PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Butyrylcholinesterase as a perioperative complication marker in patients after transcatheter aortic valve implantation: a prospective observational study
AUTHORS	Michels, Bernhard; Holzamer, Andreas; Graf, Bernhard; Bredthauer, Andre; Petermichl, Walter; Müller, Anika; Zausig, York; Bitzinger, Diane

VERSION 1 – REVIEW

REVIEWER	Keage, Hannah University of South Australia Division of Health Sciences
REVIEW RETURNED	24-Aug-2020

GENERAL COMMENTS	Thank you for asking me to review the paper "Butyrylcholinesterase as a perioperative complication marker in patients after transcatheter aortic valve implantation?". TAVI is an increasingly common procedure, so research in this area is required. I do however have major and minor concerns, as detailed below: Major: - What is the rationale and justification for the grouping of postoperative complications? They seem very heterogenous and I can't see the same biological mechanisms underlying them all. I would have liked to see the authors focus only on delirium, given the tight links with Ach. I cannot see why the other complications are grouped with delirium. - A major limitation was not assessing the effects of medications (history and those given during the TAVI), particularly, anticholinergic medications. Anticholinergic medications are known to affect circulating AChE and BChE. Were meications driving associations rather than enzyme concentrations per se.? Could you please integrate anticholinergic medication data? - The conclusion completely rests on the reliability and validity of a commercial assay device for measuring AChE and BChE. Please provide reliabilities and validities for these enzyme measurements in general and then specific to this device (preferably from peer-reviewed sources rather than corporate documents). The variabilities of both (but especially the BChE) measures are large (as illustrated in Figure), which questions the accuracy of the measurement; is such variability expected? - The statistical approach lacks detail. Were outliers assessed? Were there any missing data? Please provide a rationale and justification for no multiple comparison testing. There are an enormous number of comparisons without any correction factor; there are surely Type 1 errors. Please provide actual p values (significant and non- significant, "ns" is not enough), preferably all

	to 3 decimal places. Also, even without a correction factor, 0.058 and 0.051 are non-significant; why are they bolded in the table? Please provide effect sizes for all analyses, and discuss effect sizes rather than p values/statistically significant thresholds; are these results clinically meaningful? Minor: - All patients received elective TAVI in general anesthesia. This seems odd, the vast majority are done without general anaesthetic. Could it be due to the retrospective analytical nature of the study? When was the trial conducted? Please provide trial dates. - "Patients without postoperative complications showed a delayed decrease in BChE activity". You cannot conclude this from the statistics you have conducted. You have just compared between groups relative to time-points. - Figure 1 isn't necessary.

REVIEWER	Gasecka, A Medical University of Warsaw
REVIEW RETURNED	26-Dec-2020

GENERAL COMMENTS	BMJ Open The Authors evaluated if perioperative activity of AChE and BChE predict postoperative complications in 43 patients undergoing TAVI. Whereas AChE had no predictive value, BChE could be a useful perioperative biomarker to identify patients with a higher risk for postoperative complications after TAVI. Major comments: - As complications, the Authors defined infections, delirium and heart rhythm disturbances. Why were other complications, for example acute renal failure, stroke or TIA not taken into the analysis? - I find the statistical analysis incomplete. To prove that pre-operative BChE activity was an independent predictor of post-operative complications, I suggest to (i) determine the sensitivity and specificity and set the cut-off value based on the ROC curve, and (ii) include the low BChE activity (based on the cut-off) along with other established clinical variables in the multivariate regression model. Please present the model in the separate table. - Please have a careful look at the Figures: o There is two times "Figure 1". o Second Figure 1 has a typo (anestehsia). o Please add the exact p-values to Figures 3 and 4. - In the discussion, the authors write that BChE activity, used in combination with common preoperative evaluation procedures, could serve as a useful predictive indicator to identify high-risk patients. To state this, multivariate regression analysis as proposed before is essential. I propose minor revision.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Hannah Keage, University of South Australia Division of Health Sciences
Competing interests of Reviewer: None declared

Comments to the Author:
Thank you for asking me to review the paper “Butyrylcholinesterase as a perioperative complication marker in patients after transcatheter aortic valve implantation?”. TAVI is an increasingly common procedure, so research in this area is required. I do however have major and minor concerns, as detailed below:

Major:

- What is the rationale and justification for the grouping of postoperative complications? They seem very heterogenous and I can’t see the same biological mechanisms underlying them all. I would have liked to see the authors focus only on delirium, given the tight links with Ach. I cannot see why the other complications are grouped with delirium.

