Author’s response to reviews

Title: Clinical characterization of patients with primary aldosteronism plus subclinical Cushing’s syndrome

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Author’s response to reviews:

Zuleyha Karaca, MD
Associate Editor, BMC Endocrine Disorders

September 26, 2019

Re: submission of the revised manuscript of BEND-D-19-00205

Dear Dr. Karaca:

I appreciate your sending of an e-mail dated September 1, 2019, which conveyed the valuable comments of two reviewers on our submitted manuscript (BEND-D-19-00205).

Along with the replies to the comments on a point-by-point basis, I carefully prepared the revised manuscript in which I highlighted the modifications or additions that I made in response to the comments. Furthermore, I added line numbers to the revised manuscript for facilitating the verification by you and reviewers.
I do expect you and reviewers find the revised manuscript acceptable for publication in your journal.

Sincerely,

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Warrick J Inder (Reviewer 1):

Specific comments:

1. The authors state that they began with 187 patients, 133 with PA and 54 with SCS but neither was “definitely diagnosed” in 115. Can the authors briefly expand on this and explain how these 115 patients failed to meet the diagnostic criteria?

Replies: I appreciate your insightful comments. Patients who were not definitely diagnosed were 1) patients who underwent challenge tests for the diagnosis of either PA or SCS only; 2) patients who failed to meet both of the Japanese and US diagnostic criteria of PA; 3) and patients who failed to meet the new Japanese diagnostic criteria of SCS in 2017. Furthermore, I collated the diagnostic criteria of “subclinical hypercortisolism” described in the article of Lee et al. that you had kindly cited [Lee SH, Song KH, Kim J, Park S, Ahn SH, Kim H, et al. New diagnostic criteria for subclinical hypercortisolism using postsurgical hypocortisolism: the Co-work of Adrenal Research study. Clin Endocrinol (Oxf). 2017;86:10-8]. Consequently, one of our patients was newly found not to meet the criteria. Therefore, we excluded the relevant patient from our study, and I added the following sentences to lines 7 to 12 of page 6 of the revised manuscript: “A total of 116 patients were excluded from the study: 31 who were diagnosed with PA or SCS only because tests required for definitely diagnosing both endocrinopathies were not conducted; 61 who failed to meet the new Japanese diagnostic criteria of SCS [9]; 1 who failed to meet the new Korean diagnostic criteria of subclinical hypercortisolism [10]; and 23 who failed to meet the Japanese [11] and United States [12] diagnostic criteria of PA.”

I extensively brushed up the entire submitted manuscript with help of a physician who has linguistic proficiency and vast experience in editing the manuscripts of medical articles. The line numbers of some sentences in the revised manuscript largely changed from those in the submitted manuscript in an attempt to appropriately respond to the pertinent comments. I highlighted the modifications or additions that I made in the revised manuscript for facilitating your verification.
2. The diagnosis of subclinical Cushing’s syndrome is controversial and international bodies can’t even come up with a consensus regarding the name! It appears the authors have diagnosed SCS only in patients with a post 1 mg dexamethasone cortisol of greater than or equal to 5 mcg/dL and not the other SCS criteria as outlined in the citation from Yanase et al (ref 6). Is this correct? The fact that ACTH was not lower in the SCS group than the PA group makes me question how many of the SCS or PASCS patients truly had autonomous hypercortisolism. The authors should take into account the seminal paper by Lee et al Clin Endocrinol 2017; 86; 10-18 and include ACTH and DHEAS in their diagnostic criteria. A failed DST with cortisol of more than 5 mcg/dL without evidence of low ACTH or DHEAS might be due to chronic stress or even ACTH-dependent hypercortisolism rather than autonomous cortisol secretion from an adrenal adenoma. My suggestion is that the diagnostic criteria for SCS need further explaining and that re-analysing the data to limit the diagnosis of SCS to the cases with low ACTH or DHEAS would tighten the manuscript considerably.

