PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Preadmission use of benzodiazepines and stroke outcomes: the Biostroke prospective cohort study.
AUTHORS	Colin, Olivier; Labreuche, Julien; Deguil, Julie; Mendyk, Anne Marie; Deken, Valerie; Cordonnier, Charlotte; Deplanque, Dominique; Leys, Didier; Bordet, Régis

VERSION 1 – REVIEW

REVIEWER	Peter Knapp Department of Health Sciences, University of York, and the Hull York Medical School, UK
REVIEW RETURNED	02-Apr-2018

GENERAL COMMENTS	This is a clear, well-written account of a cohort study addressing an important question. If replicated or supported by additional analyses, the findings are important and have the potential to influence clinical practice in prescribing and/or in stroke care. The use of a cohort study design is appropriate to the question and in many ways the study has been designed and conducted so as to reduce concerns about bias and threats to validity. Patients at the single centre have been recruited consecutively, which is a strength. That the study needs replication in other, potentially multi-site studies needs acknowledgement, as does the need for replication in countries where there may be different practice in the prescribing on benzodiazepines. The exclusion criteria seem correct and unlikely to have introduced bias. It's not clear why study recruitment ended at 370: was there a prior sample size calculation? Were the patients being recruited for a study of a different question, with the benzodiazepines question being of secondary interest? This is a study of the effect of this class of drug on the mortality of patients who had a stroke and survived at least until hospital admission. The article doesn't account for two groups of patients: first, those who had a stroke but died before admission; second, those whose stroke was deemed so minor or perhaps temporary, that admission was not thought necessary. Are the authors able to estimate the size of these groups and, if so, their potential impact on the drug:excess mortality argument being proposed? If useful data aren't available, it would be important to acknowledge that this study is limited to mortality in patients admitted to hospital after stroke.

	What proportion of the patients taking benzodiazepines at admission, continued to take them after stroke? For example, did they tend to be stopped in patients with post-stroke swallowing problems? Perhaps there should be a little more clarity in the article of the relative effects of pre- and post-stroke benzodiazepines, for their effects on mortality. Patients were classified as benzodiazepines users if they had at least 15 days of use pre-stroke. Are there data on the length of time patients had been using the drug? Is it reasonable to combine patients with 15 days' use with those with several years of use? Does the potential harmful mechanism of the benzodiazepines support the possibility of an effect after 15 days? Is dosage important? Would higher daily doses of benzodiazepines carry additive risk of mortality? The authors have converted a number of measures (Bathel; NIHSS, Rankin, MMSE) into categoric variables for the analyses. Given the strength of the reported relationship with mortality, is there value in investigating what effects are seen when these measures are used as interval scales, as originally intended? There has been multiple imputation for missing data - what results are found when the data are not imputed and only participants with complete data records are included? This will reduce the available sample size, of course, but would be a more cautious form of analysis. Finally it would be helpful for the authors to comment further on possible alternative explanations for the effect reported. It's possible, as the authors acknowledge, that the indication for benzodiazepines may be the causative variable; as such it would be helpful for there to be some reference to any evidence that mood (depression or anxiety) increases mortality. I was an author on a paper that demonstrated a relationship in 2001 (http://stroke.ahajournals.org/content/32/3/696.short) and others have investigated and reported this issue since. Secondly, what evidence is there from RCTs of benzodiazepines, that they may have the harmful effects suggested by this study? This is a potentially important study - my view is that these points, if clarified, would increase the value of the study.

REVIEWER	Chian-Jue Kuo Taipei City Psychiatric Center, Taipei, Taiwan
REVIEW RETURNED	08-Apr-2018

GENERAL COMMENTS	Thanks for having the privilege to review the article. This study employed the prospective “Biostroke” cohort to estimate the association of preadmission use of benzodiazepines and post-stoke mortality. In line with previous retrospective studies in this field, this study supported the increased risk of post-stroke mortality associated with benzodiazepines use in a prospective study design. Despite this study had some findings, there existed some significant issues/problems in the design, along with the format of the paper. 1. The length of the Introduction is too short and there are no complete literature review and the proposed aims.

