PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Assessing C-reactive protein/albumin ratio as a new biomarker for Polycystic Ovary Syndrome: a case-control study of women from Bahraini medical clinics
AUTHORS	Kalyan, Shirin; Goshtesabi, Azita; Sarray, Sameh; Joannou, Angela; Almawi, Wassim

VERSION 1 – REVIEW

REVIEWER	Michael Pankhurst University of Otago, New Zealand
REVIEW RETURNED	22-Mar-2018

GENERAL COMMENTS	The study by Kalyan et al describes the use of serum CRP/albumin ratios to predict the presence of PCOS. The manuscript is well written and generally easy to follow. This is not the first study to find elevated CRP levels in individuals with PCOS but I agree with the authors that the CRP/Albumin ratio may have some use in assessing the level of inflammation in PCOS patients. However, there are serious concerns about the design of the study that invalidate the claims that CRP/albumin can be used to predict PCOS (see comment 1). The authors may be able to present the findings as a descriptive study but this manuscript does not have valid methodology to state that CRP/Albumin levels can be used to predict or diagnose PCOS. Major concerns [1] The biggest criticism is that CRP is already known to be elevated in a range of inflammatory conditions other than PCOS. One of the exclusion criteria was recent/present illness, meaning that women with other inflammatory conditions were removed from the study population. Therefore, the study design removes individuals that have the highest chance of causing a false-positive when using CRP/Albumin to predict the presence of PCOS. Therefore, the reported specificity and sensitivity are not representative of the population that such tests would be carried out on if used in clinical practice. [2] There seems to be an error in figure 2. Why are there straight lines in the scatter plot? It looks like BMI-adjusted CRP values plotted against BMI rather than age-adjusted CRP values plotted against BMI. Please check. Minor concerns [3] Insulin resistance should not be referred to as a marker of PCOS. It is also present in pre-clinical diabetes.

	[4] There is a grammatical error in line 79. [5] Line 117 – what is the “25% quartile”? 0-25% or 25-50%? [6] In Table 1, P-values for the mean differences would be useful. [7] In Table 2, the system used for the BMI intervals is not correct. The interval should be 25-30. If 25-29.9 is used, then where do individuals with a BMI of 29.95 sit? [8] In Table 2, what is the definition of “borderline insulin resistance”? [9] It would seem that a STROBE checklist is necessary for this study.

REVIEWER	Sebastião Freitas de Medeiros Department of Gynecology and Obstetrics, Medical School, Federal University of Mato Grosso, Mato Grosso, Brazil
REVIEW RETURNED	05-Apr-2018

GENERAL COMMENTS	Assessing C-reactive protein/albumin ratio as a new predictor of polycystic ovary syndrome: a case-control study A- General comments 1- A predictor of PCOS is something that could identify or predict future development of this condition. Currently, to identify/diagnosis PCOS we have three consensuses: NIH, Rotterdam, and AES. Certainly, C-reactive protein/albumin ratio will not be a diagnostic criterion to be used in the future. So, the title and objectives are inadequate. The variable CRP/albumin could be taken as a marker of risk for the development of cardiovascular disease in PCOS as it is biochemical hyperandrogenism and insulin resistance. In fact, it could be only a useful mediator of the risk. Considering these points, I think the authors should re-write the paper, redefining the objectives and using the same data. 2- English revision must be performed B- Specific comments 1- To define the cut-off for HOMA-IR in their population 2- To collect blood at any day in PCOS patients for hormone evaluation must include the measurement of progesterone to exclude eventual ovulation and validate the data. 3- To clarify data presentation and data analysis in statistical section • Non-parametric data should be analysed/compared by appropriated methods and the results must be given in median and first and third quartiles or 95% CI. • Parametric data should be compared by t test or Welch test and presented as mean and standard deviation • p value must be given for any comparison I could review this paper if authors agree to rewrite it, giving a new approach.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Michael Pankhurst

Institution and Country: University of Otago, New Zealand
Competing Interests: None declared.

The study by Kalyan et al describes the use of serum CRP/albumin ratios to predict the presence of PCOS. The manuscript is well written and generally easy to follow. This is not the first study to find elevated CRP levels in individuals with PCOS but I agree with the authors that the CRP/Albumin ratio may have some use in assessing the level of inflammation in PCOS patients. However, there are serious concerns about the design of the study that invalidate the claims that CRP/albumin can be used to predict PCOS (see comment 1). The authors may be able to present the findings as a descriptive study but this manuscript does not have valid methodology to state that CRP/Albumin levels can be used to predict or diagnose PCOS.

