PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Neuropsychiatric and cardiometabolic comorbidities in patients with previously diagnosed Cushing's disease: a longitudinal observational study.
AUTHORS	Dimopoulou, Christina; Geraedts, Victor; Stalla, Günter; Sievers, Caroline

VERSION 1 - REVIEW

REVIEWER	Susan Webb Endocrinology Hospital Sant Pau UAB Barcelona, Spian
REVIEW RETURNED	29-Sep-2014

GENERAL COMMENTS	This is the description of a declaration of interest to perform a clinical research study. So there are no Results yet or Discussion. I have the following observations: Page 1 line 42: radiological, do you mean irradiation therapy? Radiological examination doesn't seem to fit in the sentence. The same on Page 3 line 8 and line 15. Page 2 line 41: What is meant a.o.? Page 2 line 58: Add … we deem 20 patients to BE more… Page 2, lines 51-51: Since this is a protocol, how can the authors select a priori the patients for sleep EEG; cognition and MRI if they have not yet evaluated if there is a clinical indication as stated? This is not clear to me, since it would seem that all patients would be candidates, no? Page 3, line 9-13: Also here how can they select patients at risk or DXA, since it would seem that all having suffered chronic endogenous hypercortisolism would be candidates? This is not a scientific mnauscript but the description of a declaration of interest to perform a clinical research study. This is the description of a declaration of interest to perform a clinical research study. So there are no Results yet or Discussion. I have the following observations: Page 1 line 42: radiological, do you mean irradiation therapy? Radiological examination doesn't seem to fit in the sentence. The same on Page 3 line 8 and line 15. Page 2 line 41: What is meant a.o.? Page 2 line 58: Add … we deem 20 patients to BE more… Page 2, lines 51-51: Since this is a protocol, how can the authors select a priori the patients for sleep EEG; cognition and MRI if they

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REVIEWER	Giorgio Arnaldi Division of Endocrinology Ancona Hospital Ancona - ITALY
REVIEW RETURNED	30-Nov-2014

GENERAL COMMENTS	The aim of this study is to evaluate the effect of cortisol normalization on long-term neuropsychiatric and cardiometabolic comorbidities in patients with Cushing's Disease (CD). Although only partially original, the study could represent an useful addition to the literature on this important topic. The study design is well planned and the methods are appropriate. I also have specific comments: a) Population and sample size. The authors state that based on power calculation, 20 patients are sufficient for the study-targets. However, the study would be much more accurate if the authors would focus solely on patients treated with hypocortisolemic drug (pasireotide or other) or on patients in remission after surgery. In my opinion, the overall population is heterogenous and many confouders are present. In fact, a succesfull surgery require often a long-term glucocorticoid replacement therapy and pituitary radiotherapy could cause a direct neuropsychiatric damage. In addition, there is a typo in the text: "...after surgical, before/under/after radiological (?) and/or under current medical treatment". The term "radiological" should be changed to "radiotherapy". b) Bone metabolism and osteoporosis. Although osteoporosis is a frequent and important complication of hypercortisolism, I am surprised that DXA is scheduled only in a subset of patients. In addition, considering that all are CD patients, it is not clear why the authors include the long-term glucocorticoid therapy in the clinical conditions needed to perform a DXA. Furthermore, successful surgery for CD leads to transient secondary adrenal insufficiency and in most cases adrenal responsiveness is restored to normal over a period of several months to a year. Are these patients excluded from the DXA evaluation? I think no. I also suggest to include in the study, the use of FRAX algorithm with bone mineral density in active CD patients to identify patients at high fracture risk as recently proposed by our group (Trementino L, Ceccoli L, Concettoni C, Marcelli G, Michetti G, Boscaro M, Arnaldi G. Fracture risk assessment before and after resolution of endogenous hypercortisolism: Is the FRAX(®) algorithm useful? J Endocrinol Invest. 2014 Oct;37(10):957-65. doi: 10.1007/s40618-014-0126-1. Epub 2014 Jul 11. PubMed PMID: 25012159) The authors state that "Recent guidelines underline the importance of detailed fracture risk assessment in patients with CD by