Replies: I’m sorry for the insufficient descriptions of the criteria in the submitted manuscript. We conducted the present study in patients who met the Japanese diagnostic criteria of SCS described in the article of Yanase et al. [9]. Hence, I added the following sentences (line 7 of page 8 to line 1 of page 9): “Concretely, patients were required to meet the requisites 1-3) -- 1) presence of an adrenal incidentaloma; 2) lack of characteristic features of Cushing’s syndrome; and 3) normal basal serum cortisol levels, as well as to have either of the requisites 4-6) -- 4) the cutoff value of serum cortisol level for the diagnosis of SCS was ≥ 5 µg/dL after the 1-mg DST, 5) the cutoff value of serum cortisol level for the diagnosis of SCS was ≥ 3 µg/dL after the 1-mg DST, and at least one of “Low plasma levels of ACTH in the early morning,” “No diurnal changes in serum cortisol levels,” “Unilateral uptake on adrenal scintigraphy,” “Low serum levels of DHEAS,” or the presence of “Transient adrenal insufficiency or atrophy of the attached normal adrenal cortex after removal of the adrenal tumor,” or 6) the cutoff value of serum cortisol level for the diagnosis of SCS was ≥ 1.8 µg/dL after the 1-mg DST, with the presence of “Low plasma levels of ACTH in the early morning” and “No diurnal changes in serum cortisol levels,” or the presence of “Transient adrenal insufficiency or atrophy of the attached normal adrenal cortex after removal of the adrenal tumor.” In the present study, namely, we examined only patients who met the requisites 1) to 3) and either one of the requisites 4) to 6) as patients with SCS.”

We considered that the requisite “Low plasma levels of ACTH in the early morning” was met when the basal ACTH level in the early morning was &lt; 10 pg/mL or the CRH challenge test indicated an ACTH level of &lt; 1.5-fold of the basal ACTH level. The Japanese diagnostic criteria of SCS described, “If the basal ACTH level in the early morning is &lt; 10 pg/mL, then it is desirable to take more than one additional measurement. There can be a poor response of ACTH to the ACTH-stimulating test (less than 1.5-fold of the basal ACTH level). Be aware and cautious that the plasma level of ACTH is not always low when biologically inactive ACTH is secreted.”

Therefore, our study included patients whose plasma level of ACTH in the morning was not low, i.e., not being &lt; 10 pg/mL. In the CRH challenge test, however, plasma ACTH level was &lt; 1.5-fold the basal level in these patients. In consideration of your insightful comment, I added
information on the peak ACTH level in the CRH challenge test to Table 3 and to lines 8–13 of page 16.

Namely, we examined only patients who duly met the requisites of the Japanese guidelines mentioned above for the diagnosis of SCS, and we expect you find abovementioned descriptions satisfactory with respect to patients who poorly responded to the CRH challenge test.

Furthermore, I verified the diagnostic criteria of subclinical hypercortisolism in the article of Lee et al. [reference #10 in the revised manuscript] that you had kindly cited. I examined all of our patients as to whether or not they met the model VIII in the article--“the presence of 1-mg DST &gt;138 nmol/L (5.0 µg/dL) or 1-mg DST &gt; 61 nmol/L (2.2 µg/dL) with the presence of one parameter among low levels of ACTH (&lt;10 pg/mL) and DHEA-S (&lt; 80 µg/dL in males or &lt; 35 µg/dL in females).” Consequently, one of our patients failed to meet the new Korean diagnostic criteria of subclinical hypercortisolism. Therefore, we excluded the patient from our study.

3. The difference in adenoma size between the PA patients and the other two groups is of interest. The SCS with a diameter of 7 mm is a major outlier, and I suspect probably does not have autonomous cortisol secretion from this lesion, as defined using Lee’s criteria.

Replies: I appreciate your valuable comments. The patient in the SCS group, whose adenoma diameter was 7 mm—an outlier, could not be verified as having met the diagnostic criteria of SCS in the article of Lee et al. Therefore, I excluded the patient from the present study (see Fig. 1 of the revised manuscript). As you indicated insightfully, we could not demonstrate that the tumor of the patient did not have the autonomous cortisol secretion. I am sorry that the sentences of the submitted manuscript caused a confusion to you.