	2. Page 6, first paragraph: there seems to have no clear definition of time window regarding the exposure of benzodiazepines before the stroke, which could be crucial for determining the causal relationship of benzodiazepines on the mortality in such populations. 3. In this study, benzodiazepine users (n=62) were significant older than benzodiazepine non-users (n=274) (Table 1). Age is a strong and well-known predictor for mortality. Despite this study used the propensity-score method for the adjustment, it still existed the significant limitation. This study found preadmission use of benzodiazepines associated with increased post-stroke mortality, in which could not be ruled out the confounding effect of older age in benzodiazepine users. 4. The paper has many grammatical errors especially in the abstract part and strengths and limitations part, to name a few: Page 2 Line 12 …are worse than those without use of benzodiazepines Page 2 Line 14 Patients using psychoactive drugs other than benzodiazepines were excluded. Page 2 Line 18 A total number of 336 patients were included, among which 62 patients (18.5%) were using benzodiazepines before admission. Page 2 Line 22 Benzodiazepines users… Page 3 Line 12 a non-neuroprotective … Page 3 Line 23 new experimental approaches are needed to … Page 5 Line 33 either a CT scan or an MRI scan … The article is better to be edited by a native English editors. 5. The type of benzodiazepines and hypnotic drugs should be listed in supplement. Concerning the hypnotic drugs included mainly non-benzodiazepine GABA agonists, the statement on benzodiazepines in the context could be substituted with benzodiazepines and related drugs (BZDR). 6. The study included patients with benzodiazepine use for more than 15 days prior to admission and used the propensity scores to address the confounding variables. However, the treatment regimen for stroke might be different between patients with benzodiazepines and without. During the treatment period for stroke, whether to use benzodiazepines to prevent withdrawal symptoms or not to use benzodiazepines to avoid clouding the consciousness should be tailored to patients' clinical condition. The usage of benzodiazepines during treatment period could influence the outcome.

REVIEWER	Chieh-Yu Liu National Taipei University of Nursing and Health Sciences, Taiwan, R.O.C.
REVIEW RETURNED	30-May-2018

GENERAL COMMENTS	This is an interesting study, however, I have a serious statistical methodology concern. Because the outcome variable is the "mortality", which implied the authors has the time-to-event data, not just the binary outcome (death/survive) variable. Why not use the survival data analysis methods , for example, Kaplan-Meier method or Cox proportional hazard model? If the authors simplified the mortality outcome into simple a binary variable, the

	results and evidences will be much lower than results obtained from survival data analysis. Can you tell me why?

REVIEWER	William Meurer Department of Emergency Medicine, Department of Neurology, University of Michigan, Ann Arbor, U.S.A.
REVIEW RETURNED	24-Jun-2018

