PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Lower Hemoglobin-to-Red Blood Cell Distribution Width Ratio Is Independently Associated With Frailty in Community-dwelling Older Adults: a cross-sectional study
AUTHORS	Zhu, Mengpei; Wei, Chao; Yang, Xiongjun; Huang, Yumei; Xu, Yushuang; Xiong, Zhifan

VERSION 1 – REVIEW

REVIEWER	Steinmeyer, Zara CHU Toulouse, Geriatrics
REVIEW RETURNED	29-Dec-2022

GENERAL COMMENTS	Dear authors, Thankyou for providing me the opportunity to revise this article. While the subject of the study is interesting it is difficult to point out with the manuscript why HRR would be more interesting to detect frailty more than hemoglobin for example. That is one essential point that must be adressed. Concerning the abstract, the main outcome measures should be modified, the english corrected and the justification of the study should be underlined. Methods: the recruitment of the patients should be detailed in this section Discussion: the relevance of the study should be detailed in this section and how the results of the study may influence geriatric practice.

REVIEWER	Baysal, Mehmet Trakya Universitesi
REVIEW RETURNED	01-Mar-2023

GENERAL COMMENTS	It is a very well written article and a from a very interesting topic. Overall, it is adequate for the readers. Given that the uncertainty in the overall treatment modalities regarding the management of brain VMs In HHT the manuscript touches an important issue. Other than mutations that more frequently affected (Endoglin (ENG), Activin A Receptor Like Type 1 (ACVRL1) and (SMAD4). There are some other less frequently affected gene which could be mentioned in the introduction section (such as GDF2 and RASA-1) Besides it is a very good article I am happy to be able to review it. I would like to thank the authors.

REVIEWER	Setia, Maninder Karanam Consultancy

REVIEW RETURNED	29-Mar-2023

GENERAL COMMENTS	Dear Authors, You have presented useful data. I have the following comments 1) The sample size is based on the main objective. Your objective was to study the association between HRR and frailty. However, your sample size is based on the prevalence of frailty - that is not appropriate. 2) Why did you stratify the tables according to Low and Normal HRR? What does it indicate? How does it fit in your objectives? 3) Which variables are included in your Models 1 and 2 for logistic regression? Kindly specify that in the tables (as foot note or some other way). 4) In your limitations, kindly specify - what have you overestimated or underestimated? Hope these comments are useful.

VERSION 1 – AUTHOR RESPONSE

Response to the comments of Reviewer #1:

Comment 1: While the subject of the study is interesting it is difficult to point out with the manuscript why HRR would be more interesting to detect frailty more than hemoglobin for example. That is one essential point that must be addressed.

Response: Thank you for your comment. It is undeniable that previous studies have established the important role of hemoglobin in frailty. It has also been confirmed that RDW is associated with frailty in hospitalized older patients. On this basis we evaluated the relationship between HRR (combining hemoglobin and RDW) and frailty. The area under the curve of ROC of HRR, hemoglobin and RDW, was 0.802 (95% CI: 0.755 to 0.849), 0.742 (95% CI: 0.691 to 0.793), and 0.712 (95% CI: 0.651 to 0.772) respectively. At the same time, HRR appeared to be more significant by multiple logistic regression. Correlation analysis indicated that Kendall's tau-b of HRR may be better than hemoglobin (=-0.239,P<0.001 vs -0.194, P<0.001). We therefore believe that HRR may be more interesting to detect frailty more than hemoglobin in our current study population (community residents in Wuhan, China). Both from previous studies and the current findings, all of these three were associated with frailty in older adults. However, based on the results of the current study population HRR of combining hemoglobin and RDW may be better for detecting frailty. This result may only apply to local populations. Further prospective studies are needed to confirm this in the future.

Comment 2: Concerning the abstract, the main outcome measures should be modified, the English corrected and the justification of the study should be underlined.

Response: Thank you again for your great suggestion. As your suggestion, we have rewritten this part as follows,

Abstract

Objectives: The importance of blood cell markers in frailty has been studied. However, research on hemoglobin-to-red blood cell distribution width ratio (HRR) and frailty in older persons is still limited. We investigated the association between HRR and frailty in older adults.