4. Overall the findings are of interest, particularly that the PASCS group have the hypokalaemia of the PA patients and the hyperglycemia and tumor size of the SCS patients. Could the authors analyse what the probability/odds ratio and sensitivity/specificity is of a PA patient having co-existing SCS based on tumor size, and what the cut point would be. Based on the appearance of figure 1, all patients with an adenoma size of greater than approx. 2.3 cm are likely to have PASCS rather than PA alone.

Replies: In response to your very valuable and shrewd comments, I examined the potential cutoff value for tumor diameter when selecting patients with PASCS (n = 12) out of the study population of patients having the features of hyperaldosteronism (n = 53, PA + PASCS). Consequently, I added the following sentences (lines 11 to 16 of page 17): “The cutoff value of 2.4 cm for tumor size seemed to produce the largest proportion of classified patients (91.0%). Furthermore, patients with PA who had a tumor size of &gt; 2.4 cm almost certainly had the elements of PASCS (specificity 100%). Namely, the sensitivity and specificity were calculated to be 58.0% and 100%, respectively, when the cutoff point for tumor diameter was set to 2.4 cm. The odds ratio for tumor diameter when comparing PA with PASCS was 0.06 (95% CI, 0.006-0.261).
Sensitivity, specificity, and percent correct classification at different cutoff values for tumor size

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<th>Cutoff values for tumor size</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<td>27%</td>
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<td>1.2 cm</td>
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Rosa Maria Paragliola (Reviewer 2):

I read with interest the paper “Clinical characterization of patients with primary aldosteronism plus subclinical Cushing's syndrome” by Yasuda and coll. The topic is interesting, but I believe that the manuscript should be extensively improved.

Replies: I appreciate your interest in our study. I extensively brushed up the entire submitted manuscript with help of a physician who has linguistic proficiency and vast experience in editing the manuscripts of medical articles. The line numbers of some sentences in the revised manuscript largely changed from those in the submitted manuscript in an attempt to appropriately respond to the pertinent comments. I highlighted the modifications or additions that I made in the revised manuscript for facilitating your verification.

I reported my comments below:

Page 5, line 14: The definition of Cushing’s syndrome is not correct. Please distinguish between ACTH-dependent and ACTH-independent CS and provide a better definition of SCS.

Replies: In response to your valuable comments, I newly cited some articles to clearly define Cushing’s syndrome and SCS in an attempt to prepare the following sentences in lines 3-13 of page 4: “Cushing’s syndrome (CS), an endocrinopathy resulting from the prolonged, excessive adrenocortical secretion of cortisol, falls roughly into the following two categories:
adrenocorticotropic hormone (ACTH)-dependent CS and ACTH-independent CS; the former includes Cushing’s disease that is primarily caused by a pituitary ACTH-secreting tumor and ectopic ACTH syndrome resulting from extrapituitary ACTH-secreting tumors (eg, bronchial carcinoid, thymic carcinoid, and pheochromocytoma) [2], while the latter is usually caused by unilateral adenomas or carcinomas that provoke the autonomous adrenal cortical secretion [3].” Subclinical Cushing’s syndrome (SCS), an ill-defined endocrine disorder leading to the ACTH-independent secretion of cortisol from an adrenal tumor that is not fully restrained by pituitary feedback [4], is known to cause hypertension, glucose intolerance, and dyslipidemia [5].”

In the revised manuscript, I highlighted the changes or additions that I made to the submitted manuscript for facilitating your verification.

Page 5, line 31: please check again the mentioned paper and provide a better description of its content.

Replies: I am sorry for having caused a confusion to you because reference #4 in the submitted manuscript (reference #8 in the revised manuscript) alone was insufficient to provide an appropriate context. This time, I cited the articles of Piaditis et al. (reference #7) and Hiraishi et al. (reference #8) that described the incidences of 12.1% and 21.1%, respectively (line 16 of page 4 to line 1 of page 5). Furthermore, Hiraishi et al. indicated that plasma ACTH level, serum DHEAS level, and plasma aldosterone concentration were significantly lower, serum potassium concentration was significantly higher, and tumor size was significantly larger in patients with PA complicated by SCS than in patients with PA alone.

