Author’s response to reviews

Title: Adrenocortical cancer: mortality, hormone secretion, proliferation and urine steroids - Experience from a single centre spanning three decades

Authors:

Jan Calissendorff (jan.calissendorff@sodersjukhuset.se)
Freja Calissendorff (freja.s.calissendorff@gmail.com)
Henrik Falhammar (henrik.falhammar@ki.se)

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Author’s response to reviews:

Dear Editor,

We would like to thank you and the reviewers for the careful review and valuable comments on our manuscript "Adrenocortical cancer: mortality, hormone secretion, proliferation and urine steroids - Experience from a single centre spanning three decades" (BEND-D-15-00023).

We have now revised the manuscript and will here make a point-to-point comment on these alterations. We have now also added a section on Availability of data. All changes to the manuscript have also been indicated in the text by highlighting. We hope that these improvements now will make this manuscript suitable for publication in BMC Endocrine Disorders.

Sincerely

Jan Calissendorff Freja Calissendorff Henrik Falhammar
Comments to Reviewer reports:

Reviewer #1: This is an interesting and well-written manuscript on the experience of a single center on ACC.

The analysis of data was carefully planned.

Our answer: Thank you!

Comments:

i. Considering the differences in childhood and adult ACC, I don't find it advantageous to include childhood cases in the analysis, as the majority of patients were adults. I therefore propose to remove childhood cases from the overall analysis, and discuss these briefly in a separate section.

Our answer: Yes we agree that ACC in children can be a disease of its own. However, we have already omitted two children who had a child version of ACC, which histologic and biochemically was not considered as a clear-cut adrenocortical neoplasm. Thus only one child out of these 50 patients had an ACC. Excluding this child would not change the overall results and therefore we argue for this child to continue to be included.

ii. Based on novel functional genomics profiling studies, ACC can be classified in two major classes: one with better, the other with worse prognosis (e.g. de Reynies et al., 2009, J Clin Oncol, Giordano et al., 2009, Clin Cancer Res). This could be discussed, as there are also ACC cases with better and worse prognosis presented in this report.

Our answer: In the revised manuscript we now mention according to suggestion the finding by de Reyniès on prognosis in the two classes of ACC depending on gene profiling.

The following has now been added in the background section, page 3-4:
“Gene profiling of tumours could differentiate and identify two types of ACC with different prognosis, and the combined expression of BUB1B and PINK1 was the best predictor of overall survival [14].”

A comment of this is also mentioned in the Discussion, page 11:

“...gene profiling [14]...”

iii. The term "concomitant pheochromocytoma" among the laboratory findings is disturbing to me. It this supported by histological data? Were the catecholamine levels so high (over 2-3x the normal) that pheochromocytoma could really be considered? I propose to revise this.

Our answer: In the revised manuscript the phrase concomitant pheochromocytoma has been altered according to suggestion.

The following is now stated on page 7-8:

“…elevation of catecholamines (urinary noradrenaline 842 nmol/L, normal <400, and plasma normetanephrine 1.3 nmol/L, normal <0.6, respectively)…”

iv. It is interesting that 2 patients (4%) had neurofibromatosis type 1, as ACC is not typical in NF1 (in contrast to pheochromocytoma). Was the diagnosis of NF1 clear, probably based on clinical parameters? The histological analysis of adrenal tumors in these NF1 cases was clear-cut?

Our answer: The NF1 diagnosis was clinical. This was also a surprise also to us. The histological analysis of adrenal tumours in these NF1 cases was clear-cut. The following has now been added in the Discussion on page 12:

“Interestingly we found two patients also had neurofibromatosis. Previously only nine patients with ACC have been found to also have neurofibromatosis in the literature and recently a novel
germline frame shift mutation (c.5452_5453delAT) in exon 37 of the NF1 gene was described in one such patient [34].

v. The figure legends Fig 3-5 with these table like format are unusual, I propose to modify these, or include them in the figures themselves.

Our answer: Figure legends of Fig 3-5 with their table like format have now in the revised manuscript been changed so they now are placed in the Figures themselves.

vi. It is not really clear why the Weiss-score could not be determined. I suppose that the histological slides are still available, and these could be analyzed for the Weiss-score even retrospectively. This would add useful further information to the manuscript.

