

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

Lower Hemoglobin-to-Red Blood Cell Distribution Width Ratio Is Independently Associated With Frailty in Community-dwelling Older Adults: a cross-sectional study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-069141
Article Type:	Original research
Date Submitted by the Author:	11-Oct-2022
Complete List of Authors:	Zhu, Mengpei; Institute of Geriatric Medicine, Liyuan Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wei, Chao; Wuhan Geriatric Hospital Yang, Xiongjun; Huazhong University of Science and Technology Huang, Yumei; Huazhong University of Science and Technology Xu, Yushuang; Huazhong University of Science and Technology; Huazhong University of Science and Technology Xiong, Zhifan; Huazhong University of Science and Technology; Huazhong University of Science and Technology
Keywords:	GERIATRIC MEDICINE, EPIDEMIOLOGY, GENERAL MEDICINE (see Internal Medicine)

SCHOLARONE™
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1
2
3
4
5 1 **Lower Hemoglobin-to-Red Blood Cell Distribution**
6
7
8 2 **Width Ratio Is Independently Associated With Frailty**
9
10 3 **in Community-dwelling Older Adults: a cross-sectional**
11
12 4 **study**
13
14
15
16
17

18 6 Authors Name : Mengpei Zhu^{1,2}, Chao Wei³, Xiongjun Yang², Yumei Huang², Yushuang Xu^{1,2},
19
20
21 7 Zhifan Xiong^{1,2}
22

23 8 Affiliations: 1 Institute of Geriatric Medicine, Liyuan Hospital, Tongji Medical College,
24
25
26 9 Huazhong University of Science and Technology, Wuhan, China.
27

28 10 2 Division of Gastroenterology, Liyuan Hospital, Tongji Medical College, Huazhong University
29
30
31 11 of Science and Technology, Wuhan, China.
32

33 12 3 Physical Examination Center, East Lake Hospital & Wuhan Geriatric Hospital, Wuhan,
34
35
36 13 China.
37
38

39 14
40
41 15 Correspondence: Zhifan Xiong
42

43
44 16 Address: Division of Gastroenterology, Institute of Geriatric Medicine, Liyuan Hospital, Tongji
45
46
47 17 Medical College, Huazhong University of Science and Technology, 39 Lake Road, East Lake
48
49 18 Ecological Scenic, Wuhan 430077, Hubei, China
50

51
52 19 Email: xiongzhiban@126.com
53
54
55
56
57
58
59
60

1
2
3
4 23 **Abstract**

5
6 24 **Objectives:** Frailty becomes an emerging global public health burden, with the rapid growth of
7
8
9 25 the global elderly population. We investigated the association between hemoglobin-to-red
10
11
12 26 blood cell distribution width ratio (HRR) and frailty in older adults.

13
14 27 **Design:** Cross-sectional analysis of associations of HRR and frailty in older people.

15
16
17 28 **Setting:** Enrolled community-dwelling older adults older than 65 years old in Wuhan during
18
19 29 September 2021 and December 2021.

20
21
22 30 **Participants:** A total of 1296 community-dwelling older adults (age \geq 65 years) in Wuhan
23
24 31 were included in the study.

25
26
27 32 **Main outcome measures:** The main outcome measure was frailty, and the Fried Frailty
28
29 33 Phenotype Scale was used to evaluate the frailty status of the participants.

30
31
32 34 **Results:** A total of 1296 (564 man) older adults were included in this cross-sectional study.
33
34
35 35 Their mean age was 70.89 ± 4.85 years. ROC analysis showed that HRR is a good predictor
36
37 36 of frailty in older people, the area under the curve (AUC) was 0.802 (95% CI: 0.755 to 0.849),
38
39 37 and the highest sensitivity was 84.5% and the specificity was 61.9% with the optimal critical
40
41 42 values 9.97 ($P < 0.001$). Multiple logistic regression analysis indicated that lower HRR (< 9.97)
43
44 43 (OR:3.419, 1.679-6.964, $P = 0.001$) is independently associated with frailty in older people,
45
46 44 even after adjusting confounding factors.
47
48
49

50
51 41 **Conclusion:** Lower HRR is closely associated with an increased risk of frailty in the older
52
53 42 people. Lower HRR may be an independent risk factor for frailty in community-dwelling older
54
55 43 adults.

56
57
58 44 **Keywords:** Older people, Frailty, Healthy aging, Risk factor, HRR
59
60

1
2
3
4 455
6 46 **Strengths and limitations of this study**7
8
9 47 This is the first study reporting an association of HRR with the frailty in community-dwelling
10
11 48 older adults.12
13
14 49 HRR may help clinicians to identify people at high risk of frailty and take effective measures to
15
16
17 50 reduce the occurrence and development of frailty, reduce the rate of disability and mortality
18
19 51 related to frailty in the elderly.20
21
22 52 Our findings should facilitate further research to investigate any causal association of HRR
23
24 53 and frailty in older people.25
26
27 54 This was a cross-sectional study that cannot assess the cause-effect relationship.
28
29

30 55

31
32 56 **Introduction**33
34
35 57 As life expectancy increases, human societies are aging globally, in both developed and
36
37 58 developing countries.¹ By 2050, the proportion of people aged over 60 years is projected to
38
39 59 increase from 11% to 22%, and the number of aged over 60 years will increase from 605
40
41 60 million to 2.1 billion, including 425 million people aged over 80 in the world.² Frailty becomes
42
43 61 an emerging global public health burden, with the rapid growth of the global aging population.44
45
46 62 Frailty is considered to be a complex age-related clinical condition characterized by a decline
47
48
49 63 in the physiological function of multiple organs, with a resultant increased vulnerability to
50
51 64 stressors.³ It is related to adverse health-related events, including increased mortality,
52
53 65 hospitalization, falls and fractures, cognitive decline, disability, and admission to long-term
54
55
56 66 care.⁴ Therefore, early identifying modifiable risk factors of frailty is becoming increasing
57
58
59
60

1
2
3
4 67 crucial for delaying and reversing frailty and its associated adverse events in older persons.⁵
5

6 68 As an important part of CBC, hemoglobin (Hb) is usually used as an indicator of the
7
8
9 69 degree of anemia. However, previous studies showed that low Hb reflect to a decline in the
10
11
12 70 physiological function including the decreased immune response, malnutrition, and the low
13
14
15 71 resistance to external invasion.⁶ Meanwhile, there are several studies indicated that Hb is
16
17 72 related to frailty in older persons.⁷⁻¹⁰ As an indicator of heterogeneity of the erythrocyte
18
19
20 73 volume, red blood cell distribution width (RDW) is thought to be related to the prognosis of
21
22
23 74 many diseases. Hou et al. indicated that RDW is significantly associated with the risk of frailty
24
25 75 in older patients with coronary heart disease (CHD).¹¹ In addition, studies showed that RDW is
26
27 76 associated with frailty both in older inpatients and community-dwelling older people.^{12,13}
28
29

30 77 RDW can be affected by complex conditions, the effect of RDW on frailty is not only
31
32
33 78 related to inflammatory response, but also association with a decline in the physiological
34
35
36 79 function and oxygen.¹⁴ RDW alone may not provide definitive predictive information.^{14,15} The
37
38
39 80 hemoglobin-to-RDW ratio (HRR) is a cheap, rapid and readily available novel prognostic,
40
41
42 81 which combines the prognostic information of Hb and RDW and reflects a more
43
44
45 82 comprehensive health status.^{14,16} Recently, Qu et al. found that lower HRR is independent
46
47
48 83 related to the risk of frailty in older patients with CHD.¹⁴ They verified that HRR maybe a more
49
50
51 84 useful biomarker comparing with RDW or Hb alone.¹⁴
52

53 85 Studies have showed a significant association of HRR with frailty in specific
54
55
56 86 populations (patients with coronary heart disease).¹⁴ However, research on HRR and frailty in
57
58
59 87 the general older persons is still limited, and the significance of evaluating frailty is not yet
60
88 clear. In the present study, we investigated the relationship between HRR and frailty in

1
2
3
4 89 community-dwelling older adults.
5
6

7 90
8

9 91 **Material & Methods**

10 92 ***Ethical Approval***

11 93 The research protocol was approved by the Medical Ethics Committee of Liyuan Hospital,
12 94 Tongji Medical College, Huazhong University of Science and Technology (IRB ID: [2020] IEC
13 95 (A016)). All the participants gave written informed consent. The study was conducted in
14 96 accordance with the tenets of the Declaration of Helsinki.

15 97
16 98
17 99
18 100
19 101
20 102
21 103
22 104
23 105
24 106
25 107
26 108
27 109
28 110
29 111
30 112
31 113
32 114
33 115
34 116
35 117
36 118
37 119
38 120
39 121
40 122
41 123
42 124
43 125
44 126
45 127
46 128
47 129
48 130
49 131
50 132
51 133
52 134
53 135
54 136
55 137
56 138
57 139
58 140
59 141
60 142

97 ***Sampling size and sampling procedure***

98 A sample size of 1,296 was calculated using 2% error, 95% confidence interval using the

99 formula below :

100

$$101 \quad n = \frac{z^2 \times p(1-p)}{e^2}$$

102
103

104 n = minimum sample size.

105 z = confidence interval at 95%, 1.96.

106 p = estimated proportion of frailty, 10%.¹⁷

107 e = margin of error at 2%.

108 Then we kept the design effect at 1.5. n = 864 X 1.5 = 1,296

109 Totally, 1,296 participants were examined.

110 Since, it was a cluster sampling we kept the design effect at 1.5. n = 864 X 1.5 = 1296

111 ***Patient and public involvement***

1
2
3
4 112 The source population was the community-dwelling adults older than 65 living in communities
5
6 113 in Wuhan. The study population consisted of a random sample of older people from each
7
8
9 114 community.

115 ***Participants and Sociodemographic Characteristics***

116 In this present study, we recruited 1,296 community-dwelling adults older than 65 living in
117 communities in Wuhan between September 2021 and December 2021. Sociodemographic
118 characteristics, including age, gender, education years, marital status, smoking history,
119 alcohol consumption, comorbidities, including hypertension, diabetes, CHD, hyperlipidemia,
120 and cerebrovascular disease were recorded. Then body mass index (BMI), waistline , blood
121 pressure and pulse rate were measured by two professional clinicians.

122 ***Peripheral Blood Parameters***

123 Blood samples were collected, and full blood count was measured by automated hematology
124 analyzer (Mindray, BC7500, China). Other related biochemical indicators were detected by
125 automatic biochemical analyzer (Beckman, AU680, American). HRR = Hb (g/L)/RDW (%).

126 ***Fried's frailty phenotype***

127 According to the Fried's frailty phenotype, we evaluated frailty in five criteria, as follows (1)
128 Weight loss: unintentional weight loss >5% or a loss of more than 4.5 kg in the past 1 year. (2)
129 Physical weakness: dynamometer was used for participants for three trials, the maximum
130 value was recorded. Low grip strength was defined according to the standards proposed by
131 Fried et al.¹⁸ (3) Slowness: slowness was defined as when the time required to walk 4.6 meters
132 was more than 7 seconds for males (height ≤ 173 cm) and females (height ≤ 159 cm) or more
133 than 6 seconds for males (height >173 cm) and females (height >159 cm). (4) Physical activity:

1
2
3
4 134 low physical activity was defined as less than 383 kcal/week for males and less than 270
5
6 135 kcal/week for females. (5) Exhaustion: exhaustion was assessed by the following two
7
8
9 136 questions from the CES-D. "In the last week, I felt that everything I did was an effort" and
10
11 137 "Could not get going in the last week." If the participant responded "yes" to either of these
12
13
14 138 questions, the participant was considered exhausted. Participants with >3 indicators were
15
16
17 139 defined as frail, 1-2 as prefrail and none as robust.

