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Dear Editor of Gland Surgery

The authors would like to thank for the opportunity to revise the manuscript after responding to the reviewer's comments. We would also thank the reviewers for the insightful and valuable comments, which have certainly improved our manuscript. We hereby submit the list of changes incorporated on the manuscript and rebuttal against some of the questions raised.

Responses to reviewer A

1 – *“There is no appropriate ‘control arm’ (a group of patients without use of device) to show if the surgeon did indeed benefit from the use of the device developed by the authors. A more effective approach would have been to compare how the same surgeon would have fared in another set of 40 thyroidectomy patients without using the device. As in would the number of incidental parathyroidectomies been higher or unchanged without the use of the custom-built device for the same surgeon? This was a major limitation of the current study design.”*

In fact this is a limitation of the study, as it's only an observational not controlled study and for the purpose the control was the pathological examination.

However as the device was only used for post-operative inspection of the sample, it wouldn't have real influence on the surgical performance.

We are now conducting a controlled study were we use the device during surgery to help the surgeon in parathyroid identification.

2 - *The authors use strong phrases like ‘Our conviction...’ and ‘We believe...’ (Page 8, Line 15 and Line 20) despite NOT having demonstrated any correlation between postoperative hypocalcemia and incidental parathyroidectomy. The authors should modify the sentences appropriately to demonstrate an objective and scientific outlook in their manuscript.*

We modify the sentences and integrate it with the next sentences clearing the concepts:

Despite our results failing to show any relationship between the occurrence of incidental parathyroidectomy and post-operative hypocalcaemia, decreasing the necessity of auto-transplant of incidentally removed glands, there is an evident advantage in knowing if there is any parathyroid tissue in thyroidectomy specimens. Presence of more than one fragment of parathyroid tissue might become an indication for auto-transplant.

Thus the integration of intraoperative visualisation of parathyroid glands with auto-fluorescence followed by in table inspection of the specimen could decrease rates of post-operative hypocalcaemia.

3 - *Why were 4 lobectomies considered for this study? Given that the parathyroid glands of the non-operated side would compensate for post-operative hypocalcemia even if the parathyroid glands of the operated side were accidentally excised or damaged. Should not this study have been restricted to total thyroidectomies and completion thyroidectomies to truly assess the impact on post-operative hypocalcemia?*

The primary aim of the study was evaluate the utility of the device to identify incidentally removed parathyroid glands, so for the purpose we use all thyroid patients regardless the type of surgery. In the results section we clear emphasize and analyze separately the results of total and completion thyroidectomies in spite of presenting also the results for the whole sample.

Analyzing also lobectomies we were aware of an interesting phenomena that will justify attention: the occurrence of cases of post operative transient hypocalcemia on patients with at least two not disturbed parathyroid glands.

4- Were the images visualized in real-time in the operating room? The authors have not provided any figures demonstrating near infrared autofluorescence of the parathyroid glands in the excised samples (one sample with a parathyroid and one without a parathyroid). Kindly justify.

In the present cases the samples were inspected visually by the surgeon after excision and then in a separate table, inspected with the device.
We added an image with a specimen (Figure 2)

5 - Do the authors envision this device to be hand-held for the end-user as in Figure 1 or a wearable goggle-like head-set? Please elaborate.

The volume of the current acquisition system (goggle) makes it uncomfortable to be used by the surgeon with a head-mount system so we use it as showed in the figure.
We are now trying more ergonomic solutions.
For intra operative use we had to design a sterile drape.

6 - Can the authors comment on how the experience of the surgeon (years of experience) and specialization (endocrine surgery, general surgery, ENT) could have an effect on the rate of incidental parathyroidectomy? What kind of end-user would stand to benefit more from such a device as described by the authors?

Experience of the surgeon is very important in thyroid and parathyroid surgery as stated in several articles, with less complications associated with high volume surgeons.
Ideally all the procedures should be performed by experienced surgeons. Unfortunately most of them are performed by low-volume surgeons all over the world
The use of autofluorescence could help low-volume surgeons to avoid this complication and also decrease the learning curve associated with the identification of parathyroid glands .
We added the following paragraph in the end of the discussion section:

A relevant limitation of this study is the fact that all the surgeries were performed by the same surgeon, an experienced thyroid surgeon although not a high-volume surgeon, whose experience could be related to the little volume of the parathyroid fragments found.
As the majority of thyroid surgeries all-over the world are performed by low-volume surgeons, whom may benefit more of the assistance of autofluorescence for the identification of parathyroid glands, a study evolving surgeons with different levels of expertise would be useful, evaluating not only the results of postoperative hypocalcemia but also the effects on the learning curve.

