April 15, 2015

Dear Editor,

Thank you for the opportunity to revise our manuscript. We have addressed all concerns brought up by the reviewers. We have also altered the formatting of the manuscript in accordance with your recommended guidelines. Please find enclosed the edited manuscript in Word format (file name: ROC Paper Revision Draft 2.doc).

Title: Pre-lung transplant measures of reflux on impedance are superior to pH testing alone in predicting early allograft injury

Author: Wai-Kit Lo, Robert Burakoff, Hilary J. Goldberg, Natan Feldman, Walter W. Chan

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The manuscript has been improved according to the suggestions of reviewers:

1. Formatting has been updated per reviewers' comments and editors' guidelines.

2. Revision has been made according to the suggestions of the reviewers. A point-to-point summary of the changes is included below.

Reviewer 1:

Right now, ph-testing is the gold standard for the study of the function of the esophagus and the measure of acid reflux. As authors mentioned in the manuscript, the gastroesophageal reflux has been associated with poor lung transplant outcomes, including allograft injury and rejection. If these results are confirmed in posterior randomized and prospective studies, it is possible that multichannel intraluminal impedance (MII) could substitute ph-testing for preoperative study of acid reflux in patients in which a lung transplantation is going to be performed.

The quality of the manuscript's presentation and readability is appropiate.

The study has been approved by the Partners Healthcare Institutional Review Board prior to the inception.

Title: The main and short titles reflect the major topic and content of the study.

Abstract: The abstract is clear and concise. However, I believe is better to write "from January 2007 to November 2012" instead of "1/2007-11/2012".

Thank you for this comment and the suggested change has been made. The first sentence of the method section of the abstract now reads:

"This was a retrospective cohort study of lung transplant recipients who underwent pre-transplant combined MII-pH-testing at a tertiary care center from January 2007 to November 2012."

Additionally, the abstract has been reformatted to comply with submission guidelines.

Materials and Methods:
Authors do not mention if patients with reflux were in treatment with proton pump inhibitors (PPI).

As noted in Table 1, 24 out of 32 patients were treated with PPI following transplantation, and this was evenly distributed between the normal and increased distal reflux cohorts. Cox univariate analysis demonstrated no association with early allograft injury (data not shown, HR 0.85, 95% CI 0.27-2.67, p=0.78). This was clarified in the Results section by addition of the following statement:

Page 9, paragraph 1
"Post-transplant PPI use also was not associated with early allograft injury in this cohort."

The present study lacks systematic prospective data acquisition, and therefore selection of patients with both ph-testing and MII could be a selection bias.

Thank you for this comment. We cannot discount the presence of selection bias, although we have thoroughly investigated possible etiologies including pre-test GERD diagnosis, referring pulmonologist, and year of MII-pH study. We feel that the homogeneous distribution of patient factors in Table 1 by reflux severity favors minimization of bias within the cohort. In addition, all patients undergoing pre-lung transplant evaluation at our center are required to complete a form of reflux testing. The type of testing undertaken depends on whether the patient prefers to complete testing with his/her local providers and the availability of the tests. All patients who complete their pre-transplant reflux testing at our institution would have undergone MII-pH study. Nevertheless, we have added a comment on the possibility of selection bias in the Discussion section when addressing study limitations.

Page 12, paragraph 3
"The inclusion of transplant candidates receiving both MII and pH testing, as opposed to pH testing alone, may introduce external selection bias impacting generalizability, although demographics and clinical history appear homogeneous within the cohort as reported in Table 1."

Statistical analysis seems to be correct.

Maybe sample size is small.

Thank you for the comment. We agree that the sample size is small, given the limited number of lung transplantation performed and our stringent inclusion criteria to only enroll patients who completed MII-pH off PPI prior to transplant. We have mentioned sample size as a possible limitation in the Discussion section. Despite the size of the study, our cohort is comparable in size to prior publications on the same topic.

