Author's response to reviews

Title: Heat Shock Protein-27, -60 and -90 expression in gastric cancer: association with clinicopathological variables and patient survival

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Author's response to reviews: see over
Dear Editor,

Please, receive the revised version of our manuscript entitled “Heat Shock Protein-27, -60 and -90 expression in gastric cancer: association with clinicopathological variables and patient survival”. All corrections are depicted in red. We would like to thank the reviewers for their valuable comments which were carefully taken into consideration in the revision of the manuscript. We hope that the revised version and the answers provided will be satisfactory and our study will meet the criteria of the Journal of BMC Gastroenterology.

Thank you for your consideration

Sincerely yours

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Response to the reviewer’s comments

Reviewer 1

We already documented in Patients and Methods section (Immunohistochemistry) that “appropriate negative controls were performed by omitting the primary antibody and/or substituting it with an irrelevant anti-serum” and that “as positive controls paraffin-embedded liver sections with known immunoreactivity for HSPs were used”. As also noted in the Material section, HSP-27, -60 and -90 are goat anti-human primary antibodies (Santa Cruz Biochemicals, Santa Cruz, CA, USA), thus we haven not used isotypic controls. However, we have no available data so far for HSP40 and HSP70 proteins’ expression, as well as serum HSPs levels of this cohort study in order to make a link between intracellular and extracellular HSPs levels as suggested by the reviewer. We therefore added a statement in the Conclusion section emphasizing the need of such studies and making a link between the present data and future studies from our laboratory.

Reviewer 2

1] According to the useful reviewer suggestion, we added a sentence in the Results’ section for each HSP member to state the mean percentage value of HSPs expression and the incidence of tumors with high HSPs expression. We set a cut-off value based on the mean percentage value of the entire cohort for each HSP member, which produces a more balanced two groups. We further examined HSPs staining intensity in relation to clinicopathological characteristics and patients’ survival as an alternative immunohistochemical scoring that expressed different semi-quantitative information than the percentage of HSP-positively stained tumor cells, reflecting dose-dependently, the amount of HSP-proteins in the cells.

2] Although the data on “sex” and “age” do not appear to be so important as the other clinicopathological characteristics, such as tumor grading and histological type and TNM stage, we decided not to omit them. These data are clinically relevant, being used by the majority of relevant in situ studies. In fact, the age of patients constitutes an important factor in cancer, being indicative of the tumor progression and recurrence or prognosis. The sex of the patients also constitutes an important factor, being related to the estrogen status. As the expression of HSPs are induced by diverse
environmental and physiopathological stresses, the age and the sex of the patients that are related with oxidative and estrogen status, respectively may have a potential impact on HSPs expression.

3] According to the useful reviewer suggestion, we revised the relevant paragraph in the Discussion section concerning the clinical significance of HSP-60 immunostaining as “HSP-60 expression was significantly associated with patient sex, and borderline with tumor histopathological grade and proliferative capacity reflected by Ki-67 labelling index. Moreover, HSP-60 staining intensity was significantly associated with patients’ age and tumor histopathological grade”. We further stated that HSP-60 staining did not correlate with patients’ prognosis which is in contrast to another study conducted on oesophageal squamous cell carcinoma patients [25]. However, we did not omit the data concerning Ki-67 labelling index, as a trend of correlation was obtained for HSP-60 expression. Moreover, Ki-67 labelling index was widely used in such in situ studies, as it reflects the proliferation status of tumor clinical samples.

4] The reviewer stated that the Tables seem to be large. However, we aimed to include in three Tables all the data performed in our study (one Table for each HSP member) instead of six Tables (two tables for each HSP member, one for HSP expression and one for HSP intensity). It should be noted that each HSP member presented significant or borderline associations with different clinicopathological characteristics. Thus, for comparison purposes, we did not omit the non-significant data for each HSP member.

5] The reviewer rightly documented that Figure 1 could be optimized in terms of presenting staining of normal and tumor tissues of both intestinal and diffuse type cancers, and at two magnification. However, such an illustration requires more than five figures, which will cover a lot of space, since our study include three different HSP stainings in two distinct histological type of gastric cancer.

6] The reviewer suggested providing data on differences in HSP-90 client expression (HER2, EGFR) in gastric cancer tissues. We therefore added relative previous evidence in this issue in order to make a link between the present data and future studies from our laboratory.
6] We further checked throughout the manuscript in order for grammatical mistakes to be avoided. We also checked sentences formation and we changed phrasing in some cases. We further update to references.

7] We included in the Material sections (Patients) the statement that this study was approved by the ethical committee of Laikon General Hospital.

8] We included an authors’ contribution section before the Acknowledgement and Reference list.

**Reviewer 3**

The reviewer rightly documented that an exact test or continuity correction are usually recommended in order for more accurate p-value to be derived, especially in small sample size clinical material. In our study, we used Fisher’s exact test in such cases and we therefore included this information in the Patients and Method section (Statistical Analysis).