7 - The authors keep describing their device as a low-cost equipment. Please justify as to why the authors state so? Especially with respect to current devices in market such as Fluobeam and PTeye that have already been FDA-cleared for intraoperative identification of parathyroid using near infrared autofluorescence. In addition to these two, there are other systems as well – Hamamatsu PDE Neo or the Karl Storz camera that have been utilized for the same application. The authors also need to switch off the room lights with their device which impedes the surgical workflow. Fluobeam LX and PTeye can be functional without needing to switch off room lights and disrupting surgical work-flow. Therefore, what are the advantages of the

author's system compared to the mentioned devices, in terms of device features or cost-effectiveness? Please discuss in the manuscript.

Our custom-built system costs only a fraction of the FDA approved devices (3200 euros), and further research could lessen even more the costs.

There is in fact a limitation with the room lights which must be turned off, but if resources are scarce costs overwhelm this limitation.

In discussion section we added the following paragraph:

If compared with the FDA approved devices for parathyroid identification our system has the advantage of the low cost (less than 3200 euros), making it affordable even for hospitals with scarce financial resources.

*8 - The authors have custom-built this system as described in the Methods. What is the novelty or advantage of their technique compared to the several works from the Kang-Dae Lee group? The Kang-Dae Lee group from Busan had also developed a custom-built system using a Canon camera with really impressive results as demonstrated in: (a) Kim, Sung Won, et al. "Near-infrared autofluorescence image-guided parathyroid gland mapping in thyroidectomy." *Journal of the American College of Surgeons* 226.2 (2018): 165-172, (b) Kim, Sung Won, Hyoung Shin Lee, and Kang Dae Lee. "Intraoperative real-time localization of parathyroid gland with near infrared fluorescence imaging." *Gland surgery* 6.5 (2017): 516, (c) Kim, Sung Won, et al. "Real-Time Localization of Parathyroid Glands with Near Infrared Light during Thyroid and Parathyroid Surgery." *International Journal of Thyroidology* 11.2 (2018): 92-98, (d) Kim, Sung Won, et al. "Intraoperative real-time localization of normal parathyroid glands with autofluorescence imaging." *The Journal of Clinical Endocrinology & Metabolism* 101.12 (2016): 4646-4652. The authors need to discuss the novelty of their device as compared to these mentioned earlier works, to justify the significance of their findings.*

We started our research using the system described by the Busan group, and the major difficulty we had was focusing the image in real time in a narrow surgical field, situation that led to the use of the goggle handheld system, allowing the surgeon himself to inspect the area.

Another advantage of our system is that no modification is needed in the goggle device (just adding the proper filter in front of the lens) while the Camera used by the group of Kang Dae Lee was submitted to a relevant modification (remotion of the infrared filter) which also increased the costs.

Both groups share the same thought: It's not necessary an expensive technology to visualize parathyroid autofluorescence.

In addition to the paragraph added previously we add the following sentence:

and is easier to assemble than other custom-build devices that need significant technical intervention.

9- While incidental parathyroidectomy can contribute to post-operative hypocalcemia, the incidences are relatively lower. A more common cause contributing to post-operative hypocalcemia is accidental damage/devascularization of in-situ parathyroid glands. The authors admit that their study was not approved for in vivo imaging of in-situ glands by the ethics committee and hence could not be conducted. Since the authors could not investigate, they should then briefly elaborate/discuss on whether the modality would be beneficial for preventing accidental injury/devascularization of in-situ glands. Can this technique help the surgeon perform the procedure better to avoid injury to the glands? As this would then be the true benefit of this modality, and not just for minimizing incidental parathyroidectomies.

Avoiding post operative hypocalcemia is the true purpose of any parathyroid identification technique. We clarify our position stating what is the main advantage of the use of autofluorescence: helping the surgeon to recognize parathyroid glands so he can avoid their manipulation and preserve vascularization:

In spite of not providing information about the vascularization of the parathyroid glands, an early awareness of their localization may help the surgeon to avoid their manipulation and to prevent devascularization.

Responses to Reviewer B

1 - *Images of the specimen would be nice*

We added an image of the specimen (Figure 2)

2 - *Need a table to show the patients with parathyroid in the specimen and the tissue that was fluorescent on the specimen and the pathology correlates.*

A table was added (new table 2) with characteristics of the patients and specimens.

3- *Did the surgeon see the tissue on the specimen first before the camera? need to address this.*

We stated in the manuscript that in only one case the surgeon suspected first. The amount of parathyroid tissue was too low to raise high suspicion. Except for intrathyroidal parathyroid glands found in one case there were no complete glands on the samples.

4- *In the results you do not need to repeat the table results.*

We cut some repeated values.