GENERAL COMMENTS	Thank you for the opportunity to review this manuscript. At the current time, I am concerned that residual confounding is the most likely explanation for these findings. Given the extremely large effect observed, it seems relatively inconceivable that all of this excess mortality could be attributed to benzodiazepines. This is a challenge in relatively low sample size research. Several improvements could contribute to my consideration of this as a valid observation. 1. The choice to dichotomize the NIHSS as a baseline predictor of severity is questionable. While you don't have enough events to include multiple events, this would be a case where including it as a continuous predictor would be helpful. I understand you did statistical adjustment, but given the small group size and relatively small number of events, I have concerns that a few influential cases are driving the model results. 2. Similarly, there are large imbalances in age and hypertension. 3. The methods and baseline characteristics do not provide premorbid functional status. Patients who dwell in a care facility may be more likely to be getting benzos, and thus more likely to die. 4. The use of the propensity score within the logistic models is one approach to reducing bias, although I am not sure it is adequate. I would prefer a matching technique (where the non-matched non-benzo cases are actually deleted from the main comparison) similar to what was used here https://www.ncbi.nlm.nih.gov/pubmed/23033348 . The main outcome of this would be the difference in proportions across groups with the 95% confidence interval. In addition, the differences in baseline characteristics across cohorts could be given before and after matching. The benefit of this approach, given your small sample size, is that you could use an over-parameterized model to create your propensity score and you (and the readers) could directly review how well the cohorts match up after the procedure from the demographics table. 5. Since this is a small sample size, it would be beneficial to provide a table for all mortality cases (a new line for each case) that has age (this could be rounded to decade of life for privacy concerns), gender, baseline NIHSS, premorbid functional status (independent versus not at least), and benzo exposure. It would be nice to also include stroke risk factors in such a table. 6. Overall, it is possible that this unique association exists. However you need to augment your design in order to maximally account for concerns about residual confounding. 7. You could keep all of your current analyses and perform those suggested by me as sensitivity analyses. If after performing my suggested analyses, you feel mine are easier and more representative, you could shift them into the primary report. 8. You should perform additional analyses and include the fragility index (http://clincalc.com/Stats/FragilityIndex.aspx) for your mortality comparisons. If you do the more directed matching I

	request, it will be straightforward to also do this calculation in the matched (adjusted) cohort. 9. I recommend performing a sensitivity analysis to determine how large an unmeasured confounder would need to be to explain your results. The investigators in this study did a nice job of this (Seymour, Christopher W., et al. "Time to treatment and mortality during mandated emergency care for sepsis." New England Journal of Medicine 376.23 (2017): 2235-2244.) The main results of this are in their supplement.

VERSION 1 – AUTHOR RESPONSE

FOR THE REVIEWERS :
Reviewer: 1
Reviewer Name: Peter Knapp
Institution and Country: Department of Health Sciences, University of York, and the Hull York Medical School, UK Please state any competing interests or state 'None declared': None

Please leave your comments for the authors below

This is a clear, well-written account of a cohort study addressing an important question. If replicated or supported by additional analyses, the findings are important and have the potential to influence clinical practice in prescribing and/or in stroke care.

The use of a cohort study design is appropriate to the question and in many ways the study has been designed and conducted so as to reduce concerns about bias and threats to validity.

Patients at the single centre have been recruited consecutively, which is a strength. That the study needs replication in other, potentially multi-site studies needs acknowledgement, as does the need for replication in countries where there may be different practice in the prescribing on benzodiazepines.

The exclusion criteria seem correct and unlikely to have introduced bias. It's not clear why study recruitment ended at 370: was there a prior sample size calculation? Were the patients being recruited for a study of a different question, with the benzodiazepines question being of secondary interest?
Answer: There is no sample size calculation for the present ancillary analysis of BIOSTROKE cohort (Clinical Biological and Phamacological Factors Influencing Stroke Outcome). We confirm that the patients were recruited in BIOSTROKE for another aim, which was to understand the mechanisms of preventive neuroprotection by establishing link between biomarkers and preventive and neuroprotective measures. Use of benzodiazepine was one of the interests. We added this in "Methods" and "Limitations" sections.

This is a study of the effect of this class of drug on the mortality of patients who had a stroke and survived at least until hospital admission. The article doesn't account for two groups of patients: first, those who had a stroke but died before admission; second, those whose stroke was deemed so minor or perhaps temporary, that admission was not thought necessary. Are the authors able to estimate the size of these groups and, if so, their potential impact on the drug:excess mortality argument being proposed? If useful data aren't available, it would be important to acknowledge that this study is limited to mortality in patients admitted to hospital after stroke.
Answer: Unfortunately, we are not able to estimate the numbers of patients who died before admission. Those whose stroke was minor were all admitted in our stroke unit (routine clinical

practice). We acknowledge that this study is limited to mortality in patients admitted to hospital after stroke (added in “limitations of the study”).

