Author's response to reviews

Title: Unilateral cause of primary hyperaldosteronism is usual and adrenal vein sampling is mandatory in the diagnosis. Results from screening to histopathology in a Swedish population

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Author's response to reviews: see over
Correspondance to the reviewers

We thank all the reviewers for their work in order to improve our manuscript. We have considered the comments and listed the detailed response below.

Referee #1

1) The most unique finding in the present study is that unilateral PA was common and unilateral adrenal hyperplasia was a half of those cases. However, there is little discussion about the issue. Young WF reviewed the pathophysiology of PA in 2007 (YoungWF. Clin Endocrinol. 2007;66:607). He referred to 6 subtype classifications of PA: describing APA (35%) and bilateral idiopathic hyperplasia (IHA: 60%) as the most common subtypes and UAH as only 2% of PA. There is a huge discrepancy and the authors should explain and discuss it.

Our response:
This is a good point and one of the main messages from the study. The prevalence numbers of the subtypes of PA Dr. William F Young refers to in his paper (and many other papers as well) are from earlier (older studies) on the subject. We do not refer to the mentioned, otherwise very good review paper of WF Young as we refer to original papers on the subject. We discuss this in the Discussion on page 13, but obviously we do not point it out clear enough.

2) The role of AVS in the differentiation of unilateral and bilateral disease has long been discussed. The authors should explain sufficiently why they concluded ‘AVS is mandatory in the diagnosis’ from the present results.

Our response:
We thank the reviewer and in order to make our point more clear we have added a sentence at the end of the 2nd paragraph of page 14 and at the end of our conclusion in the last paragraph on page 14.

3) There is no reference in the method of histopathology (page 7).

Our response:
References have been added to page 7 (#30 and #31).

- Minor Essential Revisions
In the last two sentences in Discussion (page 14), ‘high higher’ and ‘as common as PA’ may be errors in words.

Our Response:
The reviewer is correct, this has been corrected to „higher“ and „as common as APA“ respectively.

- Discretionary Revisions
The authors had better omit the result and discussion about the clinical course of patients treated with laparoscopic adrenalectomy including Table 3.

Our response:
If the editors agree with excluding this information and Table 3, we will of course do so.

Referee #2

1. There is little correspondence between the title and the conclusion of the abstract. I suspect this is caused by addressing two very different issues: optimizing screening and the pathology causing PA.

Our response:
We thank for the good comment and have added to the abstract to make the message more clear: "Imaging diagnostic procedures with CT-scans and scintigraphy were inconclusive." To the Conclusion; AVS was mandatory in diagnosis of unilateral PA.

2. In order to determine optimal cut-off levels for the screening test, one should determine ARR in patients with proven PA and in control patients with the same entry characteristic as the patients with PA, i.e. hypertension. It is wrong to use healthy controls for reference and in fact, using these values to determine cut-off values jeopardizes the value of this part of the study considerably. Moreover, medication use at the time of screening should be well-defined. Moreover a screening test should have a very high sensitivity, and this is not given for the combination of aldosterone level and ARR.

Our response:
We are aware of that the optimal cut-off level is a subject for discussion. The much discussed fine line between low renin essential hypertension and primary hyperaldosteronism may make the control group consisting of patients with hypertension conflicting. Therefore we choose the control group from healthy volunteers. Professional statistical help was consulted on this matter as all statistical work in this paper. Concerning the medication used at the time of screening we find this to be one of the things strengthening the study; the medications used at the time of screening are very well described see Table 1.
We agree that a screening test needs to have high sensitivity, but nevertheless it needs to have good specificity. The cut-off limits used have 94% sensitivity and 91% specificity that we find very acceptable. We include in our answer the method description from our professional statistician that can be included in the paper if desired:

