

18<sup>th</sup> May 2017

RE : Manuscript 33727

Reviewed by : 02823327

Reviewer : - You chose 16 cases from a pool that has a larger number of patients. Which elements were taken into account for the selection of patients for this study?

Reply : Funding for the molecular studies was only secured after the clinical trials of ablation had begun. We were only able to collect fresh biopsies from one site. We collected fresh biopsies across all tissue types from 17 patients, and 16 of these provided good quality OpenArray data.

Reviewer : - Complete ablation of the Barrett's esophagus was achieved in 13 of those 16 cases, Why you choose patients for the study with incomplete ablation? - Did you find any difference in the molecular expression of those two groups (complete vs incomplete ablation)?

Reply : To conduct our study on neosquamous epithelium, the only requirement was that neosquamous epithelium formed after ablation. We have addressed the question regarding differences in expression in our reply to the next comment.

Reviewer : - "All patients were free of reflux symptoms by either high dose proton pump inhibitors (n=8) or a laparoscopic fundoplication (n=8)." It has been shown that Barrett patients who control symptoms with PPI continue with asymptomatic weakly acid reflux (even asymptomatic acid reflux in some cases), while patients who control their reflux symptoms with antireflux surgery have a high chance of having no reflux of any content. This observation results in that after antireflux surgery there is a lower expression of molecular markers of inflammation than after controlling symptoms with PPI. Did you find any difference in the microRNA markers studied between these two groups? It would be interesting to know these data, even if the difference was not significant considering that the number of cases is small.

Reply : We appreciate the reviewers comment, and we agree that this is a very important question. At the reviewer's suggestion we have done subset analyses for patients that were medically treated vs. surgically treated for reflux, and also for completely ablated patients vs. all patients. These analyses were done by averaging the differential expression and the Mann Whitney U test p-values across the overlapping microRNAs in each patient subgroup. Differences between the groups were tested using Welch's t-test (i.e. equal variances not assumed). We have added text to the methods, results and supplementary tables to address this. Note that with this data and only 8 patients per group for the medically treated vs. surgically treated patients comparison we estimate that the Mann Whitney U test is only able to detect differences of more than 2 fold with 80% power at an alpha of 0.05.

Reviewer : - It is necessary to clarify how long after the ablation procedure the biopsies were taken.

Reply : At a median of 6 weeks (inter quartile range 4.96 to 6.5 weeks). We have added text to the methods to address this.

Reviewer : - While reading the manuscript I struggled to identify each of the groups of biopsies that you separated (pre-ablation BE, post-ablation neosquamous, post-ablation squamous, control individuals), and many times I had to go back to reread what was referring the name of each biopsies group... I admit it may be only my problem, but I encourage you to rethink a name to identify easily each group of biopsies to make reading easier.

Reply : We apologize for this difficulty. The terminology is historical (our previous publication) and there is no consensus in the literature. We have added text to the "Terminology" section of the manuscript to try to make this more clear.

Reviewer : - I want to congratulate you again on such an interesting manuscript.

Reply : We thank the reviewer for giving their time to our manuscript, and for their constructive and kind words.

Sincerely,

Dr Damian Hussey (on behalf of all authors)

A handwritten signature in blue ink, appearing to read "Damian Hussey", written in a cursive style.