PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Serum N-terminal pro-B-type natriuretic peptide and cystatin C for acute kidney injury detection in critically ill adults in China: A prospective, observational study
AUTHORS	Deng, Jia; He, Linling; Liang, Yufan; Hu, Linhui; Xu, Jing; Fang, Heng; Li, Ying; Chen, Chunbo

VERSION 1 – REVIEW

REVIEWER	Lyu, Jun Jinan University First Affiliated Hospital
REVIEW RETURNED	27-Sep-2022

GENERAL COMMENTS	The manuscript by Jia Deng et al describes a prospective, observational study aimed at exploring the value of NT-proBNP and Cystatin c (sCystC), alone and in combination, in the diagnosis of acute kidney injury (AKI) in the intensive care unit. The topic and related results are innovative and interesting. However, the authors should pay attention to the following points: 1. Looking at the exclusion criteria, it appears that only patients having a severe chronic kidney disease (CKD) or nephrectomized patients or kidney transplant recipients should be excluded from the study. Why the authors decided that any other CKD KDIGO stage should have not been considered as an exclusion criterion? Would a GFR 60-15 ml/min and/or proteinuria and/or hematuria not be important confounders for this study? 2. As far as I can tell from Table 1, the diagnosis of AKI is made on the basis of a Scr determination on admission to ICU (related to previous Scr determinations), therefore, the AKI developed before the admission to ICU? How to show that the rise in NT-proBNP or sCystC preceded the increase in Scr in the patients that developed AKI? 3. Heart failure was present more frequently in the patients with AKI. Therefore, is not unexpected that this group had higher levels of NT-proBNP without attributing it to AKI. 4. According to clinical experience, many factors should also be related to an increase of NT-proBNP. How to prove that NT-proBNP contributes to the diagnosis of AKI and not other factors? 5. Are there any correlation of kidney prognosis and the levels of NT-proBNP or sCystC? 6. Authors are recommended to cite the following references (PMID:34380547). 7. The paper would benefit from improving the English language.

REVIEWER	Albert, Christian Diaverum Renal Services Group

REVIEW RETURNED	30-Sep-2022

GENERAL COMMENTS	Review Dear editor, thank you for the opportunity of reviewing the above manuscript. In the present study, the authors opt to identify the predictive value of a combined and single biomarker adjusted model of clinical variables enriched with NTproBNP and Cystatin C. Using established statistical methodology they conclude that both biomarkers may add benefit in risk prediction for AKI in patients admitted to a mixed ICU, even more so when measured in combination to identified clinical variables. I have the following suggestions to further improve the paper: Following on page 15 The authors used multiple clinical variables to test for independent predictors of AKI. Out of Table 3 that identified multiple variables that were independently predicting AKI in univariate logistic regression, the authors used stepwise forward integration to identify variables for their final model: Presumably: Adjusted for chronic kidney disease, heart failure, sepsis, admission type, serum creatinine at admission and acute physiology and chronic health evaluation II score. While this is a bread and butter methodology it is also considered as the statistically weakest approach as opposed to “enter” being the strongest method. It would therefore be informative to provide the threshold for variable stepwise forward integration (F or P-value). As the calculations built up, in theory any of the independent predictors should be able to increase predictive value of a biomarker when combined. This is also reflected in very high ORs in table 4 that is based on dichotomization by the biomarkers cutoff value with very strong predictors added. Accordingly, it is interesting that we see significant differences in the AUC of the models that the authors referenced as <0.05. As it is very hard to improve such high AUC values I would hypothesize that this AUC difference is barely below 0.05. Could the authors provide definite P-values please? As the final model is strong, it is no wonder that we see very high scoring reclassification metrics as well. For model characterization please include Goodness of fit. It would be interesting to see the respective reclassification metrics split into those for events and those for non-events to guide the reader where the improvement comes from as this is the strength of NRI/IDI over plain AUC. However, different samples and different model specifications will have different arbitrary scaling factors. Therefore, being a single center study, in order to overall improve the paper and the results I suggest to introduce cross-validation using a leave 10-out model as the overall patient numbers are >1000 in total. For reference please use Albert et al. Ann Lab Med 2020;40:131-141: https://doi.org/10.3343/alm.2020.40.2.131 Please additionally provide stages of AKI. Finally, it would be