"When a measurement is used to make a diagnosis, one method to assess its diagnostic value is by calculating the area under the receiver operating characteristic (ROC) curve. The ROC curve is a graphic display that gives a measure of the predictive accuracy of a logistic regression model. It is obtained by plotting the sensitivity versus 1 – specificity for each possible cut-off, and joining the points. A more complete description of classification accuracy is given by the area under the ROC curve (AUC). The AUC is a measure of overall diagnostic performance. It provides a measure of the model’s ability to discriminate between those subjects who experience the outcome of interest versus those who do not. The closer AUC is to 1, the better the overall diagnostic performance of the test. The practical lower limit for the AUC of a diagnostic test is 0.5. In this study we included 39 patients with primary aldosteronism and 36 individuals who do not have the disease. A logistic regression model was performed to study the relation between aldosterone level and risk of disease. In the analysis we plotted the ROC curve of Aldosterone for predicting primary aldosteronism obtaining an AUC of 0.975. A similar model was built using the ratio of p-aldosterone/p-renin as a predictor for primary hyperaldosteronism. The AUC of p-aldosterone/p-renin for predicting primary aldosteronism was 0.917. These results indicate that Aldosterone level is a better predictor of primary aldosteronism than the ratio of p-aldosterone/p-renin. From the analysis above, it is also possible to determine the best ‘cut-off’ for each measure of interest. If the ‘cost’ of a false negative result is the same as that of a false positive result, the best cut-off is that which maximizes the sensitivity and specificity. The choice of best cut-off is facilitated by plotting sensitivity and specificity versus all possible cutpoints. The “optimal” choice for a cut-off is where the sensitivity and specificity curves cross. In the analysis above the best cut-off for Aldosterone is 0.44 and for the ratio of p-aldosterone/p-renin is 1.28. A second analysis was performed including 16 patients with a very accurate diagnose and 36 individuals without the disease. The results showed again the same relation: the AUC of Aldosterone for predicting primary aldosteronism was 0.979 while the AUC for the ratio of p-aldosterone/p-renin was 0.969. The optimal cut-off for Aldosterone is 0.43 for p-aldosterone/p-renin is 1.65.
3. what is a standardized sphygmomanometer? P.5

Our response:
The term standardized sphygmomanometer has been used for the mercury sphygmomanometer in differentiation to the newer models of sphygmomanometers (ie the coming article by Parati G and Orchoa JE in J Hum Hypertension April 2012; Automated-auscultatory (Hybrid) sphygmomanometers for clinic blood pressure measurement: a suitable substitute to mercury sphygmomanometer as reference standard?)
In order to clear all misunderstanding we have changed this term to mercury sphygmomanometer on page 5.

4. the two PRA methods should be compared by Bland Altman plots, not by correlation.

Our response:
The reviewer points out one way of comparing the methods. We choose to keep the correlation calculation already done.

5. Coefficient of variation should be given for a specific PRA value (preferably a low one for this study)

6. I could not find what EA stands for.

Our response:
We thank the reviewer for his clear vision, EA should be LA (Laparoscopic Adrenalectomy) and has been corrected throughout the paper. We regret this wrong written shortening.

7. I doubt the prospective nature. It is unlikely for a carefully executed prospective study to have a this large loss-to-follow-up rate. In order to judge the value of diagnostic methods the only way to assess this is to determine the the benefit for the patients. There is only follow-up for five adrenalectomized patients and I think this number is far too low to draw these conclusions. These numbers cannot support in itself the recommendation in the title that adrenal vein sampling is mandatory.

Our response:
We are sorry to read the doubtfulness of the reviewer of the prospective execution of the study and would like to convince him with our good word. We are also very sorry for the large loss-to-follow-up rate and have described the causes in our paper. Nevertheless, it has been postulated that surgically correctable PA in the HT population is less than 1.5%, in this study we found it to be 2.6% and as high as 59% of those with confirmed PA (10 of 17 patients) as stated, Discussion, page 14, line 8 from below.
As stated in the end of the Introduction: „The aim of our study was screening for PA using locally produced cut-off limits of ARR and s-aldosterone, verify the PA with confirmatory testing and defining the aetiology by the use of computerised tomography, AVS and post-operative histopathology (PAD) when PA was found to be unilateral and surgically treated. “
The information in Table 3, on the follow-up of the patients after the adrenalectomy is therefore extra and not included in the aim of the study, and somewhat explains the few follow-up numbers given in the table. Table 3 can be excluded, as has been opposed by reviewer 1.