Major concerns

[1] The biggest criticism is that CRP is already known to be elevated in a range of inflammatory conditions other than PCOS. One of the exclusion criteria was recent/present illness, meaning that women with other inflammatory conditions were removed from the study population. Therefore, the study design removes individuals that have the highest chance of causing a false-positive when using CRP/Albumin to predict the presence of PCOS. Therefore, the reported specificity and sensitivity are not representative of the population that such tests would be carried out on if used in clinical practice.

The Reviewer is absolutely correct in stating that there are a broad range of indications associated with increased CRP levels, including cardiovascular disease. When making a diagnosis, the first thing one has to do is conduct a differential analysis and rule out other potential causes of the symptoms. The characteristic of ovarian cysts, which is part of the diagnostic criteria for PCOS, are actually common and most women will experience such during their life. This doesn't preclude the use of this characteristic in diagnosing PCOS. Similarly androgen access and irregular cycles can be attributed to a broad range of other conditions. Any one feature alone is thus not enough in making a diagnosis, but it is the collective presence of these and ruling out others that is necessary to accurately diagnose a condition. This is particularly true for complex syndromes like PCOS that is highly heterogeneous in its presentation. Therefore in our statistical modelling we specifically assessed how CRP/albumin, as an independent variable (i.e. predictor), performed relative to other independent variables that are known to be strongly associated with PCOS in well-defined cohorts that were selected after ensuring that the characteristics we were assessing were not likely to be attributed to other factors (see exclusion criteria starting at line 126). We did this not only for CRP/albumin, but all independent variables used in regression modelling, which included characteristics currently used to assess PCOS. This is actually a strength of the design of the study, and not a weakness.

[2] There seems to be an error in figure 2. Why are there straight lines in the scatter plot? It looks like BMIadjusted CRP values plotted against BMI rather than age-adjusted CRP values plotted against BMI. Please check.

Thank you for helping us clarify the description of this figure. Figure 2 depicts a general linear model (i.e. linear regression) of the relationship amongst PCOS diagnosis, BMI, and CRP/albumin levels that was done following univariate regression analyses of other variables that are associated with CRP/albumin. The scatter plot description is in reference to the type of graph selected for the figure in the program (it is actually composed of each data point presented as a dot instead of a continuous line). We see how this may be confusing, so we have re-labelled the figure to note it is a linear regression analysis that is being shown.

Minor concerns

[3] Insulin resistance should not be referred to as a marker of PCOS. It is also present in pre-clinical diabetes.

It is now well recognized that insulin resistance is a fundamental characteristic of PCOS (see [1]). As mentioned in the response to question 1, insulin resistance, like many other diseaseassociated symptoms/markers, is present in more than one condition. Many conditions also share considerable overlap, such as the development of diabetes and PCOS. It is true, however, that it is not yet part of the diagnostic criteria of PCOS, and we have made this clear in the revised manuscript. We have rephrased it to read "variables that strongly link to…" instead of "classical predictors".

[4] There is a grammatical error in line 79.

Thank you. We revised the rather long sentence, so it reads less awkwardly now.

[5] Line 117 – what is the "25% quartile"? 0-25% or 25-50%?

We believe the reviewer was referring to line 177 , "…and since 25% quartile of CRP value…." and not 117.

The 25% quartile encompasses those who fall within the 0-25% range of values for CRP (i.e. the lowest quarter). We have revised the wording of the manuscript to make sure that is clear now.

[6] In Table 1, P-values for the mean differences would be useful.

For presenting descriptive statistics for clinical studies, it is now generally preferred (and recommended as being more appropriate) that the data be presented with the 95% confidence interval instead of p values; the former contains a lot more useful information in addition to providing the data needed to derive at the p value. This has been extensively discussed in BMJ.
Here is a link to an article discussing this back in 1986:
https://www.bmj.com/content/292/6522/746.

[7] In Table 2, the system used for the BMI intervals is not correct. The interval should be 25-30. If 25-29.9 is used, then where do individuals with a BMI of 29.95 sit?