	informative to assess if the model would be further improving when using severe AKI (stage 2+3) or AKI with indication for dialysis as outcome measure? (The SPSS program can run the model simulation in scripted sequence for multiple outcomes once predefined including the cross validation.) Minor remarks Page 12, Line 30 Patients were not involved in the study. This does not make sense at this point. Page 14, Please explain more clearly how the AUC were derived. My understanding was that opposed to calculating standard AUC for a dichotomous outcome AKI the authors included NT-proBNP and sCysC in a univariate logistic regression model to exlude interaction. The combined BNP/sCysC was that a multivariate logistic regression containing the two markers or were the values added/calculated(+?) to a new variable? As this is a single center study the authors should tone down on generalizability: In the present cohort, simultaneous measurement of NT-proBNP and sCysC at ICU admission increasesed the early identification of AKI beyond that of biomarker in isolation…

VERSION 1 – AUTHOR RESPONSE

Reviewer 1
Comment 1:
Looking at the exclusion criteria, it appears that only patients having a severe chronic kidney disease (CKD) or nephrectomized patients or kidney transplant recipients should be excluded from the study. Why the authors decided that any other CKD KDIGO stage should have not been considered as an exclusion criterion? Would a GFR 60-15 ml/min and/or proteinuria and/or hematuria not be important confounders for this study?
Response:
Thank you for your comment. The most significant risk factor for acute kidney injury is the presence of chronic kidney disease, so chronic kidney disease excluding end-stage renal disease is also the subject of our study. For patients with existing CKD, AKI will aggravate the condition of patients with CKD and accelerate the progression of kidney disease. Early identification of AKI in patients with CKD can slow the progression of kidney disease and improve the prognosis. Considering that CKD would be a confounding factor, we added this factor into the model for correction, so as to make the model more generalizability.

Comment 2:
As far as I can tell from Table 1, the diagnosis of AKI is made on the basis of a Scr determination on admission to ICU (related to previous Scr determinations), therefore, the AKI developed before the admission to ICU? How to show that the rise in NT-proBNP or sCysC preceded the increase in Scr in the patients that developed AKI?
Response:
Thank you for your comment. We apologize for any inconvenience caused by the context of the article, for which please allow me to explain as follows. AKI is defined according to the KDIGO criteria, which defines AKI as a rise in sCr by ≥ 0.3 mg/dL (26.5μmol/L) within 48 h or a rise in sCr to ≥ 1.5 times the baseline within 1 week, or urine output < 0.5 mL/kg/h for 6 h after ICU admission. When a patient admitted to the ICU, sCr was measured and subsequently done at least once a day until discharge as part of routine clinical care. The diagnosis of AKI is made when the creatinine value meets the KDIGO criteria for defining AKI. We measured NT-proBNP and sCysC once at ICU admission to predict the occurrence of AKI. They have different criteria for defining AKI. From this

perspective, the rise in NT-proBNP or sCysC preceded the increase in Scr in the patients that developed AKI.

Comment 3:
Heart failure was present more frequently in the patients with AKI. Therefore, is not unexpected that this group had higher levels of NT-proBNP without attributing it to AKI.
Response:
Thank you for your comment. Patients with renal injury are often combined with cardiac dysfunction, but the increased level of NT-proBNP in patients with renal dysfunction is not only related to the ventricular wall tension, but also related to renal dysfunction itself, whose mechanism is mainly correlated with the clearance of NT-proBNP in vivo. In the model, NT-proBNP still has diagnostic significance for AKI after heart failure correction. Of course, a higher cut-off value of NT-proBNP should be considered for AKI patients with heart failure.