What proportion of the patients taking benzodiazepines at admission, continued to take them after stroke? For example, did they tend to be stopped in patients with post-stroke swallowing problems? Perhaps there should be a little more clarity in the article of the relative effects of pre- and post-stroke benzodiazepines, for their effects on mortality.
Answer: 57.9% of the survivor patients taking benzodiazepines at admission continued to take them after stroke at day-8, and 46.5 % at day-90. Data cannot be available for patients who died between admission and the theorical follow-up examination 8 and 90 days after stroke.

Patients were classified as benzodiazepines users if they had at least 15 days of use pre-stroke. Are there data on the length of time patients had been using the drug? Is it reasonable to combine patients with 15 days' use with those with several years of use? Does the potential harmful mechanism of the benzodiazepines support the possibility of an effect after 15 days?
Answer: Data on the length of time patients had been using the drug are unfortunately not available. As, it is reasonable to think that they had an inadequate situation with excessive duration of prescription (demographic characteristics) (Airagnes G, Pelissolo A, Lavallée M et al. Benzodiazepine Misuse in the Elderly: Risk Factors, Consequences, and Management. Curr Psychiatry Rep. 2016 Oct;18(10):89). We added this comment in the “Limitations” section.

Is dosage important? Would higher daily doses of benzodiazepines carry additive risk of mortality?
Answer: Dosage may be important in additive risk of mortality. (Wu CK, Huang YT, Lee JK, Jimmy Juang JM, Tsai CT, Lai LP et al. Anti-anxiety drugs use and cardiovascular outcomes in patients with myocardial infarction: a national wide assessment. Atherosclerosis. 2014;235:496-502.)

The authors have converted a number of measures (Barthel; NIHSS, Rankin, MMSE) into categoric variables for the analyses. Given the strength of the reported relationship with mortality, is there value in investigating what effects are seen when these measures are used as interval scales, as originally intended?
Answer: Barthel Index, Rankin and MMSE are widely converted into categoric variables considering stroke outcomes in the literature. Definitions used for variables included in the analysis have been previously defined (Deplanque D, Masse I, Lefebvre C, Libersa C, Leys D, Bordet R. Prior TIA, lipid-lowering drug use, and physical activity decrease ischemic stroke severity. Neurology. 2006;67:1403–1410.)

There has been multiple imputation for missing data - what results are found when the data are not imputed and only participants with complete data records are included? This will reduce the available sample size, of course, but would be a more cautious form of analysis.
Answer: When we repeated our previous analysis on complete case analysis (n=without missing data in baseline characteristics included in the propensity score), the adjusted OR of mortality at 90-day remained unchanged (OR, 3.98; 95%CI, 1.66 to 9.49; p=0.002).
We modified the strategy of analysis regarding reviewer’s 4 comments, who suggest to calculate the propensity score by including all-baseline characteristics (not just those with significant difference at $p<0.20$ in bivariate comparisons), and performed propensity-score matching as primary analysis. By including all-baseline characteristics, the propensity score could not be calculated for183 patients (54.5%; n=38 benzodiazepine users vs. 145 non benzodiazepine users). Those, that the sample with complete records is too small to replicate the propensity-score matched and adjusted analyses.

Finally it would be helpful for the authors to comment further on possible alternative explanations for the effect reported. It's possible, as the authors acknowledge, that the indication for benzodiazepines may be the causative variable; as such it would be helpful for there to be some reference to any

evidence that mood (depression or anxiety) increases mortality. I was an author on a paper that demonstrated a relationship in 2001 (http://stroke.ahajournals.org/content/32/3/696.short) and others have investigated and reported this issue since. Secondly, what evidence is there from RCTs of benzodiazepines, that they may have the harmful effects suggested by this study?
This is a potentially important study - my view is that these points, if clarified, would increase the value of the study.

Answer: We agree with reviewer comment. We comment further on possible alternative explanations, as the indication for benzodiazepines may be the causative variable. We added the reference that mood (depression or anxiety) increases mortality. Secondly, a recent review does not provide the evidence with a neuroprotective effect of benzodiazepines with a moderate increased mortality.