Page 6, line 37: It could be useful if Authors added information about the utility of AVS on the basis of current guidelines.

Replies: In lines 15-18 of page 7, I rephrased the sentences including references [11-13] in response to your valuable comment: “Furthermore, adrenal venous sampling (AVS), whose usefulness was well documented in the Japanese and United States guidelines, was conducted to make the differential diagnosis of uni- or bilateral aldosterone hypersecretion [11-13].”

Page 6, line 40: This section is a bit confusing. In particular, the Authors have considered the results of high dose dexamethasone suppression test, specifying that “Test results were assessed in accordance with the diagnostic criteria advocated by the Japan Endocrine Society”. However, the Japan Endocrine Society (reference 6) state that “we did not adopt a high-dose DST in the new criteria for SCS”. Furthermore, can Authors add the interpretation of CRH test results for the diagnosis? Has adrenal scintigraphy been performed only for the diagnosis of SCS or also for PA in selected cases?

Replies: I’m sorry for the insufficient descriptions of the criteria in the submitted manuscript. We conducted the present study in patients who met the Japanese diagnostic criteria of SCS
described in the article of Yanase et al. [9]. I added the following sentences (line 7 of page 8 to line 1 of page 9): “Concretely, patients were required to meet the requisites 1-3) - 1) Presence of an adrenal incidentaloma; 2) Lack of characteristic features of Cushing’s syndrome; and 3) Normal basal serum cortisol levels, as well as to have either of the requisites; 4-6) - 4) The cutoff value of serum cortisol level for the diagnosis of SCS was ≥ 5 μg/dL after the 1-mg DST. 5) The cutoff value of serum cortisol level for the diagnosis of SCS was ≥ 3 μg/dL after the 1-mg DST, and at least one of “Low plasma levels of ACTH in the early morning” or “No diurnal changes in serum cortisol levels” or “Unilateral uptake on adrenal scintigraphy” or “Low serum levels of DHEAS,” the presence of “Transient adrenal insufficiency or atrophy of the attached normal adrenal cortex after removal of the adrenal tumor,” or 6) The cutoff value of serum cortisol level for the diagnosis of SCS was ≥ 1.8 μg/dL after the 1-mg DST, with the presence of “Low plasma levels of ACTH in the early morning” and “No diurnal changes in serum cortisol levels,” or the presence of “Transient adrenal insufficiency or atrophy of the attached normal adrenal cortex after removal of the adrenal tumor.” Therefore, we diagnosed patients as having SCS when meeting all the requisites 1-3) and either one of the requisites 4-6). In the present study, namely, we examined only patients who met the abovementioned criteria as patients with SCS.”

We considered that the requisite “Low plasma levels of ACTH in the early morning” was met when the basal ACTH level in the early morning was <10 pg/mL or the CRH challenge test indicated an ACTH level of <1.5-fold the basal level. The Japanese diagnostic criteria of SCS described, “If the basal ACTH level in the early morning is <10 pg/mL, then it is desirable to take more than one additional measurement. There can be a poor response of ACTH to the ACTH-stimulating test (less than 1.5-fold the basal ACTH level). Be aware and cautious that the plasma level of ACTH is not always low when biologically inactive ACTH is secreted.” Therefore, our study included patients whose plasma level of ACTH in the morning was not low, i.e., not being <10 pg/mL. In the CRH challenge test, however, the plasma levels of ACTH were <1.5-fold the basal level in these patients. In consideration of your insightful comment, I added information on the peak ACTH level in the CRH challenge test to Table 3 and to lines 8-13 of page 16. We conducted the high-dose dexamethasone suppression test in our patients to exclude ACTH-dependent Cushing’s syndrome when diagnosing SCS. I added the following sentences regarding this fact: “Moreover, the high-dose (8-mg) DST was also conducted to rule out ACTH-dependent CS.” (lines 4-5 of page 8). As you indicate, the Japanese guideline of CS published by the Japan Endocrine Society does not include the test in the diagnostic criteria; however, the guideline admits the conduct of the test when required for the diagnosis of ACTH-dependent or ACTH-independent Cushing’s syndrome.