140 ***Statistical analysis***

141 Continuous and categorical variables were expressed as the mean \pm standard deviation and
142 numbers with percentages, respectively. The baseline characteristics of the groups were
143 compared using one-way analysis of variance and chi-squared test. The predictive value of
144 HRR for frailty was assessed by receiver operating curve (ROC) analysis. The prognostic
145 value of lower HRR on frailty was assessed using logistic regression model. Variables were
146 selected as candidates for the multivariate analysis when $p < 0.1$ in the univariate analysis.
147 After adjustment for confounding factors including age, gender, marital status, education
148 years, alone living, BMI, diabetes, RBC, albumin, triglyceride, HDL-C and LDL-C, assessing
149 the independent risk factors for frailty in older adults. Kendall's tau-b correlation analysis was
150 used to assessing the correlation between lower Hb, lower RDW, lower HRR, and frailty in
151 older adults. The p-values < 0.05 was considered statistically significant. All statistical analysis
152 were conducted using SPSS (ver. 26.0, IBM Corporation, Armonk, NY, USA).

153

154 **Result**

155 ***Characteristics of the study population***

1
2
3
4 156 A total of 1296 (564 man) older adults were included in our study. Their mean age was 70.89 ±
5
6 157 4.85 years. Of the 1296 participants, 582 (44.9%) has hypertension, 183 (14.1%) were
7
8 158 diabetes patients, 98 (7.6%) had cardiac diseases, and 43 (3.3%) had cranial vascular
9
10 159 disease. The proportions of individuals with a habit of smoking and drinking were 11.1% and
11
12 160 11.7%, respectively. And 149 (11.5%) older adults were living alone. According to Fried's
13
14 161 frailty phenotype, there were 55.09% (714) in robust group, 36.81% (477) in pre-frail group,
15
16 162 and 8.10% (105) in frail group. Baseline characteristics of three group were shown in Table 1.
17
18
19
20
21
22
23

163

164 Table1. Baseline characteristics of the study population stratified by frailty

Characteristics	Robust (n=714)	Pre-frail (n=477)	Frailty (n=105)	p-value
Age, years (SD)	69.81 ± 3.89	71.71 ± 5.34	70.89 ± 4.85	<0.001
Male gender, n (%)	347 (48.60%)	184 (38.57%)	33 (31.43%)	<0.001
Marital status Married, n (%)	622 (87.11%)	408 (85.53)	77 (73.33%)	0.001
Other*, n (%)	92 (12.89%)	69 (14.47%)	28 (26.67%)	
Education years 0-12, n (%)	466(65.27%)	337(70.65%)	85(80.95%)	0.002
>12, n (%)	248(24.73%)	140(29.35%)	20(19.05%)	
Alone living, n (%)	74 (10.36%)	53 (11.11%)	22 (20.95%)	0.006
Smoking, n (%)	90(12.61%)	44(9.22%)	10(9.52%)	0.165
Drinking, n (%)	92(12.89%)	50(10.48%)	10(9.52%)	0.345
Hypertension, n (%)	314(43.98%)	212(44.44%)	56(53.33%)	0.192
Diabetes mellitus, n (%)	81(11.34%)	82(17.19%)	20(19.05%)	0.006
Cardiac diseases, n (%)	46(6.44%)	41(8.60%)	11(10.48%)	0.194
Cranial vascular disease, n (%)	24(2.94%)	15(3.14%)	4(3.80%)	0.938
BMI, kg/m ² (SD)	24.69 ± 3.05	24.32 ± 3.14	23.83 ± 3.51	0.011
Waist circumference, cm (SD)	86.99 ± 8.66	86.32 ± 8.57	85.42 ± 9.46	0.143
SBP, mmHg (SD)	141.61 ± 18.53	142.73 ± 17.74	143.01 ± 19.37	0.516
DBP, mmHg (SD)	83.77 ± 10.44	82.87 ± 9.75	80.44 ± 10.91	0.006
Heart rate, beats/min (SD)	79.9 ± 13.4	79.8 ± 13.2	81.1 ± 13.0	0.659
WBC, 10 ⁹ /L (SD)	6.39 ± 1.51	6.36 ± 1.56	6.29 ± 1.69	0.781
Neutrophils, 10 ⁹ /L (SD)	3.89 ± 1.22	3.86 ± 1.26	3.96 ± 1.29	0.699
Lymphocytes, 10 ⁹ /L (SD)	1.95 ± 0.58	1.96 ± 0.64	1.76 ± 0.59	0.007
Eosinophils, 10 ⁹ /L (SD)	0.14 ± 0.12	0.14 ± 0.12	0.15 ± 0.18	0.628
PLT, 10 ⁹ /L (SD)	212.4 ± 52.2	216.3 ± 52.0	207.9 ± 66.1	0.251
RBC, 10 ⁹ /L (SD)	4.73 ± 0.41	4.51 ± 0.42	4.41 ± 0.65	<0.001
Hemoglobin, g/L (SD)	145.51 ± 12.15	137.29 ± 11.79	129.55 ± 13.73	<0.001

Anemia, n (%)	6 (0.84%)	17 (3.56%)	21 (20%)	<0.001
RDW, % (SD)	12.89 ± 0.55	13.15 ± 0.69	13.88 ± 1.39	<0.001
HRR	11.31 ± 1.02	10.47 ± 1.05	9.43 ± 1.41	<0.001
FBG, mmol/L (SD)	6.28 ± 1.59	6.37 ± 1.90	6.56 ± 2.13	0.278
Albumin, g/dL (SD)	46.23 ± 2.19	45.86 ± 2.47	45.13 ± 2.90	<0.001
Globulin, g/dL (SD)	30.26 ± 3.44	30.64 ± 3.65	30.55 ± 5.25	0.194
Triglyceride, mmol/L (SD)	1.49 ± 0.85	1.46 ± 0.90	1.44 ± 0.85	0.883
Total Cholesterol, mmol/L (SD)	5.17 ± 1.04	5.19 ± 1.15	4.96 ± 1.05	0.122
HDL-C, mmol/L (SD)	1.50 ± 0.39	1.54 ± 0.42	1.54 ± 0.38	0.333
LDL-C, mmol/L (SD)	3.03 ± 0.82	3.02 ± 0.90	2.82 ± 0.88	0.060

165 SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, FBG=fasting blood-glucose,
 166 WBC=white blood cell, PLT=platelets count, RBC=red blood cell, RDW=red blood cell distribution width,
 167 HRR=hemoglobin-to-RDW ratio.
 168 Other* including separated, divorced, never married or widowed

169

170 **ROC curve analysis**

171 The predictive value of HRR for frailty in older adults was assessed by ROC analysis. The
 172 AUC for HRR in the frailty older adults was 0.802 (95% CI: 0.755 to 0.849), and the highest
 173 sensitivity was 84.5% and the specificity was 61.9% with the optimal critical values 9.97
 174 (Figure 1). The AUC for Hb was 0.742 (95% CI: 0.691 to 0.793), the highest sensitivity was
 175 81% and the specificity was 57.1% with the optimal critical values 131.5 (Figure 1). The AUC
 176 for RDW was 0.712 (95% CI: 0.651 to 0.772), the highest sensitivity was 56.2% and the
 177 specificity was 81.1% with the optimal critical values 13.45 (Figure 1). Compared with Hb and
 178 RDW alone, HRR was a more strong prognostic biomarker for frailty.

179 **Differences in clinical characteristics of the study population** 180 **stratified by HRR**

181 According to ROC analysis, the optimal critical values of HRR was 9.97. Participants were
 182 grouped according to the optimal critical values of HRR, as follows: 1046 (80.71%) in the
 183 normal HRR group and 250(19.29%) in the lower HRR group (Table 2). Compared with the

184 normal HRR group, the lower HRR group had a higher lymphocytes count (P=0.002), and
 185 RDW (P<0.001), but a lower hemoglobin (P<0.001), RBC (P<0.001) and albumin (P<0.001).
 186 Compared with normal HRR group, the lower HRR group were more likely to have frailty
 187 (P<0.001).

188 Table2. Baseline characteristics of the study population stratified by HRR

Characteristics	Normal HRR (n=1046)	Lower HRR (n=250)	P-value
Age, years (SD)	70.50 ± 4.64	72.50 ± 5.40	<0.001
Male gender, n (%)	501 (47.9%)	63 (25.2%)	<0.001
Marital status Married, n (%)	901 (86.14%)	206 (82.4%)	0.133
Other*, n (%)	145 (13.86%)	44 (17.6%)	
Education years 0-12, n (%)	691 (66.06%)	197 (78.8%)	<0.001
>12, n (%)	355 (33.94%)	53 (21.2%)	
Alone living, n (%)	114 (10.90%)	35 (14.0%)	0.167
Smoking, n (%)	129 (12.33%)	15 (6.0%)	0.004
Drinking, n (%)	136 (13.0%)	16 (6.4%)	0.004
Hypertension, n (%)	456 (43.59%)	126 (50.4%)	0.052
Diabetes mellitus, n (%)	140 (13.38%)	43 (17.2%)	0.120
Cardiac diseases, n (%)	82 (7.84%)	16 (6.4%)	0.439
Cranial vascular disease, n (%)	32 (3.06%)	11 (4.4%)	0.288
BMI, kg/m ² (SD)	24.56 ± 3.07	24.15 ± 3.36	0.026
Waist circumference, cm (SD)	86.90 ± 8.51	85.45 ± 9.37	0.143
SBP, mmHg (SD)	142.3 ± 18.3	141.3 ± 18.5	0.611
DBP, mmHg (SD)	83.7 ± 10.2	80.9 ± 10.1	0.004
Heart rate, beats/min (SD)	80.2 ± 13.4	79.0 ± 12.7	0.362
WBC, 10 ⁹ /L (SD)	6.39 ± 1.49	6.28 ± 1.75	0.556
Neutrophils, 10 ⁹ /L (SD)	3.89 ± 1.19	3.86 ± 1.43	0.441
Lymphocytes, 10 ⁹ /L (SD)	1.96 ± 0.59	1.86 ± 0.69	0.002
Eosinophils, 10 ⁹ /L (SD)	0.14 ± 0.11	0.15 ± 0.17	0.621
PLT, 10 ⁹ /L (SD)	212.3 ± 50.8	218.3 ± 62.8	0.264
RBC, 10 ⁹ /L (SD)	4.71 ± 0.38	4.27 ± 0.56	<0.001
Hemoglobin, g/L (SD)	145.08 ± 10.98	124.90 ± 8.46	<0.001
RDW, % (IQR)	12.89 ± 0.51	13.79 ± 1.09	<0.001
FBG, mmol/L (SD)	6.36 ± 1.77	6.24 ± 1.70	0.166
Albumin, g/dL (SD)	46.18 ± 2.27	45.30 ± 2.69	<0.001
Globulin, g/dL (SD)	30.35 ± 3.45	30.72 ± 4.58	0.708
Triglyceride, mmol/L (SD)	1.48 ± 0.87	1.42 ± 0.83	0.755
Total Cholesterol, mmol/L (SD)	5.17 ± 1.07	5.10 ± 1.27	0.041
HDL-C, mmol/L (SD)	1.50 ± 0.39	1.58 ± 0.42	0.539
LDL-C, mmol/L (SD)	3.03 ± 0.85	2.91 ± 0.87	0.019
Frailty status Robust, n (%)	669 (63.96%)	45 (18.0%)	<0.001