Results:
Causes of death (8 patients) and postoperative complications (7 patients) were not explained.

To clarify, there were no postoperative complications. There were 8 deaths, and 7 of them were attributed to pulmonary complications. The only death not related to pulmonary complication was due to leukemia. The causes of death have been clarified in the paper.

Page 9, paragraph 1
"There were 8 deaths detected overall, with 7 attributed to pulmonary complications including pneumonia (3 subjects), pulmonary malignancy (2 subjects), pulmonary hemorrhage (1 subject), and acute rejection (1 subject)."

If subjects who did not survive beyond the first 30 days after transplant were excluded of the study, I have no clear idea about how many patients have been selected in the study for the statistical analysis (32 patients or 24 patients). Could you explain to me?

Thank you for this comment. To clarify, all 32 patients were included in the statistical analysis, which was a Cox proportional hazards time-to-event analysis. Subjects not reaching the early allograft injury endpoint were censored at death, post-transplant anti-reflux surgery, or date of last clinic evaluation, whichever was earliest, to account for the impact of time. By study design, all included subjects (32) survived at least 30 days after transplant and contributed data toward the analysis.

Discussion:
The discussion is well organized and the conclusions are appropriated. It is very interesting the section related to limitations of the study, which adds more value to the investigation.

Thank you for this comment.

References:
References have to be revised according to manuscript guidelines for authors.
Some references are cited with 3 authors and et al, and others with six authors.
Maybe 11 references are not enough for an original article.

Thank you for the suggestion. We have revised our references for formatting in accordance with the submission guidelines. We have also increased the number of references included to 30.

Tables and Figures:

Table 1: The abbreviations of the text are not explained in the bottom of the table.

Thank you for the suggestion. The explanations for the abbreviations have been appended to the Table 1 description:

"Table 1: Baseline characteristics demonstrating homogeneity of the pulmonary transplant study population. BMI=body mass index; IPF=idiopathic pulmonary fibrosis; COPD=chronic obstructive pulmonary disease; CF=cystic fibrosis; COP=cryptogenic organizing pneumonia; AAT=alpha-1-antitrypsin deficiency; LVH=left ventricular hypertrophy; LVEF=left ventricular ejection fraction; PCWP=pulmonary capillary wedge pressure; PVR=pulmonary vascular resistance; FVC=forced vital capacity; %-pred=percent of predicted value; FEV1=forced expiratory volume in 1 second; PPI=proton pump inhibitor; BOS=bronchiolitis obliterans syndrome"

Finally, I have some questions for authors:

As you cited in the manuscript, gastroesophageal reflux has been associated with poor lung transplant outcomes, including allograft injury and rejection. Although proton pump inhibitors is less likely to have an effect on non-acid reflux, Is there any protocol in lung transplantation that administrates PPI to the patients with clinical reflux or in patients with reflux measure on multichannel intraluminal impedance and ph-testing in order to improve the outcomes of this kind of transplantation?
In the same way, could be indicated in these patients an antireflux technique during lung transplantation or a postoperative treatment with PPI?

Thank you for the comments and important questions, which highlight the need for additional research. At present, there is no standard protocol for medical or surgical anti-reflux treatment in lung transplant recipients. As indicated in our manuscript, there is not even standard recommendation regarding reflux testing in these patients. Some prior studies have suggested that anti-reflux treatment in these patients may lead to better allograft outcome, but the optimal candidate selection, modality of treatment, mode of testing, and timing of intervention remain unclear. We do believe that early testing for reflux among lung transplant candidates is predictive of outcome, as suggested by our results. In patients identified with abnormal reflux, early intervention would likely improve outcome. However, the best timing and mode of treatment remain to be determined. Our group is currently exploring these areas in our follow-up studies, and will hopefully be addressing both issues in forthcoming manuscripts.

Reviewer 2:

Important study. Some questions:
1. How many of the patients had gastroparesis and how did this influence the impedance findings.

