**Author’s response to reviews**

**Title:** Evaluation of Risk Factors for Perforated Peptic Ulcer

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**Author’s response to reviews:**

Dear Editor

Subject: Submission of revised paper “Evaluation of Risk Factors for Perforated Peptic Ulcer”, ID: BMGE-D-17-00333

We thank referees for careful reading our manuscript and for giving useful comments. We feel the comments will significantly improve our paper. In response to the Referees' comments, we have revised the manuscript. Our responses are given in a point by point manner below. Changes to the manuscript are shown in underline and yellow marker.

We hope the revised version is now suitable for publication.

Sincerely,

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Our responses to the referees' reports are as follows:

RESPONSE TO REVIEWER 1:

We thank you for your comments, which have helped us to substantially improve the manuscript.

Comment 1: article is not well written and doesn't deep analyzed all obtained results, focusing only on some of them.

Response: We corrected the manuscript, in accordance with Reviewer’s specific comments in below. If we need further correction, would you please let us know.

Comment 2: there may be same limitation in sample examined (e.g. single institution, same ethnic group..) that have to be highlighted.

Response: We thank the Reviewer for this pertinent comment.

In accordance with the Reviewer’s comment, the following sentence has been inserted and changed the following text from (P 10, line 175).

“There are some limitations in our study. For steroids and PPI, these are known risk and protective factors for PUD, respectively; therefore, we expected to observe the same result, but the result was outside of our expectations. However, the study is still significant because the AUCs of all models are very high. In addition, our study used four different multivariate analyses. These can reinforce the reliability of our data.”

to

“There are some limitations in our study. Our study is a research at a single institution and targets only one ethic group. These could be a selection bias. Moreover, there are some missing data because initial encounter with patients and diagnostic process are conducted by different doctors each time. For example, it could be an interesting research by comparing X-ray and CT scan to patients with abdominal pain. Since X-ray is not gold standard for diagnosing acute abdomen, thus unperformed each case, we could not gather enough data for X-ray. Furthermore, there may be a group of false negative. Some outpatients with abdominal disturbance may not be taken CT scan, and may not be diagnosed as PPU. However in our hospital, CT scan is available
24 hours every day, every year, so that barrier for taken CT scan is very low. In addition to the low barrier, board certificated radiologists are within reach at all times, false negative is limited.

Comment 3: There are some missing data and repetitions in the Methods section (e.g. number of patients).

Response: Specific part such as number of patients is written in Result section. We only wrote methodological part in Method section for this study. About repetition, if there are any redundancies, would you please let us know.

Comment 4: Did you performed a chest/abdominal RX to patients? In this case it could be of interest compare RX vs CT (although RX isn't gold standard, CT is time-consuming and not always available).

Response: We appreciate the Reviewer’s comment.

We understand that it could be an interesting research by comparing X-ray and CT scan, however, each initial diagnostic process is conducted by different doctors. Thus, we could not gather enough data for X-ray. We think this is a limitation of our study and added as our limitation part.

Comment 5: Finally it's necessary to amplify Introduction and Discussion sections.

Response: In terms of Introduction and Discussion section, other Reviewers also comment to amplify these sections, so that in accordance with comments, we extend these sections. Please refer to the revised text.

RESPONSE TO REVIEWER 2:

We wish to express our strong appreciation to the Reviewer for insightful comments on our paper. We feel the comments have helped us significantly improve the paper.
Comment 1: Abstract: All data such as past medical histories, physical findings, and laboratory data were collected [though?] chart reviews. I conjecture that though may be through?

Response: In accordance with the Reviewer's comment, we have changed the following text in the abstract from (p.2, line 36):

“All data such as past medical histories, physical findings, and laboratory data were collected though chart reviews.”

to

“All data such as past medical histories, physical findings, and laboratory data were collected through chart reviews.”

Comment 2: Introduction: The introduction is somewhat complicated. Please make it more concise, for example, you do not need to narrate all details accompanied with PUD. You need to focus on PPU.

Response: In accordance with the Reviewer's comment, I have changed introduction as the following text and deleted some about PUD. (p.3, line 65):

As a result, several clinical features, such as diffuse abdominal pain, muscular defense and a change in symptoms are unique for PPU, which is not shown in patients with PUD 2. Therefore, we should distinguish PPU from PUD upon diagnosis of PPU.