Pre-frail, n (%)	337 (32.22%)	140 (56.0%)
Frailty, n (%)	40 (3.82%)	65 (26.0%)

189 SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, FBG=fasting blood-glucose,
 190 WBC=white blood cell, PLT=platelets count, RBC=red blood cell, RDW=red blood cell distribution width,
 191 HRR=hemoglobin-to-RDW ratio.
 192 Normal HRR=HRR above 9.97, Lower HRR: HRR below 9.97 (Optimal cut-off value of the ROC curve)
 193 Other* including separated, divorced, never married or widowed

195 **Logistic regression analysis**

196 Among 1296 older adults, 105 (8.10%) were considered frail. Multiple logistic regression
 197 analysis was conducted to assessing the associations of the lower HRR with frailty.
 198 Unadjusted model1 showed that lower Hb (OR:2.129,1.133-4.001, p=0.019) and lower
 199 HRR (OR:3.285,1.676-6.440, p=0.001) were risk factors related to frailty, whereas lower
 200 RDW (OR:0.310, 0.193-0.497, p<0.001) was a protective factor. After adjustment for
 201 confounding factors (including age, gender, marital status, education years, living alone,
 202 BMI, diabetes, RBC, albumin, triglyceride, HDL-C and LDL-C), there was significant
 203 association of lower HRR (OR:3.419, 1.679-6.964, p=0.001) and lower RDW (OR:0.285,
 204 0.170-0.477, p< 0.001) with frailty (Table 3). Lower HRR was independently related to
 205 frailty in older adults.

207 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

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

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

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

214 **Correlation Analysis**

215 Correlation analysis indicated that there was an obvious positive correlation between
216 RDW (Kendall's tau-b=0.173, P<0.001) and frailty. Nevertheless, HRR (Kendall's
217 tau-b=-0.239, P<0.001) and Hb (Kendall's tau-b=-0.194, P<0.001) were a negative
218 correlation with frailty (Table 4).

220 Table4. Correlation analysis of Hb, RDW, HRR, and frailty in older adults

	Kendall's tau-b	p
Hb	-0.194	<0.001
RDW	0.173	<0.001
HRR	-0.239	<0.001

222 **. Correlation is significant at the 0.01 level (2-tailed).

224 **Discussion**

225 Frailty, as a geriatric syndrome, has attracted more and more scientific attention in the
226 background of continuously increase global population ageing.¹⁹ In this cross-sectional study
227 including 1,296 community-dwelling older adults, we found that lower HRR is independently
228 related to frailty in older people, even after adjusting confounding factors. Multiple logistic
229 regression analysis showed that lower HRR is associated with to a 3-fold more likelihood or

1
2
3
4 230 odds of frailty. ROC analysis showed that AUC and highest sensitivity of HRR are higher than
5
6
7 231 RDW. Thus, HRR may be a more powerful marker for frailty than RDW.

8
9 232 HRR is cost-effective, common, and accessible laboratory parameter for clinicians. As
10
11 233 a novel inflammatory factor, Qu et.al found that HRR is a significant associated with frailty in
12
13
14 234 older patients with CHD.¹⁴ In there study the AUC for HRR in the frailty patients was exceed
15
16
17 235 Hb and RDW, and after adjusting confounding factors lower HRR was a risk factor for frailty in
18
19
20 236 older patients with CHD. These findings are consistent with our results. Now the
21
22 237 pathophysiological mechanism has not been fully understood. We try to provide a possible
23
24
25 238 explanation for the association between HRR and frailty in older adults.

26
27 239 A decreased in HRR may be due to low Hb, high RDW, or both. As we all know, low
28
29
30 240 Hb indicates a condition of anemia, which is one of the acknowledged risk factors for
31
32
33 241 hospitalization, morbidity, and mortality in older people.²⁰ Anemia decreases the
34
35
36 242 oxygen-carrying capacity, leading to tissue hypoxia and even organ failure, especially in older
37
38
39 243 patients, increasing the risk of frailty.²¹ Besides, anemia can cut down submaximal and
40
41
42 244 maximal aerobic capacity, leading to several adverse outcomes including loss of muscle
43
44
45 245 strength, cognitive decline and development of frailty.¹⁰ In addition, chronic conditions and
46
47
48 246 comorbidities leading to a low grade of inflammation reducing hemoglobin level,²² also known
49
50
51 247 as chronic diseases anemia, which is the most common type of anemia in older adults.²¹ And a
52
53
54 248 state of chronic inflammation has been suggested as contributors to frailty.²³ Furthermore,
55
56
57 249 anemia caused by malnutrition is also a significance health-affecting factor among older
58
59
60 250 adults.²¹

251 Anemia reaching a prevalence of 17% in older people, is a threat to healthy aging.²⁴

1
2
3
4 252 Corona et.al found that lower hemoglobin and anemia were related to frailty in Brazilian older
5
6 253 adults.⁸ In their study, anemia was related to low physical activity, weakness(weaker) and
7
8
9 254 walked more slowly. Another study from Spain indicated that anemia is independently
10
11 255 associated with frailty in older people.²⁵ Moreover, Xu et.al found that Hb is closely associated
12
13
14 256 with frailty in elder patients in hospital.⁹ A systematic review and meta-analysis including 19
15
16
17 257 studies indicated that older adults with anemia have more than a two-fold increased odds of
18
19 258 frailty.²⁶ Silva et.al suggested that lower Hb level should be considered a significant
20
21
22 259 component of frailty in older persons.²⁷ Similarly, another meta-analysis including 32,934
23
24
25 260 robust participants and 6864 frail participants found that Hb is a useful biomarker of frailty.⁷
26
27 261 However, there were no significant association of lower Hb and frailty after adjusting
28
29
30 262 confounding factors in our study. The discrepancy of results may be due to the definition of
31
32
33 263 lower Hb is determined by the optimal cut-off value of the ROC curve in this research.
34
35 264 Therefore, further studies are needed to confirm the relationship between Hb and frailty in
36
37
38 265 older people.

39
40 266 A increasing RDW also can lead to low HRR. Studies have proved that increased
41
42
43 267 RDW is related to inflammation. Inflammatory cytokines reduce erythropoietin gene
44
45 268 expression and erythropoietin receptor expression, which lead to the release of immature
46
47
48 269 erythrocytes and heterogeneity of the erythrocyte volume increasing.^{28,29} In addition, metabolic
49
50
51 270 abnormalities including shortened telomere length, oxidative stress, and malnutrition may also
52
53 271 contribute to increased RDW.^{30,31} What's more, others have suggested that RDW may be a
54
55
56 272 potential biomarker for biological aging.²⁹ Study has indicated that a high RDW was related to
57
58
59 273 a high sarcopenia risks.³² Sarcopenia, which plays a key role in frailty, is a progressive loss of
60

1
2
3
4 274 skeletal muscle mass and strength.²⁷

5
6 275 RDW is a biomarker of inflammation, oxidative stress, poor nutritional status, aging,
7
8
9 276 and sarcopenia, and all of these could be underlying reasons of development of frailty. A study
10
11 277 enrolled 3,635 community-dwelling older men indicated that participants with a high RDW are
12
13
14 278 more likely to have functional limitations and frailty.²⁹ Li et.al indicated that increased RDW
15
16
17 279 may be closely related to frailty through inflammation.¹³ Hou et.al proved that frailty is closely
18
19
20 280 associated with RDW in elder patients with CHD.¹¹ Another study including 2,932
21
22
23 281 community-dwelling older adults found RDW is independently associated with high frailty risk
24
25 282 even after adjusting for potential confounding factors.¹²

26
27 283 To sum up, a large number of researches have verified the association between frailty
28
29
30 284 and a low Hb and a high RDW among older persons. However, both RDW and Hb are
31
32
33 285 susceptible to many other diseases conditions and sub-health states, HRR may provide a
34
35
36 286 more powerful parameter than a single parameter alone. Moreover, ROC analysis showed that
37
38
39 287 the AUC and highest sensitivity of HRR are higher than RDW and Hb. It may be a more
40
41
42 288 reliable and effective marker than Hb and RDW alone.

43 289 There were also some limitations of our present study. Firstly, this was a
44
45
46 290 cross-sectional study that cannot assess the cause-effect relationship. Secondly, our
47
48
49 291 participants are limited to local participants, these findings need to be validated in different
50
51
52 292 populations around the world. Finally, we did not assess iron, folic, and vitamin B12, which
53
54 293 may affect RDW and Hb level.

55
56 294

57 58 295 **Conclusion**

1
2
3
4 296 In conclusion, a low HRR is independent associated with higher frailty risk in
5
6 297 community-dwelling older adults. This inexpensive and common laboratory parameter may
7
8
9 298 provide useful information to identify the risk of frailty in older adults. Furthermore, use of the
10
11 299 HRR may help clinicians to identify people at high risk of frailty and take effective measures to
12
13
14 300 reduce the occurrence and development of frailty, reduce the rate of disability and mortality
15
16
17 301 related to frailty in the elderly, and reduce the waste of medical resources, and promote
18
19 302 healthy ageing. Evidence is needed from cohort studies to verify this conclusions in the future.
20
21
22
23
24
25
26
27
28
29

303

304

305 **Author contributions**

306 Conceptualization: Zhifan Xiong, Mengpei Zhu

307 Data curation: Mengpei Zhu, Chao Wei, Yushuang Xu

308 Formal analysis: Mengpei Zhu, Chao Wei

309 Visualization: Xiongjun Yang, Yumei Huang

310 Writing – original draft: Mengpei Zhu

311 Writing – review and editing: Zhifan Xiong, Mengpei Zhu, Chao Wei, Xiongjun Yang, Yumei

312 Huang, Yushuang Xu. All authors read and approved the final manuscript.

313

314 **Acknowledgements**

315 The authors thank all the volunteers that participated in this study.

316

317 **Funding**

318

1
2
3
4 318 This study was supported by the National Key Research and Development Program of China
5
6 319 (2018YFC2002000).
7
8
9

10 320

11 321 **Data availability**

12
13
14 322 Data for this study are available from the corresponding author.
15
16
17 323

18
19 324 **Declaration** The authors report no conflicts of interest in this work.
20
21
22 325

