Author’s response to reviews

Title: Association between HER2 status in gastric cancer and clinicopathological features: a retrospective study using whole-tissue sections

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Author’s response to reviews:

Cover letter

Response to Reviewers’ Comments

Date: 20th September 2015

To: Matteo Fassan, M.D., Ph.D.
Editor, BMC Gastroenterology

Dear Editor Matteo Fassan,

We would like to thank you and the reviewers for their time and comments on our paper “Association between HER2 status in gastric cancer and clinicopathological features: a retrospective study using whole-tissue sections” (BMGE-D-15-00063).

We appreciate the opportunity to submit our revised manuscript containing changes according to all reviewers' suggestions. We have uploaded two documents: a “Revised Manuscript” incorporating the revised changes, which are highlighted in the text, and a “Cover Letter” with a point-by-point description of changes, based on each reviewer’s comment, detailed as follow:
Reviewer #1:

- Line 82: We included a reference for the sentence in line 82:

- Line 133: The sentence was rephrased to make it more clear to readers: “Three pathologists (RSL, HG and CBN) all at once analyzed immunostained slides using a multi-head microscope and obtained a consensus score for HER2 expression according to Hofmann's and Rüschoff's recommendations for GC surgical specimens”. Therefore, intra and interobserver agreement coefficients (kappa-value) could not be assessed at this point. Although the authors agree with the reviewer’s comment that this would be important to corroborate the reproducibility of the methods, it was not the purpose of this study to evaluate agreement coefficients between different pathologists.

- Line 220: The word "cytoplasmatic" was replaced by "cytoplasmic" across the whole text. We also added a sentence for clarification: “Non specific staining was seen in both metaplastic and dysplastic epithelium, despite the nature of the metaplasia or the degree of dysplasia.”

- Line 287: Changes were made and sentences were added to the text concerning HER2 heterogeneity, but we prefer to have done it on line 279, by the time this subject was first addressed in the text: “HER2 genetic heterogeneity by ISH has been previously demonstrated on breast cancer, but it seems to be a more complex issue on GC specimens [39]. Protein expression heterogeneity by IHC, usually referred as variability in immunohistochemical intensity and extension of HER2-positive areas, is also a problem which could greatly impact on sample selection for HER2 testing in GC. In fact, HER2 expression heterogeneity has been demonstrated not only by our study but also by other authors [39,40], including a study comprising 2,727 gastrectomies [32]. As a result, whole-tissue sections obtained from resected primary gastric tumors may offer a larger representation of different sub clonal cancer cell population which could help avoid false-negative results due to HER2 heterogeneity. Since patients with locally advanced or metastatic GC might not undergo primary gastrectomy, whole-tissue sections are not always available and other GC samples ought to be used instead, raising the question as to which are the best suited for HER2 testing: endoscopic biopsies versus metastatic lesions. For endoscopic biopsies, the recommendation is a viable number of representative tumor fragments (ideally 6-8) and, if possible, the pathologist should also perform HER2 testing on another specimen when a negative result is found on a tumor endoscopic biopsy [40].”

- Figure legends: We added the grade of the dysplasia (Figure 2) and the field magnification (Figures 1 and 2) as suggested by the reviewer.
Reviewer #2:

- Table 2: We added a table summarizing data obtained from some of the most relevant studies concerning HER2 positivity rates in gastric cancer in populations from different parts of the world in order to compare the results to our findings. Whenever possible, we have included the correlation between HER2 status and clinicopathological features that were found among different studies. Please check the attached files for a better view of Table 2. Line 362: “Table 2 summarizes results from various studies concerning HER2 positivity rates and the association between HER2 expression with clinicopathological features in patients with GC from different parts of the world, in comparison to our findings.”

