Peer Review File

Reviewer A:

Comment 1:This is an interesting work...some minor revision are needed to improve the manuscript: n for number...it should be in italic.

a little English revision, some mistake throughout the entire manuscript.

Reply1: Thank you for your feedback. I changed the *n* in italics and modified some of the parts in the manuscript.

Changes in the text: Result and Table 1-3 parts

The presence of anaerobic GN in blood is detected when the cancer is already present...Could it be used as prognostic value? or it is used to perform further examinations as gastroscopy or colonoscopy??

Reply2: Thank you for your feedback. Anaerobic Gram-negative bacteria may be a marker of gastrointestinal cancer. We may recommend gastroscopy and colonoscopy to patients who develop Gram-negative rod anaerobic bacteremia.

Reviewer B:

Abe et al aimed to investigate risk factors for gastrointestinal malignancies in Japanese patients with GNR bacteremia.

While a very interesting topic of highly importance there are several issues that require attention.

Major comment: It is a limitation that cancers before/after are combined into one variable, even without providing information on numbers before/after. In principle the data could be 90 patients with prevalent cancer and 4 with cancer diagnosed after bacteremia. Previous studies referred to

(7 and 11) excluded all prior cancers and only focused on subsequent diagnoses which would make more sense to do in a similar way.

Reply3: Thank you for your feedback. As you pointed out, the use of anaerobic GNR bacteremia for the search of malignancies is intended for the diagnosis of undiagnosed gastrointestinal cancers. This retrospective study aimed to determine the usefulness of anaerobic GNR as a marker for gastrointestinal cancer, necessitating the accumulation of cases. Whether the cancer or the bacteremia occurred first, the critical factor is that the presence of cancer and a compromised gastrointestinal mucosal barrier may provide a portal for anaerobic GNR present in the gastrointestinal tract to enter. In light of these results, we hope to conduct prospective studies in patients with anaerobic GNR bacteremia who have not been diagnosed with cancer, performing endoscopic examinations and other tests to verify the accuracy of this method.

As a consequence of this patient mix the analysis of BMI also makes little sense and does not contribute much. Also the "significant" result should be evaluated as to clinical relevance (20.5 vs. 21.8). Only because you achieve a significant result as deemed by p testing does not mean that you can conclude on causality.

If an analysis of BMI should be included (after prevalent cancers are excluded), I would suggest making categories: low BMI/underweight, normal BMI (reference group), high BMI/obese.

Reply4: Thank you for your feedback. As you pointed out, even though the difference in BMI was statistically significant (p-value), it was clinically insignificant, so the discussion of BMI has been removed.

Changes in text 1: I removed the "Additionally, our results indicated that the patients with gastrointestinal cancer show a lower BMI than the non-cancer group (20.5 kg/m^2 vs. 21.8 kg/m^2). However, it has been reported that gastrointestinal cancers are more common in obese patients 13-15. Therefore, the relationship between BMI and cancer reported in these studies contradicts our results. Several studies with a focus on the relationship between BMI and the incidence of sepsis have also been reported 16. However, the results are still debatable. Additionally, given that BMI is affected by several confounding factors such as age and comorbidities, we could not conclude that a lower BMI alone would reduce the risk of cancer." in the discussion part.

Changes in the text 2: 114-116, Table 4a, Table 4b

The sentence 165-170 in the conclusion does not make sense. When the authors include prevalent diagnoses they cannot conclude on occult cancer.

Reply5: Thank you for your feedback. I modified the sentence including the reply 3.

Change In this study, we included data on cases of gastrointestinal cancer that occurred within 1 year of onset of bacteremia. Biomarkers are generally used to detect previously undiagnosed cancers. This retrospective study aimed to assess the efficacy of anaerobic GNR as a marker for gastrointestinal cancer, which required the collection of a substantial number of samples. Based on these results, we plan to conduct prospective studies in patients with anaerobic GNR bacteremia who have not been diagnosed with cancer, performing endoscopic examinations and other tests to confirm the validity of this method.

Changes in the text: in Line 153-158

A priori the majority of the GNR cohort are patients with E.coli urosepsis. As they authors are attempting to examine risk of GI cancer, I would suggest to exclude patients with urosepsis/urinary tract focus, as this would dilute any association. Or at least do a sensitivity analysis excluding urosepis patients.

Reply6: Thank you for your feedback. As you pointed out, the patient with Enterobacteriaceae in GNR bacteremia may indeed include a majority of cases of urinary tract infections. If gastrointestinal cancer is associated with a breakdown of barriers in the membrane, then Enterobacteriaceae other than anaerobes might also be useful as markers. However, in this study, Enterobacteriaceae did not show a significant risk as a marker for gastrointestinal cancer but acted as a protective factor in multivariate analysis (refer to table 4). It is possible that urinary tract infections were included, but the current data did not allow us to extract those cases from our chart system. On the other hand, anaerobic GNRs are rare in urinary tract infections, so it's unlikely they were included in this study. Moving forward, we intend to perform prospective studies to exclude GNR bacteremia caused by urinary tract infections and to investigate whether intestinal bacteria show a significant difference.

Changes in the text: in Line 160-162

I am not sure it makes sense to include in the logistic regression both "enterobacteriacea" and "anaerobic" - this is the same variable just opposite 0/1 1/0. Maybe consider making a stratified analysis?

Reply7: Thank you for your feedback. As you pointed out, it was incorrect to analyze anaerobic GNR and Enterobacterales as confounders in the multivariate analysis. In a sub-analysis, when we conducted multivariate analysis with age and gender as covariates, anaerobic GNR were significantly associated with an increased risk of gastrointestinal cancer (odds ratio, 3.440; 95% CI, 2.085–5.675, p <0.001).

Changes in the text: Line 36, 116-119, Table 4

The study 11 is not a cross sectional study, it is a cohort study which includes cancer diagnoses after bacteremia with up to 10 years of follow-up.

Reply8:>Thank you for your feedback. I changed the study design to a cohort study

Changes in the text: In a cohort study, in line 131.

Minor comment. Please use updated taxonomy for enterobacterales.

Reply9:>Thank you for your feedback. I changed the "Enterobacterales" in the manuscript.

Minor comment. In the version for download there is use of different fonts within a table (but maybe it is only a formatting problem, and it is correct in the uploaded file?)

Reply10:>Thank you for your feedback. I modified the font in the table you pointed out.