	assessment of bone mineral density by dual X-ray absorptiometry (DEX)" but the citation number 15 is inaccurate. In fact, the reference quoted is based on exogenous glucocorticoid-induced osteoporosis and it is unclear whether such recommendations may be adopted to patients with endogenous hypercortisolism. At the moment, there are no available guidelines for the management of osteoporosis induced by endogenous hypercortisolism. On the contrary, there are expert opinions and suggestions recently published that I suggest to include in the protocol: 1) Scillitani A, Mazziotti G, Di Somma C, Moretti S, Stigliano A, Pivonello R, Giustina A, Colao A; ABC Group. Treatment of skeletal impairment in patients with endogenous hypercortisolism: when and how? Osteoporos Int. 2014 Feb;25(2):441-6. doi: 10.1007/s00198-013-2588-y. Epub 2013 Dec 6. PubMed PMID: 24311114. 2) Arnaldi G, Mancini T, Tirabassi G, Trementino L, Boscaro M. Advances in the epidemiology, pathogenesis, and management of Cushing's syndrome complications. J Endocrinol Invest. 2012 Apr;35(4):434-48. Review. PubMed PMID: 22652826. 3) Tóth M, Grossman A. Glucocorticoid-induced osteoporosis: lessons from Cushing's syndrome. Clin Endocrinol (Oxf). 2013 Jul;79(1):1-11. doi: 10.1111/cen.12189. Epub 2013 Apr 13. Review. PubMed PMID: 23452135. Neuropsychiatric alterations. I suggest to add the following paper recently published on the topic: 1) Crespo I, Esther GM, Santos A, Valassi E, Yolanda VG, De Juan-Delago M, Webb SM, Gómez-Ansón B, Resmini E. Impaired decision-making and selective cortical frontal thinning in Cushing's syndrome. Clin Endocrinol (Oxf). 2014 Dec;81(6):826-33. doi: 10.1111/cen.12564. Epub 2014 Sep 1. PubMed PMID: 25052342. 2) Resmini E, Santos A, Gómez-Anson B, López-Mourelo O, Pires P, Vives-Gilabert Y, Crespo I, Portella MJ, de Juan-Delago M, Webb SM. Hippocampal dysfunction in cured Cushing's syndrome patients, detected by (1) H-MR-spectroscopy. Clin Endocrinol (Oxf). 2013 Nov;79(5):700-7. doi: 10.1111/cen.12224. Epub 2013 May 27. PubMed PMID: 23594250. 3) Nelson LM, Forsythe A, McLeod L, Pulgar S, Maldonado M, Coles T, Zhang Y, Webb SM, Badia X. Psychometric evaluation of the Cushing's Quality-of-Life questionnaire. Patient. 2013;6(2):113-24. doi: 10.1007/s40271-013-0012-5. PubMed PMID: 23575965. Page 2, line 41: The paper by Dimopoulou et al EJE 2014 was already published. Please quote it: Dimopoulou C, Athanasoulia AP, Hanisch E, Held S, Sprenger T, Toelle TR, Roemmler-Zehrer J, Schopohl J, Stalla GK, Sievers C. Clinical characteristics of pain in patients with pituitary adenomas. Eur J Endocrinol. 2014 Nov;171(5):581-91. doi: 10.1530/EJE-14-0375. Epub 2014 Aug 12. PubMed PMID: 25117460.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Susan Webb

This is the description of a declaration of interest to perform a clinical research study. So there are no Results yet or Discussion. I have the following observations:

1. Page 1 line 42: radiological, do you mean irradiation therapy? Radiological examination doesn't

seem to fit in the sentence. The same on Page 3 line 8 and line 15.

Thank you for your comment. “Radiological” has been replaced by “irradiation therapy” throughout the whole manuscript.

2. Page 2 line 41: What is meant a.o.?

Thank you for your comment. A.o. was a typographical error and has been deleted from the text.

3. Page 2 line 58: Add … we deem 20 patients to BE more…

Thank you for your comment. We have modified the text accordingly (page 3, line 1).