We have revised the range in the Table to read 25-30.

[8] In Table 2, what is the definition of "borderline insulin resistance"?

We have now included in the methods section the following in response to this question:

"HOMA-IR values were characterized as Normal (insulin-sensitive) if <2.40; Borderline if between 2.40-3.50, and High (insulin-resistant) if > 3.50."

[9] It would seem that a STROBE checklist is necessary for this study.
Yes, thank you. We have provided one now.

Reviewer: 2
Reviewer Name: Sebastião Freitas de Medeiros
Institution and Country: Department of Gynecology and Obstetrics, Medical School, Federal University of
Mato Grosso, Mato Grosso, Brazil
Competing Interests: The reviewer declares no conflict of interest

A- General comments

1- A predictor of PCOS is something that could identify or predict future development of this condition. Currently, to identify/diagnosis PCOS we have three consensuses: NIH, Rotterdam, and AES. Certainly, C-reactive protein/albumin ratio will not be a diagnostic criterion to be used in the future. So, the title and objectives are inadequate. The variable CRP/albumin could be taken as a marker of risk for the development of cardiovascular disease in PCOS as it is biochemical hyperandrogenism and insulin resistance. In fact, it could be only a useful mediator of the risk.

In statistical modelling, the predictor variable is synonymous to "independent variable". This is the context in which the term predictor is used in this work. CRP/albumin was used in regression modelling as the independent variable (i.e. predictor) in a well-defined cohort of premenopausal women to assess its relationship to the diagnosis of PCOS. In best practice, any diagnostic criterion that is currently in place, be it for PCOS or another condition, should be amendable to change with the acquisition of more evidence through research. There are known issues with the current diagnostic criteria for PCOS, and there is an acknowledged need to bring in a more evidence-based process to the development of a better criteria [2]. This is the purpose of the work presented. The reviewer may be right in that the value of CRP/albumin could be in its ability to predict the risk of developing certain co-morbidities associated with PCOS, such as cardiovascular disease or diabetes. Future investigations that use our suggested variable of CRP/albumin in prognostic studies in women with PCOS are needed to determine if this is true.

Considering these points, I think the authors should re-write the paper, redefining the objectives and using the same data.

2- English revision must be performed

B- Specific comments

1- To define the cut-off for HOMA-IR in their population

In response to the above, we have corrected any grammatical error(s) that may have been present.

HOMA-IR values were characterized as Normal (insulin-sensitive) if <2.40; Borderline if between 2.40-3.50, and High (insulin-resistant) if > 3.50. This information has now been added to the manuscript in the methods section.

2- To collect blood at any day in PCOS patients for hormone evaluation must include the measurement of progesterone to exclude eventual ovulation and validate the data.

This was a cross-sectional retrospective case-control study, which precludes the ability to collect blood samples for hormonal analysis for progesterone. Regardless, the diagnosis of PCOS was based on the 2003 Rotterdam Criteria, so a single ovulatory cycle is unlikely to change the results of the study/analysis.

3- To clarify data presentation and data analysis in statistical section

- Non-parametric data should be analysed/compared by appropriated methods and the results must be given in median and first and third quartiles or 95% CI.
- Parametric data should be compared by t test or Welch test and presented as mean and standard deviation
- p value must be given for any comparison

We thank the reviewer for this suggestion. We had used the Shapiro-Wilk test to evaluate the distribution of the variables. Many variables, particularly for the PCOS cohort, were flagged as being non-normally distributed. However, upon testing for skewness, we found the values for asymmetry and kurtosis fell between -2 to +2 for all. Given the sample sizes were >30, we followed the

recommendation that parametric tests could be used for such data [3]. This was deemed appropriate as we could not guarantee that the assumptions for using non-parametric tests could be met, particularly that the data have equal variances for the two groups being compared. We reassessed the validity of our analysis by running the Mann–Whitney U test in addition to student t test for variables we had detected as being non-normal and confirmed that the results were similar. We have thus included this explanation in the statistical methods.

Please see our response to Reviewer 1 regarding the use of p values. The 95% confidence interval we provide gives a lot more information regarding the distribution of the data and contains information regarding the p value within it.

I could review this paper if authors agree to rewrite it, giving a new approach.

