Author's response to reviews

Title: Oxidative stress mediated arterial dysfunction in patients with obstructive sleep apnoea. Effect of continuous positive airway pressure treatment

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Author's response to reviews: see over
To the Editor

Please, find enclosed a carefully revised version of our manuscript. The manuscript has been extensively revised taking in to consideration all the comments, questions, criticisms and suggestions of the 4 Reviewers. We have also better ranked the strength of the evidence supporting the individual points discussed. A point to point reply to the criticisms of the Reviewer has been prepared and appropriate changes have been done in the text. All changes have been highlighted in red.

Yours sincerely

Francesco Angelico

1. Reviewer's report

Title: Oxidative stress mediated arterial dysfunction in patients with obstructive sleep apnoea. Effect of continuous positive airway pressure treatment

Version: 2 Date: 25 September 2011

Reviewer: Seockhoon Chung

Reviewer's report:

Major Compulsory Revisions

This article is a well-written and well-designed study. However, there are several issues which should be discussed.

1. Unattended NPSG (home PSG) and home nCPAP titration by using an automated pressure setting device are ashould be considered as a major limitation of this study. The severity of OSAS in this study dependes on the result of home PSG, however, the reliability of home PSG is usually thought to be low. And CPAP titration should be done by using the attended NPSG rather that home auto-PAP device.

We acknowledge that the "gold standard" diagnostic test for OSA is a PSG performed in a sleep laboratory. Therefore, we have stressed in the “Discussion” that home monitoring is a major limitation of our study. However, when properly utilized and operated by skilled professionals, unattended portable recording for the assessment of OSA is an acceptable alternative to standard polysomnogram, especially in patients with a high pretest probability of OSA. Moreover, current literature indicates that evaluation of OSA should be by clinical evaluation and overnight monitoring, either by attended PSG or by portable unattended home monitoring under qualified supervision. Indeed, PSG and home sleep tests use the same respiratory equipment, pulse oximetry equipment, and movement and position sensors and data generated from each test is analyzed in the same manner. Home unattended PSG has also the ability to record in a natural sleep environment and patients are tested in the comfort and privacy of their home. Multiple studies have demonstrated excellent correlation between the results of PSG and home monitoring. Studies published in recent years confirmed the performance and efficacy of home studies in the clinical diagnosis and management of sleep apnea and documented acceptable sensitivity and specificity of tests, a high degree of patient acceptance and low failure rates, as well as comparable outcomes to PSG. Finally, home unattended PSG in studies published in high rank journals (see The Sleep Heart Health Study in Diabetes Care, 2008;31:1001-1006). We have added reference no. 50.
2. Just 10 patients were compliant to CPAP treatment over a 6 months. Small number of CPAP patients also should be presented as a limitation of this study. The CPAP trial study was planned and performed in the first 10 consecutive patients with severe OSA who complained to CPAP treatment over a period of six months. Patient selection has been better described in the “Methods”. The small number of patients in the CPAP study has been indicated in the “Discussion” as a limitation of the study.

3. CPAP-related data must be added.
   1) How many patients were done the CPAP titration?
   CPAP titration was performed in all patients with severe OSA

2) How many patients used nCPAP after nCPAP titration?
   All patients with severe OSA started nCPAP therapy

3) The authors presented "adherence to CPAP was defined as > 4hours". However, the author should define the compliance rate, and the compliance rate to CPAP should be presented. 
   EX: CPAP > 4 hours in all days? nCPAP > 4 hours just in nCPAP using day? 
   Adherence to CPAP was defined as nCPAP use for at least 4 hours per night and 5 days per week. These criteria have been detailed in the “Methods”.

Others
1) Title of this article should be "one sentence"
   As suggested, we have combined the two sentences in one sentence.

2) Abstract - Background
   Several studies suggest an increase of oxidative stress and a reduction of endothelial dysfunction in OSAS --> "reduction of endothelial dysfunction" is it right?
   We made a mistake. The sentence has been corrected in the “Abstract – Background”.

3) The aim of the authors was to assess the association of OSAS with endothelial dysfunction and oxidative stress in a "large sample" of patients...
   However, the number of enrolled subjects was small.
   We removed the word “large” from the text.