4. Page 2, lines 51-51: Since this is a protocol, how can the authors select a priori the patients for sleep EEG; cognition and MRI if they have not yet evaluated if there is a clinical indication as stated? This is not clear to me, since it would seem that all patients would be candidates, no?

We thank the reviewer for her comment and apologise for not clarifying this point. Indeed, all patients with CD are potential candidates for performing sleep EEG, cognition testing and functional MRI studies. Clinical indication was set upon patient evaluation within the cross-sectional part of the study (part I). We have modified the text accordingly.

5. Page 3, line 9-13: Also here, how can they select patients at risk or DXA, since it would seem that all having suffered chronic endogenous hypercortisolism would be candidates?

We thank the reviewer for this comment and apologise for this mistake. This point has been also underlined by reviewer 2. It is common knowledge that osteoporosis is a frequent and important complication of hypercortisolism, this is why - as suggested by both reviewers - ALL CD patients included in this study will undergo DXA evaluation. Additionally, fracture risk within the study population will be assessed by FRAX algorithm. We have modified the text accordingly and included the recent work by Trementino et al. 2014 in the reference list (page 4, lines 8-12).

Reviewer 2: Giorgio Arnaldi

The aim of this study is to evaluate the effect of cortisol normalization on long-term neuropsychiatric and cardiometabolic comorbidities in patients with Cushing’s Disease (CD). Although only partially original, the study could represent a useful addition to the literature on this important topic. The study design is well planned and the methods are appropriate. I also have specific comments:

1. Population and sample size. The authors state that based on power calculation, 20 patients are sufficient for the study-targets. However, the study would be much more accurate if the authors would focus solely on patients treated with hypocortisolemic drug (pasireotide or other) or on patients in remission after surgery. In my opinion, the overall population is heterogenous and many confouders are present. In fact, a succesfull surgery require often a long-term glucocorticoid replacement therapy and pituitary radiotherapy could cause a direct neuropsychiatric damage. In addition, there is a typo in the text: “...after surgical, before/under/after radiological (?) and/or under current medical treatment”. The term “radiological” should be changed to “radiotherapy”.

Thank you for your comment. The reviewer is right that is a rather heterogenous study population. Regarding glucocorticoid replacement therapy, all patients will be evaluated under optimal substitution therapy. If possible – dependent on the included patient numbers- we will move on to perform a subgroup analysis e.g. in patients after TSS, patients after radiotherapy etc. in order to rule out this

confounder. 20 is the minimum patient number.
“Radiological” has been replaced by “irradiation therapy” throughout the whole manuscript.

2. Bone metabolism and osteoporosis.

Although osteoporosis is a frequent and important complication of hypercortisolism, I am surprised that DXA is scheduled only in a subset of patients. In addition, considering that all are CD patients, it is not clear why the authors include the long-term glucocorticoid therapy in the clinical conditions needed to perform a DXA. Furthermore, successful surgery for CD leads to transient secondary adrenal insufficiency and in most cases adrenal responsiveness is restored to normal over a period of several months to a year. Are these patients excluded from the DXA evaluation? I think no. I also suggest to include in the study, the use of FRAX algorithm with bone mineral density in active CD patients to identify patients at high fracture risk as recently proposed by our group (Trementino L, Ceccoli L, Concettoni C, Marcelli G, Michetti G, Boscaro M, Arnaldi G. Fracture risk assessment before and after resolution of endogenous hypercortisolism: Is the FRAX(®) algorithm useful? J Endocrinol Invest. 2014 Oct;37(10):957-65. doi: 10.1007/s40618-014-0126-1. Epub 2014 Jul 11. PubMed PMID: 25012159)

We thank the reviewer for this useful comment and apologise for this mistake. It is common knowledge that osteoporosis is a frequent and important complication of hypercortisolism, this is why - as suggested by the reviewer - ALL CD patients included in this study will undergo DXA evaluation. Additionally, fracture risk within the study population will be assessed by FRAX algorithm. We have modified the text accordingly and included the recent work by Trementino et al. 2014 in the reference list (page 4, lines 8-12).

