April, 18th 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (ESPS manuscript No -10768- review)

Title: **Endoscopic Ultrasound Fine-needle aspiration evaluating adrenal gland enlargement or mass**

Author: Melissa Martinez, MD, Julia LeBlanc, MD, Al-Haddad Mohammad, MD, Stuart Sherman, MD, John DeWitt, MD.

Name of Journal: *World Journal of Nephrology*

ESPS Manuscript NO: 10768

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers:
(1) Reviewer 0035938
(2) Reviewer 01209300
(3) Reviewer 00039518
(4) Reviewer 02544637

Attached to this document you will find the detailed responses to each one of the reviewer's comments.

Thank you again for publishing our manuscript in the *World Journal of Nephrology*.

Sincerely yours,

Melissa Martinez Mateo, MD
Indiana University School of Medicine
Gastroenterology Department

Indianapolis, IN
46202

Fax: (317) 278-1949

E-mail: melamart@iupui.edu

Reviewer Comments and Responses:

Reviewer 1 (0035938)

1- How is the size of the adrenal gland measured?
The size of the adrenal gland for the purposes of this manuscript was the maximal cross-sectional diameter of the gland.

2- What is the difference between adrenal gland size and adrenal enlargement?
Adrenal gland mass: focal enlargement of the gland with a notable discrete mass
Adrenal gland enlargement: diffusively increased size of the adrenal gland without a visible discrete mass

3- How often could the right adrenal gland be visualized in this study?
The EUS procedure done for the patients in this study was focused on a known or suspected mass/enlargement of the adrenal gland. The endoscopist did not routinely intentionally attempt to visualize the adrenal gland.

4- Did you observe any trend regarding the use of 19G, 22G, 25G needles or the number of passes and the diagnostic yield?
No trend was noted. Most fine-needle aspirations were performed with a 22G needle. All the non-diagnostic specimens were obtained with 22G needles. Only 3 and 1 biopsies on these series were obtained with a 25G and a 19G needle, respectively.

5- Table 2: The differentiation of the indications is confusing

Table 2 (Indications for EUS) has been changed for:

INDICATION	N	%
Cancer staging *	26	28%
Suspected cancer recurrence**	5	6%
Abnormal CT/ PET-CT or MRI		
Pancreatic mass	20	21%
Mediastinal mass	10	11%
Lung mass	7	7%
Adrenal mass	7	7%
Gastric mass	2	2%
Liver mass	3	3%
Kidney mass	1	1%
Retroperitoneal mass	1	1%
Other ***	12	13%

*Esophageal cancer (n=3), gastric cancer (n=2), breast (n=1), jejunal adenocarcinoma (n=1), renal cell cancer (n=2), cholangiocarcinoma (n=1), lung cancer (n=16)

**Suspected recurrence of oral cancer (n=1), breast cancer (n=1), hepatoma (n=1), lung adenocarcinoma (n=1), esophageal adenocarcinoma (n=1).

***Chronic pancreatitis (n=3), abnormal upper endoscopy (n=3), common bile duct stricture (n=2), celiac nerve block (n=1), suspected metastatic disease on imaging (n=1), Barrett's esophagus with high grade dysplasia (n=1), ectatic

pancreatic duct (n=1).

6- Why are Barrett's esophagus or planned celiac block indications to perform EUS-FNA of the adrenal gland?

Malignancy was being considered in these patients, and because of the clinical implications of stage IV cancer, risks of adrenal gland EUS-FNA were considered minimal in comparison with a missed diagnosis of cancer.

Reviewer 2 (01209300)

1- Main outcome measurements need to be better defined

We are uncertain about what the reviewer means with this question. If this refers to the objective of these case series, or if the question is addressing to better define malignant or benign biopsies.

2- Calculation of sensitivity, specificity, positive predictive value and negative predictive value and diagnostic accuracy need to be done for both malignant and benign lesions.