We appreciate this important annotation. In previous retrospective studies with more than 800 transfemoral- and transapical TAVI patients we could show that delirium, pneumonia, acute renal failure and indication for permanent pacemaker were the most frequently reported perioperative complications (Goldfuss S et al., BMJ Open 2019; Würschinger F et al., PLOS ONE 2018). So our intention was to find a complication marker for TAVI-patients, which includes these most frequently reported perioperative complications. Recent studies have shown that AChE and BChE serve as diagnostic markers of low-grade systemic inflammation and have remarkable predictive value for mortality in critically ill patients (Chiarla C et al., Minerva Chir 2011; Zivkovic AR et al., Mediators Inflamm 2015). In the present study we could show that BChE could be a useful perioperative marker to identify patients with a higher risk for postoperative complications after TAVI. One possible biological mechanism which underlies these complications might be local/systemic inflammation processes. However, further studies focusing on the underlying biological mechanism and including more patients would be needed to validate our findings. To address to this important issue we have revised our limitations section.

- A major limitation was not assessing the effects of medications (history and those given during the TAVI), particularly, anticholinergic medications. Anticholinergic medications are known to affect circulating AChE and BChE. Were medications driving associations rather than enzyme concentrations per se.? Could you please integrate anticholinergic medication data?

This is a very important annotation. However, in the CESARO study with more than 600 adult surgical patients an association between the occurrence of postoperative delirium and the so-called anticholinergic load (measured by the ADS, anticholinergic drug scale) could not be found in multiple logistic regression analysis (Müller A et al., European Journal of Anaesth, 2018). In addition, there was no association between anticholinergic burden and BChE or AChE activities in any time point (Spearman’s rank correlation). The colleagues suggested that anticholinergic drugs are not solely responsible for the anticholinergic load. Regarding these findings, we did not focus on anticholinergic drugs in this secondary analysis. Furthermore, during the TAVI procedure all patients received the same medication (material and methods section), so we can offer a standardized setting for this patient population.

- The conclusion completely rests on the reliability and validity of a commercial assay device for measuring AChE and BChE. Please provide reliabilities and validities for these enzyme measurements in general and then specific to this device (preferably from peer-reviewed sources rather than corporate documents). The variabilities of both (but especially the BChE) measures are large (as illustrated in Figure), which questions the accuracy of the measurement; is such variability expected?

We used the CE certified and validated test system (ChE Check, Securetec Detektions-Systeme AG, Neubiberg, Germany; In-Vitro-Diagnostics Guideline 98/79/EG; DIN EN ISO 18113-2 and -3), which is based on a latter method and is the most widely used method to measure the activity of AChE/BChE. The great advantage of this enzymatic assay is that it provides rapid and precise determination of AChE and BChE activities in whole blood without any pre-treat of the samples. The test system has been shown to be a suitable model for studying AChE and BChE activity with increased accuracy and reproducibility and with saving of time and materials. Many clinical trials with large patient populations already used this point-of-care-system (Müller A et al., European Journal of Anaesth, 2018; Zivkovic AR et al., Mediators Inflamm 2015; John M et al., J Intensive Care, 2017 etc.). Furthermore the small effect sizes of our results do not indicate high variances of cholinesterase activity values. To address to this important issue we have revised our manuscript.

- The statistical approach lacks detail. Were outliers assessed? Were there any missing data? Please provide a rationale and justification for no multiple comparison testing. There are an enormous number of comparisons without any correction factor; there are surely Type 1 errors.

Please provide actual p values (significant and non- significant, “ns” is not enough), preferably all to 3 decimal places. Also, even without a correction factor, 0.058 and 0.051 are non-significant; why are they bolded in the table? Please provide effect sizes for all analyses, and discuss effect sizes rather than p values/statistically significant thresholds; are these results clinically meaningful?
Thank you for your important annotation. Some p-values were mistakenly bolded. We apologize for this misunderstanding. This has been changed. We have changed the “statistic section”, “tables” and “figures” and have provided all needed information.To reduce any kind of misinterpretation we have extensively revised the manuscript.

