Reviewer's report

**Title:** The Renin-Angiotensin-Aldosterone system in patients with depression compared to controls - a sleep-endocrine study

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**Reviewer:** Florian Chapotot

**Level of interest:** A paper of considerable general medical or scientific interest

**Advice on publication:** Unable to decide on acceptance or rejection until the authors have responded to the compulsory revisions

The manuscript entitled The Renin-Angiotensin-Aldosterone system in patients with depression compared to controls - a sleep-endocrine study submitted by MURK H, HELD K, ZIEGENBEIN M, KUNZEL H, KOCH K and STEIGER A presents the results of a study comparing sleep and HPA-RAA endocrine changes in depressed patients versus healthy controls. The authors partly confirmed in depression the previously described hypercortisolemia and they further described during sleep an increased aldosteronemia in depressed patients compared to controls. This finding, sign of an increased activity of the RAA system in depression, has never been described precisely. The collection of data seems correct but the analysis and presentation need compulsory revisions. This is an interesting paper and the results of the study could be clinically significant. However, the quality of the paper could be improved significantly by addressing the points below before publication.

1. The question investigated by the authors is new, only two previous reports based on infrequent hormonal measurements being available on the considered topic.

1.1 Compulsory: From the literature, the authors reported that depression is accompanied by HPA axis overactivity and sleep-EEG changes and that the RAAS is closely related to sleep and HPA axis activity. In this study, the authors just focus on the RAAS in connection with the HPA system activation and sleep EEG in depressed patients compared to controls. However, from the information provided in the introduction, one may expect putative changes of the RAAS in depression, toward either an increase or a decrease. Rather than to prescribe a blind exploration, it is asked that the question posed by the authors be more precisely defined by stating a clear hypothesis in conformity with the literature on sleep-endocrine changes related to depression.

2. The study design and the methods sound appropriate to evaluate concomitant sleep-endocrine activities but not enough details are given in the manuscript to replicate the study.
2.1 Discretionary: In addition to age, it is suggested to provide the body-mass index for each group of subjects and patients.

2.2 Compulsory: Because endocrine systems are very sensitive to environmental changes, climatic conditions in the sleep chambers must be documented (ambient temperature, light intensity). In addition, access to food and water must have been controlled. This aspect must be described in the manuscript, in terms of schedule, quantity and frequency, and discussed if necessary since differences in fluid intake between depressed patients and controls may introduce a bias in the evaluation of hydromineral metabolism.

2.3 Discretionary: Considering that sleep is likely to be outside the field of expertise of the majority of the BMC Psychiatry readership, the introduction needs to provide a better overview of the neurophysiological processes thought to underlie sleep and sleep EEG activity, as well as of their typical changes in depression. A very limited amount of information is resumed in the first sentence of the third paragraph of the introduction and this should be expanded.

2.4 Compulsory: The procedures and methods for PSG recording (electrode sites, fixation technique, montage, recording apparatus, sampling rate, amplification and filtering settings) and analysis (R&K sleep-wake stage scoring: blinding, epoch length, sleep parameter description) need to be described precisely, as well as the methods for blood sampling (use of a peristaltic pump, heparinisation, centrifugation, quantity of blood removal) and hormonal measurement (for each hormone: reference of the RIA kit, detection limit, CV). As a general rule, each time a commercial product is listed in the manuscript, the authors should provide a reference for this product indicating its brand name, model, distributor and distributor country. Similarly, if the investigation used home-made devices or methods, their technical specifications must be indicated in the manuscript.

2.5 Compulsory: It is not indicated how night sleep quality was monitored and evaluated. It is possible that some of the subjects and patients did not get a good night sleep. Sleep efficiency must be calculated and indicated in the manuscript to confirm this aspect.

2.6 Compulsory: The authors must indicate the software used for statistical analysis and give a reference for each method (MANOVA, cross-correlation). What was the method used to compute cross-correlation coefficient and their significance (on individual or group data, parametric or non-parametric estimation, with or without temporal lag between series)? Were the cross-correlation coefficients homogeneous between subjects and groups to allow for coefficient averaging and group comparison?

2.7 Discretionary: It is suggested to the authors to separate the Methods paragraph in different sub-paragraphs such as for example Subjects, Experimental design, PSG recording and analysis, Blood sampling and hormonal measurement, Statistical analysis.

3. The data lost significance since the pathognomonic sleep alterations classically described in depression are not confirmed from the study presented by the authors. The sample heterogeneity and the small number of subjects may account for this discrepancy. However, this aspect does not preclude the publication of the paper since it is clearly stated and discussed in the manuscript that the observed results concerned the study sample only.

3.1 Discretionary: It is suggested to apply the decomposition in two half-nights to the analysis of the selected sleep parameters (all but latencies), as it was done with hormonal measures. If this has already been tested by the authors but didn't bring significant results, it should be mentioned in the manuscript.

3.2 Discretionary: As a suggestion to the authors, the use of a quantitative EEG analysis (power
spectral analysis of EEG slow-wave activity in the delta frequency range) or of a larger sample may help improving the findings of the study.

3.3 Discretionary: A figure illustrating the principal result of the study (mean aldosterone night-sleep profiles in depressed patient vs controls) should make the manuscript more attractive.

4. The manuscript adheres to the relevant standards for reporting and data deposition.

4.1 Discretionary: The study has been conducted by the investigators in respect with the ethical concerns, however, even if this is included in the Declaration of Helsinki, it should be stated in the Methods that a written informed consent has been obtained from each participant of the study before enrolment.

5. The discussion and conclusion are well balanced and are adequately supported by the data except for the aspect detailed below.