Level of interest: An article of importance in its field

2. Reviewer’s report
   Title: Oxidative stress mediated arterial dysfunction in patients with obstructive sleep apnoea. Effect of continuous positive airway pressure treatment
   Version: 2 Date: 4 October 2011
   Reviewer: Konstantinos Kostikas

Reviewer’s report:
The study de Ben Maria and colleagues deals with the always interesting topic of oxidative stress and endothelial dysfunction in patients with obstructive sleep apnea (OSA) syndrome. However, the study is not original and several of the
findings of this manuscript have been previously reported. My specific comments are the following:

Major Comments

• The study does not have a clear aim and several parameters and correlations have been evaluated. The presentation of the results is not clear also. I would suggest the use of subtitles to help the reader identify the different parts of the study analyses.

• The use of unattended polysomnography and unattended home CPAP titration are, at least to my point of view, unacceptable in research studies that evaluate biomarkers for OSA and the effects of CPAP treatment.

We acknowledge that the "gold standard" diagnostic test for OSA is a PSG performed in a sleep laboratory. Therefore, we have stressed in the “Discussion” that home monitoring is a major limitation of our study. However, when properly utilized and operated by skilled professionals, unattended portable recording for the assessment of OSA is an acceptable alternative to standard polysomnogram, especially in patients with a high pretest probability of OSA. Moreover, current literature indicates that evaluation of OSA should be by clinical evaluation and overnight monitoring, either by attended PSG or by portable unattended home monitoring under qualified supervision. Indeed, PSG and home sleep tests use the same respiratory equipment, pulse oximetry equipment, and movement and position sensors and data generated from each test is analyzed in the same manner. Home unattended PSG has also the ability to record in a natural sleep environment and patients are tested in the comfort and privacy of their home. Multiple studies have demonstrated excellent correlation between the results of PSG and home monitoring. Studies published in recent years confirmed the performance and efficacy of home studies in the clinical diagnosis and management of sleep apnea and documented acceptable sensitivity and specificity of tests, a high degree of patient acceptance and low failure rates, as well as comparable outcomes to PSG. Finally, home unattended PSG in studies published in high rank journals (see The Sleep Heart Health Study in Diabetes Care, 2008;31:1001-1006). We have added reference no. 50.

• The fact that only 10 patients received acceptable CPAP treatment does allow for proper conclusions regarding the effect of CPAP on FMD and oxidative stress biomarkers.

The CPAP trial was planned from the beginning in the first 10 patients with severe OSA adhering to CPAP therapy for a period of six months (at least 4 hours per day and 5 days per week). The small number of patients in the CPAP study has been indicated in the “Discussion” as a limitation of the study

• The authors have found significant associations of FMD only with the presence of metabolic syndrome and urine 8-isoprostane, but not with AHI. This is not supportive of their title.

In the title we report of an association between oxidative stress and arterial dysfunction in patients with OSA, No association between OSA severity (AHI) and FMD is mentioned in the title.

Minor Comment

• In Tables 1 & 2 the second columns probably refer to mild/moderate OSA.

You are right. It was our mistake. The second columns of tables 1 and 2 refer to mild/moderate OSA.
3. **Reviewer’s report**

**Title:** Oxidative stress mediated arterial dysfunction in patients with obstructive sleep apnoea. Effect of continuous positive airway pressure treatment

**Version:** 2  **Date:** 9 October 2011

**Reviewer:** Frank Reichenberger

**Reviewer’s report:**

Review
“Oxidative stress mediated arterial dysfunction in patients with obstructive sleep apnoea. Effect of continuous positive airway pressure treatment.” by Del Ben Maria et al.

The authors studied markers of oxidative stress and endothelial dysfunction in a mixed population of 138 patients without, mild or severe OSA assessed by home polysomnography.

They describe a higher rate of oxidative stress (measured by markers of lipoxidation and NADPH activity) and a higher rate of endothelial dysfunction (measured by flow mediated dilatation) in patients with severe OSAS characterised with an AHI > 30/h.

In a subset of patients a repeat measurement showed improvement in some oxidative stress mediators and endothelial dysfunction after 4 month successful treatment with nCPAP.

**Comments**

The submitted manuscript deals with the interesting subject of systemic consequences of OSAS. However, there are several significant comments to be made.

A main problem arises from the unclear patient population. The authors mention that the main purpose of the work is the study on OSAS patients with comorbidities. However, it is not clear how many do have e.g. full metabolic syndrome or only diabetes et.c, and how does the presence of comorbidities affects the measurements. The concept of “healthy OSAS patients” and “real world OSAS patients” is somehow difficult, as most of these patients are at least obese with subsequent complications. Table 2 is unclear in this respect.