23
24
25 326

26
27 327

28
29
30 328

31 32 33 329 **References**

- 34
35 330 1. Kameda M, Teruya T, Yanagida M, Kondoh H. Frailty markers comprise blood metabolites
36 331 involved in antioxidation, cognition, and mobility. *Proc Natl Acad Sci U S A*.
37 332 2020;117(17):9483-9489.
38
39 333 2. Travers J, Romero-Ortuno R, Bailey J, Cooney M-T. Delaying and reversing frailty: a
40 334 systematic review of primary care interventions. *Br J Gen Pract*. 2019;69(678):e61-e69.
41
42 335 3. Dent E, Martin FC, Bergman H, Woo J, Romero-Ortuno R, Walston JD. Management of frailty:
43 336 opportunities, challenges, and future directions. *Lancet*. 2019;394(10206):1376-1386.
44
45 337 4. Coelho-Junior HJ, Marzetti E, Picca A, Cesari M, Uchida MC, Calvani R. Protein Intake and
46 338 Frailty: A Matter of Quantity, Quality, and Timing. *Nutrients*. 2020;12(10).
47
48 339 5. Ward RE, Orkaby AR, Chen J, et al. Association between Diet Quality and Frailty Prevalence in
49 340 the Physicians' Health Study. *J Am Geriatr Soc*. 2020;68(4):770-776.
50
51 341 6. Zhai Z, Gao J, Zhu Z, et al. The Ratio of the Hemoglobin to Red Cell Distribution Width
52 342 Combined with the Ratio of Platelets to Lymphocytes Can Predict the Survival of Patients
53 343 with Gastric Cancer Liver Metastasis. *Biomed Res Int*. 2021;2021:8729869.
54
55 344 7. Mailliez A, Guilbaud A, Puisieux F, Dauchet L, Boulanger É. Circulating biomarkers
56 345 characterizing physical frailty: CRP, hemoglobin, albumin, 25OHD and free testosterone as
57 346 best biomarkers. Results of a meta-analysis. *Exp Gerontol*. 2020;139:111014.
58
59 347 8. Pires Corona L, Drumond Andrade FC, de Oliveira Duarte YA, Lebrao ML. The Relationship
60 348 between Anemia, Hemoglobin Concentration and Frailty in Brazilian Older Adults. *J Nutr*
349 349 *Health Aging*. 2015;19(9):935-940.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 350 9. Xu L, Zhang J, Shen S, et al. Clinical Frailty Scale and Biomarkers for Assessing Frailty in Elder
351 Inpatients in China. *J Nutr Health Aging*. 2021;25(1):77-83.
- 352 10. Ruan Y, Guo Y, Kowal P, et al. Association between anemia and frailty in 13,175
353 community-dwelling adults aged 50 years and older in China. *BMC Geriatr*. 2019;19(1):327.
- 354 11. Hou P, Xue H-P, Mao X-E, Li Y-N, Wu L-F, Liu Y-B. Inflammation markers are associated with
355 frailty in elderly patients with coronary heart disease. *Aging (Albany NY)*.
356 2018;10(10):2636-2645.
- 357 12. Li C-M, Chao C-T, Chen S-I, Han D-S, Huang K-C. Elevated Red Cell Distribution Width Is
358 Independently Associated With a Higher Frailty Risk Among 2,932 Community-Dwelling Older
359 Adults. *Front Med (Lausanne)*. 2020;7:470.
- 360 13. Li Q, Chen X, Han B. Red blood cell distribution width is associated with frailty in older
361 inpatients in China: Sex differences in a cross-sectional study. *Exp Gerontol*.
362 2021;150:111392.
- 363 14. Qu J, Zhou T, Xue M, et al. Correlation Analysis of Hemoglobin-to-Red Blood Cell Distribution
364 Width Ratio and Frailty in Elderly Patients With Coronary Heart Disease. *Front Cardiovasc*
365 *Med*. 2021;8:728800.
- 366 15. Su Y-C, Wen S-C, Li C-C, et al. Low Hemoglobin-to-Red Cell Distribution Width Ratio Is
367 Associated with Disease Progression and Poor Prognosis in Upper Tract Urothelial Carcinoma.
368 *Biomedicines*. 2021;9(6).
- 369 16. Sun P, Zhang F, Chen C, et al. The ratio of hemoglobin to red cell distribution width as a novel
370 prognostic parameter in esophageal squamous cell carcinoma: a retrospective study from
371 southern China. *Oncotarget*. 2016;7(27):42650-42660.
- 372 17. Ma L, Tang Z, Zhang L, Sun F, Li Y, Chan P. Prevalence of Frailty and Associated Factors in the
373 Community-Dwelling Population of China. *J Am Geriatr Soc*. 2018;66(3):559-564.
- 374 18. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. *J*
375 *Gerontol A Biol Sci Med Sci*. 2001;56(3):M146-M156.
- 376 19. Kojima G, Taniguchi Y, Iliffe S, Jivraj S, Walters K. Transitions between frailty states among
377 community-dwelling older people: A systematic review and meta-analysis. *Ageing Res Rev*.
378 2019;50:81-88.
- 379 20. Lee CT, Chen MZ, Yip CYC, Yap ES, Lee SY, Merchant RA. Prevalence of Anemia and Its
380 Association with Frailty, Physical Function and Cognition in Community-Dwelling Older
381 Adults: Findings from the HOPE Study. *J Nutr Health Aging*. 2021;25(5):679-687.
- 382 21. Röhrig G. Anemia in the frail, elderly patient. *Clin Interv Aging*. 2016;11:319-326.
- 383 22. Steinmeyer Z, Delpierre C, Soriano G, et al. Hemoglobin concentration; a pathway to frailty.
384 *BMC Geriatr*. 2020;20(1):202.
- 385 23. Wilson D, Jackson T, Sapey E, Lord JM. Frailty and sarcopenia: The potential role of an aged
386 immune system. *Ageing Res Rev*. 2017;36.
- 387 24. Stauder R, Valent P, Theurl I. Anemia at older age: etiologies, clinical implications, and
388 management. *Blood*. 2018;131(5):505-514.
- 389 25. Esquinas-Requena JL, García-Nogueras I, Hernández-Zegarra P, Atienzar-Núñez P,
390 Sánchez-Jurado PM, Abizanda P. [Anemia and frailty in older adults from Spain. The FRADEA
391 Study]. *Rev Esp Geriatr Gerontol*. 2021;56(3):129-135.
- 392 26. Palmer K, Vetrano DL, Marengoni A, et al. The Relationship between Anaemia and Frailty: A
393 Systematic Review and Meta-Analysis of Observational Studies. *J Nutr Health Aging*.

- 1
2
3 394 2018;22(8):965-974.
4 395 27. Silva JC, Moraes ZVd, Silva C, et al. Understanding red blood cell parameters in the context of
5 396 the frailty phenotype: interpretations of the FIBRA (Frailty in Brazilian Seniors) study. *Arch*
6 397 *Gerontol Geriatr.* 2014;59(3):636-641.
7 398 28. Pierce CN, Larson DF. Inflammatory cytokine inhibition of erythropoiesis in patients
8 399 implanted with a mechanical circulatory assist device. *Perfusion.* 2005;20(2):83-90.
9 400 29. Kim KM, Lui L-Y, Browner WS, et al. Association Between Variation in Red Cell Size and
10 401 Multiple Aging-Related Outcomes. *J Gerontol A Biol Sci Med Sci.* 2021;76(7):1288-1294.
11 402 30. Ghaffari S. Oxidative stress in the regulation of normal and neoplastic hematopoiesis.
12 403 *Antioxid Redox Signal.* 2008;10(11):1923-1940.
13 404 31. Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: A simple
14 405 parameter with multiple clinical applications. *Crit Rev Clin Lab Sci.* 2015;52(2).
15 406 32. Kim J, Im J-S, Choi CH, et al. The Association between Red Blood Cell Distribution Width and
16 407 Sarcopenia in U.S. Adults. *Sci Rep.* 2018;8(1):11484.
17
18
19
20
21
22 408
23
24
25 409
26
27 410
28
29
30 411
31
32 412
33
34
35 413
36
37
38 414 **Figure legends**
39
40 415 **Figure1** ROC curve for HRR (A), Hb (B) and RDW (C)
41
42
43 416
44
45 417 **Notes:**
46
47
48 418 A. ROC curve indicated that the best intercept value for HRR was 9.97 (sensitivity 84.5%,
49
50 419 specificity 61.9%, AUC = 0.802, P<0.001)
51
52
53 420 B. ROC curve indicated that the best intercept value for Hb was 131.5 (sensitivity 81% ,
54
55 421 specificity 57.1%, AUC = 0.742, P<0.001)
56
57
58 422 C. ROC curve indicated that the best intercept value for RDW was 13.45 (sensitivity 56.2% ,
59
60

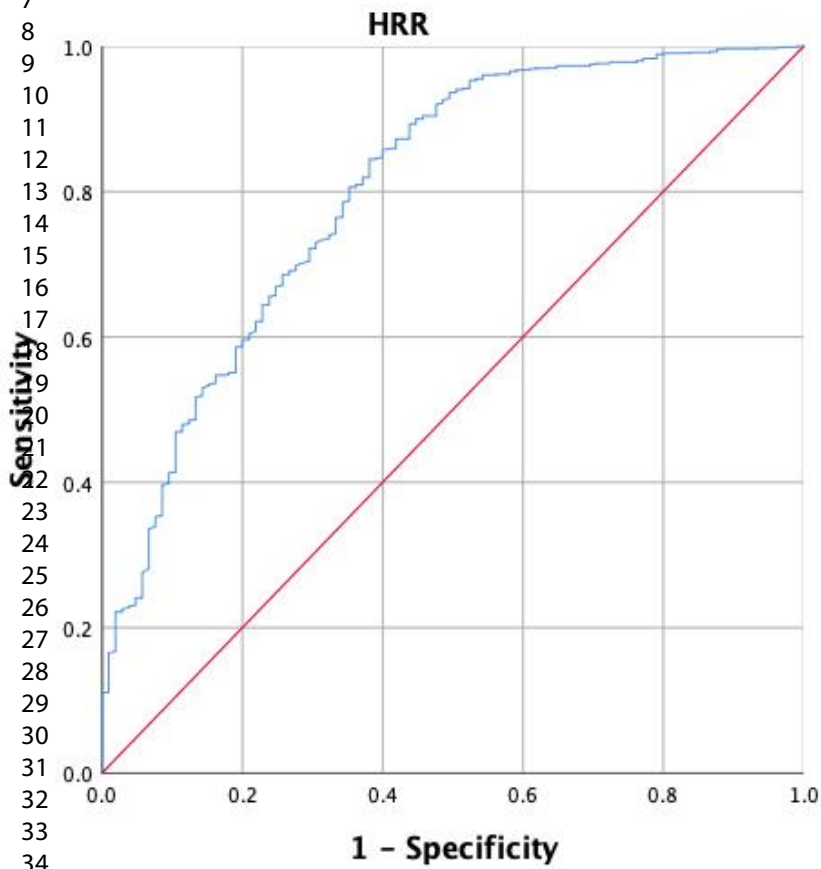
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

423 specificity 81.1%, AUC = 0.712, P<0.001)

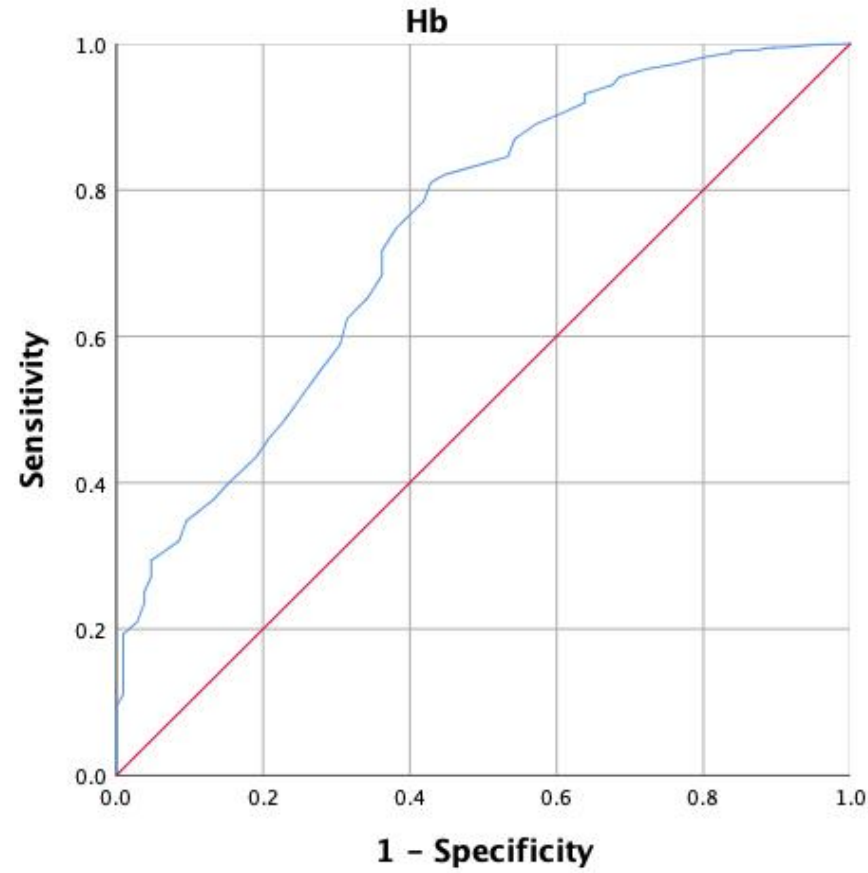
For peer review only

Figure 1.