VERSION 2 – REVIEW

REVIEWER	Michael Pankhurst University of Otago, New Zealand
REVIEW RETURNED	02-Jul-2018

GENERAL COMMENTS	Some of the concerns have been addressed but my main concern is that the claims of the study go beyond what the results can reasonably demonstrate. This does not mean that the data is not worthy of publication but the manuscript needs to be re-written so that the outcomes are not exaggerated. Specific comments below. [1] Original Comment: The biggest criticism is that CRP is already known to be elevated in a range of inflammatory conditions other than PCOS. One of the exclusion criteria was recent/present illness, meaning that women with other inflammatory conditions were removed from the study population. Therefore, the study design removes individuals that have the highest chance of causing a false-positive when using CRP/Albumin to predict the presence of PCOS. Therefore, the reported specificity and sensitivity are not representative of the population that such tests would be carried out on if used in clinical practice. Authors' Response: The Reviewer is absolutely correct in stating that there are a broad range of indications associated with increased CRP levels, including cardiovascular disease. When making a diagnosis, the first thing one has to do is conduct a differential analysis and rule out other potential causes of the symptoms. The characteristic of ovarian cysts, which is part of the diagnostic criteria for PCOS, are actually common and most women will experience such during their life. This doesn't preclude the use of this characteristic in diagnosing PCOS. Similarly androgen access and irregular cycles can be attributed to a broad range of other conditions. Any one feature alone is thus not enough in making a diagnosis, but it is the collective presence of these and ruling out others that is necessary to accurately diagnose a condition. This is particularly true for complex syndromes like PCOS that is highly heterogeneous in its presentation. Therefore in our statistical modelling we specifically assessed how CRP/albumin, as an independent variable (i.e. predictor), performed relative to other independent variables that are known to be strongly associated with PCOS in well-defined cohorts that were selected after ensuring that the characteristics we were assessing were not likely to be attributed to other factors (see exclusion criteria starting at line 126). We did

	this not only for CRP/albumin, but all independent variables used in regression modelling, which included characteristics currently used to assess PCOS. This is actually a strength of the design of the study, and not a weakness. Reviewer's response: This reviewer does not disagree that PCOS is a complex disorder that requires the exclusion of other criteria for diagnosis. The problem is that individuals with a range of conditions that may cause an increase in CRP levels have been excluded from the study. Hence, one can diagnose PCOS by excluding a range of other conditions, CRP/albumin ratios can be used to predictor PCOS. There isn't much utility in a predictor that can only be used after diagnosis. Furthermore, the authors state in the introduction that they are testing whether CRP/albumin ratios can be used as a predictor "in itself". Furthermore the used ROC analysis which is generally used to determine diagnostic efficacy which does not match the authors' claim that the term predictor is only used as a synonym for "independent variable". This study does show that elevated CRP/albumin ratios are present in women with PCOS relative to normal women but the design is not sufficient to evaluate whether they can be used as a predictor. The manuscript needs to acknowledge this. [2] Original comment: There seems to be an error in figure 2. Why are there straight lines in the scatter plot? It looks like BMI-adjusted CRP values plotted against BMI rather than age-adjusted CRP values plotted against BMI. Please check. Author's response: Thank you for helping us clarify the description of this figure. Figure 2 depicts a general linear model (i.e. linear regression) of the relationship amongst PCOS diagnosis, BMI, and CRP/albumin levels that was done following univariate regression analyses of other variables that are associated with CRP/albumin. The scatter plot description is in reference to the type of graph selected for the figure in the program (it is actually composed of each data point presented as a dot instead of a continuous line). We see how this may be confusing, so we have re-labelled the figure to note it is a linear regression analysis that is being shown. Reviewer's comment: It is still not clear what is going on in this figure. Based on the axes, it still looks like a scatter plot of BMI vs. CRP/albumin. Is this a graph showing where the residuals map to along the regression line? The figure legend mentions that the CRP/albumin levels have been adjusted. How have they been adjusted? The Results text seems to indicate that Spearman correlation has been used but there is no indication of this in the figure legend. More explanation of this figure is needed. It is unlikely that a single sentence for the figure legend will suffice. [6] Original comment: In Table 1, P-values for the mean differences would be useful. Author's response: For presenting descriptive statistics for clinical studies, it is now generally preferred (and recommended as being more appropriate) that the data be presented with the 95% confidence interval instead of p values; the former contains a lot more useful information in addition to providing the data needed to derive at the p value. This has been extensively discussed in BMJ. Here is a link to an article discussing this back in 1986: https://www.bmj.com/content/292/6522/746. Reviewer's response: Thanks for alerting me to this paper, I hadn't read it previously. However, it does clearly state in the paper that p-values should also be used; ". However, the actual P value is helpful in addition to the confidence interval, and preferably both should be

	presented.". Furthermore, BMJ Open instructions to authors also state; "results: main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm."