5.1 Compulsory: The discussion concerning cross-correlations between hormone concentrations in controls and depressed patients must be entirely revised after a strict methodology is applied to this complex analysis. From the few details given in the Methods paragraph, it is impossible to assess the validity of these results.

6. The title and abstract accurately convey what has been found.

7. The writing of the manuscript needs revisions as indicated below.

7.1 Abstract: In the first sentence, introduce the abbreviation for hypothalamus-pituitary-adrenocortical axis by replacing HPA-axis by hypothalamus-pituitary-adrenocortical (HPA) axis. Replace sleep-EEG by sleep EEG. Replace mineralocorticoid-agonistic stimulation by mineralocorticoid agonistic stimulation. In the sentence as 1. Adrenocorticotropic hormone (ACTH) ... , the A of Adrenocorticotropic must be lower case. Replace nonREM by NREM. Replace REM-nonREM cycle by REM-NREM cycle. Use +/- throughout the manuscript and not +/-.

Background:

7.2 Page 3: In the first sentence of the first paragraph, introduce the abbreviation for hypothalamus-pituitary-adrenocortical system by replacing hypothalamus-pituitary-adrenocortical system by hypothalamus-pituitary-adrenocortical (HPA) system. In the second sentence of the first paragraph, introduce the abbreviation for adrenocorticotropic hormone by replacing ACTH by adrenocorticotropic hormone (ACTH).

7.3 In the sentence of the first paragraph It has been suggested that the pathophysiology of the hypercortisolism is related to a change in function of the mineralocorticoid-receptors (MR), but both decreased [10] and increased function [11] has been reported, it is not clear what the authors suggested by increased or decreased MR function since both studies [10,11] reported an increased cortisol secretion after MR blockade. It is suggested to rewrite this sentence avoiding confusion for the readers. In addition, the term mineralocorticoid-receptors (MR) must be replaced by mineralocorticoid receptors (MR).

7.4 It is not necessary to abbreviate short words such as aldosterone. Using the term aldosterone
instead of its abbreviation aldo would appear more pleasant.

7.5 It would be advantageous to provide a reference of a review covering the physiological regulation of the renin-angiotensine-aldosterone system.

7.6 The sentence Spironolactone, a MR agonist, ... must be replaced by Spironolactone, a MR antagonist, ... !!!

7.8 The sentence These findings suggest an inhibitory action of peripheral MR blockade on HPA-system activity is very unclear and contradictory with what one should logically conclude from the information previously given. Indeed, the references provided by the authors indicate that the inhibitory action caused by MR antagonists affects the negative feedback loop and thus activates the HPA axis. These sentence must be rewritten replacing the term blockade by the term stimulation, for example, to stay in line with the idea of the authors. In addition, the term HPA-system must be replaced by HPA system. This stands throughout the complete manuscript.

7.9 The sentence Angiotensin II has a direct ... in the first paragraph must be replaced by Angiotensin II (ATII) has a direct ...

7.10 Page 4: In the second paragraph of the introduction, replace the term dexamethasone by its abbreviation Dex. It is also suggested not to abbreviate this word which doesn't appear frequently in the manuscript.

7.11 In the third paragraph of the introduction, replace the term slow-wave-sleep (SWS) by slow-wave sleep (SWS).

7.12 Methods, page 6: The sentence Values are expressed as mean +/- standard deviation (SD) should be replaced by All values are expressed as mean +/- standard deviation (SD). In addition, since it is more conventional, it is suggested to expressed the results as mean +/- standard error of the mean (SEM).

7.13 Results: In the first paragraph, the term sleep-EEG parameter must be replaced by the term sleep parameter as sleep EEG parameters are obtained by the use of a quantitative EEG signal analysis, which is not the case of the present study. This stands for the entire manuscript were the expression sleep EEG refers to polysomnography.

7.14 The hypothesis tested in the third paragraph using cross-correlation between hormone concentrations must be introduced earlier in the manuscript, the detailed methodology of this analysis being ideally described in the Methods paragraph and the results only given in the Results paragraph.

Discussion:

7.15 Page 7: In the third paragraph, the term sleep-EEG changes must be replaced by the term sleep changes for the same reason as cited above.

7.16 The term i.e. must be preceeded and followed by a comma.

7.17 In the sentence However, we have no evidence for the occurrence of atypical depressive features in our population, in fact 5 of the patients reported a decreased appetite at the beginning of the hospitalisation, it is not clear what the authors mean.

7.18 Page 8: The last sentence of the fourth paragraph is repeated twice.
7.19 Reference 31 and 32 doesn't correspond to the original article or review covering the indicated topics. It is suggested to find two more adequate references.

7.20 Authors' contributions, page 10: KC does not figure in the authors' list. We suppose it is KK.

7.21 Acknowledgements: It is suggested to replace I by The authors.

7.22 References: Check them thoroughly while taking account of the guidelines for authors of the journal. In references 4, 6, 7, 13, 28, there are some supernumerary spaces in the page numbers. Abbreviated journal names does not require dot (in ref 3: J Affect.Disord. 1989, must be replaced by J Affect Disord 1989, ; in ref 15: J Clin Endocrinol & Metab must be replaced by J Clin Endocrinol Metab ; in ref 16: J Psychiatr.Res. must be replaced by J Psychiatr Res ; in ref 18: Front Neuroendocrinol. must be replaced by Front Neuroendocrinol ; in ref 23: Sleep Research Online must be replaced by Sleep Res Online ; in ref 35: Fed.Proc. must be replaced by Fed Proc ).

7.23 Table 2: The term REM-density must be replaced by REM density. The term REM-latency must be replaced by REM latency.

**Competing interests:**

None declared.