Our answer: We do agree that reporting the Weiss score would be useful. However, the Weiss score was not reported in many of the cases and to analyse the histological slides would not be easy and would take considerable time since many of these cases are from many years ago. Thus we would prefer not to comply with this.

Reviewer #4: In this paper, the authors report on the outcome of 50 patients treated at their Institution for ACC.

The paper has the particular merit to include data on urinary Steroid profiling.

Our answer: Thank you!

Overall, the paper is written in an acceptable style and the conclusions are well supported by the results. I strongly support that the authors clearly state the limitations, where applicable, of their work. It may be perceived as a disadvantage that the paper covers an overly long period of time rendering treatment regimens and Management difficult to compare.
Our answer: As indicated by the reviewer we already comment on the limitations of the long follow-up period in the Discussion on page 12.

I have a few comments to improve the paper:

- in the abstract, I would include the number of patients in each stage and provide their prognosis to make this more meaningful

Our answer: As suggested by the reviewer we now include in the abstract data on ENSAT stage and median survival.

The following has been added in the abstract:

“In ENSAT stage II 25/48 patients had a median survival of 7.0 years (0.7 – 15.), in stage III 8/48 this was 1.9 (0.4 – 19.8) and in stage IV 15/48 it was 1.2 (0.3 – 3.6) years.”

- p 3 l 10: weight loss at present is seldomly found in ACC patients, many patients in contrast present with pain or abdominal discomfort

Our answer: We agree that symptoms can vary from those commonly seen with other malignancies such as weight loss. In the revised manuscript we have now made the alteration suggested by the reviewer.

The beginning of the sentence on page 3 has now been changed to:

“Symptoms can vary from abdominal pain and fatigue to hormonal symptoms…”

- p 3 l 22: Mitotane have -> has
Our answer: Has been changed according to suggestion.

- p 3 l 39 ff: Here citations are not completely correct. The lymph node dissection does not refer to repeat surgery but to the primary intervention.

Our answer: In the revised manuscript we have now removed the part “including lymph node dissection” together with former reference 7.

- p 4 l 24ff: The citations provided here may be perceived as outdated but I admit that there has been published little re LC-MS in urine for ACC. Maybe rephrase.

Our answer: We agree that there has been published little on LC-MS in urine for ACC. In the revised manuscript part of the sentence has now been rephrased as suggested.

The following has been added on page 4:

“…but further studies are required.”

- p 5 l 4: and other occurrences throughout the paper: etiocholanolone would be correct

Our answer: Has now been changed according to suggestion throughout the manuscript.

- p 5 l 44: Proliferation index is the preferred term

Our answer: Has now been changed according to suggestion.

- p5 l 50ff: how was catecholamine excess defined?
Our answer: In the revised manuscript the following is now added on page 7-8:

“…elevation of catecholamines (urinary noradrenaline 842 nmol/L, normal <400, and plasma normetanephrine 1.3 nmol/L, normal <0.6, respectively)…”

- p 6 l 5: why was survival calculated from time of surgery? Should be from date of diagnosis. If unavailable, please comment.

Our answer: We have chosen the date of surgery as the date of diagnosis to be sure that all biochemical samples are analysed prior to adrenalectomy and in some cases it is difficult to say exactly when the diagnosis of ACC was done. Some patients were, e.g., referred from other hospitals with a diagnosis or suspect diagnosis of ACC, which then was clarified at our centre. The date of imaging and biochemistry could of course also be used. In our experience these dates comes within weeks. Thus, we prefer date of surgery as the date of diagnosis as it is more precise and consistent.

- p 6 l 10: rephrase sentence "As well as . . ."

Our answer: The sentence on page 6 has now been rephrased as suggested to:

“All data was retrieved from the electronic medical records, and the National Population Register…”

- p 6: disease specific death should be given as a term for the Primary end Point since the description of the methods suggests this and not all cause

Our answer: To require disease specific death we would have to in detail analyze medical records from other hospitals, palliative care facilities, GPs, surgeries and hospital in the home facilities. Unfortunately we only had the medical records from the Karolinska University Hospital. There is also the The Swedish Cause of Death Registry (held by the National Board of
Health and Welfare) however we do not have ethical approval to access this or medical records from other care givers. Thus unfortunately disease specific death is unavailable for many of the patients but most can be presumed to be caused by the ACC.