REVIEWER	Sebastião Freitas de Medeiros Federal University of Mato Grosso, Cuiabá, MT, Brazil
REVIEW RETURNED	05-Jun-2018

GENERAL COMMENTS	Assessing C-reactive protein/albumin ratio as a new predictor of polycystic ovary syndrome: a case-control study of women from Bahraini medical clinics General comments Please, put dots or commas after the reference number(s) Please, present tables and figures in separated pages English language should be reviewed by a native English Specific comments Methods section Subjects. What does active thyroid disease mean? Biochemical analysis. Adiponectin, progesterone, estradiol, DHEAS assays were not described. Statistical analysis. This section is unclear. When t-test was used? When Mann-Whitney test was used? When x^2 test was used? The data in table 1 are given in mean and standard deviation and how they were compared? Results Page 11, line 236. Total testosterone levels and other androgens should be given in table 1. Tables Please, withdraw vertical lines p values should be given for all comparisons in table 1. Table 1 does not show any link between adiponectin and visceral adiposity Figures Figures 1 and 2 were changed.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Michael Pankhurst
Institution and Country: University of Otago, New Zealand
Competing Interests: None

Some of the concerns have been addressed but my main concern is that the claims of the study go beyond what the results can reasonably demonstrate. This does not mean that the data is not worthy of publication but the manuscript needs to be re-written so that the outcomes are not exaggerated. Specific comments below.

[1] Original Comment: The biggest criticism is that CRP is already known to be elevated in a range of inflammatory conditions other than PCOS. One of the exclusion criteria was recent/present illness, meaning that women with other inflammatory conditions were removed from the study population. Therefore, the study design removes individuals that have the highest chance of causing a false-positive when using CRP/Albumin to predict the presence of PCOS. Therefore, the reported specificity and sensitivity are not representative of the population that such tests would be carried out

on if used in clinical practice.
Authors' Response: The Reviewer is absolutely correct in stating that there are a broad range of indications associated with increased CRP levels, including cardiovascular disease. When making a diagnosis, the first thing one has to do is conduct a differential analysis and rule out other potential causes of the symptoms. The characteristic of ovarian cysts, which is part of the diagnostic criteria for PCOS, are actually common and most women will experience such during their life. This doesn't preclude the use of this characteristic in diagnosing PCOS. Similarly androgen access and irregular cycles can be attributed to a broad range of other conditions. Any one feature alone is thus not enough in making a diagnosis, but it is the collective presence of these and ruling out others that is necessary to accurately diagnose a condition. This is particularly true for complex syndromes like PCOS that is highly heterogeneous in its presentation. Therefore in our statistical modelling we specifically assessed how CRP/albumin, as an independent variable (i.e. predictor), performed relative to other independent variables that are known to be strongly associated with PCOS in well-defined cohorts that were selected after ensuring that the characteristics we were assessing were not likely to be attributed to other factors (see exclusion criteria starting at line 126). We did this not only for CRP/albumin, but all independent variables used in regression modelling, which included characteristics currently used to assess PCOS. This is actually a strength of the design of the study, and not aweakness.
Reviewer's response: This reviewer does not disagree that PCOS is a complex disorder that requires the exclusion of other criteria for diagnosis. The problem is that individuals with a range of conditions that may cause an increase in CRP levels have been excluded from the study. Hence, one can diagnose PCOS by excluding a range of other conditions, CRP/albumin ratios can be used to predictor PCOS. There isn't much utility in a predictor that can only be used after diagnosis. Furthermore, the authors state in the introduction that they are testing whether CRP/albumin ratios can be used as a predictor "in itself". Furthermore the used ROC analysis which is generally used to determine diagnostic efficacy which does not match the authors' claim that the term predictor is only used as a synonym for "independent variable". This study does show that elevated CRP/albumin ratios are present in women with PCOS relative to normal women but the design is not sufficient to evaluate whether they can be used as a predictor. The manuscript needs to acknowledge this.