Minor:
- All patients received elective TAVI in general anesthesia. This seems odd, the vast majority are done without general anaesthetic. Could it be due to the retrospective analytical nature of the study? When was the trial conducted? Please provide trial dates.

This is an important annotation. TAVI is performed under local anesthesia or general anesthesia. Both ways are comparable in procedure-related outcomes (Fröhlich GM et al. BMC Med 2014)). Local anesthesia in TAVI has the advantage of a shorter induction time, more stable haemodynamic conditions and a faster postoperative mobilisation. General anesthesia provides a better patient comfort with more stable surgical conditions and periprocedural complications can be better controlled. Continuous TEE monitoring is possible under general anesthesia and thus patients have a reduced incidence of aortic regurgitation. In a previous study we could show that anesthesia-related complications are relatively rare, and side effects can be avoided by adequate prophylaxis (Goldfuss S et al., BM Open 2019) These are the reasons why TAVI is mostly performed under general anesthesia in our center (even today), which is in accordance with the German guidelines for TAVI procedures (https://www.g-ba.de/richtlinien/84/). The study was conducted from March 2014 until June 2016. To address to this important issue we have revised the material and methods section.
- “Patients without postoperative complications showed a delayed decrease in BChE activity”. You cannot conclude this from the statistics you have conducted. You have just compared between groups relative to time-points.

Your are right. We have revised our statistics as stated before.
- Figure 1 isn’t necessary.

Figure 1 was removed

Reviewer: 2
Dr. A Gasecka
Competing interests of Reviewer: None declared
Comments to the Author:
BMJ Open
The Authors evaluated if perioperative activity of AChE and BChE predict postoperative complications in 43 patients undergoing TAVI. Whereas AChE had no predictive value, BChE could be a useful perioperative biomarker to identify patients with a higher risk for postoperative complications after TAVI.

Major comments:
- As complications, the Authors defined infections, delirium and heart rhythm disturbances. Why were other complications, for example acute renal failure, stroke or TIA not taken into the analysis?

We appreciate this important annotation. In previous retrospective studies with more than 800 transfemoral- and transapical TAVI patients we could show that delirium, pneumonia, acute renal failure and indication for permanent pacemaker were the most frequently reported perioperative complications (Goldfuss S et al., BMJ Open 2019; Würschinger F et al., PLOS ONE 2018). Cerebrovascular events occurred only in 2 % of the TAVI-patients in this retrospective analysis (Würschinger F et al., PLOS ONE 2018). However, in the present study with a small population size we focused on the most frequently reported perioperative complications. A larger, possibly multicenter study, would be needed to evaluate more complications.
- I find the statistical analysis incomplete. To prove that pre-operative BChE activity was an independent predictor of post-operative complications, I suggest to (i) determine the sensitivity and specificity and set the cut-off value based on the ROC curve, and (ii) include the low BChE activity

(based on the cut-off) along with other established clinical variables in the multivariate regression model. Please present the model in the separate table.
Thank you for your important annotation and your valuable suggestion. We have carefully revised our statistics and stated effect sizes as indicated by reviewer 1. The effect sizes are much bigger compared to the CESARO study, which may rule out a great variance of enzyme activities in this patient group. To prove that pre-operative BChE activity is an independent predictor of postoperative complications we need further studies with much greater patient populations. So we have revised our discussion and limitations sections.
- Please have a careful look at the Figures:
o There is two times “Figure 1”.
You are right. We apologize for this misunderstanding. This has been corrected.
o Second Figure 1 has a typo (anestehsia).
This has been corrected.
o Please add the exact p-values to Figures 3 and 4.
This has been changed.
- In the discussion, the authors write that BChE activity, used in combination with common preoperative evaluation procedures, could serve as a useful predictive indicator to identify high-risk patients. To state this, multivariate regression analysis as proposed before is essential.
That’s correct. This has been changed.

I propose minor revision.