Comment 4:
According to clinical experience, many factors should also be related to an increase of NT-proBNP. How to prove that NT-proBNP contributes to the diagnosis of AKI and not other factors?
Response:
Thank you for your comment. To demonstrate the ability of NT-proBNP for AKI detection, we calculated its AUC-ROC, which was 0.821. For AKI detection, NT-proBNP had a sensitivity of 78% and a specificity of 75%. We stratified patients based on the cutoff value of serum NT-proBNP levels into two categories. Compared with those with serum NT-proBNP < 204 pg/mL, patients with serum NT-proBNP ≥ 204 pg/mL on admission exhibited a higher incidence of AKI (7.2% versus 45.1%, $P < 0.001$). As we all know, the value of NT-proBNP is affected by age, obesity, comorbidities and other factors, so we corrected the factors influencing NT-proBNP. After adjustment, high NT-proBNP level of patients with AKI was associated with 3.5-fold higher odds for AKI compared with low NT-proBNP level ($P < 0.001$). Therefore, we believe that NT-proBNP contributes to the diagnosis of AKI, rather than other factors.

Comment 5:
Are there any correlation of kidney prognosis and the levels of NT-proBNP or sCystC?
Response:
Thank you for your comment. In the present study, we opt to identify the predictive value of a combined and single biomarker adjusted model of clinical variables enriched with NTproBNP and Cystatin C. Using established statistical methodology, we conclude that both biomarkers may add benefit in risk prediction for AKI in patients admitted to a mixed ICU, even more so when measured in combination to identified clinical variables. We supposed that there is correlation of kidney prognosis and the levels of NT-proBNP or sCysC. However, future studies are needed to explore the relationship linking these renal markers to kidney prognosis, and that is also what we will do next.

Reviewer 2
Major comments
Comment 1:
Following on page 15 The authors used multiple clinical variables to test for independent predictors of AKI. Out of Table 3 that identified multiple variables that were independently predicting AKI in univariate logistic regression, the authors used stepwise forward integration to identify variables for their final model: Presumably: Adjusted for chronic kidney disease, heart failure, sepsis, admission type, serum creatinine at admission and acute physiology and chronic health evaluation II score.
While this is a bread and butter methodology it is also considered as the statistically weakest approach as opposed to “enter” being the strongest method.
It would therefore be informative to provide the threshold for variable stepwise forward integration (F or P-value).
Response:
Thank you for your comment. We apologize for any inconvenience caused by the context of the article, for which please allow me to explain as follows. Logistic analysis was utilized to identify the independent risk factors of AKI, with a forward stepwise method, in which the clinical variables with $P < 0.10$ in univariate analysis were incorporated into the multivariate logistic model. The threshold for variable stepwise forward integration was $P<0.10$, this threshold was stated in the statistical analysis of the revised manuscript. (Page 12, Line 9, the part of **statistical analysis** in main document-marked copy)

Comment 2:
As the calculations built up, in theory any of the independent predictors should be able to increase predictive value of a biomarker when combined. This is also reflected in very high ORs in table 4 that is based on dichotomization by the biomarkers cutoff value with very strong predictors added.
Accordingly, it is interesting that we see significant differences in the AUC of the models that the authors referenced as <0.05. As it is very hard to improve such high AUC values I would hypothesize that this AUC difference is barely below 0.05. Could the authors provide definite P-values please?

