Author's response to reviews

Title: Chromogranin A Is a Reliable Serum Diagnostic Biomarker for Pancreatic Neuroendocrine Tumors but Not for Insulinomas

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Author's response to reviews: see over
Dear Editor, Dr Malaguarnera and Dr Strimpakos,

Thank you for giving us the opportunity to revise our manuscript. We appreciate the helpful comments and suggestions regarding the MS. We have carefully revised the MS in response to reviewer’s comments, all changes are highlighted in yellow for ease of reference.

In the general, we have made following important revision and changes:
1. Followed Dr Malaguarnera’s suggestion, we added information on chromogranin A in the introduction, mainly the diagnostic and prognostic value of CgA in endocrine and non-endocrine tumors/ cancers.
2. Increased 10 cases of PNETs (4 insulinomas and 6 localized non-insulinomas), right now 89 patients were studied (in previous MS, 79 patients were studied).
3. Increased 3 figures, i.e. Fig 4, Fig 5C and Fig 6 as well as several references.
4. According to ENETS guidelines, we added the stage information in table 1, the CgA levels were correlated with the stage (table 2) as well as primary location of tumors (Fig 5C).
5. Followed Dr Strimpakos’ criticisms, we not only increased 10 patients with PNETs but also compared the serum CgA levels in 2 subgroups of patients, one group of patients with metastases and another group of patients with localized tumors (Fig 4). Moreover, blood levels of CgA in patients with benign insulinomas were compared with the levels in patients with localized non-insulinomas (Fig. 6).
6. Modified the discussion.

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Referee 1: Michele Malaguarnera

Many studies reported that chromogranin is a reliable diagnostic and prognostic biomarker for various type of tumors which affect liver, pancreas and prostate (Biondi et al., BMC surg 2012; Malaguarnera et al., Arch Gerontol Geriatr. 2009 ; Ranno et al., Arch Gerontol Geriatr. 2006). The authors evaluated a group of pancreatic neuroendocrine tumors (PNETs).
The manuscript contains new information to justify publications, furthermore, results and data are really interesting and well discussed.

Minor essential revisions
It would be appropriate to add in the introduction adequate information on chromogranin.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.

Response: We appreciate Dr Malaguarnera’s comments and suggestions. We added information on chromogranin A in the introduction (please see the second paragraph, line 84 – line 90), mainly the diagnostic and prognostic value of CgA in endocrine and non-endocrine tumors/ cancers. We also discussed the relationship between the blood levels of CgA and tumor metastasis in Discussion (line 311 - 313).

Referee 2: Alexios S Strimpakos
1. Major Compulsory Revision. This work addresses a significant clinical question and the researchers should be congratulated for their effort. Nevertheless, there is a crucial imbalance between the supposed comparators (insulinomas and non-insulinomas) which is the differences in the stage of the disease. Only 1 out of the 53 patients with insulinomas appeared to have metastatic/unresectable disease as opposed to 17 out of 26 patients with Non-Insulinoma. This difference might encounter the lower plasma level of ChrA in insulinomas (pre and post operatively) despite similar to non-insulinomas expression levels. I would suggest authors to compare subgroups with non metastatic disease, as there is only 1 patient with metastatic insulinoma, insufficient for comparison.

Taking the above into account, one can assume that the sample sizes will be small to draw safe conclusions, but it will be a step to the right direction.

Level of interest: An article whose findings are important to those with closely related research interests. Quality of written English: Acceptable.
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Comments 1: There is a crucial imbalance between the supposed comparators (insulinomas and non-insulinomas) which is the differences in the stage of the disease. Only 1 out of the 53 patients with insulinomas appeared to have metastatic/unresectable disease as opposed to 17 out of 26 patients with Non-Insulinoma. This difference might encounter the lower plasma level of ChrA in insulinomas (pre and post operatively) despite similar to non-insulinomas expression levels. I would suggest authors to compare subgroups with non metastatic disease, as there is only 1 patient with metastasic insulinoma, insufficient for comparison.

Response 1: We appreciate Dr Strimpakos’ criticisms and suggestions. We agree with reviewer’s point that few metastases in insulinomas (only one patient) might be one of the reasons for low serum level of CgA in insulinomas. Many previous studies confirmed that CgA levels in patients with NETs metastases were higher than that in patients with localized NETs. We correlated the serum CgA levels with tumor metastases and stage. As Dr Strimpakos expected, the higher serum levels of CgA were significantly associated with metastases ($P = 4.1 \times 10^{-5}$, Fig 4) and advanced stage ($P = 0.003$, table 2). However, absent metastasis in majority of insulinomas is one of the main characteristics of this unique tumor [de Herder W, et al: Well-differentiated pancreatic tumor/carcinoma: insulinoma. Neuroendocrinology 2006, 84:183-188], and in our hospital, more than 95% of insulinomas are benign [Zhao YP, et al. Surgical management of patients with insulinomas: Result of 292 cases in a single institution. Journal of Surgical Oncology 2011; 103:169-174].

We further followed Dr Strimpakos’ suggestion that we should compare subgroups of non-metastatic tumors with benign insulinomas. We added 10 cases, 6 localized non-insulinomas and 4 benign insulinomas in order to increase the cases of both groups. The data showed that CgA levels was not significantly different between 2 groups ($P = 0.693$, Fig. 6). However, we found that the rate of elevated CgA levels in patients with localized non-insulinomas was significantly higher than that in patients with localized insulinomas, 4/12 vs. 3/56, $P = 0.015$ (please see results in detail, line 215 - 230). In addition, one study demonstrated that CgA levels were elevated in 9 of 10 patients with gastrinoma, in the absence of metastasis [Tomassetti, et al. Diagnostic value of plasma
Collectively, all of the data above suggested that metastasis could be one of the determinant reasons for high levels of CgA in PNETs, but not the only determinant factor. The tumor subtype (gastrinoma, insulinoma) could be another determinant for CgA serum levels.

It's true that more patients with metastatic insulinomas were needed to validate the low levels of CgA in insulinomas but it might be quite difficult for us to do so due to the limited numbers of malignant insulinomas. As in our hospital, more than 95% of insulinomas are benign, and according to many literatures, most of insulinomas (> 90%) are benign. We wish several clinical centers would pool the blood samples of malignant insulinomas together, and measure their CgA levels in future.

Comments 2: Taking the above into account, one can assume that the sample sizes will be small to draw safe conclusions, but it will be a step to the right direction.

Response 2: We agree with Dr Strimpakos' opinion that more samples would make the conclusion more reliable. We tried our best to increase 10 cases (4 insulinomas and 6 localized non-insulinomas) in present study, and reanalyzed the data. The data were very similar to (or same as) the previous results and we got some new findings. Because pancreatic neuroendocrine tumors are a group of rare tumors, its incidence is around 1-2 new cases / million populations, it's hard for us to get more serum samples at moment.

Finally, we appreciate all reviewers and editors' criticisms, suggestions and your valuable time.