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Patients’ place of origin</th>
<th>Number of patients</th>
<th>HER2 positive rate (%)</th>
<th>Association with clinicopathological features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bang YJ [6]</td>
<td>2010</td>
<td>ToGA trial (Multicenter)</td>
<td>3,665</td>
<td>16.6</td>
<td>Topography (GEJ) Intestinal-type Low-grade</td>
</tr>
<tr>
<td>Begnami MD [19]</td>
<td>2011</td>
<td>Brazil (Single center)</td>
<td>221</td>
<td>8.0</td>
<td>Intestinal-type Low-grade</td>
</tr>
<tr>
<td>Cruz-Reyes C [47]</td>
<td>2013</td>
<td>Mexico (Single center)</td>
<td>269</td>
<td>3.7</td>
<td>Intestinal-type Low-grade</td>
</tr>
<tr>
<td>Cho J [32]</td>
<td>2013</td>
<td>South Korea (Single center)</td>
<td>2,798</td>
<td>7.3</td>
<td>Older age</td>
</tr>
<tr>
<td>Matsusaka S [35]</td>
<td>2015</td>
<td>Japan (Multicenter)</td>
<td>1,461</td>
<td>15.6</td>
<td>Intestinal-type Hepatic metastasis Absence of peritoneal metastasis</td>
</tr>
<tr>
<td>Cappellesso R [34]</td>
<td>2015</td>
<td>Europe (Multicenter)</td>
<td>1,040</td>
<td>11.0</td>
<td>Intestinal-type Low-grade</td>
</tr>
<tr>
<td>Laboissiere RS</td>
<td>2015</td>
<td>Brazil (Single Center)</td>
<td>124</td>
<td>10.5</td>
<td>Intestinal-type Low-grade</td>
</tr>
</tbody>
</table>

GEJ gastroesophageal junction.


- Although we agree with the reviewer's comment that the paper could be improved by the addition of images of all techniques adopted for this study (SISH), we thought that, since all cases were negative for gene amplification by SISH, adding the picture could represent
unnecessary costs to the publication with less valuable information added to the paper. Nevertheless, we might as well add the figure below (please check the attached files for a better view of the figure) to the manuscript, illustrating a SISH negative result, if the reviewer still thinks we should do it.

Reviewer #3:

- Line 261: Even though we agree with the reviewer's opinion that there is evidence in the literature which sustain the lack of difference between HER2 expression between proximal and distal gastric cancers, we think the literature can be quite controversial about the topic. We decided to add a paragraph in order to emphasize the different results published to date, including references cited by the reviewer. Since there is also evidence supporting that proximal gastric tumors and those arising at the gastroesophageal junction are associated with higher HER2 expression, we believe it may partially explain the lower HER2 positivity rate found in our series: “Similar to our results, in a recent large European series [34], as well as in a Japanese cohort of 1,461 patients [35], there was no difference in HER2 positivity between proximal and distal gastric tumors. However, two large Chinese series [17,33] recently showed significantly higher HER2 expression in proximal GC, in agreement with ToGA trial findings [30] and with results from a series of 2,798 South Korean patients [32]. The authors have implied that intestinal-type cancers are usually more frequent in the GEJ and, as different etiologies may play a role in carcinogenesis of tumors from these two locations, this could partially justify distinct HER2 expression rates according to tumor topography [30].” [33] Fan XS, Chen JY, Li CF, Zhang YF, Meng FQ, Wu HY, et al. Differences in HER2 over-expression between proximal and distal gastric cancers in the Chinese population. World J Gastroenterol. 2013;19:3316-23. [34] Cappellesso R, Fassan M, Hanspeter E, Bornschein J, d'Amore ES, Cuorvo LV, et al. HER2 status in gastroesophageal cancer: a tissue microarray study of 1040 cases. Hum Pathol. 2015;46:665-72. [35] Matsusaka S, Nashimoto A, Nishikawa K, Miki A, Miwa H, Yamaguchi K, et al. Clinicopathological factors associated with HER2 status in gastric cancer: results from a prospective multicenter observational cohort study in a Japanese population (JFMC44-1101). Gastric Cancer. 2015 Aug 12. [Epub ahead of print]

- Line 269: A short comment about gastric carcinogenesis, influenced by differences in ethnicity and also by dietary habits was added to the manuscript, as suggested by the reviewer: “In addition to the genetic ancestry according to ethnicity, multiple gastric carcinogenesis pathways may be greatly influenced by dietary habits and chronic Helicobacter pylori infection [3]. Although this probably also has some influence on HER2 positivity rates found in different populations with GC, the relation between these factors and HER2 expression remains unknown, with few papers published [36,37].” [36] Menendez JA, Papadimitropoulou A, Vellon L, Lupu R. A genomic explanation connecting "Mediterranean diet", olive oil and cancer: oleic acid, the main monounsaturated fatty acid of olive oil, induces formation of inhibitory "PEA3 transcription factor-PEA3 DNA binding site" complexes at the Her-2/neu (erbB-2) oncogene promoter in breast, ovarian and stomach cancer cells. Eur J Cancer. 2006;42:2425-32. [37] Yoo MW, Han HS, Kim SY, Cho YH, Lee HG, Kim JH, et al. Is Helicobacter pylori associated with Her2/neu Overexpression in Gastric Cancer Patients who Underwent Curative Resection? Hepatogastroenterology. 2014;61:858-62.
Yours truly,

Renato Santos Laboissière, M.D.