Summary

This study examines data from the WHO SAGE study in 3 countries to examine prevalence and correlates of anemia among older adults. Information on anemia prevalence and etiology among older adults is indeed a gap in the literature in low- and middle-income countries; therefore the study is a useful contribution to the literature. There are several notable limitations to the analysis, including the method of Hb measurement in the SAGE studies and lack of detail in the available data to examine predictors of anemia. For example, information on biomarkers of inflammation or micronutrient status would provide more certainty about the potential causes of anemia in this population. I also have some questions about the statistical analysis, as noted below. Lastly, the paper could be more concise, particularly the introduction. While some of the limitations reflect the underlying data, the latter two points could be addressed and would make the manuscript much stronger.

Major points

The introduction is very thorough but somewhat repetitive. For example, definitions of anemia are given in two different places, and examples of the potential consequences of anemia among older adults are given in multiple places as well. It also includes some basic information on anemia that is not necessary for this type of article (eg lines 9-13 on page 5 of the proof). Introduction would be strengthened by streamlining (cutting the word count by 1/3 to 1/2) to include only the core information needed to interpret the study.

Reply1: We have modified the introduction so that it is more streamlined.
Changes in text: It does not include the section "Anemia definition and Prevalence" (see page 4). Thus, there is less repetition of information. We instead moved the definitions to all be together at the start of the introduction (see page 3 lined 5-10).

The analysis includes a very limited subset of factors that could help to understand anemia etiology or inform targeting of programs. It would be ideal to have more information on biomarkers of nutritional status or inflammation, information on dietary intake, etc. The authors mention adjusting anemia cutoffs for smoking – was smoking also considered as a potential predictor variable in regression models (smoking may artificially increase Hb, but is also associated with chronic diseases which may contribute to anemia via inflammation, ie anemia of chronic disease)?

Reply 2: Unfortunately, the relevant biomarker data is not available. However, survey measures including smoking, alcohol use, and servings of fruits and vegetables were added to the regression models as predictors.

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The manuscript would benefit from including more detail on the sampling and data collection methods for each survey. Although some information is available through the SAGE study website, it would be helpful to have some key information in the paper. For example, on Page 7, line 9: please clarify the sampling strategy for each study. Were these meant to be population representative samples? I see on page 9 that the study used a cluster design. Was the design also stratified (for example into regions)? Were sampling weights applied in the analyses? In addition, what were the inclusion/exclusion criteria for participating households? For example, were all the women confirmed to be nonpregnant?

Reply 3: Manuscript was edited to add clarity on the sample data.

Changes in the text: "Sampling weights could not be used to create representative samples in these analyses since biomarker analyses were only performed on a convenience sample of the overall study cohorts" (see page 9 line 5-7). Additionally, "For the SAGE study, participants were randomly selected from population census data to create nationally representative samples using a stratified multistage cluster sampling design. Not all SAGE participants had biomarker values, a convenience sample of dried blood spots for biomarker analysis have been run as a preliminary analysis. Comparative samples of younger adults (aged 18 to 49 years old) were also obtained from each country, but were not included in the present analysis." (see page 6 line 12-17)

The statistical analysis section does not appear to be consistent with some of the analyses that were conducted, and some sections were confusing. On Page 9, lines 11-13: It is not clear what the 6 models were: is this men/women in 3 countries, or something else? Does Table 4 present results from a multivariable model or are these all bivariate associations? Also, Table 4 presents associations stratified by sex but this is not clear from the statistical analysis section. It would be helpful to explain the rationale for stratification by sex in the statistical analysis section. For tables 4 and 5, prevalence ratios are preferable to odds ratios for a common outcome, which would be the case for anemia in China and Mexico.

Reply 4: manuscript was edited to provide clarity on analyses that were run and why we chose to split up analysis based on gender. Tables have also been changed with updated model statistics

Changes in the text: "*Both descriptive and inferential statistics were run on women and men separately since they often have different rates of anemia and different anemia etiologies. To explore this relationship in older adults, Hb levels and anemia rates will be compared between women and men in each of the three countries (China, Mexico, and South Africa). Six mixed effect binomial logistic regressions using maximum likelihood estimation were used to predict the presence of anemia, modeling women and men separately for each of the three countries. The predictors of interest in these models were socioeconomic status, wealth, and education, and covariates included health, cigarette smoking, alcohol use, servings of fruit and servings of vegetables.*" (see page 9 line 10-12).

Additionally, on page 9, lines 21-23 suggest that covariates are included, but this is not

clear from the methods. Suggest to clarify the details of which models were run and which variables were included in each model.

Reply 5: Methods section was updated to include headings that matched the results section for clarity. It was also clarified which models were run in the statistics section on page ___ lines ___ through ___.

Changes in the text: "*Covariates included self-rated health, cigarette smoking frequency, frequency of alcohol consumption, average number of servings of fruit in a day, and average number of servings of vegetables in a day.*" (seen on page 8 line 8-9)

Finally, in Table 3: It is not clear what "t" refers to. Is this a t statistic? If so, this is not mentioned in the analysis section, and a standard t test would not be appropriate for clustered data. Suggest to include 95% confidence intervals instead of SD.

Reply 6: Edits have been made to clarify reading apprehension of table 3.

Changes to the text: Changed to multilevel model in order to control community level clustering and added 95% CI to Table 3.

