PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Folic acid and methotrexate use and their association with COVID-19 diagnosis and mortality: a case-control analysis from the UK Biobank
AUTHORS	Topless, Ruth; Green, Ralph; Morgan, Sarah; Robinson, Philip; Merriman, Tony; Gaffo, Angelo

VERSION 1 – REVIEW

REVIEWER	Smith, David University of Oxford, Pharmacology
REVIEW RETURNED	09-May-2022

GENERAL COMMENTS	A very clearly presented report of an observational study that may well have public health implications. I have very minor comments. Page 7, line 16: UK has not yet actually implemented the decision to fortify food with folic acid and so this sentence needs to be modifed to say that UK has decided to introduce fortification. Page 19: in the Discussion about possible limitations the authors might consider whether the indication(s) for prescribing folic acid might have influenced the Covid outcomes. I assume that these indications are not available to the authors? If they are, they shoud be listed. Page 19, line 51: insert 'RA' before 'disease activity'

REVIEWER	Lee, Eun Bong Seoul National University College of Medicine, Internal Medicine
REVIEW RETURNED	29-May-2022

GENERAL COMMENTS	This report is very provocative and interesting report on the association between folic acid replacement and Covid-19 infection. The authors assert that the persons who take folate supplementation show higher Covid-19 infection and mortality. Following is my concerns. Method Medication can always be changed. How can the authors confirm that folic acid and methotrexate were continuously used in the enrolled patients? Results The reasons why the patients take folic acid or methotrexate may be different. That is to say, specific diseases may be associated with taking each medicine. Therefore, it's not clear that current difference of Covid-19 infection or its mortality is truly the result of folic acid supplementation. From the statistical point, is there any possibility of multicollinearity among the independent variables during your logistic regression? Table 1

	In case-control studies, it is more understandable when numbers of both case(+) and case(-) are described in one table.

REVIEWER	Lee, Peter N. PN Lee Stat
REVIEW RETURNED	02-Jun-2022

GENERAL COMMENTS	I have been asked to review the paper as a statistician, having no specialist medical knowledge of methotrexate or folic acid prescription. I see no general problem with the way that the statistical analysis has been carried out, and the paper is well written and clear.

REVIEWER	Xu, Hongyan Augusta University
REVIEW RETURNED	06-Jun-2022

GENERAL COMMENTS	The authors used logistic regression to test the association of risk factors with COVID19 diagnosis and death. The approach is fine, but it is not described clearly. 1. In the Statistical Analysis section, the model should include the main variables of interest, namely, prescription of folic acid, and prescription of MTX, for both model 1 and model 2. The corresponding results should also be presented, not just the results for the main variable of interest. 2. Table S2 contains the analysis results by sex, which is not described in the Statistical Analysis section, 3. It is unclear if the authors used one variable for the main variable of interest with 4 levels representing the prescription of folic acid and/or MTX or two variables, i.e. one for folic acid and one for MTX. 4. In Table 3, the result for MTX only is missing.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Prof. David Smith, University of Oxford
Comments to the Author:
A very clearly presented report of an observational study that may well have public health implications. I have very minor comments.

1. Page 7, line 16: UK has not yet actually implemented the decision to fortify food with folic acid and so this sentence needs to be modified to say that UK has decided to introduce fortification.

We have modified this statement as follows (requested change underlined) – Page 6, line 6:

"The oxidized form, folic acid, is presently added to fortified foods in the USA and over 80 other countries. Recently, <u>a decision has been taken in the UK to introduce fortification</u>"

2. Page 19: in the Discussion about possible limitations the authors might consider whether the indication(s) for prescribing folic acid might have influenced the Covid

outcomes. I assume that these indications are not available to the authors? If they are, they should be listed.

Unfortunately, we do not have access to information about indications for prescriptions in the UKBB. We reviewed data from the UKBB again in response to this comment and could not find information about specific indications for the prescription. Common published indications for supplemental folate prescription (besides ameliorating methotrexate toxicity) include pregnancy, particularly in women with a history of previous neural tube defect complications, seizure disorders in patients taking anticonvulsant medications, sickle cell anaemia and other hemoglobinopathies or chronic haemolytic anaemia (e.g.hereditary spherocytosis) and folate-deficient megaloblastic anaemia . We agree with Professor Smith that the indication for folic acid prescription could have introduced a situation of confounding by indication in which the underlying reason for the prescription is the real risk factor for COVID19 diagnosis and associated mortality.

We attempted to address this by including indicators of prescription for RA, sickle cell disease, and use of anticonvulsants (which could also serve as a surrogate for convulsive disorders) in the modeling. However, there can be a degree of residual confounding. We include this as another item in the limitations as follows (page 19, first paragraph):

"Finally, residual confounding conferred by the underlying indications for folate prescriptions (besides the ones addressed in our analysis) is a possibility."