Reviewer: 2
Reviewer Name: Chian-Jue Kuo
Institution and Country: Taipei City Psychiatric Center, Taipei, Taiwan
Please state any competing interests or state 'None declared': I have declared that no competing interests exist.

Please leave your comments for the authors below

Thanks for having the privilege to review the article. This study employed the prospective "Biostroke" cohort to estimate the association of preadmission use of benzodiazepines and post-stoke mortality. In line with previous retrospective studies in this field, this study supported the increased risk of post-stroke mortality associated with benzodiazepines use in a prospective study design. Despite this study had some findings, there existed some significant issues/problems in the design, along with the format of the paper.

1. The length of the Introduction is too short and there are no complete literature review and the proposed aims.
Answer: We expand our Introduction section, to include more background context and a better explained rationale for performing this study.

2. Page 6, first paragraph: there seems to have no clear definition of time window regarding the exposure of benzodiazepines before the stroke, which could be crucial for determining the causal relationship of benzodiazepines on the mortality in such populations.
Answer: Definition of time window regarding the exposure of benzodiazepines before the stroke was defined by benzodiazepine drugs administered orally for more than fifteen days before stroke, as it was a routine clinical practice cohort. Data on the length of time patients had been using the drug are so not available. Considering their demographic characteristics, it is reasonable to think that they had an inadequate situation with excessive duration of prescription (Airagnes G, Pelissolo A, Lavallée M et al. Benzodiazepine Misuse in the Elderly: Risk Factors, Consequences, and Management. Curr Psychiatry Rep. 2016 Oct;18(10):89)

3. In this study, benzodiazepine users (n=62) were significant older than benzodiazepine non-users (n=274) (Table 1). Age is a strong and well-known predictor for mortality. Despite this study used the propensity-score method for the adjustment, it still existed the significant limitation. This study found

preadmission use of benzodiazepines associated with increased post-stroke mortality, in which could not be ruled out the confounding effect of older age in benzodiazepine users.
Answer: We agree with reviewer comment, that the difference in age in strong. However, as suggested by the reviewer 4, we now assemble two matched groups using a propensity score (now calculated using all baseline characteristics). As show in figure 1, the difference in age was strongly reduced with an absolute standardized difference of 55.7% before matching and 0.2 after. In propensity-score matched, the association of benzodiazepines and 90-day mortality was of borderline of significance, with however, comparable estimates in term of odd ratio (propensity-score matched OR, 3.98 vs. propensity-score adjusted OR, 3.50). We caution that our sample is limited to perform matched analysis with adequate statistical power. We added in discussion, a limitation on this issue.

4. The paper has many grammatical errors especially in the abstract part and strengths and limitations part, to name a few:
Page 2 Line 12 …are worse than those without use of benzodiazepines
Page 2 Line 14 Patients using psychoactive drugs other than benzodiazepines were excluded.
Page 2 Line 18 A total number of 336 patients were included, among which 62 patients(18.5%) were using benzodiazepines before admission.
Page 2 Line 22 Benzodiazepines users…
Page 3 Line 12 a non-neuroprotective …
Page 3 Line 23 new experimental approaches are needed to …
Page 5 Line 33 either a CT scan or an MRI scan …
The article is better to be edited by a native English editors.
Answer : We have check for errors in language.

5. The type of benzodiazepines and hypnotic drugs should be listed in supplement. Concerning the hypnotic drugs included mainly non-benzodiazepine GABA agonists, the statement on benzodiazepines in the context could be substituted with benzodiazepines and related drugs (BZDR).
Answer: We agree with reviewer comment, but hypnotic drugs also act on GABAa receptors in the brain. They were so included as benzodiazepines due to their same pharmacodynamic properties. A classification of benzodiazepines on the basis of elimination half-life could represent a more rational comparison of the potential durations of effect and reflect their relative potentials for accumulation at the site of action (Pierce and Franklin. The classification of benzodiazepine hypnotics. Br J Clin Pharmacol. 1983 Sep; 16(3): 345–347.) Unfortunately, these data are missing.