We find Adrenal Vein Sampling to be mandatory in order to diagnos unilateral disease. In this study it was very clear that results of imaging research (CT-scan of the adrenals) and scintigraphy were inconclusive and and therefor not helpful in deteting unilateral disease.
We agree that a better follow up of the adrenalectomized patients would have added great information. Nevertheless, we find our study to add information on screening, verification and treatment of PA.

8. The supplementary table 1 should contain data on potassium levels.
   Our response:
   Data on serum potassium levels have been added to Table 1.

9. Adrenal vein sampling is rarely available. What should a physician do if he/she has no access to AVS?
   Our response:
   Adrenal vein sampling (AVS) should be executed by an experienced radiologist, due to the anatomy of the right adrenal vein, that often causes a failing procedure. This indicates that AVS should only be performed in specialized centers. The question raised by the reviewer is therefore more than qualified. Our opinion is that in case a physician does not have access to AVS he/she should either refer the patient to a qualified center or treat the patient medically. Studies have indicated that patients with primary hyperaldosteronism may be at higher risk for target organ damages of the heart and kidney compared to patients with essential hypertension (Stowasser et al JCEM 2005, and Milliez et al J Am J College of Cardiology 2005), it is therefore desirable to find curable causes for PA.

Referee #3

Major Compulsory Revisions
Identification of the cutoffs of ARR and S-aldosterone for PA. I appreciated the willingness of establishing local cutoff values, but the sample is too small to provide solid data. Moreover, ROC curves and Youden index should be shown.
Our response:
ROC curves are easily shown and we include them below. We believe that including them in the paper does not explain more to the reader than the text already does. This can of course be changed if the editor agrees with the reviewer.

![ROC curves](image-url)
Screening for PA. It was based on S-aldosterone and ARR measured in patients also assuming antihypertensive drugs that can interfere with the renin-angiotensin system, as shown in Table 1. The second measurements of S-aldosterone and ARR after withdrawal of interfering drugs were used to confirm PA.

1. The values obtained after appropriate pharmacological wash-out (which the Authors identified as follow-up visit), not the values obtained under unrestricted drug treatment, should be used for case detection of PA. Thus, the values obtained at follow-up visit should be shown in the Table.

Our response:
This study was constructed with the aim of finding the cut-off limits for PA and use them to screen for PA in a selected hypertensive population (from specialised hypertensive units) and an unselected hypertensive population (from primary care centers). Therefor was the anti-hypertensive treatment the patient was already recieving not changed during screening. To verify the diagnosis, the pharmacological treatment was changed and all medication interfering with the Renin Angiotensin Aldosterone System stopped for 4-6 weeks (ie 6 weeks if spironolactone). Correlation calculation did on the other hand not find any difference between the serum values of aldosterone and renin before and after wash-out as stated on page 4 (Verification of PA).

2. It is not clear how long was the wash-out period before the follow-up visit. This is a crucial issue.
Our response:
This is already described on page 6, line 6 in Subjects and methods to verify PA.

3. Use of S-aldosterone in conjunction with ARR should be discussed. Use of a ‘formal’ cutoff of S-aldosterone for case detection of PA is debated, as also reported in the 2008 Guidelines.
Our response:
This is already discussed in our Introduction, line 3 from below.

4. PRA values lower than 0.2 ng/ml/h are conventionally set to 0.2 to avoid inflation of ARR. Did the Authors adopt this strategy?
Our response:
The detection values for PRA were set at 0.15 ng/ml/h as already stated on page 8, 2nd line from below in Analytical methods.

5. Was hypokalaemia corrected before measuring ARR?
Our response:
Yes, after screening and during the wash-out period hypokalaemia was corrected as described on page 6, line 3-6 in Subjects and methods to verify PA.

AVS. A rapid determination of cortisol was done in RAV before catheterization of LAV. It is of utmost relevance mention the interval time elapsing from the collection of plasma in RAV and collection in LAV. Moreover, what method was used for the rapid measurement of cortisol?