Design: Cross-sectional population-based study.

Setting: Community-dwelling older adults older than 65 years old were recruited from September 2021 to December 2021.

Participants: A total of 1296 community-dwelling older adults (age ≥ 65 years) in Wuhan were included in the study.

Main outcome measures: The main outcome was the presence of frailty. The Fried Frailty Phenotype Scale was used to evaluate the frailty status of the participants. Multivariable logistic regression analysis was performed to determine the relationship between HRR and frailty.

Results: A total of 1296 (564 man) older adults were included in this cross-sectional study. Their mean age was 70.89 ± 4.85 years. ROC analysis showed that HRR is a good predictor of frailty in older people, the area under the curve (AUC) was 0.802 (95% CI: 0.755 to 0.849), and the highest sensitivity was 84.5% and the specificity was 61.9% with the optimal critical values 9.97 (P<0.001). Multiple logistic regression analysis indicated that lower HRR (<9.97) (OR:3.419, 1.679-6.964, P=0.001) is independently associated with frailty in older people, even after adjusting confounding factors.

Conclusion: Lower HRR is closely associated with an increased risk of frailty in the older people. Lower HRR may be an independent risk factor for frailty in community-dwelling older adults.

Keywords: Older people, Frailty, Healthy aging, Risk factor, HRR

Comment 3 Methods: the recruitment of the patients should be detailed in this section.

Response: Following these suggestions, we revised this part of methods as follows,

Inclusion criteria were the community-dwelling adults older than 65 living in communities in Wuhan. Exclusion criteria were malignant disease or advanced organic diseases, hematologic diseases, acute stage of disease, and participants with missing the key parameters. (lines101 -104)

Comment 4 Discussion: the relevance of the study should be detailed in this section and how the results of the study may influence geriatric practice.

Response: As your suggestion, we have detailed the relevance of the study and how the results of the study may influence geriatric practice. We have carefully revised discussion section as follows,

In this cross-sectional study including 1,296 community-dwelling older adults, we found that lower HRR is independently related to frailty in older people, even after adjusting confounding factors (P=0.001). Multiple logistic regression analysis showed that lower HRR is associated with to a 3-fold more likelihood or odds of frailty (OR = 3.419, 95%CI 1.679-6.964). ROC analysis showed that the AUC for HRR in the frailty older adults was 0.802, and the highest sensitivity was 84.5% and the specificity was 61.9% with the optimal critical values 9.97. The results of the present study confirmed that HRR was also significantly associated with frailty in the general older people, not only in patients with coronary heart disease in previous studies. (lines219 - 227)

This inexpensive and common laboratory parameter may provide useful information to identify the risk of frailty in older adults. Furthermore, use of the HRR may help clinicians to identify people at high risk of frailty and take effective measures to reduce the occurrence and development of frailty, reduce the rate of disability and mortality related to frailty in the elderly, and reduce the waste of medical resources, and promote healthy aging. (lines285 - 289)

Response to the comments of Reviewer #3

Comment 1: The sample size is based on the main objective. Your objective was to study the association between HRR and frailty. However, your sample size is based on the prevalence of frailty - that is not appropriate.

Response: Your comments are highly appreciated. The current calculation is based on the previous literature[1-3]. We are sorry about the inappropriate calculation, we have removed this part. Since there has been no prior research on the association between HRR and frailty in community-dwelling older adults , the best estimate (P) would be 50%. With the following considerations in mind, sample size was calculated using the single population proportion formula with a marginal error of 0.05, a 95% confidence interval, and a p-value of 0.5. After adjusting for sampling error with a 10% non-response rate, the estimated sample size was 423. Then kept the design effect at 2, the final sample size of 846 participants was estimated.

The present study comprised 1296 participants, which showed an adequate sample size.

And we will avoid similar errors in future calculations, thank you again for your great suggestion.