Authors' reply: When studying a new biomarker (this is the first study to look at this relationship between CRP/albumin and PCOS), it is critical to ensure that the groups being studied are well-defined. The exclusion criteria we implemented are the standard used for studies measuring CRP relationships in women with PCOS; please see: https://www.ncbi.nlm.nih.gov/pubmed/17489777. As noted in the reference study, "The patients were carefully interviewed, clinically examined, and laboratory tested to eliminate conditions, probable to provoke an inflammatory response which was an exclusion criterion...." We are thus implementing the standard in the field. It is expected that when assessing an inflammatory marker as either a correlate or predictor of a condition, study subjects would not be on any medication that would influence its expression or have acute infectious illness. This is true for any condition that CRP has been studied as a predictive biomarker, such as heart disease (https://www.health.harvard.edu/heart-health/c-reactive-protein-test-to-screen-for-heart-disease). The foundational studies that established CRP as a predictor of heart disease also used similar exclusion criteria strategy for their study cohorts. The ROC curve analysis in our study serves to: 1) distinguish CRP/albumin from CRP alone in its relationship to PCOS, and 2) provides an evaluation of the utility of the potential of CRP/albumin to serve as a predictive biomarker that distinguishes women with PCOS. This is a recommendation that came from a critical overview of studies that propose the use of CRP as a predictor of heart disease that found most studies did not include such a measure of sensitivity and specificity (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC400721/).

We hope that our manuscript does not leave the impression that we are proposing that CRP/albumin alone be used to detect the presence of PCOS. When we stated in the introduction, "...we hypothesized that CRP/albuminratio would, in itself, serve as a strong predictor of PCOS in a cohort of similarly aged women....", we were using "in itself" for emphasis, not to mean "by itself". To remove

any confusion on that front, we have deleted “in itself”. We have also followed the recommendation to use less emphatic/conclusive language throughout the manuscript (for example using the terms “association”, “biomarker”, and “correlate” in place of “predictor” where more appropriate). We recognize that follow-on prospective studies are needed now to test how well CRP/albumin serves as a predictor of PCOS, which is likely a point the Reviewer is trying to make. Our discussion emphasizes the need for prognostic studies to determinethe utility of CRP/albumin as a predictive biomarker for women with PCOS, and we hope this work serves as the foundation for such. The latter being an aim of this work.

[2] Original comment: There seems to be an error in figure 2. Why are there straight lines in the scatter plot? It looks like BMI-adjusted CRP values plotted against BMI rather than age-adjusted CRP values plotted against BMI. Please check.
Author’s response: Thank you for helping us clarify the description of this figure. Figure 2 depicts a general linear model (i.e. linear regression) of the relationship amongst PCOS diagnosis, BMI, and CRP/albumin levels that was done following univariate regression analyses of other variables that are associated with CRP/albumin. The scatter plot description is in reference to the type of graph selected for the figure in the program (it is actually composed of each data point presented as a dot instead of a continuous line). We see how this may be confusing, so we have re-labelled the figure to note it is a linear regression analysis that is being shown.
Reviewer’s comment: It is still not clear what is going on in this figure. Based on the axes, it still looks like a scatter plot of BMI vs. CRP/albumin. Is this a graph showing where the residuals map to along the regression line? The figure legend mentions that the CRP/albumin levels have been adjusted. How have they been adjusted? The Results text seems to indicate that Spearman correlation has been used but there is no indication of this in the figure legend. More explanation of this figure is needed. It is unlikely that a single sentence for the figure legend will suffice.

Authors’ reply: A correlation analysis was first performed to identify variables that linked significantly with CRP/albumin. The following variables were identified: BMI, Status (PCOS diagnosis), insulin, free testosterone, progesterone, and adiponectin. A univariate GLM was then computed investigating the relationship between CRP/albumin and BMI, adjusting for the variables found to associate with CRP/albumin plus age and stratified by PCOS diagnosis. Figure 2 shows the predicted values of CRP/albumin according to BMI for women with and without PCOS. We have now expanded upon the figure legend to include this information. Thank you for prompting greater clarity for this.