6. The study included patients with benzodiazepine use for more than 15 days prior to admission and used the propensity scores to address the confounding variables. However, the treatment regimen for stroke might be different between patients with benzodiazepines and without. During the treatment period for stroke, whether to use benzodiazepines to prevent withdrawal symptoms or not to use benzodiazepines to avoid clouding the consciousness should be tailored to patients' clinical condition. The usage of benzodiazepines during treatment period could influence the outcome.
Answer: 57.9% of the survivor patients taking benzodiazepines at admission continued to take them after stroke at day-8, and 46.5 % at day-90. Data cannot be available for patients who died between admission and the theorical follow-up examination 8 and 90 days after stroke (routine clinical practice cohort).

Reviewer: 3
Reviewer Name: Chieh-Yu Liu
Institution and Country: National Taipei University of Nursing and Health Sciences, Taiwan, R.O.C.
Please state any competing interests or state 'None declared': No

Please leave your comments for the authors below

This is an interesting study, however, I have a serious statistical methodology concern. Because the outcome variable is the "mortality", which implied the authors has the time-to-event data, not just the binary outcome (death/survive) variable. Why not use the survival data analysis methods , for example, Kaplan-Meier method or Cox proportional hazard model? If the authors simplified the mortality outcome into simple a binary variable, the results and evidences will be much lower than results obtained from survival data analysis. Can you tell me why?

Answer : We did not use survival analysis since the time of occurrence of all-cause mortality is not of interest, as in done in most of the randomized clinical trial on acute stroke treatment, with 90-day all-cause mortality as an endpoint and analyzed as binary variable. We disagree that results and evidences will be much lower than results obtained from survival data analysis, since we limited the follow-up time to 3-month.

Reviewer: 4
Reviewer Name: William Meurer
Institution and Country: Department of Emergency Medicine, Department of Neurology, University of Michigan, Ann Arbor, U.S.A.
Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below

Thank you for the opportunity to review this manuscript. At the current time, I am concerned that residual confounding is the most likely explanation for these findings. Given the extremely large effect observed, it seems relatively inconceivable that all of this excess mortality could be attributed to benzodiazepines. This is a challenge in relatively low sample size research. Several improvements could contribute to my consideration of this as a valid observation.

1. The choice to dichotomize the NIHSS as a baseline predictor of severity is questionable. While you don’t have enough events to include multiple events, this would be a case where including it as a continuous predictor would be helpful. I understand you did statistical adjustment, but given the small group size and relatively small number of events, I have concerns that a few influential cases are driving the model results.
Answer: To acknowledge the reviewer comment, we now analyzed the baseline NIHSS as a continuous variable.

2. Similarly, there are large imbalances in age and hypertension.
Answer: We caution that there is a strong imbalance in age and hypertension between study groups, which were taking to account in propensity score adjustment. As suggested by reviewer, we now performed a propensity-score matched analysis, and as shown in figure the difference in age and hypertension were strongly reduced after matching.

3. The methods and baseline characteristics do not provide premorbid functional status. Patients who dwell in a care facility may be more likely to be getting benzos, and thus more likely to die.
Answer: Unfortunately, we don’t’ have information on premorbid functional status. We added this in the “limitations” section.

4. The use of the propensity score within the logistic models is one approach to reducing bias, although I am not sure it is adequate. I would prefer a matching technique (where the non-matched non-benzo cases are actually deleted from the main comparison) similar to what was used here