The sensitivity, specificity, positive predictive value and negative predictive value of adrenal gland EUS-FNA for malignant lesions was 86%, 97%, 96% and 89%, respectively. For this calculation, only diagnostic biopsies were included.

The sensitivity, specificity, positive predictive value and negative predictive value of adrenal gland EUS-FNA for benign lesions was 97%, 86%, 89% and 96%, respectively. For this calculation, only diagnostic biopsies were included.

The diagnostic accuracy of adrenal gland EUS-FNA was 92% for both benign and malignant lesions.

3- I would shorten the results section to make it more clear because it is difficult to be followed

The result section has been shortened by 9 lines (1 paragraph). One table (Table 5.) has been added to better illustrate EUS-FNA cytologic diagnosis.

EUS-FNA Cytologic Diagnosis	N
Malignant EUS-FNA cytology (26%, n=25)	
- Metastatic lung cancer	10
- Metastatic esophageal adenocarcinoma	5
- Metastatic colon adenocarcinoma	2
- Metastatic renal cell carcinoma	2
- Metastatic breast adenocarcinoma	1
- Metastatic pancreatic adenocarcinoma	1
- Metastatic melanoma	1
- Metastatic oral squamous cell carcinoma	1
- Metastatic hepatocellular carcinoma	1
- Undifferenciated carcinoma	1
Benign EUS-FNA cytology (64%, n=60)	
- Benign adrenal tissue	57
- Aldosteronoma	1
- Paraganglioma	1
- Pheochromocytoma *	1

*Previously negative normal plasma catecholamines and, 24-hour urine normetanephrines, vanillylmandelic acid and metanephrines.

4- The discussion need to be shortened and be more focused on the results obtained
The discussion has been shortened.

5- Any comments about the 15 patients with benign EUS-FNA cytology who died before the repeat imaging could be performed? (6 month by the study definition). Could have been possible to consider them false negative results and if this would be the case how would this affect your results?
For patients with benign adrenal gland EUS-FNA cytology for whom follow-up imaging was not available in our hospital system, the referring physician was contacted to inquire records of repeat imaging and/or death. The Social Security Death index database was reviewed for each one of these patients. Fifteen patients had died within a mean of 27.93 (±36.18) months from the procedure before a CT abdomen/pelvis to follow-up on the adrenal abnormality was ever done. If these patients would have all died within 6 months of the EUS-FNA we could have potentially included them in our test performance calculations. Only 2 of the 15 patients died within 6 months of the procedure; including those to the false negative results would change the test measures to: sensitivity 80%, specificity 97%, positive predictive value 96% and negative predictive value to 84%.

6- Can you discuss the false positive result in the patient with melanoma? This should also be reported in the clinical impact of EUS-FNA paragraphs.
False positive results EUS-FNA has been described in the literature. Its incidence varies anywhere from 1% to 15%, and can be due to cytological misinterpretation. This has been included in the discussion.

Reviewer 3 (0039518)

1- The abstract should be organized following the editorial guidelines of WJG (Aim, Materials and Methods, Results, Conclusions).
The abstract has been formatted. Thank you.

2- EUS Technique: The authors should specify the minimal clotting parameters required to perform EUS-FNA and the maximum number of biopsy attempts allowed for each procedure. In particular, it is interesting to know how many attempts were performed in the 9 non-diagnostic cases in their series. Furthermore, the authors should comment if the operators' learning curve may have affected this result; indeed, in Table 4, the rate of non-diagnostic procedures was reduced after 2004 even if the difference did not reach statistical significance.

Minimal clotting parameters required to perform EUS-FNA were Platelet count of > 50,000 and INR of < 1.5.

There was no maximum number of biopsy attempts allowed. Biopsy attempts were done until the endosonographer considered that useful clinical information was provided vs. further attempts would be futile. The maximum number of needle passes was 7. There was no statistical difference between the number of needle passes for diagnostic vs. non-diagnostic biopsies *(p=0.98)*.