VERSION 2 – REVIEW

REVIEWER	Keage, Hannah University of South Australia Division of Health Sciences
REVIEW RETURNED	04-Feb-2021

GENERAL COMMENTS	Thank you for your responses and updates to the manuscript. I would like some further clarifications. Major 1. Regarding the rationale and justification for the grouping of postoperative complications. It is stated in the response to review document that “In previous retrospective studies with more than 800 transfemoral- and transapical TAVI patients we could show that delirium, pneumonia, acute renal failure and indication for permanent pacemaker were the most frequently reported perioperative complications (Goldfuss S et al., BMJ Open 2019; Würschinger F et al., PLOS ONE 2018)”. However, Würschinger et al. (2018) does not appear to include delirium and Goldfuss et al. (2019) is a paper relating to anesthesia-related complications and anesthesia-related side post-TAVI, not general complications. Further, Goldfuss et al. (2019) lists side effects and complications that are not included here yet have higher incidence (e.g. hypothermia) or a larger clinical importance (e.g. death). The rationale for the inclusion and grouping of postoperative complications is still unclear. 2. Regarding the effects (possible residual confounding) of anticholinergic medications. The Muller et al. paper did in fact report “significantly higher ADS [anticholinergic medication burden] score in patients with delirium in comparison with those without delirium”. It was only in multivariable analyses that this association was not statistically significant; however, that will occur when there are colinear factors in the model. Further, this paper looked at all

	surgical patients, not TAVI patients only, who are older and therefore have a much higher risk of current of previous anticholinergic medication use. Historical anticholinergic load can affect delirium and AChE/BChE (e.g. https://bmjopen.bmj.com/content/10/1/e031212). Medication effects are a major limitation and need to be noted. Given these ADS data are already available in this cohort, please (1) report in participant characteristic table and (2) run a partial correlation with BChE analysis to test if effects remain. 3. It is stated that “Effect sizes were particularly high for BChE measurements in this homogeneous patient group, which may rule out a great variance of enzyme activities”. There is however large variability (from data presented in tables). Could this statement please be corrected? Minor: 4. I believe the literature has shown small-medium effect sizes between AChE and BChE in critically ill patients; using the term “remarkable” may be overstating it. 5. There still is no justification for no correction for multiple comparisons. 6. It is stated that “Men showed a higher incidence of postoperative complications (p = 0.095)”; they did not show a significantly higher incidence (i.e. p>.05); please correct. 7. Statements such as “no difference regarding…” should be avoided. There was no statistically significant difference, but there was a difference. 8. They are not “high effect sizes” rather “large effect sizes”. 9. Please reference all these linked statements: “BChE activity could be regarded as an inflammatory parameter in this context. In literature, lower levels of BChE activity have already been described during inflammatory processes, stress and malnutrition. Therefore, lower levels of BChE activity in complication group might reflect perioperative inflammation, which is known to promote complications like delirium or infections. Conventional markers like CRP and leucocytes did not differ in both groups.” 10. Please include sample size and related statistical power as a major limitation in the Discussion.

REVIEWER	Gasecka, A Medical University of Warsaw
REVIEW RETURNED	04-Mar-2021

GENERAL COMMENTS	Previously, I suggested to complete the statistical analysis by (i) determining the sensitivity and specificity and set the cut-off value based on the ROC curve, and (ii) including low BChE activity (based on the cut-off) along with other established clinical variables in the multivariate regression model. In the statistical description the authors write that “A multivariate logistic regression analysis was performed to investigate the association between cholinesterase activity and postoperative complications”. However, the results of this analysis are not presented in modified version. Please provide the results.

	I propose minor revision.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Dr. Hannah Keage, University of South Australia Division of Health Sciences

Comments to the Author:

Thank you for your responses and updates to the manuscript. I would like some further clarifications.

Major

1. Regarding the rationale and justification for the grouping of postoperative complications. It is stated in the response to review document that “In previous retrospective studies with more than 800 transfemoral- and transapical TAVI patients we could show that delirium, pneumonia, acute renal failure and indication for permanent pacemaker were the most frequently reported perioperative complications (Goldfuss S et al., BMJ Open 2019; Würschinger F et al., PLOS ONE 2018)”. However, Würschinger et al. (2018) does not appear to include delirium and Goldfuss et al. (2019) is a paper relating to anesthesia-related complications and anesthesia-related side post-TAVI, not general complications. Further, Goldfuss et al. (2019) lists side effects and complications that are not included here yet have higher incidence (e.g. hypothermia) or a larger clinical importance (e.g. death). The rationale for the inclusion and grouping of postoperative complications is still unclear.