https://www.ncbi.nlm.nih.gov/pubmed/23033348 . The main outcome of this would be the difference in proportions across groups with the 95% confidence interval. In addition, the differences in baseline characteristics across cohorts could be given before and after matching. The benefit of this approach, given your small sample size, is that you could use an over-parameterized model to create your propensity score and you (and the readers) could directly review how well the cohorts match up after the procedure from the demographics table.
Answer: We thanks the reviewer for his suggestion which improve the quality of analyses. As suggested, we now reported the propensity-score matched analysis as primary analysis. We also now included all baseline characteristics in propensity score calculation (which is clearly an over-parameterized model), and provided baseline characteristics according to study group before and after propensity score matching. To illustrate the confusion bias reduction by using propensity-score matching, we plotted the absolute standardized differences (ASD) before and after matching; most of the imbalances were reduced, with ASD <10%.
Although the difference in 90-day mortality between study groups did not reach the significance level in propensity-score matched cohort, the effect size (propensity-score matched OR, 3.98, 95%CI, 0.91 to 16.98, p=0.067) was comparable to the effect size estimated in propensity-score adjusted cohort (propensity-score adjusted OR, 3.50; 95%CI, 1.57 to 7.76, p=0.002). We caution that our sample is limited to perform matched analysis with adequate statistical power. We added in discussion, a limitation on this issue.

5. Since this is a small sample size, it would be beneficial to provide a table for all mortality cases (a new line for each case) that has age (this could be rounded to decade of life for privacy concerns), gender, baseline NIHSS, premorbid functional status (independent versus not at least), and benzo exposure. It would be nice to also include stroke risk factors in such a table.
Answer: As suggested we added a supplemental table on all mortality cases at 90-day including demographics and main vascular risk factors.

6. Overall, it is possible that this unique association exists. However you need to augment your design in order to maximally account for concerns about residual confounding.
Answer: We caution that this association is derived from observational analyses which are subject to well-known limitations (selection bias, residual confounding), which should be interpreted with caution and replicated in further studies. We more emphases in the discussion the limitations.

7. You could keep all of your current analyses and perform those suggested by me as sensitivity analyses. If after performing my suggested analyses, you feel mine are easier and more representative, you could shift them into the primary report.
Answer: We thanks the reviewer for your suggestion which improve the quality of analyses and we decided to consider the propensity-score matched analysis as a primary analysis, since we agree that this method reduce the impact of imbalance characteristics more than the propensity-score adjusted analysis (although the former had a lower power to detect difference since the sample was reduced).

8. You should perform additional analyses and include the fragility index (http://clincalc.com/Stats/FragilityIndex.aspx) for your mortality comparisons. If you do the more directed matching I request, it will be straightforward to also do this calculation in the matched (adjusted) cohort.
Answer: Since the fragility index was developed for clinical trial, we believe that calculating such index does not bring useful information. We caution that regarding the study design, the study sample size, the limited number of death, our results should be interpreted as hypothesis generating (without possibility of concluding that there is a causal effect) and should be replicated in further studies. We use the unique opportunity to address the potential risk of benzodiazepines in the BIOSTROKE cohort study.

9. I recommend performing a sensitivity analysis to determine how large an unmeasured confounder would need to be to explain your results. The investigators in this study did a nice job of this (Seymour, Christopher W., et al. "Time to treatment and mortality during mandated emergency care for sepsis." New England Journal of Medicine 376.23 (2017): 2235-2244.) The main results of this are in their supplement.
Answer: We read with interest this reference. We believe that the quantitative bias analysis which was based on hypothetical unmeasured confounder is difficult to understand for most of readers. Only randomized clinical trial can address such bias. To acknowledge the reviewer comments, we added a paragraph of limits.

VERSION 2 – REVIEW

REVIEWER	Peter Knapp University of York & the Hull York Medical School, UK
REVIEW RETURNED	07-Aug-2018

GENERAL COMMENTS	The authors have responded to all 4 reviewers' comments and the article is improved.

REVIEWER	Chian-Jue Kuo Taipei City Psychiatric Center, Taipei, Taiwan
REVIEW RETURNED	14-Aug-2018

GENERAL COMMENTS	None.