[6] Original comment: In Table 1, P-values for the mean differences would be useful.
Author’s response: For presenting descriptive statistics for clinical studies, it is now generally preferred (and recommended as being more appropriate) that the data be presented with the 95% confidence interval instead of p values; the former contains a lot more useful information in addition to providing the data needed to derive at the p value. This has been extensively discussed in BMJ. Here is a link to an article discussing this back in 1986: https://www.bmj.com/content/292/6522/746.
Reviewer’s response: Thanks for alerting me to this paper, I hadn’t read it previously. However, it does clearly state in the paper that p-values should also be used; “. However, the actual P value is helpful in addition to the confidence interval, and preferably both should be presented.”. Furthermore, BMJ Open instructions to authors also state; “results: main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm.”

Authors’ reply: We wave the white flag! We have included p values to Table 1. Although we reiterate the information regarding statistical significance is contained within the confidence intervals provided. The inclusion of p values is most appropriate when hypothesis testing – i.e. within the context of an experimental set-up (for example, when testing the influence of an intervention) and not when describing study cohorts. However, we acquiesced as it does not seem that many are aware of this

and there is still an expectation that p values be included in thecontext of descriptive statistics (even though there is no expectation to correct for multiple comparisons when doing so...).

Reviewer: 2
Reviewer Name: Sebastião Freitas de Medeiros
Institution and Country: Federal University of Mato Grosso, Cuiabá, MT, Brazil
Competing Interests: None declared

General comments
Please, put dots or commas after the reference number(s)
Please, present tables and figures in separated pages
English language should be reviewed by a native English

Authors' reply: We followed the instructions for formatting for BMJ Open. Any alterations required in that regard, we will make with direction from the Editorial Office. Dr. Shirin Kalyan, despite her exotic sounding name, is a native English speaker who was born, raised and educated in Canada. Being her primary language of use, Dr. Kalyan's English skills are well honed; she has authored book chapters and a number of lay and professional manuscripts, serves as an Editor for a major publishing company, and edits works for those who are not "native English speakers". She wrote most of this manuscript and reviewed all of it.

Specific comments
Methods section
Subjects. What does active thyroid disease mean?

Authors' reply: Active thyroid disease refers to overt, central and subclinical hypothyroidism or hyperthyroidism. Although this is generally known in the medical field, we have included the definition in the methods.

Biochemical analysis. Adiponectin, progesterone, estradiol, DHEAS assays were not described.

Authors' reply: Details on adiponectin, progesterone, estradiol, and DHEAS measurements have now been added to the manuscript.

Statistical analysis. This section is unclear. When t-test was used? When Mann-Whitney test was used? When x^2 test was used? The data in table 1 are given in mean and standard deviation and how they were compared?

Authors' reply: As noted in the methods, we used t-tests for all variables. Mann-Whitney was used to assess variables with non-normal distribution, which included adiponectin and most measures of steroidal hormones (totaltestosterone, free testosterone, bioavailable testosterone, free androgen index, estradiol and progesterone). After confirming that parametric and non-parametric analyses yielded similar results, we used parametric analysis (shown in Table 1) as is recommended for sample sizes >30 (please see methods for reference and details). As is the expectation, chi square analysis was used for the categorical variables we describe in the Results (i.e. parity, number of live births, educational attainment).

Results
Page 11, line 236. Total testosterone levels and other androgens should be given in table 1.

Authors' reply: We wanted to make the Table less cumbersome by providing the androgen measure of interest, but upon your request have included all the androgen measures we have.

Tables
Please, withdraw vertical lines

Authors' reply: As noted above – formatting changes will be left to the discretion of the editorial office.

p values should be given for all comparisons in table 1.

Authors' reply: Confidence intervals contain the information provided by p values. Please see response to Reviewer 1 for the use of p values and their use being most appropriate for hypothesis testing. For those who are unfamiliar with extracting the relevant information from confidence intervals, we have now included the p values to Table 1.

Table 1 does not show any link between adiponectin and visceral adiposity

Authors' reply: Table 1's objective was not to show a link between adiponectin and visceral adiposity. It is describing the characteristics of the cohort.

Figures
Figures 1 and 2 were changed.

VERSION 3 – REVIEW

REVIEWER	Michael Pankhurst University of Otago, New Zealand
REVIEW RETURNED	30-Aug-2018

GENERAL COMMENTS	My concerns have been satisfied in the latest revision.