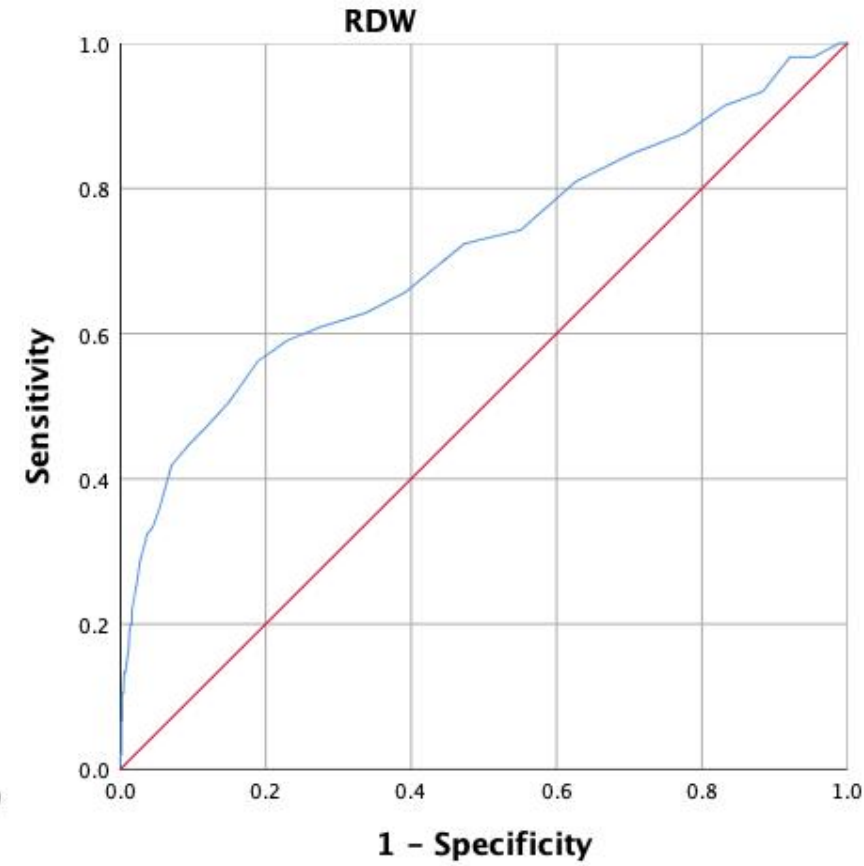
A



B



C



STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	5

Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7
		(b) Describe any methods used to examine subgroups and interactions	7
		(c) Explain how missing data were addressed	7
		(d) If applicable, describe analytical methods taking account of sampling strategy	7
		(e) Describe any sensitivity analyses	7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7-8
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	8
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-11
		(b) Report category boundaries when continuous variables were categorized	9-11

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Lower Hemoglobin-to-Red Blood Cell Distribution Width Ratio Is Independently Associated With Frailty in Community-dwelling Older Adults: a cross-sectional study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-069141.R1
Article Type:	Original research
Date Submitted by the Author:	26-Apr-2023
Complete List of Authors:	Zhu, Mengpei; Institute of Geriatric Medicine, Liyuan Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wei, Chao; Wuhan Geriatric Hospital Yang, Xiongjun; Huazhong University of Science and Technology Huang, Yumei; Huazhong University of Science and Technology Xu, Yushuang; Liyuan Hospital of Tongji Medical College of Huazhong University of Science and Technology Xiong, Zhifan; Huazhong University of Science and Technology; Huazhong University of Science and Technology
Primary Subject Heading:	Geriatric medicine
Secondary Subject Heading:	Global health
Keywords:	GERIATRIC MEDICINE, EPIDEMIOLOGY, GENERAL MEDICINE (see Internal Medicine)

SCHOLARONE™
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1
2
3
4
5 1 **Lower Hemoglobin-to-Red Blood Cell Distribution**
6
7
8 2 **Width Ratio Is Independently Associated With Frailty**
9
10 3 **in Community-dwelling Older Adults: a cross-sectional**
11
12 4 **study**
13
14
15
16
17

18 6 Authors Name : Mengpei Zhu^{1,2}, Chao Wei³, Xiongjun Yang², Yumei Huang², Yushuang Xu^{1,2},
19
20
21 7 Zhifan Xiong^{1,2}
22

23 8 Affiliations: 1 Institute of Geriatric Medicine, Liyuan Hospital, Tongji Medical College,
24
25
26 9 Huazhong University of Science and Technology, Wuhan, China.
27

28 10 2 Division of Gastroenterology, Liyuan Hospital, Tongji Medical College, Huazhong University
29
30
31 11 of Science and Technology, Wuhan, China.
32

33 12 3 Physical Examination Center, East Lake Hospital & Wuhan Geriatric Hospital, Wuhan,
34
35
36 13 China.
37
38

39 14
40
41 15 Correspondence: Zhifan Xiong
42

43
44 16 Address: Division of Gastroenterology, Institute of Geriatric Medicine, Liyuan Hospital, Tongji
45
46
47 17 Medical College, Huazhong University of Science and Technology, 39 Lake Road, East Lake
48
49 18 Ecological Scenic, Wuhan 430077, Hubei, China
50

51
52 19 Email: xiongzhiban@126.com
53
54
55
56
57
58
59
60

1
2
3
4 23 **Abstract**

5
6 24 **Objectives:** The importance of blood cell markers in frailty has been studied. However,
7
8 25 research on hemoglobin-to-red blood cell distribution width ratio (HRR) and frailty in older
9
10 26 persons is still limited. We investigated the association between HRR and frailty in older
11
12 27 adults.

13
14 28 **Design:** Cross-sectional population-based study.

15
16
17 29 **Setting:** Community-dwelling older adults older than 65 years old were recruited from
18
19 30 September 2021 to December 2021.

20
21
22 31 **Participants:** A total of 1296 community-dwelling older adults (age \geq 65 years) in Wuhan
23
24 32 were included in the study.

25
26
27 33 **Main outcome measures:** The main outcome was the presence of frailty. The Fried Frailty
28
29 34 Phenotype Scale was used to evaluate the frailty status of the participants. Multivariable
30
31 35 logistic regression analysis was performed to determine the relationship between HRR and
32
33 36 frailty.

34
35
36
37 37 **Results:** A total of 1296 (564 man) older adults were included in this cross-sectional study.
38
39 38 Their mean age was 70.89 ± 4.85 years. ROC analysis showed that HRR is a good predictor
40
41 39 of frailty in older people, the area under the curve (AUC) was 0.802 (95% CI: 0.755 to 0.849),
42
43 40 and the highest sensitivity was 84.5% and the specificity was 61.9% with the optimal critical
44
45 41 values 9.97 ($P < 0.001$). Multiple logistic regression analysis indicated that lower HRR (< 9.97)
46
47 42 (OR:3.419, 1.679-6.964, $P = 0.001$) is independently associated with frailty in older people,
48
49 43 even after adjusting confounding factors.

50
51
52 44 **Conclusion:** Lower HRR is closely associated with an increased risk of frailty in the older
53
54 45 people. Lower HRR may be an independent risk factor for frailty in community-dwelling older
55
56
57
58
59
60

1
2
3
4 46 adults.

5
6 47 **Keywords:** Older people, Frailty, Healthy aging, Risk factor, HRR

7
8
9 48

10
11 49 **Strengths and limitations of this study**

12
13
14 50 Frailty was diagnosed in accordance with the Fried' s frailty phenotype.

15
16
17 51 This cross-sectional analysis was performed in a medium volume population.

18
19 52 Some variables were self-reported, but best available measures were used.

20
21
22 53 The study reflects the situation of older adults in Wuhan, China, and the generalizability needs
23
24
25 54 to be further verified.

26
27 55 This was a cross-sectional study that cannot assess the cause-effect relationship.

28
29
30 56

31
32 57 **Introduction**

33
34
35 58 As life expectancy increases, human societies are aging globally, in both developed and
36
37
38 59 developing countries.¹ By 2050, the proportion of people aged over 60 years is projected to
39
40
41 60 increase from 11% to 22%, and the number of aged over 60 years will increase from 605
42
43
44 61 million to 2.1 billion, including 425 million people aged over 80 in the world.² Frailty becomes
45
46
47 62 an emerging global public health burden, with the rapid growth of the global aging population.

48
49
50 63 Frailty is considered to be a complex age-related clinical condition characterized by a decline
51
52
53 64 in the physiological function of multiple organs, with a resultant increased vulnerability to
54
55
56 65 stressors.³ It is related to adverse health-related events, including increased mortality,
57
58
59 66 hospitalization, falls and fractures, cognitive decline, disability, and admission to long-term
60
61
62 67 care.⁴ Therefore, early identifying modifiable risk factors of frailty is becoming increasing

1
2
3
4 68 crucial for delaying and reversing frailty and its associated adverse events in older persons.⁵
5

6
7 69 As an important part of CBC, hemoglobin (Hb) is usually used as an indicator of the
8
9 70 degree of anemia. However, previous studies showed that low Hb reflect to a decline in the
10
11 71 physiological function including the decreased immune response, malnutrition, and the low
12
13 72 resistance to external invasion.⁶ Meanwhile, there are several studies indicated that Hb is
14
15 73 related to frailty in older persons.⁷⁻¹⁰ As an indicator of heterogeneity of the erythrocyte
16
17 74 volume, red blood cell distribution width (RDW) is thought to be related to the prognosis of
18
19 75 many diseases. Hou et al. indicated that RDW is significantly associated with the risk of frailty
20
21 76 in older patients with coronary heart disease (CHD).¹¹ In addition, studies showed that RDW is
22
23 77 associated with frailty both in older inpatients and community-dwelling older people.^{12,13}
24
25
26
27
28
29

30 78 RDW can be affected by complex conditions, the effect of RDW on frailty is not only
31
32 79 related to inflammatory response, but also association with a decline in the physiological
33
34 80 function and oxygen.¹⁴ RDW alone may not provide definitive predictive information.^{14,15} The
35
36 81 hemoglobin-to-RDW ratio (HRR) is a cheap, rapid and readily available novel prognostic,
37
38 82 which combines the prognostic information of Hb and RDW and reflects a more
39
40 83 comprehensive health status.^{14,16} Recently, Qu et al. found that lower HRR is independent
41
42 84 related to the risk of frailty in older patients with CHD.¹⁴ They verified that HRR maybe a more
43
44 85 useful biomarker comparing with RDW or Hb alone.¹⁴
45
46
47
48
49

50 86 Studies have showed a significant association of HRR with frailty in specific
51
52 87 populations (patients with coronary heart disease).¹⁴ However, research on HRR and frailty in
53
54 88 the general older persons is still limited, and the significance of evaluating frailty is not yet
55
56 89 clear. In the present study, we investigated the relationship between HRR and frailty in
57
58
59
60

1
2
3
4 90 community-dwelling older adults.
5
6
7 91

92 **Material & Methods**

93 ***Patient and public involvement***

94 The source population was the community-dwelling adults older than 65 living in communities
95 in Wuhan. The study population consisted of a random sample of older people from each
96 community. Inclusion criteria were the community-dwelling adults older than 65 living in
97 communities in Wuhan. Exclusion criteria were malignant disease or advanced organic
98 diseases, hematologic diseases, acute stage of disease, and participants with missing the key
99 parameters.