Early detection and rapid intervention are crucial for treating PPU and affecting prognosis of patients. PPU is a rare disease condition that only occurs in 2% to 10% of patients with PUD 3, therefore PPU is not a top of differential diagnosis of acute abdomen, leading to delay of intervention. In fact, time of intervention is one of the known prognostic values, known as the Boey score. Boey et al. stated that a delay of more than 24 hours in diagnosis and management greatly worsened predicted outcomes and increased post-operative complications 6,7. PPU is important to diagnose because the mortality rate increases drastically with delayed diagnosis and intervention. And, prognosis itself is poor; a study prior to 1997 reported the mortality rate of PPU as 12% 4. Later, the mortality rate gradually decreased as time progressed, but it is still high, and even a recent review article reported determined that the high mortality rate for PPU, ranged from 1.3% to 20% 1. Possible reasons for high mortality are pan peritonitis and consequences of bacterial infection following PPU. Perforation allows gastric contents into the
peritoneal cavity, initiating chemical peritonitis, and if left untreated, bacterial contamination, intra-abdominal abscess formation and sepsis are results of continuous leakage. Another reason for high mortality is delayed treatment. Therefore, misdiagnosis and delay of treatment must be prevented.

Comment 3: Method: Please describe method section as delicately as possible. It is very important

Response: We appreciate the Reviewer’s comment.

- We inserted the title; “Study design and setting” in (P6 line 98)

- Following text are inserted in (p.6, line 100); “St. Luke’s international Hospital is a general and teaching hospital in Tokyo, Japan. Approximately 2,550 outpatients are coming daily and the hospital provides 539 beds in total. “ for describing about our hospital.

- The word “randomly” was inserted (p.6, line 116) and changed text as “Two control subjects were randomly recruited for each case subject.”

- Following text (p.7, line 117) are changed as “We obtained following patient data from electronic charts. Age, gender, and social history were obtained as demographic data. History of the present illness, past conditions and comorbidities such as PUD, gastroesophageal reflux disease, Crohn’s disease, ulcerative colitis, H. pylori infection, hypertension, diabetes mellitus, dyslipidemia and among other things were collected. Physical examinations, Medications, including findings of vital signs and laboratory measures such as location of pain, loss of appetite, vomiting, nausea, hematemesis, diarrhea, muscular defense, use of antiplatelet, anticoagulant, NSAIDs proton pump inhibitor, spironolactone, steroid, white blood cell count, hemoglobin, platelet count, total bilirubin, AST, C-reactive protein, etc. were also evaluated.”

- Following text (p.7, line 127) are changed as “First, we compared baseline characteristics between cases and controls using a chi-squared test and t-test. Then, multivariate analyses were conducted. To confirm our results, we performed the following four 4 multivariate analyses: logistic regression with variables that had a p value of less than 0.2 through univariate analyses
or clinically important variables; logistic regression with stepwise methods (significance level for entry: 0.05, for removal: 0.10); and conditional logistic regressions with the same two methods. Four different models are used because this is an exploratory study. Use of only one model may arise an incidental significance. In order to adjust covariance and confirm consistent results, sensitive analysis are conducted.”

Comment 4: Authors described "In contrast, we considered those patients who were subjected to an abdominal CT scan at dates close to the scans of case subjects but were diagnosed with diseases other than PPU and had the same demographics, including exact age and gender, as potential control subjects.” Have authors ever thought of 1:3 case-control comparison? You used 1:2 (136:272) situation. It is Ok, but 1:3 might increase the statistical power. Did you select 1:2 due to not enough PPU patients? Please let me know.

Response: In Cancer Epidemiology: Principles and Methods, chapter 9, it said “the optimal control to case ratio is 1:1. However, when the number of cases available for the study is small, the control to case ratio can be altered.” PPU is a rare disease, seen only 10 patients per year. Only small number of cases is collected in our study, thus I altered control to case ratio. Furthermore, even though we used 1:2 case-control ratios, enough significant variables were obtained via multivariate analysis.

Comment 5: Why did you use four models?

Response: We appreciate the Reviewer’s comment.

The reason for using four models is because this study is exploratory study. If we only use one model, there may be an incidental significance due to the variables which affect one another. Thus, we use sensitive analysis instead, in order to adjust covariance and confirm consistent results.

In accordance with Reviewer’s comment, we revised the text and explained the reason for using four models in Method section.

Comment 6: I would like to listen to authors' opinion about ROC (Fig 1). Would you please describe the accurate title of ROC? I cannot understand what this curve means when I watch this tile. As you know ROC curve needs two responsive variables and one explanatory variable. Is
ROC curve about muscular defense or PUD history? The suggested significant factors were muscular defense and PUD history. Do you have any scale about this variables in common clinical practice in authors' hospital? I understand that this is a retrospective study "A retrospective case control study was conducted between August 2004 and March 2016 at St. Luke's International Hospital"

Response: We inserted following title and figure legends in Figure 1.

“The comparison of ROCs models between four different multivariate analyses.

Figure legends: ROCs of four models are shown above and area under the curves (AUCs) and 95% confidence interval (CI) are also calculated. Both AUC and 95% CI from all models are high enough to confirm the results. Especially, AUC of conditional step-wise method shows the highest value, indicating its high reliability.”

