48, 48a-48d.

Comment 8. Additionally, limitations of the study should be identified.
Reply: According to your suggestion, we have supplemented the limitations of our study in the manuscript (Manuscript Line 355-365). Please review.

Limitation section: There are limitations merit mentioned in our study. This study needs to be interpreted within the context of the study design. This was a single-centre, retrospective study, so our results may be biased by the potential confounding factors inherent in the nature of such studies. Due to the low incidence of ICI-associated myocarditis, the analysis was affected by a small sample size. Since this was a retrospective study, we did not prospectively perform ECG and CMR in ICI-associated myocarditis before patients started ICI and in patients without ICI-associated myocarditis. Therefore, we lacked data to compare the imaging differences between myocarditis group and the control group. In addition, although we observed that early and sufficient steroid administration may improve outcomes in patients with ICI-associated myocarditis, we did not establish this recommendation with a randomized study, hence detailed larger multicenter investigations should be performed to better determine the role of steroid timing and dose on prognosis of ICI-associated myocarditis.

Reviewer B

Comment 1. Could you please specify how the myocarditis was diagnosed in the patient with normal troponin? You could also consider providing the specific arguments for the diagnosis for each of the 14 patients instead of the general designations "definite", "probable", "possible" of Table 3.

Reply: All patients in our study were diagnosed by clinical oncologists according to Chinese expert consensus on the surveillance and management of immune checkpoint inhibitor-related myocarditis (2020 version). Detailed criteria for diagnosis are supplemented in the Online Supplement section (Online Supplement Line 1-42).

Only one patient had a normal troponin in our cases. Although troponin level in this was normal, creatine kinase and pro-B-type natriuretic peptide levels were significantly higher than normal value. In addition, the patient had an abnormal ECG and echocardiogram and presented with pericardial effusion, myalgia, and myasthenia consistent with the clinical features of myocarditis. Therefore, the patient was diagnosed with probable ICI-associated myocarditis, in accordance with the diagnostic criteria recommended by the guidelines.

In our viewpoint, for patients with normal troponin, attention should be paid to changes in cardiac imaging and other cardiac indicators. Although troponin level is normal, if there are clinical symptoms and imaging abnormalities, myocarditis may also occur.

Online supplement: Diagnosis criteria of immune checkpoint inhibitor-associated myocarditis

Immune checkpoint inhibitor (ICI)-associated myocarditis was diagnosed based on Chinese expert consensus on the surveillance and management of immune checkpoint inhibitor-related myocarditis (2020 version) (1). Diagnosis criteria were as followed:

Definite Myocarditis: Any of the following:

1. Tissue pathology diagnostic of myocarditis (e.g. on biopsy or autopsy)
2. Cardiac magnetic resonance imaging (CMR) diagnostic of myocarditis, a clinical syndrome, and one of following:

a. Elevated biomarker of cardiac myonecrosis
b. Electrocardiographic (ECG) evidence of myo-pericarditis

3. New wall motion abnormality (WMA) on echocardiogram not explained by another diagnosis (e.g. acute coronary syndrome ruled out by angiography, trauma, stress induced cardiomyopathy, sepsis) and all of the following:

a. Clinical syndrome consistent with myocarditis
b. Elevated biomarker of cardiac myonecrosis
c. ECG evidence of myo-pericarditis
d. Negative angiography or other testing to exclude obstructive coronary disease

Probable Myocarditis: Any of the scenarios below that are not explained by another diagnosis (e.g. acute coronary syndrome, trauma, stress induced cardiomyopathy)

1. CMR with findings diagnostic of myocarditis without any of the following (when screening CMR is being performed routinely as in the context of trial procedure)

a. Clinical syndrome consistent with myocarditis

b. Elevated biomarker of cardiac myonecrosis
c. ECG evidence of myo-pericarditis
2. Non-specific CMR findings suggestive of myocarditis with any 1 or more of the following:
a. Clinical syndrome consistent with myocarditis
b. Elevated biomarker of cardiac myonecrosis
c. ECG evidence of myo-pericarditis
3. New WMA on echocardiogram with a clinical syndrome consistent with myocarditis and either:
a. Elevated biomarker of cardiac myonecrosis
b. ECG evidence of myo-pericarditis
4. A scenario meeting criteria for Possible Myocarditis (see below) with 18F-Fluorodeoxyglucose (FDG) Positron Emission Tomography (PET) imaging showing patchy cardiac FDG uptake without another explanation
Possible Myocarditis: Any of the scenarios below that are not explained by another diagnosis (e.g. acute coronary syndrome, trauma, stress induced cardiomyopathy)
1. Non-specific CMR findings suggestive of myocarditis with none of the following:
a. Clinical syndrome consistent with myocarditis
b. Elevated biomarker of cardiac myonecrosis
c. ECG evidence of myo-pericarditis
2. New WMA on echocardiogram and 1 of the following:
a. Clinical syndrome consistent with myocarditis
b. ECG evidence of myo-pericarditis
3. New elevated biomarker (beyond baseline) and 1 of the following:
a. Clinical syndrome consistent with myocarditis
b. ECG evidence of myo-pericarditis
Subclinical myocardial injury
1. Only cardiac injury biomarkers were elevated (excluding other diseases)
2. Without clinical symptoms, electrocardiogram, echocardiogram, or CMR changes
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Comment 2. Abstract (and also Results): could you please provide the response rate of myocarditis to steroids (like you provide the % of patients with abnormal troponin etc) and the lethality of myocarditis?