Our response:
While the cortisol levels from the RAV were beeing measured the cortisol levels from the LAV were sampled directly, thus only minutes elapsed between the sampling of the cortisol levels from the RAV and the LAV, probably less then 5 minutes in most cases. Only in the cases when the cortisol levels from the RAV were less than 10 fold increased compared to the values from the infra-renal inferior vena cava, a re-cathetarisation of the RAV was performed. In order to make this more clear the following paragraph has been added to the methods, last line page 6: „(performed in the mean-time while the cortisol level from the RAV was being measured)“.
We regret to have left out the method description of the cortosol analysis, that is now included; Analytical methods, lowest line, page 8.

Histology. The boundary between adenoma and hyperplasia can be difficult to establish. A more detailed description of the criteria used for discriminating the two forms is needed. This is a crucial issue to understand why the Authors found a high percentage of unilateral hyperplasia.
Our response:
We agree with the reviewer, not only is this a very difficult diagnosis to establish but also a one of the major findings of our study. In response to reviewer no 1 we have added references see reviewer #1 point 3. Diagnostic criteria differentiating between adenoma and hyperplasia are needed. This study uses the accepted criteria used by the Pathological department, Sahlgrenska University Hospital that we believe are used by many other hospitals. As this will inevitable cause a further debate we believe our study is of importance to everyone interested in Primary Hyperaldosteronism.

Statistical analysis.
1. Two methods were used for PRA measurement. Comparison between methods needs Bland-Altman based approach.
Our response:
The reviewer points out one way of comparing the methods. We choose to keep the correlation calculation already done.

2. How the correction factor was calculated?
Seventy-four samples were run in parallel using both methods, and there was a highly significant correlation between the two measurements (r = 0.97, p<0.0001). The first 227 PRA values were corrected for the subsequent method inorder to minimize the effect of changing methods. PRA had a within-run coefficient of variation of 8.8 % and the lower detection limit was 0.15 ng/ml/h

3. Were aldosterone levels log transformed before being analysed?
Our response:
Yes, this work was performed by the consulted statician.

4. It is not clear why a non parametric test was used also for variables with a normal distribution.
Cutoff value for s-aldosterone. The value 0.44 mmol/L is too high to be as cutoff. Please check aldosterone units throughout the text.
Our response:
We thank the reviewer for the very good comment. In spite of everything nmols/l were wrongly written mmol/l for serum levels of aldosterone in the Results, page 10, highest section. This has now been corrected throughout. On the other hand the gathered results of the ROC curves found the cut-off limit for the serum aldosterone to be 0.43 nmol/l and the ARR 1.28 (both values used). After re-checking the aldosterone values a wrongly written aldosteron value was found in Table 2, patient 2069 that has been corrected.

Identification of PA subtype. AVS was not performed in 2 of 20 PA patients with ‘negative’ CT. It is well known that very small APAs may be not detected with CT or RM. Hence, these two patients might have unilateral, not bilateral, form of PA.
Our response:
We totally agree with the reviewer patients might as well have unilaterla hyperplasia, and we have not stated otherwise. The 2 patients not undergoing AVS were diagnosed with AH due to results in the verification process as stated on page 11, line 7 from below. We admit that, if we were performing the study by now all patients would be invited further examination with AVS. We hope our study clarifies this point of view to sceptical readers.
Primary from secondary aims should be distinguished. Moreover, when describing aims, the object of the screening work-up, i.e. the local population of Sweden, should be specified.

Our response:
We believe this is well described on page 5, *Subjects and methods used for screening for PA*.

Reference for cosyntropin dose and time of infusion should be added.

Our response:
The dose of cosyntropin used in AVS and time of infusion has been a debate lately. This study is designed before the recent papers on the subject were published. Reference for the AVS also concerning the dose and time of infusion is already found in our paper (Reference 26-29).

Please add a reference for the lateralization criteria and explain why you used these criteria. Could the high per cent rate of hyperplasia be related to the choice of these criteria or the criteria adopted for case detection?

Our response:
We refer to the answer right above. We believe that further studies are needed on the dose of cosyntropin used and the time of infusion, to answer the questions raised by the referee. This cannot be answered by our study.

Minor Essential Revisions
Some references were erroneously reported. E.g.: the paper quoted in ref 15 was published in JACC, not Hypertension.

Our response:
We really regret this error that has now been corrected.