Comment 2: Why did you stratify the tables according to Low and Normal HRR? What does it indicate? How does it fit in your objectives?

Response: Thank you for your great suggestion. In our study, lower HRR may be an independent risk factor for frailty in community-dwelling older adults. And referring to previous literature[4-6], we divided HRR into low and normal HRR groups. So that the reader can visibly see the correlation of every parameter with HRR. Low HRR group may have a higher age, more female, less education years, more smoking and drinking, lower BMI, lower diastolic blood pressure, lower hemoglobin, higher RDW, lower albumin and more pre-frail and frail patients. And, this may help the reader understand why lower HRR is associated with frailty.

Comment 3: Which variables are included in your Models 1 and 2 for logistic regression? Kindly specify that in the tables (as foot note or some other way).

Response: We are sorry that this part was not clear in the original manuscript, and thank you very much for your careful examination. As your suggestion, we have revised it as follows,

Table3. Multiple logistic regression analysis of blood parameters and frailty in older adults

	Model1		Model2	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Categorical variable				
Lower Hb	2.129 (1.133-4.001)	0.019	1.163 (0.562-2.409)	0.684
Lower RDW	0.310(0.193-0.497)	<0.001	0.285 (0.170-0.477)	<0.001
Lower HRR	3.285 (1.676-6.440)	0.001	3.419 (1.679-6.964)	0.001

Hb=hemoglobin, RDW=red blood cell distribution width, HRR=hemoglobin-to-RDW ratio.

Lower Hb: <131.5g/L, Lower RDW: <13.45%, Lower HRR: <9.97. (Optimal cut-off value of the ROC curve)

OR: odds ratio, CI: confidence interval, Model 1, unadjusted; Model 2, adjusted for age, gender, marital status, education years, alone living, BMI, diabetes, RBC, albumin, triglyceride, HDL-C and LDL-C.

Comment 4: In your limitations, kindly specify - what have you overestimated or underestimated?

Response: Thank you for your comments. We have revised it as follows,

There were also some limitations of our present study. Firstly, because cross-sectional studies measure the outcome and the exposures in the study participants at the same time, it is difficult to assess the cause-effect relationship. Secondly, our participants are limited to local participants, these findings need to be validated in different populations around the world. What is more, we unable to investigate the temporal relation between outcomes and risk factors. In addition, despite the inevitable selection bias and information bias in cross-sectional studies, we improved this problem through more rational statistical methods and interviewer training. Finally, we did not assess iron, folic, and vitamin B12, which may affect RDW and Hb level. (lines290 - 297)

References

1. Wassie LA, Azagew AW, Bifftu BB. Depression and its associated factors among primary caregivers of adult cancer patients at Northwest Amhara Regional States Referrals Hospitals oncology treatment units, Northwest Ethiopia, 2021. *BMC Psychiatry.* 2022;22(1):533.
2. Mamun MA, Al-Mamun F, Islam J, Muhit M. Prevalence and associated factors of suicidal behaviors among Bangladeshi rural community people: Findings from the 'BD ComMen Study'. *PLoS One.* 2022;17(12):e0279271.
3. Sewalem J, Kassaw C, Anbesaw T. Sexual dysfunction among people with mental illness attending follow-up treatment at a tertiary hospital, Jimma University Medical Center: A cross-sectional study. *Front Psychiatry.* 2022;13:999922.
4. Su Y-C, Wen S-C, Li C-C, et al. Low Hemoglobin-to-Red Cell Distribution Width Ratio Is Associated with Disease Progression and Poor Prognosis in Upper Tract Urothelial Carcinoma. *Biomedicines.* 2021;9(6).
5. Wang J, Chen Z, Yang H, Li H, Chen R, Yu J. Relationship between the Hemoglobin-to-Red Cell Distribution Width Ratio and All-Cause Mortality in Septic Patients with Atrial Fibrillation: Based on Propensity Score Matching Method. *J Cardiovasc Dev Dis.* 2022;9(11).
6. Bozkaya Y, Kurt B, Gürler F. A prognostic parameter in advanced non-small cell lung cancer: the ratio of hemoglobin-to-red cell distribution width. *Int J Clin Oncol.* 2019;24(7):798-806.