Response:
Thank you for your comment. According to your suggestions, we have made corresponding changes in the revised manuscript. As shown in table 2, we used AUC-ROCs to calculate the two biomarkers, respectively, and in combination to demonstrate the ability of these biomarkers for AKI detection. AUC-ROCs for NT-proBNP and sCysC were computed for AKI detection (0.821 and 0.766, respectively). The AUC-ROCs for AKI presented a better performance by NT-proBNP plus sCysC than by any individual biomarker (*P*=0.0145, *P*<0.0001, respectively). We provide definite P-value for differences in the AUC of the models in the table 2 of the revised manuscript. (Page17, Table 2 in main document-marked copy)

Comment 3:
As the final model is strong, it is no wonder that we see very high scoring reclassification metrics as well. For model characterization please include Goodness of fit.
Response:
Thank you for your comment. According to your suggestions, we have made corresponding changes in the revised manuscript. Considering the effect of adding NT-proBNP and sCysC, or any of them, to a clinical model as categorical variables for AKI detection, logistic regression analysis was employed. As shown in Table 5, we had four models, adding NT-proBNP and sCysC to a clinical model for AKI detection further increased the AUC-ROC to 0.859 beyond that of the clinical model with or without sCysC ($P < 0.05$) (Figure 3). Moreover, this panel addition to the clinical model significantly enhanced the risk reclassification of AKI beyond that of the clinical model with or without any individual biomarkers ($P < 0.05$), with maximum NRI (0.531) and IDI (0.038). Hosmer–Lemeshow goodness-of-fit test: for clinical model, chi-squared value=14.249(P=0.075); for clinical model + sCysC, chi-squared value=6.971 (P=0.54); for clinical model + NT-proBNP, chi-squared value=9.362 (P=0.313); or clinical model + NT-proBNP +sCysC, chi-squared value=4.245 (P=0.834). We provide the result of Hosmer–Lemeshow goodness-of-fit test for model characterization in footnote of table 5. (Page 20, Table 5 in main document-marked copy)

Comment 4:
It would be interesting to see the respective reclassification metrics split into those for events and those for non-events to guide the reader where the improvement comes from as this is the strength of NRI/IDI over plain AUC.
However, different samples and different model specifications will have different arbitrary scaling factors. Therefore, being a single center study, in order to overall improve the paper and the results I suggest to introduce cross-validation using a leave 10-out model as the overall patient numbers are >1000 in total. For reference please use Albert et al. Ann Lab Med 2020;40:131-141: https://doi.org/10.3343/alm.2020.40.2.131
Response:
Thank you for making this valuable suggestion. We apologize for any inconvenience caused by the context of the article, for which please allow me to explain as follows. In the original paper, the calculation of NRI and IDI for AKI prediction models was internally validated by a bootstrap method with 1000 replications, the statistical significance alpha was set at 0.05. According to your suggestions, we have made corresponding changes in the revised manuscript. We adopted the new method you have mentioned, using 10-fold cross-validation as the overall patient numbers are >1000 in total. The cross-validated baseline performance characterized by accuracy and Kappa for the clinical model was 0.844 and 0.448. Cross-validation baseline performance characterized by accuracy and Kappa was 0.848 and 0.475 for clinical model enriched with NTproBNP and Cystatin C. The results of cross validation show that the models we finally selected was reliable and stable. Thank you very much for your valuable advice and great help. (Page 12, Line 19 and Page 15, Line 1 and 10 in main document-marked copy)

Comment 5:
Please additionally provide stages of AKI. Finally, it would be informative to assess if the model would be further improving when using severe AKI (stage 2+3) or AKI with indication for dialysis as outcome measure? (The SPSS program can run the model simulation in scripted sequence for multiple outcomes once predefined including the cross validation.)
Response:
Thank you for this very insightful comment. We are very sorry that we cannot completely revise the manuscript according to your suggestion. We conducted a prospectively-recruited, observational study that were designed to predict the diagnosis of total AKI. From your suggestion, we also realized that it would make sense to assess whether the model would be further improving, when adding severe AKI (stage 2+3) or AKI with dialysis indications as outcome measure. Future studies are needed to explore the relationship linking these renal markers to different outcome measure, and that is also which necessitate to do next.

VERSION 2 – REVIEW

REVIEWER	Lyu, Jun Jinan University First Affiliated Hospital
REVIEW RETURNED	21-Nov-2022

GENERAL COMMENTS	It is OK.