In all of patients who had SCS or PASCS, we conducted 131I-adosterol adrenal scintigraphy to make the differential diagnosis of uni- or bilateral aldosterone hypersecretion. Therefore, I added the following sentences to the “Materials and Methods” Section (lines 2-4 of page 9): “which indicates the uni- or bilateral accumulation of the tracer, was conducted in all of patients who had SCS or PASCS to specify the laterality of the adrenal tumor.” Furthermore, we also conducted 131I-adosterol adrenal scintigraphy in only 33 of 45 patients who had PA; 12 patients with PA did not undergo adrenal scintigraphy because 1) the affected kidney had been specified by CT and AVS, and 2) SCS had been denied by hematologic tests (1-mg DST, CRH challenge test, plasma ACTH levels, serum DHEAS levels, and diurnal changes in serum cortisol levels). However, we did not reflect this information in the revised manuscript because we considered it
not essential for the present study. In addition, I added the following sentences to the “Results” Section: “¹³¹I-adosterol adrenal scintigraphy was conducted in all patients in the SCS and PASCs groups. Consequently, the unilateral accumulation of the tracer was detected in the affected adrenal gland of 12 patients each in the SCS and PASCs groups. Among two patients in the PASCs group who had bilateral adrenal tumors detected by CT, one showed the accumulation in the left adrenal gland harboring a larger tumor, but not in the right adrenal gland; another showed the accumulation in the right adrenal gland harboring a larger tumor, but not in the left adrenal gland.” (line 19 of page 14 to line 4 of page 15). We could not investigate, through ¹³¹I-adosterol adrenal scintigraphy, postsurgical hypoadrenalism that may cause abnormalities in glucose metabolism, blood pressures, and lipid metabolism. I added this point as the fourth limitation of our study (lines 3 to 6 of page 25), and we will intend to address this point in the future.

Page 9, line 14: the section should be rephrased. Please better specify the diagnostic criteria of PA after AVS.

Replies: I rephrased the sentences in response to your valuable comments as follows: “To specify the source of aldosterone hypersecretion by AVS, the following diagnostic criteria were used: 1) the laterality ratio (LR) and the contralaterality ratio (CR) calculated before and after the ACTH challenge test in reference to the Japanese guidelines of PA [11]; 2) the absolute PAC value of ≥ 14,000 pg/mL in reference to the articles of Ohmura [17] and Makita [18]; and 3) the aldosterone ratio of the right and left adrenal veins. According to the Japanese guidelines of PA [11], an LR of &gt; 4 and a CR of &lt; 1 after the ACTH challenge test were used as the cutoff values. Tumor laterality was determined based on a CR of &lt; 1 and the absolute PAC value of ≥ 14,000 pg/mL when the ACTH challenge test indicated an LR of 2 to 4 or a discrepancy occurred in tumor laterality before and after the ACTH challenge test. Since serum cortisol levels considerably differed in the adrenal veins of PASCs patients, the adrenal gland secreting cortisol predominantly was determined based on the aldosterone ratio and on the right-to-left ratio of aldosterone and cortisol in the adrenal veins in reference to the article of Hiraishi et al. [8]. Moreover, tumor laterality was determined based on the results from ¹³¹I-adosterol adrenal scintigraphy in reference to the article of Späth et al [34] and on the absolute value of PAC in reference to the articles of Funder et al. [12] and Minami et al. [13].” (line 10 of page 10 to line 6 of page 11).

Page 12, line 1: The term “Hematology” is not appropriate in this context.

Reply: I replaced “Hematology” with “Results from laboratory tests” in response to your comment (line 1 of page 14).