VERSION 2 – REVIEW

REVIEWER	Steinmeyer, Zara CHU Toulouse, Geriatrics
REVIEW RETURNED	15-May-2023

GENERAL COMMENTS	Dear Authors, I find the subject of your article very interesting. The objective is well described. The english should be reviewed by an english speaker. In the introduction, it would be nice if the authors could describe what is RDW, and what influences its variation. The authors

	should underline why RDW is more interesting than other biological markers to date? in the methods section, it should be explained how the patients are recruited? in the discussion section: the authors should explain more the reasons of decrease in hrr thankyou

REVIEWER	Setia, Maninder Karanam Consultancy
REVIEW RETURNED	06-May-2023

GENERAL COMMENTS	Thanks for your response.

VERSION 2 – AUTHOR RESPONSE

Response to the comments of Reviewer #1:

Comment 1: The english should be reviewed by an english speaker

Response: We apologize for the grammatical mistakes. The manuscript has been reviewed by an english speaker. The manuscript has been carefully proofread.

Comment 2: In the introduction, it would be nice if the authors could describe what is RDW, and what influences its variation. The authors should underline why RDW is more interesting than other biological markers to date?

Response: Your comments are highly appreciated. As your suggestions, We have revised introduction section as follows,

Red blood cell distribution width (RDW) is a simple parameter of CBC, which reflects the degree of heterogeneity of the erythrocyte volume, and is traditionally used for the differential diagnosis of anemia.[1] However, with the deepening of the study, it was found to be related to the prognosis of many diseases. Increased RDW reflects dysregulation of erythrocyte homeostasis, which may be attributed to various underlying metabolic abnormalities such as shortened telomere length, oxidative stress, inflammation, malnutrition, dyslipidemia, hypertension, erythrocyte fragmentation and altered erythropoietin function.[1]

Inflammation has been identified as a potential cause of frailty.[2] Inflammation in response to elevated RDW may be highly correlated with frailty. (Red markings in the introduction section)

Comment 3: In the methods section, it should be explained how the patients are recruited?

Response: Thank you very much for your suggestion on this section. Following these suggestions, we revised this part of methods as follows,

In this present study, we recruited 1,296 community-dwelling adults older than 65 living in communities in Wuhan between September 2021 and December 2021.

Inclusion criteria were the community-dwelling adults older than 65 living in communities in Wuhan.

Exclusion criteria were malignant disease or advanced organic diseases, hematologic diseases, acute stage of disease, and participants with missing the key parameters. (Red markings in the Material & Methods section)

Comment 4: In the discussion section, the authors should explain more the reasons of decrease in hrr.

Response: Thank you again for your great suggestion. As your suggestion, we have carefully revised discussion section as follows,

Increased RDW combined with anemia is more likely to lead to decreased HRR. Elevated RDW suggests chronic inflammation, malnutrition and ageing.[1] Anemia is the cause of reduced tissue oxygenation and the consequent increase in fatigue, weakness and functional impairment.[3] Also anemia may affect muscle mass and strength loss through inflammatory pathways.[3] Therefore, decreased HRR may be associated with sarcopenia, slowness, weakness, inflammation, malnutrition, and weight loss in frailty patients. (Red markings in the discussion section)

Response to the comments of Reviewer #3

Comments to the Author: Thanks for your response.

Response: Thank you.

References

1. Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: A simple parameter with multiple clinical applications. *Crit Rev Clin Lab Sci.* 2015;52(2).
2. Dent E, Lien C, Lim WS, et al. The Asia-Pacific Clinical Practice Guidelines for the Management of Frailty. *J Am Med Dir Assoc.* 2017;18(7):564-575.
3. Picca A, Coelho-Junior HJ, Calvani R, Marzetti E, Vetrano DL. Biomarkers shared by frailty and sarcopenia in older adults: A systematic review and meta-analysis. *Ageing Res Rev.* 2022;73:101530.