3. The authors state that “Recent guidelines underline the importance of detailed fracture risk assessment in patients with CD by assessment of bone mineral density by dual X-ray absorptiometry (DEX)”, but the citation number 15 is inaccurate. In fact, the reference quoted is based on exogenous glucocorticoid-induced osteoporosis and it is unclear whether such recommendations may be adopted to patients with endogenous hypercortisolism. At the moment, there are no available guidelines for the management of osteoporosis induced by endogenous hypercortisolism. On the contrary, there are expert opinions and suggestions recently published that I suggest to include in the protocol:

1) Scillitani A, Mazziotti G, Di Somma C, Moretti S, Stigliano A, Pivonello R, Giustina A, Colao A; ABC Group. Treatment of skeletal impairment in patients with endogenous hypercortisolism: when and how? Osteoporos Int. 2014 Feb;25(2):441-6. doi: 10.1007/s00198-013-2588-y. Epub 2013 Dec 6. PubMed PMID: 24311114.
2) Arnaldi G, Mancini T, Tirabassi G, Trementino L, Boscaro M. Advances in the epidemiology, pathogenesis, and management of Cushing's syndrome complications. J Endocrinol Invest. 2012 Apr;35(4):434-48. Review. PubMed PMID: 22652826.
3) Tóth M, Grossman A. Glucocorticoid-induced osteoporosis: lessons from Cushing's syndrome. Clin Endocrinol (Oxf). 2013 Jul;79(1):1-11. doi: 10.1111/cen.12189. Epub 2013 Apr 13. Review. PubMed PMID: 23452135.

Thank you for your comment. The reviewer is right, reference 15 concerns exogenous glucocorticoid-induced osteoporosis and not osteoporosis caused by endogenous hypercortisolism and has been therefore removed from the reference list. Instead, we have added expert opinions and suggestions 1-3 to the study protocol and modified the text accordingly (page 4, lines 36-39).

4. Neuropsychiatric alterations. I suggest to add the following paper recently published on the topic:

1) Crespo I, Esther GM, Santos A, Valassi E, Yolanda VG, De Juan-Delago M, Webb SM, Gómez-

Ansón B, Resmini E. Impaired decision-making and selective cortical frontal thinning in Cushing's syndrome. Clin Endocrinol (Oxf). 2014 Dec;81(6):826-33. doi: 10.1111/cen.12564. Epub 2014 Sep 1. PubMed PMID: 25052342.

2) Resmini E, Santos A, Gómez-Anson B, López-Mourelo O, Pires P, Vives-Gilabert Y, Crespo I, Portella MJ, de Juan-Delago M, Webb SM. Hippocampal dysfunction in cured Cushing's syndrome patients, detected by (1) H-MR-spectroscopy. Clin Endocrinol (Oxf). 2013 Nov;79(5):700-7. doi: 10.1111/cen.12224. Epub 2013 May 27. PubMed PMID: 23594250.

3) Nelson LM, Forsythe A, McLeod L, Pulgar S, Maldonado M, Coles T, Zhang Y, Webb SM, Badia X. Psychometric evaluation of the Cushing's Quality-of-Life questionnaire. Patient. 2013;6(2):113-24. doi: 10.1007/s40271-013-0012-5. PubMed PMID: 23575965.

We thank the reviewer for his suggestions, which have been added to the study protocol.

5. Page 2, line 41: The paper by Dimopoulou et al EJE 2014 was already published. Please quote it: Dimopoulou C, Athanasoulia AP, Hanisch E, Held S, Sprenger T, Toelle TR, Roemmler-Zehrer J, Schopohl J, Stalla GK, Sievers C. Clinical characteristics of pain in patients with pituitary adenomas. Eur J Endocrinol. 2014 Nov;171(5):581-91. doi: 10.1530/EJE-14-0375. Epub 2014 Aug 12. PubMed PMID: 25117460.

Thank you for your comment. The paper by Dimopoulou et al EJE 2014 has been quoted in the reference list.