Non-diagnostic aspirations occurred mostly before 2004, but this was found not to be statistically

significant *(p=0.14)*. However, it was considered that this was related to operator's learning curve.

3- Results: The authors claim that the 94 patients enrolled in the study were consecutive. This implies that all patients addressed to adrenal gland EUS-FNA could undergo the procedure and that there were no cases in which the target adrenal mass could not be visualized or biopsied. If so, this should be specified in the text.
There were 94 consecutive patients who had an attempted EUS-FNA of the right and /or left adrenal gland.

4- "Prior attempt with percutaneous CT-guided approach for adrenal biopsy was attempted and unsuccessful in 3 patients, two of them subsequently had a diagnostic adrenal EUS-FNA (1 malignant, 1 benign). What about the third patient? Why he did not undergo EUSFNA? Please specify.
The third patient had a non-diagnostic EUS-FNA. However, follow-up imaging showed that the adrenal gland mass was stable at 3 years.

5- "Diagnostic cytology was obtained in 86 biopsies after a mean of 3.2 (+1.4) needle passes". Considering that the number of non-diagnostic procedures is 9 and the total number of patients in 94, diagnostic cytology was probably obtained in 85 patients. Is it right?
There were 94 patients, but, one patient had both EUS-FNA of the right and left adrenal glands, reason why there are 95 EUS-FNA biopsies. The statement on the manuscript is correct, there were 9 biopsies that were non diagnostic and 86 biopsies that were diagnostic for a total of 94 patients.

6- Clinical follow up: Among the 36 patients with benign adrenal cytology with available follow up, 5 underwent adrenalectomy and surgical pathology was benign in 4 and demonstrated adrenocortical carcinoma in 1. It is not clear to me why these 5 patients with biopsy diagnosis of benign disease underwent surgery. Did the lesions increase in size? Was there a clinical suspicion of a false negative result of EUS-FNA? I think the Authors should clarify this point
Three of the 5 patients that underwent adrenalectomy within 2weeks-2months from adrenal EUS-FNA without repeat imaging. From chart review, we consider that this was related to surgeon's preference as these patients were undergoing surgical resection of a primary malignancy (i.e. lung cancer) anyways.
Two additional patients had adrenalectomy within 11 and 20 months from EUS-FNA. Repeat imaging showed tumor thrombus and concern for adrenocortical carcinoma in one, and was not available for the second patient.

7- It has been proposed that PET-CT should be routinely performed in case of a nodule detected in a normal appearing adrenal gland. This could avoid the puncture of PT-CT nodules that are usually benign (Eloubeidi et al, Gastrointestinal Endoscopy 2010, cited among the references). However, the Authors did not mention if in their series there were patients with imaging suspicion of a benign adrenal lesion and if these patients were submitted to PET-CT before EUS-FNA. Furthermore, I think that the Authors should comment about the role of this technique in the diagnostic flow chart of adrenal masses. This is only partially addressed in the Discussion.
During several years of the study time period, PET scan was not available and therefore is not applicable. With the advent of PET, any decision to pursue a biopsy for a positive or indeterminate PET scan is at the

discretion of the referring physician. With widespread metastatic disease, a positive scan within either adrenal is likely considered as diagnostic for metastatic disease and therefore a biopsy would not be necessary. However, in a patient with malignancy, a positive adrenal gland in isolation may signify novel metastatic disease. In that case, a biopsy may be required to consider additional or novel chemotherapy or possibly adrenalectomy.

8- I think that the legend of figure 1 could be changed as follows: Final diagnosis in patients who underwent EUS-FNA of either gland.
This has been changed. Thank you.

Reviewer 4 (02544637)

1- In these series, EUS is demonstrated to be safe in adrenal mass biopsy. As a retrospective research, authors should compare the outcomes in these series to those from percutaneous biopsy. Besides, there are some type errors (p8, line3, P 0.98, etc)
Additions have been made to the discussion about this request.
Type errors have been corrected. Thank you.