REVIEWER	CHIEH-YU LIU National Taipei University of Nursing and Health Sciences, Taiwan, R.O.C.
REVIEW RETURNED	06-Aug-2018

GENERAL COMMENTS	The relationship between preadmission use of benzodiazepines and mortality (no matter 8 days or 90 days) should be interpreted with care. In this manuscript, the authors did not provide the dosage information of preadmission use of benzodiazepines, which is my major concern of this manuscript. Also, the operational definition of "preadmission use of benzodiazepines" was still too vague: "Drug exposition was defined by benzodiazepine drugs administered orally for more than fifteen days before stroke regardless of length of treatment period and dosage of treatment". If stroke patients who have received benzodiazepines for a long time before stroke onset, such patients may have higher risk to have dysphagia or bucking which may also result in higher risk of mortality.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Peter Knapp
Institution and Country: University of York & the Hull York Medical School, UK
Please state any competing interests or state ‘None declared’: None

Please leave your comments for the authors below
The authors have responded to all 4 reviewers' comments and the article is improved.

Reviewer: 2
Reviewer Name: Chian-Jue Kuo
Institution and Country: Taipei City Psychiatric Center, Taipei, Taiwan
Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below
None.

Reviewer: 3
Reviewer Name: CHIEH-YU LIU
Institution and Country: National Taipei University of Nursing and Health Sciences, Taiwan, R.O.C. Please state any competing interests or state 'None declared': No. Please leave your comments for the authors below

The relationship between preadmission use of benzodiazepines and mortality (no matter 8 days or 90 days) should be interpreted with care. In this manuscript, the authors did not provide the dosage information of preadmission use of benzodiazepines, which is my major concern of this manuscript.

Also, the operational definition of "preadmission use of benzodiazepines" was still too vague: "Drug exposition was defined by benzodiazepine drugs administered orally for more than fifteen days before stroke regardless of length of treatment period and dosage of treatment". If stroke patients who have received benzodiazepines for a long time before stroke onset, such patients may have higher risk to have dysphagia or bucking which may also result in higher risk of mortality.

We agree with the reviewer's comment. In our cohort, the dosage information of preadmission use of benzodiazepines was unfortunately not reported *(see "discussion section)*. Our definition was pragmatic, based on prescriptions at admission in this routine cohort. We hypothesize that therapeutic doses of benzodiazepines were prescribed and that their use was predominantly chronic in older people, as suggested by epidemiological studies *(Billioti de Gage S et al. Benzodiazepine use and risk of dementia: prospective population-based study. BMJ. 2012;345:e6231; Airagnes G et al. Benzodiazepine Misuse in the Elderly: Risk Factors, Consequences, and Management. Curr Psychiatry Rep. 2016 Oct;18(10):89)* (see references 20-21). Further research should definitely explore possible correlations between dosage or cumulative length of exposure and post-stroke mortality. We added this in the manuscript.

Concerning the risk to have dysphagia, our results suggest no impact of benzodiazepines. As dysphagia may result in higher risk of pneumonia, benzodiazepines users have a similar early

pneumonia risk than non-users, at an early stage. The swallowing disorders are more often caused by neuroleptics agents more than benzodiazepines *(Dziewas R, Warnecke T, Schnabel M et al. Neuroleptic-induced dysphagia: case report and literature review. Dysphagia. 2007 Jan;22(1):63-7. Epub 2006 Oct 6.)*

VERSION 3 – REVIEW

REVIEWER	
REVIEW RETURNED	

GENERAL COMMENTS	

REVIEWER	
REVIEW RETURNED	

GENERAL COMMENTS	

REVIEWER	
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GENERAL COMMENTS	

VERSION 3 – AUTHOR RESPONSE

VERSION 4 – REVIEW

REVIEWER	
REVIEW RETURNED	

GENERAL COMMENTS	

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REVIEW RETURNED	

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GENERAL COMMENTS	

VERSION 4 – AUTHOR RESPONSE

VERSION 5 – REVIEW

REVIEWER	
REVIEW RETURNED	

GENERAL COMMENTS	

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REVIEW RETURNED	

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GENERAL COMMENTS	

VERSION 5 – AUTHOR RESPONSE