Reply: According to your suggestion, we have added the response rate to steroids and the mortality of myocarditis patients in the Abstract and Results section (Manuscript Line 35-36, 178, and 187-188). In our study, 92.9% of patients with ICI-associated myocarditis were treated with steroids timely, of which the response rate to steroids was

76.9% and the mortality rate was 7.1%. Please review.

Comment 3. "Factors associated with myocarditis include a history of smoking „„ and previous use of beta-blockers and ACEI or ARB" -> this raises the suspicion that myocarditis could be associated with preexisting heart disease (as beta-blockers-ACEI-ARB are typical heart failure/hypertension etx drugs). Any such association?

Reply: In our study, the proportion of patients with pre-existing heart disease was also compared between ICI-associated myocarditis group and control group, and there remained no statistically significant difference in the univariate analysis results (7.1% vs. 6.7%, *P*=1.000; Manuscript Table 1). To gain a deeper understanding of the relationship between pre-existing heart disease, previous use of beta-blockers and ACEI or ARB and ICI-associated myocarditis, we have reviewed some literature and cited them in Discussion section of the manuscript (Manuscript Line 216-222). Please review.

Discussion section: Since beta-blockers, ACEI, and ARB are all cardiovascular drugs, it is suggested that cardiovascular disease may be related to ICI-associated myocarditis. In a retrospective study discussing the relationship between cardiovascular factors and ICI-associated myocarditis, history of heart failure and history of acute coronary syndrome help identify patients at higher risk of ICI-induced myocarditis. In a meta-analysis, all cases that experienced cardiovascular irAEs presented cardiovascular risk factors. However, our study did not show a difference in previous heart history between the myocarditis and control groups.

1. Oren O, Yang EH, Molina JR, et al. Cardiovascular Health and Outcomes in Cancer Patients Receiving Immune Checkpoint Inhibitors. Am J Cardiol 2020;125:1920-6.
2. Rubio-Infante N, Ramírez-Flores YA, Castillo EC, et al. Cardiotoxicity associated with immune checkpoint inhibitor therapy: a meta-analysis. Eur J Heart Fail 2021;23:1739-47.

Comment 4. line 55 on page 5 "life-support therapy" -> this means reanimation. Please consider rephrasing according to what is actually meant here.

Reply: Thank you for your careful checking. We have revised this word mistake (Manuscript Line 63), please review.

Comment 5. line 137 on page 9: "could reach 653 itmes the normal value" -> the normal value of Troponin assays varies (high sensitivity, ultra high sensitiivty etc) -> please consider providing the max value in absolute terms.

Reply: Thanks very much for your valuable advice. We have added the normal range of troponin in absolute terms (Manuscript Line 150-151), please review.

Comment 6. lines 147-151: please consider describing also the baseline EKG of these patients (before ICI start). As some of these patients may have preexisting heart disease (see point 3 above), it is important whether there were any changes compared to the baselines associated with the onset of myocarditis (and not the

mere "pathologic EKG", which could be preexisting). Could you please please clarify whether the analysis was indeed comparative with the baseline EKG as reference and how it was exactly performed / how the findings at baseline were?
Reply: We also retrieved and collected the baseline ECG of patients with ICI-associated myocarditis, but unfortunately, no patients had received an ECG examination before the use of ICI, and only 2 patients had received an ECG examination before the occurrence of myocarditis after ICI treatment. One patient had a normal ECG (patient #13) and the other had an ECG abnormality of premature beat because he had a history of premature beat (patient #12), and this data was incorporated into our analysis of pre-existing heart disease, of which there was no statistical difference in pre-existing heart disease between the ICI-myocarditis and control groups. Therefore, we only analyzed the ECG during the onset of myocarditis, aiming to describe the imaging features during the onset of ICI myocarditis. Indeed, as you suggested, we should compare the ECG before ICI treatment with the ECG during the onset of myocarditis to better illustrate the changes of ECG during the onset of ICI-associated myocarditis, which may require further studies with a larger sample size. It enlightens us a lot. Thanks for your constructive suggestion.

Comment 7. What was the median patient survival from the onset of myocarditis and from the treatment starT? How did this survival compare to the survival of the control patients? One could also make the point that myocarditis (due to the high lethality, see point 2 above) as an irAE is an exception to the rule that irAE are generally associated with better prognosis and longer survival (which is true for other irAE, there are many possible references for this, e.g. https://pubmed.ncbi.nlm.nih.gov/34268127/).
Reply: Thanks very much for your very meaningful suggestion. In this study, the median overall survival of patients with myocarditis was 178 (interquartile range: 21.8-527) days. We have compared the survival in myocarditis cases in another study, please review.
1. Xie X, Wang L, Li Y, et al. Multi-organ Immune-Related Adverse Event Is a Risk Factor of Immune Checkpoint Inhibitor-Associated Myocarditis in Cancer Patients: A Multi-center Study. Front Immunol 2022;13:879900.