Measurement of hemoglobin from dried blood spots, rather than whole blood, is a limitation, as is the use of capillary rather than venous samples in some countries. Page 8, lines 14-20: Suggest to comment on the validity of Hb as measured by dried blood spots. Were there any validation tests done within the SAGE study, or if not, what literature was used to justify use of dried blood spots (was Hb the primary outcome or were these collected for another purpose)? The results for South Africa (>90% prevalence) in particular are very surprising and it is not clear whether this is the true prevalence or if the methods may underestimate Hb content (other literature suggests that capillary blood samples may have lower Hb content compared to venous, but I am not sure how this translates to dried blood spots).

Reply 7: A validation test was conducted through SAGE however we do not have access to it.

Changes in the text: Modifications were added in order to speak on why dried blood spot collection was conducted (see page 12-13 line 22-3).

Specific

Comment 1: Page 2, Lines 6 and elsewhere: Suggest to refer to anemia prevalence (vs rate) consistently.

Reply1: We have modified our test as advised (see page 2, line 1) throughout the paper.

Comment 2: Page 2, Lines 17-19: "highlighting the importance of global research to target public health interventions." Suggest to be more specific about how the results would inform program targeting.

Reply2: This paper has been modified to better describe how the results would inform program targeting (see page 2 line 17-19).

Comment 3: Page 3, Line 3-Suggest to reword, as referring to anemia as a "common micronutrient disorder" is misleading given the many possible causes of anemia.

Reply 3: This paper has been modified to describe anemia as a blood condition (see

page 3 line 3).

Comment 4: Page 4, line 7: "However, there are serious health concerns related to anemia among older adults as it increases the likelihood of osteoporosis and other comorbidities (8)." Is this a causal relationship? If so, what is the mechanism?

Reply 4: This paper has been modified to address the relationship of osteoporosis and anemia status. (see page 4 line 13).

Comment 5: Page 7, line 1: Not clear what checklist is referred to.

Reply 5: This checklist title has been modified to more accurately identify the information it is referring to (see page 6 line 5-7)

Comment 6: Page 7, line 13-14: Does the convenience sample refer to the entire sample included in this analysis? Or is this something else? Which biomarkers are referred to here?

Reply 6: Page 6 lines 12 and 13 were updates with, "*For the SAGE study, households were randomly selected from population census data to create nationally representative samples using a stratified multistage cluster sampling design. Not all SAGE participants had Hb biomarker values, a convenience sample of dried blood spots for biomarker analysis have been run as a preliminary analysis.*"

Comment 7: Page 8, line 8-9: Please provide more detail on what variables were included in the wealth index, eg were participants asked about income directly? Were these the same across countries?

Reply 7: More detail was provided on page 7 lines 15 and 16, "*Questions asked about various aspects of socioeconomic status such as income (wages, earnings from selling products, and income from rental property, pension, interest, and other), assets (land/property, jewellery, books, art, and other valuable items), and housing characteristics (e.g., washing machine, hot running water, a refrigerator, internet access, household employees) and were adapted to each country. The responses were modeled as independent observations of wealth then combined using item response theory*."

Comment 7: Page 9, line 16: suggest to present confidence intervals in addition to prevalence.

Reply 7: Added confidence intervals to country wide prevalence estimates: "*The prevalence rates of anemia were 28% (95% CI [27%, 29%]), 24% (95% CI [22%, 25%]), and 91% (95% CI [90%, 93%]) in China, Mexico, and South Africa, respectively*" (see page 9 line 20).

Comment 8: Page 9, line 23: "increase in the risk of anemia" – suggest to reword (perhaps likelihood, or odds) as these are not risk ratios. Same thing for page 10, line 3-5.

Reply 8: This paper has been modified to streamline this analysis (see both page 9 line 15).

Comment 9: Page 11, lines 10-11: Please clarify how this might explain the direction of association between education and anemia prevalence.

Reply 9: This weird association went away once you controlled smoking. One possibility is women w more education could afford cigarettes, but also it could still

have been a weird effect from education being grouped by community and community being controlled for. Either way, it is not a part of the updated model.

Comment 10: Page 11, lines 14-16: Consider commenting on anemia of chronic disease – may correlate with higher SES in LMICs.

Reply 10: Good thought! This is no longer relevant to the updated model but we appreciate your insight.

Comment 11: Page 12, line 10: unfinished sentence

Reply 11: This sentence has been edited due to editors' comment (see page 11).

Comment 12: Page 13, line 18-19: Note that identifying access to iron supplements as a contributor to anemia implies that the anemia is due to iron deficiency.

Suggest to clarify specifically how the India study informs interpretation of the results of this analysis.

Reply 12: Modifications to this paper have been made upon editors' comments. Further clarification was provided (see page xx, line xxx).

Comment 13: Table 1: suggest to report confidence intervals instead of mean (SD). Typically SD is not reported for survey data; rather SE or 95% CI. Were any sampling weights applied?

Reply 13: Table 1 was updated with confidence intervals and the statistics section was updated to clarify why sampling weights could not be applied on page 9 lines 5-6: "Sampling weights could not be used to create representative samples in these analyses since biomarker analyses were only performed on a convenience sample of the overall study cohorts"

Comment 14: Table 4: The table needs footnotes to explain the asterisks, and a more explanatory title.

Reply 14: Table 4 was updated with notes to explain the asterisk and the title was changed to "Mixed Methods Logistic Regression Prevalence Ratios predicting Anemia and Model Fit Statistics"