100 ***Participants and Sociodemographic Characteristics***

101 In this present study, we recruited 1,296 community-dwelling adults older than 65 living in
102 communities in Wuhan between September 2021 and December 2021. Sociodemographic
103 characteristics, including age, gender, education years, marital status, smoking history,
104 alcohol consumption, comorbidities, including hypertension, diabetes, CHD, hyperlipidemia,
105 and cerebrovascular disease were recorded. Then body mass index (BMI), waistline , blood
106 pressure and pulse rate were measured by two professional clinicians.

107 ***Peripheral Blood Parameters***

108 Blood samples were collected, and full blood count was measured by automated hematology
109 analyzer (Mindray, BC7500, China). Other related biochemical indicators were detected by
110 automatic biochemical analyzer (Beckman, AU680, American). HRR = Hb (g/L)/RDW (%).

111 ***Fried's frailty phenotype***

1
2
3
4 112 According to the Fried's frailty phenotype, we evaluated frailty in five criteria, as follows (1)
5
6 113 Weight loss: unintentional weight loss >5% or a loss of more than 4.5 kg in the past 1 year. (2)
7
8
9 114 Physical weakness: dynamometer was used for participants for three trials, the maximum
10
11 115 value was recorded. Low grip strength was defined according to the standards proposed by
12
13
14 116 Fried et al.¹⁷ (3) Slowness: slowness was defined as when the time required to walk 4.6 meters
15
16
17 117 was more than 7 seconds for males (height ≤ 173 cm) and females (height ≤ 159 cm) or more
18
19 118 than 6 seconds for males (height >173 cm) and females (height >159 cm). (4) Physical activity:
20
21
22 119 low physical activity was defined as less than 383 kcal/week for males and less than 270
23
24
25 120 kcal/week for females. (5) Exhaustion: exhaustion was assessed by the following two
26
27 121 questions from the CES-D. "In the last week, I felt that everything I did was an effort" and
28
29 122 "Could not get going in the last week." If the participant responded "yes" to either of these
30
31
32 123 questions, the participant was considered exhausted. Participants with >3 indicators were
33
34
35 124 defined as frail, 1-2 as prefrail and none as robust.

125 ***Patient and public involvement statement***

126 Patients and/or the public were not involved in the design, conduct, reporting or dissemination
127 of this research.

128 ***Statistical analysis***

129 Continuous and categorical variables were expressed as the mean ± standard deviation and
130 numbers with percentages, respectively. The baseline characteristics of the groups were
131 compared using one-way analysis of variance and chi-squared test. The predictive value of
132 HRR for frailty was assessed by receiver operating curve (ROC) analysis. The prognostic
133 value of lower HRR on frailty was assessed using logistic regression model. Variables were

134 selected as candidates for the multivariate analysis when $p < 0.1$ in the univariate analysis.

135 After adjustment for confounding factors including age, gender, marital status, education

136 years, alone living, BMI, diabetes, RBC, albumin, triglyceride, HDL-C and LDL-C, assessing

137 the independent risk factors for frailty in older adults. Kendall's tau-b correlation analysis was

138 used to assessing the correlation between lower Hb, lower RDW, lower HRR, and frailty in

139 older adults. The p-values < 0.05 was considered statistically significant. All statistical analysis

140 were conducted using SPSS (ver. 26.0, IBM Corporation, Armonk, NY, USA).

141

142 **Result**

143 ***Characteristics of the study population***

144 A total of 1296 (564 man) older adults were included in our study. Their mean age was $70.89 \pm$

145 4.85 years. Of the 1296 participants, 582 (44.9%) has hypertension, 183 (14.1%) were

146 diabetes patients, 98 (7.6%) had cardiac diseases, and 43 (3.3%) had cranial vascular

147 disease. The proportions of individuals with a habit of smoking and drinking were 11.1% and

148 11.7%, respectively. And 149 (11.5%) older adults were living alone. According to Fried's

149 frailty phenotype, there were 55.09% (714) in robust group, 36.81% (477) in pre-frail group,

150 and 8.10% (105) in frail group. Baseline characteristics of three group were shown in Table 1.

151

152 Table 1. Baseline characteristics of the study population stratified by frailty

Characteristics	Robust (n=714)	Pre-frail (n=477)	Frailty (n=105)	p-value
Age, years (SD)	69.81 \pm 3.89	71.71 \pm 5.34	70.89 \pm 4.85	<0.001
Male gender, n (%)	347 (48.60%)	184 (38.57%)	33 (31.43%)	<0.001
Marital status Married, n (%)	622 (87.11%)	408 (85.53)	77 (73.33%)	0.001
Other*, n (%)	92 (12.89%)	69 (14.47%)	28 (26.67%)	
Education years 0-12, n (%)	466(65.27%)	337(70.65%)	85(80.95%)	0.002

60

1					
2					
3		>12, n (%)	248(24.73%)	140(29.35%)	20(19.05%)
4		Alone living, n (%)	74 (10.36%)	53 (11.11%)	22 (20.95%)
5		Smoking, n (%)	90(12.61%)	44(9.22%)	10(9.52%)
6		Drinking, n (%)	92(12.89%)	50(10.48%)	10(9.52%)
7		Hypertension, n (%)	314(43.98%)	212(44.44%)	56(53.33%)
8		Diabetes mellitus, n (%)	81(11.34%)	82(17.19%)	20(19.05%)
9		Cardiac diseases, n (%)	46(6.44%)	41(8.60%)	11(10.48%)
10		Cranial vascular disease, n (%)	24(2.94%)	15(3.14%)	4(3.80%)
11		BMI, kg/m ² (SD)	24.69 ± 3.05	24.32 ± 3.14	23.83 ± 3.51
12		Waist circumference, cm (SD)	86.99 ± 8.66	86.32 ± 8.57	85.42 ± 9.46
13		SBP, mmHg (SD)	141.61 ± 18.53	142.73 ± 17.74	143.01 ± 19.37
14		DBP, mmHg (SD)	83.77 ± 10.44	82.87 ± 9.75	80.44 ± 10.91
15		Heart rate, beats/min (SD)	79.9 ± 13.4	79.8 ± 13.2	81.1 ± 13.0
16		WBC, 10 ⁹ /L (SD)	6.39 ± 1.51	6.36 ± 1.56	6.29 ± 1.69
17		Neutrophils, 10 ⁹ /L (SD)	3.89 ± 1.22	3.86 ± 1.26	3.96 ± 1.29
18		Lymphocytes, 10 ⁹ /L (SD)	1.95 ± 0.58	1.96 ± 0.64	1.76 ± 0.59
19		Eosinophils, 10 ⁹ /L (SD)	0.14 ± 0.12	0.14 ± 0.12	0.15 ± 0.18
20		PLT, 10 ⁹ /L (SD)	212.4 ± 52.2	216.3 ± 52.0	207.9 ± 66.1
21		RBC, 10 ⁹ /L (SD)	4.73 ± 0.41	4.51 ± 0.42	4.41 ± 0.65
22		Hemoglobin, g/L (SD)	145.51 ± 12.15	137.29 ± 11.79	129.55 ± 13.73
23		Anemia, n (%)	6 (0.84%)	17 (3.56%)	21 (20%)
24		RDW, % (SD)	12.89 ± 0.55	13.15 ± 0.69	13.88 ± 1.39
25		HRR	11.31 ± 1.02	10.47 ± 1.05	9.43 ± 1.41
26		FBG, mmol/L (SD)	6.28 ± 1.59	6.37 ± 1.90	6.56 ± 2.13
27		Albumin, g/dL (SD)	46.23 ± 2.19	45.86 ± 2.47	45.13 ± 2.90
28		Globulin, g/dL (SD)	30.26 ± 3.44	30.64 ± 3.65	30.55 ± 5.25
29		Triglyceride, mmol/L (SD)	1.49 ± 0.85	1.46 ± 0.90	1.44 ± 0.85
30		Total Cholesterol, mmol/L (SD)	5.17 ± 1.04	5.19 ± 1.15	4.96 ± 1.05
31		HDL-C, mmol/L (SD)	1.50 ± 0.39	1.54 ± 0.42	1.54 ± 0.38
32		LDL-C, mmol/L (SD)	3.03 ± 0.82	3.02 ± 0.90	2.82 ± 0.88

153 SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, FBG=fasting blood-glucose,

154 WBC=white blood cell, PLT=platelets count, RBC=red blood cell, RDW=red blood cell distribution width,

155 HRR=hemoglobin-to-RDW ratio.

156 Other* including separated, divorced, never married or widowed

157

158 **ROC curve analysis**

159 The predictive value of HRR for frailty in older adults was assessed by ROC analysis. The

160 AUC for HRR in the frailty older adults was 0.802 (95% CI: 0.755 to 0.849), and the highest

161 sensitivity was 84.5% and the specificity was 61.9% with the optimal critical values 9.97

60

(Figure 1). The AUC for Hb was 0.742 (95% CI: 0.691 to 0.793), the highest sensitivity was 81% and the specificity was 57.1% with the optimal critical values 131.5 (Figure 1). The AUC for RDW was 0.712 (95% CI: 0.651 to 0.772), the highest sensitivity was 56.2% and the specificity was 81.1% with the optimal critical values 13.45 (Figure 1). Compared with Hb and RDW alone, HRR was a more strong prognostic biomarker for frailty.

Differences in clinical characteristics of the study population stratified by HRR

According to ROC analysis, the optimal critical values of HRR was 9.97. Participants were grouped according to the optimal critical values of HRR, as follows: 1046 (80.71%) in the normal HRR group and 250(19.29%) in the lower HRR group (Table 2). Compared with the normal HRR group, the lower HRR group had a higher lymphocytes count (P=0.002), and RDW (P<0.001), but a lower hemoglobin (P<0.001), RBC (P<0.001) and albumin (P<0.001). Compared with normal HRR group, the lower HRR group were more likely to have frailty (P<0.001).