Page 16, line 5-8: “Of special note was the fact that the PASCs group involving both hyperaldosteronism and hypercortisolism did not show any greater increase in serum potassium concentration as compared with the PA group”. This finding is very interesting, and it should be better argued, also proposing physiopathological hypothesis.
Replies: In response to your valuable suggestion, I enriched the argument and described the potential physiopathological hypothesis as described below:

“The MRs bind both mineralocorticoids and glucocorticoids with high affinity (deoxycorticosterone = corticosterone ≥ aldosterone = cortisol) [26]. On the other hand, a cortisol-degrading enzyme--11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2)--is expressed in renal epithelial cells and regulates the binding of aldosterone to the MRs by impeding cortisol binding to the MRs through the inactivation of cortisol to cortisone [26, 27]. Namely, this physiological event explains the MR-mediated renal excretion of potassium that is enhanced by both cortisol and aldosterone. We hypothesize that the renal potassium excretion-enhancing activity is greater for aldosterone than for cortisol due to the 11β-HSD2-induced, extensive inactivation of cortisol and that the hyperaldosteronism-enhanced renal excretion of potassium in patients with PASCS becomes more apparent, with the less effect of hypercortisolism on renal potassium excretion. Zallocchi et al. [28] described that renal 11β-HSD2 activity is regulated by glucocorticoids, is downregulated following adrenalectomy, and is restored by corticosterone replacement. These findings lead us to hypothesize that 11β-HSD2 may suppress the binding of corticosteroids to the MRs almost completely in subclinical hypercortisolism or that the expression or activity of renal 11β-HSD2 may be increased in PA. However, this hypotheses require further research for its demonstration.”

Page 16, line 44: Please rephrase the sentence.

Replies: In response to your valuable comment, I rephrased the sentence for better readability as follows: “The fact that the risk for DM is augmented in PA patients with mild glucocorticoid excess has been reported [30–32].”

Other general comments:

The discussion section is substantially well written. However, the references are limited and the Authors often cite the same papers. I suggest to consult other papers about the topics debated (see especially page 17). Data about BMD are lacking in this study. The Authors should add more comments about this point in the discussion section. The studies performed by Chiodini and coll. can be useful in this context. Preoperative diagnosis of SCS is very important, in particular to establish the correct management and follow-up. I suggest to add data about the use of adrenal scintigraphy in the diagnosis of SCS, aimed to predict postsurgical hypoadrenalism. (see Ricciato MP, WJS 2014). The English language should be carefully reviewed by a native English speaker.

Replies: I increased the number of references for the entire revised manuscript (i.e., from 31 references in the submitted manuscript to 40 references in the revised manuscript), especially 4 new references in the “Discussion” Section in consideration of your valuable suggestions. Furthermore, I discussed bone metabolism as follows: “Regarding bone metabolism impairment in SCS, the risk of developing osteoporosis is enhanced by the overproduction of cortisol in SCS [37,38]. On the other hand, hyperaldosteronism is also known to increase the risk for
osteoporosis [39]. SCS and PA are the risk factors for a reduction in bone mineral density (BMD) and an increase in vertebral fracture [37–39]. In the present study, serum ALP level was significantly greater in the PASCS group than in the PA group (p < 0.01). No significant difference was found in serum ALP level between the SCS group and the PASCS group. If this ALP represents bone alkaline phosphatase (BAP), the deleterious effects of hyperaldosteronism on bone metabolism might be masked by the severe abnormalities of bone metabolism caused by hypercortisolism in patients with PASCS. However, the relevant effects are difficult to assess by means of bone metabolism markers [eg, BAP] in patients with hypercortisolism as found in SCS [37]. Unfortunately, we neither used bone metabolism markers, nor measured BMD. Therefore, we will intend to investigate these variables in the future. (line 12 of page 23 to line 5 of page 24)” In line 19 of page 14 to line 4 of page 15 of the revised manuscript, moreover, I described the results from ¹³¹I-adosterol adrenal scintigraphy that we had conducted to diagnose SCS. We are fully agree with you in that the association between adrenal scintigraphy results and postsurgical hypoadrenalism is a critical issue to investigate. Unfortunately, however, we could not address the issue in the present study. Therefore, I referred to these facts as the fourth limitation as follows: “Fourth, ¹³¹I-adosterol adrenal scintigraphy is not only useful for the diagnosis of SCS, but also is a very important imaging modality to predict postsurgical hypoadrenalism [40]. However, we could not investigate the latter.” (lines 3 to 6 of page 25).