1. Page 19, line 51: insert 'RA' before 'disease activity'

This edit is done – Page 18, second paragraph

Reviewer: 2
Dr. Eun Bong Lee, Seoul National University College of Medicine Comments to the Author:
This report is very provocative and an interesting report on the association between folic acid replacement and Covid-19 infection. The authors assert that the persons who take folate supplementation show higher Covid-19 infection and mortality. Following are my concerns.

Methods

2. Medication can always be changed. How can the authors confirm that folic acid and methotrexate were continuously used in the enrolled patients?

We cannot confirm that the prescriptions were used continuously during the study period or if there was compliance with the prescriptions. We amended the fifth limitation in our discussion that alluded to this, in the following way:

"Fifth, prescription data were single script from General Practitioners only, and it was not possible to ascertain compliance or whether participants were taking the prescribed medication during the COVID-19 pandemic or continuously during the study period although we attempted to account for this by restricting our use of prescription information to the years 2019 and 2020 only."

Results
The reasons why the patients take folic acid or methotrexate may be different. That is to say, specific diseases may be associated with taking each medicine. Therefore, it's not clear that current difference of Covid-19 infection or its mortality is truly the result of folic acid supplementation.

We agree with the observation by the reviewer and we have tried to address this point in the limitations of our study as stated in our response to a similar point made by Reviewer 1. Please refer to item#2 in this document.

3. From the statistical point, is there any possibility of multicollinearity among the independent variables during your logistic regression?

In response to this request, we ran collinearity tests. There are mild correlations in ways we would expect. Statin prescription is correlated with age groups (0.32), sex (0.16) and BMI (0.19). Sickle cell disease is correlated with ethnicity (0.14). Methotrexate and/or folate medication variable is correlated with RA (0.17) and iron prescription (0.13), not surprisingly as folate and iron often prescribed as a combination medication. Tolerance and Variance Inflation Factors were calculated. Tolerance values ranged between 0.84 and 1.00, and VIF ranged between 1.00 and 1.19. This suggests that we do not have multicollinearity.

4. Table 1

In case-control studies, it is more understandable when numbers of both case(+) and case(-) are described in one table.

In response to this comment, we have expanded Table 1 to include the following columns:

Demographic	All N= 380,380	COVID-19 diagnosis N= 26,033	No COVID-19 diagnosis N= 354,347	COVID-19-related death N= 820	No COVID-19-related death N= 379,560

Reviewer: 3
Dr. Peter N. Lee, PN Lee Stat
Comments to the Author:
I have been asked to review the paper as a statistician, having no specialist medical knowledge of methotrexate or folic acid prescription. I see no general problem with the way that the statistical analysis has been carried out, and the paper is well written and clear.

Reviewer: 4
Dr. Hongyan Xu, Augusta University
Comments to the Author:
The authors used logistic regression to test the association of risk factors with COVID19 diagnosis and death. The approach is fine, but it is not described clearly.

5. In the Statistical Analysis section, the model should include the main variables of interest, namely, prescription of folic acid, and prescription of MTX, for both model 1 and model 2. The corresponding results should also be presented, not just the results for the main variable of interest.

In response to this comment, we have included the detailed models including the main variables of interest and the adjustors for the model as supplementary tables S2 and S4. We have modified the results section to refer to those tables along with the abbreviated tables presented in the main manuscript.

6. Table S2 contains the analysis results by sex, which is not described in the Statistical Analysis section

We now have added the following to the statistical methods section (Page 10):

"Sex-stratified analyses were done using the same approach to explore any differential association with COVID-19 diagnosis or associated mortality. "

7. It is unclear if the authors used one variable for the main variable of interest with 4 levels representing the prescription of folic acid and/or MTX or two variables, i.e. one for folic acid and one for MTX.

One variable for the main variable of interest with 4 levels was used. This is added to the statistical methods (page 10, first paragraph) as follows:

"For methotrexate and folate use, a single variable with 4 levels was used for statistical modeling (no methotrexate or folate, methotrexate only, folate only, methotrexate and folate)."

8. In Table 3, the result for MTX only is missing.

As there were no study participants in the UKBB who were taking only methotrexate (without folate) and died from COVID 19, we could conduct this specific analysis. This is mentioned in the first item in the limitations section of the discussion and we added a footnote to Table 3 to remind the readers.

"*There were no deaths in the group of participants taking only methotrexate without folate"

VERSION 2 – REVIEW

REVIEWER	Smith, David University of Oxford, Pharmacology
REVIEW RETURNED	01-Jul-2022

GENERAL COMMENTS	The revisions have satisfied my concerns.

REVIEWER	Lee, Peter N. PN Lee Stat
REVIEW RETURNED	27-Jun-2022

GENERAL COMMENTS	My concerns are well addressed.

REVIEWER	Xu, Hongyan Augusta University
REVIEW RETURNED	11-Jul-2022

GENERAL COMMENTS	This is a revision. The authors have addressed my previous concerns sufficiently. I have no further comments.