5 - *The authors fail to refer to two randomized trials using image based NIR Parathyroid autofluo imaging use during total thyroidectomy (Jama surg and Jacs, Benmiloud and Dip). These studies have pertinent findings regarding removal of parathyroid tissue and the use of fluo imaging need to add and discuss.*

In fact those are very pertinent studies that show the validity of fluorescence and must be referred, in spite of none of them show the results of post operative inspection of the sample with the device. That inspection is the third step of the stepwise approach described by Kim (JACS).

We reformulate the paragraph, incorporating those works in the discussion section:

Thus the integration of intraoperative visualisation of parathyroid glands with auto-fluorescence followed by in table inspection of the specimen could decrease rates of post-operative hypocalcaemia as shown by the works of Kim et al and the randomized controlled trials conducted by Dip et al and Benmilloud et al.

6 - *The study may not be powered to detect hypocalcemia.*

Absolutely agree. This study is underpowered to detect hypocalcemia ($\pi = 0.085$) and we stated it in the results section:

The study was however underpowered to detect hypocalcemia ($\pi = 0.085$).

7 - I agree with the authors that looking for parathyroid tissue in the specimen with this technology may help particularly in cases that are associated with higher removal of parathyroid tissue.

Thank you. In fact one of the advantages of this technology is the possibility to identify parathyroid tissue in amount that justifies autotransplantation. Fortunately for your patients it wasn't the case.

Responses to reviewer C

1 -The numbers in the study are relatively low, therefore should focus more on the details of the removed parathyroids, and not on their correlation with hypocalcemia. This is really a feasibility study.

We added a table with characteristics of the removed parathyroid glands.

2- The sensitivity and specificity of the technique should be emphasized, not the correlation with calcium.

We emphasized the specificity and sensitivity in discussion and conclusions:

The high sensitivity and specificity rates obtained with the utilization of autofluorescence showed the utility of the system that may function as an "optical biopsy" after proper validation.

Although in this study we could not demonstrate a relationship between incidental parathyroidectomy and postoperative hypocalcaemia, the high sensitivity and specificity rates make autofluorescence of parathyroid glands an useful tool for identification of incidentally removed glands during thyroidectomy

3 - Most other studies on IP categorize the removed parathyroid better – partial or whole, and intra-thyroidal (completely surrounded by thyroid tissue), intracapsular of the thyroid, or extra-capsular. The authors only categorize it as intra-thyroidal or not. More information is needed. For the intra-thyroidal, how deep into the thyroid tissue was it. How many were extra or intra-capsular? How many were partial glands or complete parathyroid glands.

In the new table we characterized the removed parathyroid glands with the information available on pathology.

We also added a sentence clarifying the localization of the intrathyroidal parathyroid:

The system failed to identify one of the two intrathyroidal parathyroid glands present in a patient, identifying only the most superficial (intracapsular), missing the deep situated one, localized 3 mm into the parenchyma.

4 - Localization of high fluorescent areas were recorded – how? Was a stitch placed to correlate with the final pathology, or a picture taken?

A silk stitch was placed (vicryl is cause of false positive) to point the localization.

We could only take pictures in a few cases and the quality of the pictures allowed by the device is poor.

5 - Please describe Figure 1 better. Presumably the room lights are on. Why is the assistant shining the LED off of the thyroid gland?

Figure 1 was taken during the focusing of the device, before lights were turned off. The LED was still pointing out thyroid because we prefer to focus without the interference of near infrared light.

6- It is surprising that none of the identified parathyroids were auto-transplanted. In the results section, it states that the volume of tissue was too small, but in the discussion section, it notes that ethics committee did not approve the device for parathyroid identification. Could not a frozen section be done of the tissue, and it then re-implanted?

We were prepared to re-implant, however the amount of tissue was too small to allow a frozen section. In the only case it would be possible (case 7) we decided not to do so, since a the visual inspection of the field by the surgeon identified well vascularized parathyroid glands.

7 - Was there variability in the amount of autofluorescence seen in the thyroid gland, and could this explain the false negative?

The amount of autofluorescence in thyroid gland is variable and heterogeneous and can contribute to false negatives, however the principal factor in this case was the deep situation of the intrathyroidal parathyroid gland in the thyroid parenquima. As stated, NIR light has some capacity of penetration in the tissues (until 5 mm) , but surely is a limitation of the technique.

8- The first line of the abstract states that incidental excision “does not demonstrate obvious clinical significance” which I think is not accurate. Although the data is mixed, the largest studies and a recent well done multivariate analysis (14) shows that it is associated with hypocalcemia.

We changed the paragraph :

Incidental excision of parathyroid glands is a common event during thyroid surgery and in spite the divergent results that can be obtained from the literature about its clinical significance, all efforts must be used to preserve parathyroids.