As clearly reported in the “Discussion”, in contrast to many previous studies performed in otherwise healthy OSA who are poorly representative of the real world OSA patients, we also included patients with co-morbidities usually linked to overweight and obesity, i.e. metabolic syndrome, dyslipidemia, hypertension and diabetes mellitus. The prevalence of these co-morbidities is reported in table 2 (i.e. the prevalence of metabolic syndrome in severe OSA is 70%).

The authors do not explain why they choose these distinct parameters to assess oxidative stress. Especially the role of NOx and sNOX-dp should be shortly explained in the introduction.
As reported in the “Discussion”, this is the first study where sNOX-dp was measured in OSA patients to assess systemic oxidative stress. This is a marker of NOX2 activation, a member of NADPH oxidase family, responsible for ROS generation and NO inactivation, which is a key mechanism for the development of endothelial dysfunction and cardiovascular disease. In a previous paper by our group we have already demonstrated an improvement of endothelial dysfunction via sNOX-dp generated oxidative stress down regulation.

Table 3: A correlation of the apnoea-hypopnoea index with a coefficient minor than r= -0.3 or so might be statistically significant, but questionable biologically relevant. Therefore the presented results should be interpreted very cautiously.

We agree that -0.3 statistically significant correlation may be questionable biologically relevant and therefore should be interpreted cautiously.

Surprisingly, the authors did only include 10 patients in the follow up cohort resulting in an acceptance rate of long term nCPAP treatment for severe OSAS of only 30%. What happened with the patient with moderate OSAS? It is not true that only 10 out of 30 patients with severe OSA adhered

Unfortunately, in the Methods” we were unclear. In fact, the CPAP trial was planned from the beginning only in the first 10 patients with severe OSA adhering to CPAP therapy for a period of six months (at least 4 hours per day and 5 days per week). Therefore, it is not true that acceptance rate of nCPAP treatment was only 30%. When the first 10 patients adhered to 4 months nCPAP treatment the other patients on treatment were not anymore followed-up. The “Methods” have been amended accordingly.

The conclusions of the presented work are not new. Several previous works describe similar findings in OSAS patients but also controversial results, which is sufficiently discussed in the presented manuscript.

In general, the manuscript is not targeted on a distinct subject
It should be focussed on the core findings e.g. new parameter to assess oxidative stress or OSAS patients with metabolic syndrome etc.
We agree with the Reviewer that previous results are controversial. Our study reports that patients with OSAS have increased oxidative stress, assessed for the first time by the measurement of urinary isoprostanes and serum NOX2,

Level of interest: An article of limited interest
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests.

4.
Reviewer’s report
Title: Oxidative stress mediated arterial dysfunction in patients with obstructive sleep apnoea. Effect of continuous positive airway pressure treatment
Version: 2 Date: 19 October 2011
**Reviewer:** In-Young Yoon

**Reviewer’s report:**

**Material and Methods**

**FMD**
Before FMD investigation, at least 8 hour fasting is required. Did the subjects fast at least 8 hours before FMD evaluation? Description on this issue should be included in the text.

FMD assessment was performed in a temperature-controlled room (22°) with the subjects in a resting, supine state between 8 a.m. and 10 a.m. after at least 8 hours fasting. The “Methods” have been amended.

**Results**

In the stepwise multiple regression analysis of FMD, r or beta of MS and PGF2# should be presented with r2 implying the contribution of MS and PGF2# to FMD. We have added in the “Results” beta for MS and PGF2 and R squared of MS and PGF2 to FMD prediction.

The process should be described in which only 10 out of 30 patients with severe OSA participated in the CPAP trial study.

Unfortunately, in the Methods” we were unclear in the description of criteria for the selection of patients to enrol in the CPAP trial. The CPAP trial was planned from the beginning only in the first 10 patients with severe OSA adhering to CPAP therapy for a period of six months.

Data on the CPAP compliance should be presented.

Compliance was defined as CPAP therapy at least 4 hours per day and 5 days per week over a for months period.

**Discussion**

Limitation of this study should be included.

Limitations of the study have been described in the “Discussion”.

Discussion is somewhat long. Reviews on oxidative stress in OSAS and FMD in OSAS could be shortened.

We believe that a detailed discussion of the controversial literature is important.

**Tables**

In Table 1 and 2, moderate / severe should be changed with mild / moderate.

You are right. It was our mistake. The second columns of tables 1 and 2 refer to mild/moderate OSA.