Comment 7: Result: Please describe result as delicately as possible.

Response: We corrected the manuscript, in accordance with Reviewer’s specific comments in below.

Comment 8: In table 1 and table 2, I found out that some variables are significant in Multivariate analysis but not significant in Univariate analysis. This phenomenon might be caused by following four reasons; (1) the effect of unbalanced sample size; (2) the influence of missing data; (3) an extremely large within group variation, relative to between group variation; and (4) the presence of interaction. Please describe authors' view on this statistical phenomenon in authors' paper.

Response: In accordance with the Reviewer’s comment, the following sentence has been inserted and changed the following text from (P 11, line 201).

“Moreover, the phenomenon which some variables are significant in multivariate analyses but not significant in univariate analysis is mainly coming from confounding factors.”

Comment 9: In table 2, many statistical results are missing. I would like to ask authors why?
Response: Table 2 shows multivariate analyses using different models. Blank data are simply not included and calculated in the model. Categories with data are risk factors included in the model. In order not to mislead as missing results, we inserted oblique line in blank categories.

Comment 10: Authors described "There were some differences in the prediction factors between PUD and PPU. For one, anticoagulants were thought of as a prediction factor for PUD, increasing the OR to 1.98 15, but anticoagulants did not increase the risk of PPU in our study. We hypothesized that the insignificance of anticoagulant drugs was due to their mechanism. Unlike Cox-1 inhibitors, anticoagulants are medications which inhibit the productions of fibrin at the end of the blood coagulation reaction 16 ; they may promote the bleeding risk, but may not cause damage to the gastric mucous membrane."

In my opinion, authors' explanation seems not so persuasive. I agree that anticoagulant does not cause mucous membrane damage directly, however it can prohibit rapid healing of damaged mucosa and consequently can contribute perforation process because coagulation is also the defense mechanism prohibiting progression of PUD. As you know, our body is homeostatic status; damage & repair, damage & repair, & damage & repair… I do believe the breakdown of homeostasis may substantially contribute from ulcer to perforation. I would like to listen to authors' opinion.

Response: We wish to thank the Reviewer for this comment. In accordance with a Reviewer’s comment, we inserted following comment in (p.9, line 174)

“Certainly, in molecular biological point of view, coagulation is a defense mechanism that can protect gastric mucous membrane, and by the use of anticoagulants, homeostasis of reproductive process can be disrupted. However, in clinical setting, by the perspective of our findings we consider that anticoagulants alone do not cause significant influence on biological setting.”

Comment 11: Authors described "increase in platelet count was statistically significant in PPU, and could be used as a prediction factor. Platelets are component of blood, necessary to arrest bleeding, thus thrombocytopenia is known cause of bleeding. In our PPU study, however, platelet counts were significantly increased. We presumed that peritonitis caused inflammation and perforation stimulated coagulation; therefore, platelet counts were increased especially in the PPU patients."

Do you think this is the logical explanation? You said that thrombocytopenia is known cause of bleeding, AND said stimulated coagulation is the cause of thrombocytopenia. Would you please show logically missed explanation to make me understand?
Response: We meant for platelet aggregation. Thus, we have changed the following text from (p.2, line 36):

“We presumed that peritonitis caused inflammation and perforation stimulated coagulation; therefore, platelet counts were increased especially in the PPU patients.”

to

“We presumed that peritonitis caused inflammation and perforation stimulated platelet aggregation; therefore, platelet counts were increased especially in the PPU patients.”

Comment 12: Conclusion: I do believe that the conclusion should be concise with gist information, but authors’ conclusion is very obscure. I believe that most physicians know that they need APCT when PPU is suspicious. I think the reasonable concise conclusion from authors’ paper would be "When a physician find out muscular defense and PUD history APCT would be necessary for diagnosing PPU" if I accept all method and results of this paper. Please keep in mind that the conclusion should not be obscure or too long.

Response: In accordance with the Reviewer’s comment, the following sentence has been inserted and changed the following text from (P 11, line 175).

“In the clinical setting, even though perforation is a rare disease, when the patients present with muscular defense and PUD history, a physician should take abdominal CT scan without any hesitation for diagnosing PPU.”

RESPONSE TO REVIEWER 3:

Comment 1: The article is well organized and developed. I really appreciated the topic and the aim of this study. The tables are clear and explicit. I accept the article without revisions.

Response: We thank the Reviewer for this insightful comment.

RESPONSE TO REVIEWER 4:
Comment 1: While the topic is interesting, the paper needs an extensive revision and formal language modification prior to even initial consideration

Response: The paper has been edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English speaking editors at American Journal Experts Certificate Verification

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