Table2. Baseline characteristics of the study population stratified by HRR

Characteristics	Normal HRR (n=1046)	Lower HRR (n=250)	P-value
Age, years (SD)	70.50 ± 4.64	72.50 ± 5.40	<0.001
Male gender, n (%)	501 (47.9%)	63 (25.2%)	<0.001
Marital status			
Married, n (%)	901 (86.14%)	206 (82.4%)	0.133
Other*, n (%)	145 (13.86%)	44 (17.6%)	
Education years			
0-12, n (%)	691 (66.06%)	197 (78.8%)	<0.001
>12, n (%)	355 (33.94%)	53 (21.2%)	
Alone living, n (%)	114 (10.90%)	35 (14.0%)	0.167
Smoking, n (%)	129 (12.33%)	15 (6.0%)	0.004
Drinking, n (%)	136 (13.0%)	16 (6.4%)	0.004
Hypertension, n (%)	456 (43.59%)	126 (50.4%)	0.052
Diabetes mellitus, n (%)	140 (13.38%)	43 (17.2%)	0.120
Cardiac diseases, n (%)	82 (7.84%)	16 (6.4%)	0.439
Cranial vascular disease, n (%)	32 (3.06%)	11 (4.4%)	0.288
BMI, kg/m ² (SD)	24.56 ± 3.07	24.15 ± 3.36	0.026

1				
2				
3	Waist circumference, cm (SD)	86.90 ± 8.51	85.45 ± 9.37	0.143
4	SBP, mmHg (SD)	142.3 ± 18.3	141.3 ± 18.5	0.611
5	DBP, mmHg (SD)	83.7 ± 10.2	80.9 ± 10.1	0.004
6	Heart rate, beats/min (SD)	80.2 ± 13.4	79.0 ± 12.7	0.362
7	WBC, 10 ⁹ /L (SD)	6.39 ± 1.49	6.28 ± 1.75	0.556
8	Neutrophils, 10 ⁹ /L (SD)	3.89 ± 1.19	3.86 ± 1.43	0.441
9	Lymphocytes, 10 ⁹ /L (SD)	1.96 ± 0.59	1.86 ± 0.69	0.002
10	Eosinophils, 10 ⁹ /L (SD)	0.14 ± 0.11	0.15 ± 0.17	0.621
11	PLT, 10 ⁹ /L (SD)	212.3 ± 50.8	218.3 ± 62.8	0.264
12	RBC, 10 ⁹ /L (SD)	4.71 ± 0.38	4.27 ± 0.56	<0.001
13	Hemoglobin, g/L (SD)	145.08 ± 10.98	124.90 ± 8.46	<0.001
14	RDW, % (IQR)	12.89 ± 0.51	13.79 ± 1.09	<0.001
15	FBG, mmol/L (SD)	6.36 ± 1.77	6.24 ± 1.70	0.166
16	Albumin, g/dL (SD)	46.18 ± 2.27	45.30 ± 2.69	<0.001
17	Globulin, g/dL (SD)	30.35 ± 3.45	30.72 ± 4.58	0.708
18	Triglyceride, mmol/L (SD)	1.48 ± 0.87	1.42 ± 0.83	0.755
19	Total Cholesterol, mmol/L (SD)	5.17 ± 1.07	5.10 ± 1.27	0.041
20	HDL-C, mmol/L (SD)	1.50 ± 0.39	1.58 ± 0.42	0.539
21	LDL-C, mmol/L (SD)	3.03 ± 0.85	2.91 ± 0.87	0.019
22	Frailty status			
23	Robust, n (%)	669 (63.96%)	45 (18.0%)	<0.001
24	Pre-frail, n (%)	337 (32.22%)	140 (56.0%)	
25	Frailty, n (%)	40 (3.82%)	65 (26.0%)	

177 SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, FBG=fasting blood-glucose,
178 WBC=white blood cell, PLT=platelets count, RBC=red blood cell, RDW=red blood cell distribution width,
179 HRR=hemoglobin-to-RDW ratio.
180 Normal HRR=HRR above 9.97, Lower HRR: HRR below 9.97 (Optimal cut-off value of the ROC curve)
181 Other* including separated, divorced, never married or widowed

182

183 **Logistic regression analysis**

184 Among 1296 older adults, 105 (8.10%) were considered frail. Multiple logistic regression
185 analysis was conducted to assessing the associations of the lower HRR with frailty.
186 Unadjusted model1 showed that lower Hb (OR:2.129,1.133-4.001, p=0.019) and lower
187 HRR (OR:3.285,1.676-6.440, p=0.001) were risk factors related to frailty, whereas lower
188 RDW (OR:0.310, 0.193-0.497, p<0.001) was a protective factor. After adjustment for
189 confounding factors (including age, gender, marital status, education years, living alone,
190 BMI, diabetes, RBC, albumin, triglyceride, HDL-C and LDL-C), there was significant

191 association of lower HRR (OR:3.419, 1.679-6.964, p=0.001) and lower RDW (OR:0.285,
 192 0.170-0.477, p< 0.001) with frailty (Table 3). Lower HRR was independently related to
 193 frailty in older adults.

194
 195 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

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

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

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

201

202 **Correlation Analysis**

203 Correlation analysis indicated that there was an obvious positive correlation between
 204 RDW (Kendall's tau-b=0.173, P<0.001) and frailty. Nevertheless, HRR (Kendall's
 205 tau-b=-0.239, P<0.001) and Hb (Kendall's tau-b=-0.194, P<0.001) were a negative
 206 correlation with frailty (Table 4).

207

208 Table4. Correlation analysis of Hb, RDW, HRR, and frailty in older adults

209

	Kendall's tau-b	p

Hb	-0.194	<0.001
RDW	0.173	<0.001
HRR	-0.239	<0.001

210 **. Correlation is significant at the 0.01 level (2-tailed).

211

212 Discussion

213 Frailty, as a geriatric syndrome, has attracted more and more scientific attention in the
214 background of continuously increase global population aging.¹⁸ In this cross-sectional study
215 including 1,296 community-dwelling older adults, we found that lower HRR is independently
216 related to frailty in older people, even after adjusting confounding factors (P=0.001). Multiple
217 logistic regression analysis showed that lower HRR is associated with to a 3-fold more
218 likelihood or odds of frailty (OR = 3.419, 95%CI 1.679-6.964). ROC analysis showed that the
219 AUC for HRR in the frailty older adults was 0.802, and the highest sensitivity was 84.5% and
220 the specificity was 61.9% with the optimal critical values 9.97. The results of the present study
221 confirmed that HRR was also significantly associated with frailty in the general older people,
222 not only in patients with coronary heart disease in previous studies.

223 HRR is cost-effective, common, and accessible laboratory parameter for clinicians. As
224 a novel inflammatory factor, Qu et.al found that HRR is a significant associated with frailty in
225 older patients with CHD.¹⁴ In there study the AUC for HRR in the frailty patients was exceed
226 Hb and RDW, and after adjusting confounding factors lower HRR was a risk factor for frailty in
227 older patients with CHD. These findings are consistent with our results. Now the
228 pathophysiological mechanism has not been fully understood. We try to provide a possible
229 explanation for the association between HRR and frailty in older adults.

1
2
3
4 230 A decreased in HRR may be due to low Hb, high RDW, or both. As we all know, low
5
6
7 231 Hb indicates a condition of anemia, which is one of the acknowledged risk factors for
8
9 232 hospitalization, morbidity, and mortality in older people.¹⁹ Anemia decreases the
10
11 233 oxygen-carrying capacity, leading to tissue hypoxia and even organ failure, especially in older
12
13
14 234 patients, increasing the risk of frailty.²⁰ Besides, anemia can cut down submaximal and
15
16
17 235 maximal aerobic capacity, leading to several adverse outcomes including loss of muscle
18
19 236 strength, cognitive decline and development of frailty.¹⁰ In addition, chronic conditions and
20
21
22 237 comorbidities leading to a low grade of inflammation reducing hemoglobin level,²¹ also known
23
24
25 238 as chronic diseases anemia, which is the most common type of anemia in older adults.²⁰ And a
26
27 239 state of chronic inflammation has been suggested as contributors to frailty.²² Furthermore,
28
29
30 240 anemia caused by malnutrition is also a significance health-affecting factor among older
31
32
33 241 adults.²⁰

34
35 242 Anemia reaching a prevalence of 17% in older people, is a threat to healthy aging.²³
36
37
38 243 Corona et.al found that lower hemoglobin and anemia were related to frailty in Brazilian older
39
40 244 adults.⁸ In their study, anemia was related to low physical activity, weakness(weaker) and
41
42
43 245 walked more slowly. Another study from Spain indicated that anemia is independently
44
45
46 246 associated with frailty in older people.²⁴ Moreover, Xu et.al found that Hb is closely associated
47
48 247 with frailty in elder patients in hospital.⁹ A systematic review and meta-analysis including 19
49
50
51 248 studies indicated that older adults with anemia have more than a two-fold increased odds of
52
53
54 249 frailty.²⁵ Silva et.al suggested that lower Hb level should be considered a significant
55
56 250 component of frailty in older persons.²⁶ Similarly, another meta-analysis including 32,934
57
58
59 251 robust participants and 6864 frail participants found that Hb is a useful biomarker of frailty.⁷
60

1
2
3
4 252 However, there were no significant association of lower Hb and frailty after adjusting
5
6 253 confounding factors in our study. The discrepancy of results may be due to the definition of
7
8
9 254 lower Hb is determined by the optimal cut-off value of the ROC curve in this research.
10
11
12 255 Therefore, further studies are needed to confirm the relationship between Hb and frailty in
13
14 256 older people.

15
16
17 257 A increasing RDW also can lead to low HRR. Studies have proved that increased
18
19 258 RDW is related to inflammation. Inflammatory cytokines reduce erythropoietin gene
20
21
22 259 expression and erythropoietin receptor expression, which lead to the release of immature
23
24 260 erythrocytes and heterogeneity of the erythrocyte volume increasing.^{27,28} In addition, metabolic
25
26
27 261 abnormalities including shortened telomere length, oxidative stress, and malnutrition may also
28
29
30 262 contribute to increased RDW.^{29,30} What's more, others have suggested that RDW may be a
31
32 263 potential biomarker for biological aging.²⁸ Study has indicated that a high RDW was related to
33
34
35 264 a high sarcopenia risks.³¹ Sarcopenia, which plays a key role in frailty, is a progressive loss of
36
37 265 skeletal muscle mass and strength.²⁶

38
39
40 266 RDW is a biomarker of inflammation, oxidative stress, poor nutritional status, aging,
41
42 267 and sarcopenia, and all of these could be underlying reasons of development of frailty. A study
43
44 268 enrolled 3,635 community-dwelling older men indicated that participants with a high RDW are
45
46
47 269 more likely to have functional limitations and frailty.²⁸ Li et.al indicated that increased RDW
48
49
50 270 may be closely related to frailty through inflammation.¹³ Hou et.al proved that frailty is closely
51
52 271 associated with RDW in elder patients with CHD.¹¹ Another study including 2,932
53
54
55 272 community-dwelling older adults found RDW is independently associated with high frailty risk
56
57
58 273 even after adjusting for potential confounding factors.¹²
59
60

1
2
3
4 274 To sum up, a large number of researches have verified the association between frailty
5
6 275 and a low Hb and a high RDW among older persons. However, both RDW and Hb are
7
8
9 276 susceptible to many other diseases conditions and sub-health states, HRR may provide a
10
11 277 more powerful parameter than a single parameter alone. Moreover, ROC analysis showed that
12
13
14 278 the AUC and highest sensitivity of HRR are higher than RDW and Hb. It may be a more
15
16
17 279 reliable and effective marker than Hb and RDW alone.

