

First of all we would like to thank the reviewers for the opportunity to improve our paper

Reviewer #1

**You should check article on English grammar rule, scientific writing and punctuation, there are many.**

The manuscript has now been edited by a native English speaker

**Figures and tables should be simplified and focused on the subject. They should be comparing the endoscopic ultrasound staging and clinical staging in clinical characteristics and in analysis of factors influencing disease-free survival.**

We agree that the text, figures and table reported too many factors related to anal cancer prognosis and did not focus on the purpose of the article, we therefore simplified the article accordingly, and unrelated factors have been removed.

Reviewer #2

**I suppose that whether the authors could establish a model to assess the survival of patients by ultrasound staging, adding clinical staging and ASA scores if necessary. For example, how long will the patients of ultrasound T1-2 staging live, and how will the anal cancer of ultrasound T3-4 staging affect a patient's survival time?**

This is a very attractive suggestion: combining multiple prognostic variables to form a prognostic index. Since the 2 prognostic significant variables, in our group of patients, were ASA score and ultrasonographic Stage, we combined them and we found an adjoined result; this analysis allowed for the identification of four groups of patients with different prognosis.

Reviewer #3

**Was sample size calculated?**

We calculated the required sample size to detect a difference between the two staging systems, and found that more than 300 patients would have been needed. However it was impossible to reach such a great number of individuals in a single center in a reasonable timeframe; moreover we found that the ultrasound staging was significantly associated with DFS, even with a limited number of people. Since our results were in line with a previous study, we decided to present our results in order to stimulate a multicenter study or a meta-analysis or to implement the use of ultrasonographic staging in the clinical setting.

**Of 63 patients, why only 48 underwent endoscopic us? Did the others not meet the inclusion criteria?**

During the study period 63 patients with anal canal cancer were observed, 15 of them did not undergo ultrasound examination, either for unavailability of the ecograph or because they were referred from other Institution after completion of the staging, or for refusal due to painful lesions; this statement has been added in the text

**As all patients underwent MRI, were they compared with sonographic findings? - Could the discrepancy between staging relate to incorrect clinical staging?**

The clinical staging was based on RMI and CT examination with exact measurement of tumor diameters and evaluation of clinically involved lymph nodes; this stage was compared with the ultrasound staging which is based not on tumor size but on tumor penetration through the anal wall and sphincters (as previously described by Giovannini, ref 5) therefore the discrepancy between the two staging is related to a different classification system

**Obvious restricted role of sonography in identification of metastases has not been pointed out**

We agree that ultrasonography has no role in the identification of liver and lung metastasis or nodes that are outside its field of vision, this has now been pointed out in the discussion. However the focus of this paper is not on the role of MRI and ultrasound in the diagnosis and staging of anal tumors but on the different prognostic significance of two staging system, one based on tumor dimension, the other based on tumor depth. At present this tumor depth is better assessed with ultrasound than with MRI, this last examination has undoubtedly several other advantages that are now better underlined in the Discussion.

**As sonography is operator dependent, and requires training, are they not limiting factors?**

In the present study the ultrasounds were all performed by the same operator who has extensive experience in this diagnostic method (over than 3.000 ano-rectal ultrasound performed during the last 15 years); however we agree that this examination requires training and it is not always available in the clinical settings; advantages and disadvantages of the ultrasound system have been now underlined in the Discussion

**Conclusion is not based on data from the manuscript ("..could help to a more correct identification of risk factors for anal cancer ")**

Conclusions have been modified according to the suggestion of the reviewer

**References are not according to standard format**

References have now be formatted, the PubMed numbers and DOI citation numbers have been added and all authors of the references have been listed



We thank the reviewer for the opportunity to improve the article. Here a point-by-point response to the questions raised by reviewer #027336328:

**1- The article should be revised on medical article writing rules**

The article has been revised according to the Journal's writing rules

**2- Abbreviations should be checked.**

All the abbreviations have been defined upon first appearance in text, tables, and figure

**3- Sentences written by authors in red color should be corrected.**

Sentences in red color were the sentences modified after the first revision these were the sentences highlighted because modified according to the other reviewers; they are now in black color.

**4- Authors compare the sphincter invasiveness by cancer determined by anal US to tumor size determined by anal MR and effect on prognosis of anal cancer. In fact they compare different things. MR can also show anal sphincter invasiveness.**

In this article we compared 2 different staging system, one based on tumor dimension and the other on tumor penetration into the anal wall. At present this tumor depth is better assessed with ultrasound than with MRI, MRI provides a good assessment of tumor size, position, extent, of the disease, infiltration of adjacent organs but is less precise in defining invasion confined to the mucosa or submucosa; on the other hand, with technique improvement and possibly dedicated radiologists, MRI could also be employed for the evaluation of depth of invasion in early tumor and employed for a different staging system. In summary it is not a matter of technique, is a matter of different classification, we employed ultrasound but the same staging system could be based on MRI. This observation has been added in the Discussion

**5- In addition they grouped the patients up to US found N stages although they wrote that US could not see all the area LN metastases could be.**

Ultrasound classification takes into account involvement of the anal canal wall and perirectal LN (as reported in Materials and Methods); endoscopic ultrasound can efficiently evaluate lymph nodes located in the mesorectum, although it cannot visualize lymph nodes located in the internal iliac basin or the para-aortic nodes.

**6- There is really difference in the number of metastatic LNs between US found and MR found.**

Indeed in the present series MRI detected more abnormal nodes than ultrasound however, apparently, this disagreement did not influence outcome. This has been incorporated in the Discussion.

**7- They found different groups according to uT, ASA and LN metastasis. They should describe these groups more and even give their prognostic values with comparing AJCC on tables and I think those differences in prognosis due to ASA score. Authors should discuss on these points.**

We agree with the reviewer that most of the differences in prognosis observed can be due to the ASA score alone. This is clearly reflected in the p-value distributions observed with the compound group having higher, although still significant p-values, than those obtained when the stratification was made using exclusively the ASA score. However, this part was included in the final version of the manuscript as this was specifically asked during the revision process. The median survival for the four groups has been added in Results, and a sentence has been added in the Discussion.

**8- Tables have no meanings. They should be restructured.**

Table 1 and 2 have been deleted. A table reporting the number of patients classified with the two staging and factors potentially influencing the prognosis has been added. The table has been structured according to the Journal's rules

Here are the revision according to "53826" list of issues that need to be addressed in conditionally accepted manuscript

*Style and format:* page number has been included

*Abbreviations:* all the abbreviations have been defined upon first appearance in text, table, figure legends

*Ethics:* IRB approval is not needed for a retrospective observational study. We provided the informed consent form of the first patient. STROBE statement with specific page number has been added

*Manuscript organization:* manuscript has been organized and ARTICLE HIGHLIGHTS has been added

*Citation:* name of the Journal and year has been added in the citation

*Article highlights:* article highlights has been added following the guidelines

Figure and tables: figure and tables have been formatted, sub-tables and sub-figures eliminated, data with statistical significance denoted following the examples

*Answering reviewers:* a point-to-point response has been provided