Thank you for your comment. Gastric emptying study and barium swallow with small bowel follow through are not in the routine pre-transplant assessment. We re-reviewed the records of all subjects and no included subjects carried a diagnosis of gastroparesis. A comment regarding gastroparesis has been included in the Discussion section.

Page 11, paragraph 2
"Review of medical records did not reveal a diagnosis of esophageal dysmotility or gastroparesis in any subjects in our cohort, though neither esophageal manometry nor gastric emptying study were routinely performed prior to transplantation."

2. The advantage of impedance over pH measurement is clear;

While the theoretical advantage of impedance has been widely suggested and discussed given the additional information collected, its advantage over pH-only measurements with regards to predicting clinical and treatment outcomes has not been as clear. Earlier studies such as the one from Bredenoord et al (Am J Gastroenterol 2006) showed that addition of impedance to pH monitoring increased the yield of the test. However, more recent studies such as those from Patel et al (Am J Gastroenterol 2014 and Clin Gastroenterol Hepatol 2015) suggested that acid-based parameters on pH study performed better than impedance-based parameters in predicting treatment outcomes for gastroesophageal reflux disease. Therefore, there is continued debate regarding the utility and advantage of impedance with regards to management of typical symptoms of reflux. In this context, the amount of data available for extraesophageal manifestations of reflux with regards to impedance vs pH measurement is even more scarce. We believe that our study is unique in comparing impedance and pH measurements directly with regards to lung transplantation outcomes. We have added a statement in the Discussion section regarding the current debate regarding impedance vs pH measurement in management of typical reflux, and included the above-mentioned studies as references.

Page 11, paragraph 3
"Moreover, the value of impedance testing continues to be debated even in the management of typical GERD symptoms in non-transplant patients[24,25,26]."

what the clinician will want to know are:
(a) how predictive, in concrete terms was MII?

The closer the c-statistic to 1 the better the prediction value. Values greater than 0.7 are generally thought to be reasonable, though there is no concrete cutoff. Three of the MII parameters had c-statistics greater than 0.7, while pH testing variables had c-statistics exceeding 0.7. We have also calculated the hazard ratio for development of early allograft injury for each pH and MII parameter. Significant results were shown for recumbent bolus reflux exposure [HR 1.25 (1.04-1.50), p=0.01], total bolus reflux exposure [HR 1.18 (1.01-1.36), p=0.03], and abnormal bolus clearance [HR 1.09 (1.01-1.17), p=0.02], indicating that these parameters on MII are associated with early allograft injury post-transplant. These results are listed in table 2.

(b) which parameter or group of parameters was/were most predictive?

Taking the univariate analysis together with the c-statistic, we believe that the impedance measures of greatest impact are recumbent bolus reflux exposure, and bolus clearance, given their significant hazard ratios and high c-statistics on the ROC curve. We have included this conclusion in the discussion section.

Page 10, paragraph 1
"Taken together, the MII parameters of bolus reflux exposure in the recumbent position and bolus clearance time are the best predictors of early allograft injury, based on the significant univariate results and the higher c-statistic values."

(c) should pH alone be abandoned?

Based on our investigation, we would argue that MII-pH is preferable where available, in the setting of pre-transplant evaluation. This conclusion has been reinforced in the Discussion section.

Page 13, paragraph 2
"MII-pH appears to be more valuable than pH-monitoring alone in pre-lung transplant reflux assessment, outcome risk stratification, and planning of peri-transplant care, and these findings would support the use of MII-pH rather than pH testing alone in this setting."

Thank you again for your consideration to publish our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Walter W. Chan, MD, MPH
Brigham and Women's Hospital
Division of Gastroenterology, Hepatology, and Endoscopy
75 Francis Street
Boston, MA 02115
USA
Telephone: +1-617-732-6389
Fax: +1-617-525-0338
E-mail: wwchan@partners.org