- p 6: the paper has the shortcoming of not providing multivariate analyses. This may be linked to unavailability of suitable software (Graph Pad does not provide this function), however some obvious clinical parameters may be accounted for and lead to different results (e.g. Ki67 <-> stage; age might be reasonable to include as well; this is also true at other instances throughout the manuscript.

Our answer (to this and the question below about cox regression): We have examined our cohort with uni- and multivariable analyses using SPSS. We do not find this particularly useful as our cohort is quite small. Statistically the power is weak when multiple variables are used. The confidence interval is broad and with this only ENSAT 4 stands out as significant. Moreover, the first reviewer states “The analysis of data was carefully planned.” This indicates that he was happy with the current analysis. Thus we would prefer not to use these suggested analysis by reviewer nr 2.

- p 7 l 7: it is very unusual to have two patients with NF1 in a series who both develop ACC. This should be commented on.

Our answer: We agree. In the revised manuscript we have commented on this in the Discussion. The following has now been added on page 12:

“Interestingly we found two patients also had neurofibromatosis. Previously only nine patients with ACC have been found to also have neurofibromatosis in the literature and recently a novel germline frame shift mutation (c.5452_5453delAT) in exon 37 of the NF1 gene was described in one such patient [34].”
the fact that two patients are reported to have pheochromocytoma at the same time with ACC is at least very surprising. Is it possible that these were either not pheos or that pheos were misdiagnosed as ACC?

Our answer: In both patients with elevated cathecholamines the final pathology diagnosis was ACC. In the revised manuscript the sentence of patients with concomitant pheochromocytoma on page 7-8 has been altered to:

“…elevation of catecholamines (urinary noradrenaline 842 nmol/L, normal <400, and plasma normetanephrine 1.3 nmol/L, normal <0.6, respectively)…”

- p 7 l 54: was it laboratory evidence of Primary aldosteronism? 15% is quite high for ACC.

Our answer: The following has now been added on page 8:

“(all had more than double the upper reference limit of plasma aldosterone and urinary aldosterone with hypokalaemia and low renin).”

- p 7 l 56: was the Patient with elevated testosterone male?? Should be provided. Low testosterone in my opinion does not contribute to the diagnosis of ACC. This is not helpful for the reader

Our answer: The patient was a woman. This has now been clarified in the manuscript with the level. We agree that low testosterone does not contribute to the ACC diagnosis. We have now omitted the sentence about low testosterone

The following has also been added on page 8:

“…, a woman, … (14 nmol/L, normal <3).”
The following sentence has also been altered to:

“In all,…”

- p 8 l 10: the patients with missing values should be excluded before reporting abnormal/normal lab.

Our answer: The patients with missing values have already been excluded before reporting abnormal/normal lab as suggested by the reviewer.

- p 8 l 51: doxorubicin would be correct

Our answer: Has now been changed according to suggestion throughout the manuscript.

- p 9 l 19: Mortality -> Survival

Our answer: Has now been changed according to suggestion.

In this entire paragraph, adjustment for above mentioned factors in a Cox proportional hazards model may much improve the results and strengthen the viewpoint of the authors that USP significantly adds to the Management of ACC.

Our answer: Please see our answer above. Moreover only 5 patients had normal USP making it difficult to adjust for this.

- p11 l 53: the steroid panel reported here has yet been proposed for diagnostic purposes. How do the authors imagine it could impact on prognosis? It may be a tool to discover recurrence . . .
Our answer: We agree that it could be used as a tool to discover recurrence and is in fact also used in this capacity by us and others. We have already discussed that a pathological USP may have a worse prognosis.

The following has now been added on page 12:

"USP could also potentially be used as a tool to discover recurrence."

Authors’ own corrections: Some minor linguistic corrections have been done in the revised manuscript, all highlighted.