We appreciate this annotation and make a hopefully clearer explanation. Goldfuss et al. (2019) did in fact include more complications e.g. death or side effects like hypothermia. Since recent studies have shown that AChE and BChE serve as diagnostic markers of low-grade systemic inflammation, we focused on postoperative complications, which might be explained by local/systemic inflammation processes. A larger, possibly multicenter study would be needed to evaluate more complications. Therefore, we revised our limitation section.

2. Regarding the effects (possible residual confounding) of anticholinergic medications. The Muller et al. paper did in fact report “significantly higher ADS [anticholinergic medication burden] score in patients with delirium in comparison with those without delirium”. It was only in multivariable analyses that this association was not statistically significant; however, that will occur when there are colinear factors in the model. Further, this paper looked at all surgical patients, not TAVI patients only, who are older and therefore have a much higher risk of current of previous anticholinergic medication use. Historical anticholinergic load can affect delirium and AChE/BChE (e.g. https://bmjopen.bmj.com/content/10/1/e031212). Medication effects are a major limitation and need to be noted. Given these ADS data are already available in this cohort, please (1) report in participant characteristic table and (2) run a partial correlation with BChE analysis to test if effects remain.

As suggested, we included pre-operative medication in the characteristic table. Further, we conducted a partial correlation and a regression analysis. The results are indicated in the manuscript.

3. It is stated that “Effect sizes were particularly high for BChE measurements in this homogeneous patient group, which may rule out a great variance of enzyme activities”. There is however large variability (from data presented in tables). Could this statement please be corrected?

This has been corrected.

Minor:

4. I believe the literature has shown small-medium effect sizes between AChE and BChE in critically ill patients; using the term “remarkable” may be overstating it.

That’s correct. This has been corrected.

5. There still is no justification for no correction for multiple comparisons.

In our repeat-measurements post-hoc Bonferroni-correction was done to rule out alpha error accumulation. We revised therefore the material and methods section.

6. It is stated that “Men showed a higher incidence of postoperative complications ($p = 0.095$)”; they did not show a significantly higher incidence (i.e. $p>.05$); please correct.

We apologize for this misunderstanding. This has been corrected.

7. Statements such as “no difference regarding…” should be avoided. There was no statistically significant difference, but there was a difference.

This has been corrected.

8. They are not “high effect sizes” rather “large effect sizes”.

This has been corrected.

9. Please reference all these linked statements: “BChE activity could be regarded as an inflammatory parameter in this context. In literature, lower levels of BChE activity have already been described during inflammatory processes, stress and malnutrition. Therefore, lower levels of BChE activity in complication group might reflect perioperative inflammation, which is known to promote complications like delirium or infections. Conventional markers like CRP and leucocytes did not differ in both groups.”

References were included as suggested.

10. Please include sample size and related statistical power as a major limitation in the Discussion.

The limitation section has been revised.

Reviewer: 2

Dr. A Gasecka

Comments to the Author:

The authors corrected the manuscript to all my suggestions. I recommend accepting it in the present form.

Congratulations to the authors for the nice study.

Thank you for your constructive review of our manuscript.

Reviewer: 1

Competing interests of Reviewer: None declared

Reviewer: 2

Competing interests of Reviewer: None declared

VERSION 3 – REVIEW

REVIEWER	Keage, Hannah University of South Australia Division of Health Sciences
REVIEW RETURNED	04-May-2021

GENERAL COMMENTS	Thank you for responding to my comments. A very small point - please state p values consistently, regardless of statistical significance. E.g. do not us "n.s".

REVIEWER	Gasecka, A Medical University of Warsaw
REVIEW RETURNED	01-May-2021

GENERAL COMMENTS	Dear Authors, I recommended to accept the paper already at the previous review rounds. All my previous comments have been addressed. Congratulations with your research.