18
19 280 This inexpensive and common laboratory parameter may provide useful information to
20
21
22 281 identify the risk of frailty in older adults. Furthermore, use of the HRR may help clinicians to
23
24 282 identify people at high risk of frailty and take effective measures to reduce the occurrence and
25
26
27 283 development of frailty, reduce the rate of disability and mortality related to frailty in the elderly,
28
29
30 284 and reduce the waste of medical resources, and promote healthy ageing.

31
32 285 There were also some limitations of our present study. Firstly, because cross-sectional
33
34
35 286 studies measure the outcome and the exposures in the study participants at the same time, it
36
37
38 287 is difficult to assess the cause-effect relationship. Secondly, our participants are limited to local
39
40
41 288 participants, these findings need to be validated in different populations around the world.
42
43 289 What is more, we unable to investigate the temporal relation between outcomes and risk
44
45
46 290 factors. In addition, despite the inevitable selection bias and information bias in cross-sectional
47
48
49 291 studies, we improved this problem through more rational statistical methods and interviewer
50
51
52 292 training. Finally, we did not assess iron, folic, and vitamin B12, which may affect RDW and Hb
53
54
55 293 level.

56 294

58 295 **Conclusion**

1
2
3
4 296 In conclusion, a low HRR is independent associated with higher frailty risk in
5
6 297 community-dwelling older adults. And this relationship is not affected by confounding factors.
7
8
9 298 However, the causal relationship and the specific mechanism between the HRR and frailty is
10
11 299 unclear. Evidence is needed from prospective studies to verify this conclusions in the future.
12
13

14 300

15 301

16 302 **Author contributions**

17 303 Conceptualization: Zhifan Xiong, Mengpei Zhu

18 304 Data curation: Mengpei Zhu, Chao Wei, Yushuang Xu

19 305 Formal analysis: Mengpei Zhu, Chao Wei

20 306 Visualization: Xiongjun Yang, Yumei Huang

21 307 Writing – original draft: Mengpei Zhu

22 308 Writing – review and editing: Zhifan Xiong, Mengpei Zhu, Chao Wei, Xiongjun Yang, Yumei

23 309 Huang, Yushuang Xu. All authors read and approved the final manuscript.

24 310

25 311 **Acknowledgements**

26 312 The authors thank all the volunteers that participated in this study.

27 313

28 314 **Funding**

29 315 This study was supported by the National Key Research and Development Program of China

30 316 (2018YFC2002000).

31 317

1
2
3
4 318 **Data availability**
5

6 319 Data for this study are available from the corresponding author.
7
8

9 320

10
11 321 **Declaration**
12

13
14 322 The authors report no conflicts of interest in this work.
15
16

17 323

18
19 324 **Ethics statements**
20

21
22 325 **Patient consent for publication**
23

24 326 Not applicable.
25
26

27 327 **Ethical approval**
28

29
30 328 The research protocol was approved by the Medical Ethics Committee of Liyuan Hospital,
31

32 329 Tongji Medical College, Huazhong University of Science and Technology (IRB ID: [2020] IEC
33

34 330 (A016)). All the participants gave written informed consent. The study was conducted in
35
36

37 331 accordance with the tenets of the Declaration of Helsinki.
38
39

40 332

41 333

42 334

43 335
44
45
46
47
48
49

50
51 336 **References**
52

53 337 1. Kameda M, Teruya T, Yanagida M, Kondoh H. Frailty markers comprise blood metabolites
54 338 involved in antioxidation, cognition, and mobility. *Proc Natl Acad Sci U S A*.
55 339 2020;117(17):9483-9489.

56 340 2. Travers J, Romero-Ortuno R, Bailey J, Cooney M-T. Delaying and reversing frailty: a
57 341 systematic review of primary care interventions. *Br J Gen Pract*. 2019;69(678):e61-e69.

58 342 3. Dent E, Martin FC, Bergman H, Woo J, Romero-Ortuno R, Walston JD. Management of frailty:
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- opportunities, challenges, and future directions. *Lancet*. 2019;394(10206):1376-1386.
- 344 4. Coelho-Junior HJ, Marzetti E, Picca A, Cesari M, Uchida MC, Calvani R. Protein Intake and
345 Frailty: A Matter of Quantity, Quality, and Timing. *Nutrients*. 2020;12(10).
- 346 5. Ward RE, Orkaby AR, Chen J, et al. Association between Diet Quality and Frailty Prevalence in
347 the Physicians' Health Study. *J Am Geriatr Soc*. 2020;68(4):770-776.
- 348 6. Zhai Z, Gao J, Zhu Z, et al. The Ratio of the Hemoglobin to Red Cell Distribution Width
349 Combined with the Ratio of Platelets to Lymphocytes Can Predict the Survival of Patients
350 with Gastric Cancer Liver Metastasis. *Biomed Res Int*. 2021;2021:8729869.
- 351 7. Mailliez A, Guilbaud A, Puisieux F, Dauchet L, Boulanger É. Circulating biomarkers
352 characterizing physical frailty: CRP, hemoglobin, albumin, 25OHD and free testosterone as
353 best biomarkers. Results of a meta-analysis. *Exp Gerontol*. 2020;139:111014.
- 354 8. Pires Corona L, Drumond Andrade FC, de Oliveira Duarte YA, Lebrao ML. The Relationship
355 between Anemia, Hemoglobin Concentration and Frailty in Brazilian Older Adults. *J Nutr
356 Health Aging*. 2015;19(9):935-940.
- 357 9. Xu L, Zhang J, Shen S, et al. Clinical Frailty Scale and Biomarkers for Assessing Frailty in Elder
358 Inpatients in China. *J Nutr Health Aging*. 2021;25(1):77-83.
- 359 10. Ruan Y, Guo Y, Kowal P, et al. Association between anemia and frailty in 13,175
360 community-dwelling adults aged 50 years and older in China. *BMC Geriatr*. 2019;19(1):327.
- 361 11. Hou P, Xue H-P, Mao X-E, Li Y-N, Wu L-F, Liu Y-B. Inflammation markers are associated with
362 frailty in elderly patients with coronary heart disease. *Aging (Albany NY)*.
363 2018;10(10):2636-2645.
- 364 12. Li C-M, Chao C-T, Chen S-I, Han D-S, Huang K-C. Elevated Red Cell Distribution Width Is
365 Independently Associated With a Higher Frailty Risk Among 2,932 Community-Dwelling Older
366 Adults. *Front Med (Lausanne)*. 2020;7:470.
- 367 13. Li Q, Chen X, Han B. Red blood cell distribution width is associated with frailty in older
368 inpatients in China: Sex differences in a cross-sectional study. *Exp Gerontol*.
369 2021;150:111392.
- 370 14. Qu J, Zhou T, Xue M, et al. Correlation Analysis of Hemoglobin-to-Red Blood Cell Distribution
371 Width Ratio and Frailty in Elderly Patients With Coronary Heart Disease. *Front Cardiovasc
372 Med*. 2021;8:728800.
- 373 15. Su Y-C, Wen S-C, Li C-C, et al. Low Hemoglobin-to-Red Cell Distribution Width Ratio Is
374 Associated with Disease Progression and Poor Prognosis in Upper Tract Urothelial Carcinoma.
375 *Biomedicines*. 2021;9(6).
- 376 16. Sun P, Zhang F, Chen C, et al. The ratio of hemoglobin to red cell distribution width as a novel
377 prognostic parameter in esophageal squamous cell carcinoma: a retrospective study from
378 southern China. *Oncotarget*. 2016;7(27):42650-42660.
- 379 17. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. *J
380 Gerontol A Biol Sci Med Sci*. 2001;56(3):M146-M156.
- 381 18. Kojima G, Taniguchi Y, Iliffe S, Jivraj S, Walters K. Transitions between frailty states among
382 community-dwelling older people: A systematic review and meta-analysis. *Ageing Res Rev*.
383 2019;50:81-88.
- 384 19. Lee CT, Chen MZ, Yip CYC, Yap ES, Lee SY, Merchant RA. Prevalence of Anemia and Its
385 Association with Frailty, Physical Function and Cognition in Community-Dwelling Older
386 Adults: Findings from the HOPE Study. *J Nutr Health Aging*. 2021;25(5):679-687.

- 1
2
3 387 20. Röhrig G. Anemia in the frail, elderly patient. *Clin Interv Aging*. 2016;11:319-326.
4 388 21. Steinmeyer Z, Delpierre C, Soriano G, et al. Hemoglobin concentration; a pathway to frailty.
5 389 *BMC Geriatr*. 2020;20(1):202.
6 390 22. Wilson D, Jackson T, Sapey E, Lord JM. Frailty and sarcopenia: The potential role of an aged
7 391 immune system. *Ageing Res Rev*. 2017;36.
8 392 23. Stauder R, Valent P, Theurl I. Anemia at older age: etiologies, clinical implications, and
9 393 management. *Blood*. 2018;131(5):505-514.
10 394 24. Esquinas-Requena JL, García-Nogueras I, Hernández-Zegarra P, Atienzar-Núñez P, Sánchez-Jurado PM, Abizanda P. [Anemia and frailty in older adults from Spain. The FRADEA Study]. *Rev Esp Geriatr Gerontol*. 2021;56(3):129-135.
11 395
12 396
13 397 25. Palmer K, Vetrano DL, Marengoni A, et al. The Relationship between Anaemia and Frailty: A
14 398 Systematic Review and Meta-Analysis of Observational Studies. *J Nutr Health Aging*.
15 399 2018;22(8):965-974.
16 400 26. Silva JC, Moraes ZVd, Silva C, et al. Understanding red blood cell parameters in the context of
17 401 the frailty phenotype: interpretations of the FIBRA (Frailty in Brazilian Seniors) study. *Arch*
18 402 *Gerontol Geriatr*. 2014;59(3):636-641.
19 403 27. Pierce CN, Larson DF. Inflammatory cytokine inhibition of erythropoiesis in patients
20 404 implanted with a mechanical circulatory assist device. *Perfusion*. 2005;20(2):83-90.
21 405 28. Kim KM, Lui L-Y, Browner WS, et al. Association Between Variation in Red Cell Size and
22 406 Multiple Aging-Related Outcomes. *J Gerontol A Biol Sci Med Sci*. 2021;76(7):1288-1294.
23 407 29. Ghaffari S. Oxidative stress in the regulation of normal and neoplastic hematopoiesis.
24 408 *Antioxid Redox Signal*. 2008;10(11):1923-1940.
25 409 30. Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: A simple
26 410 parameter with multiple clinical applications. *Crit Rev Clin Lab Sci*. 2015;52(2).
27 411 31. Kim J, Im J-S, Choi CH, et al. The Association between Red Blood Cell Distribution Width and
28 412 Sarcopenia in U.S. Adults. *Sci Rep*. 2018;8(1):11484.
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52

419 Figure legends

420 **Figure1** ROC curve for HRR (A), Hb (B) and RDW (C)

421

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

422 **Notes:**

423 A. ROC curve indicated that the best intercept value for HRR was 9.97 (sensitivity 84.5%,
424 specificity 61.9%, AUC = 0.802, P<0.001)

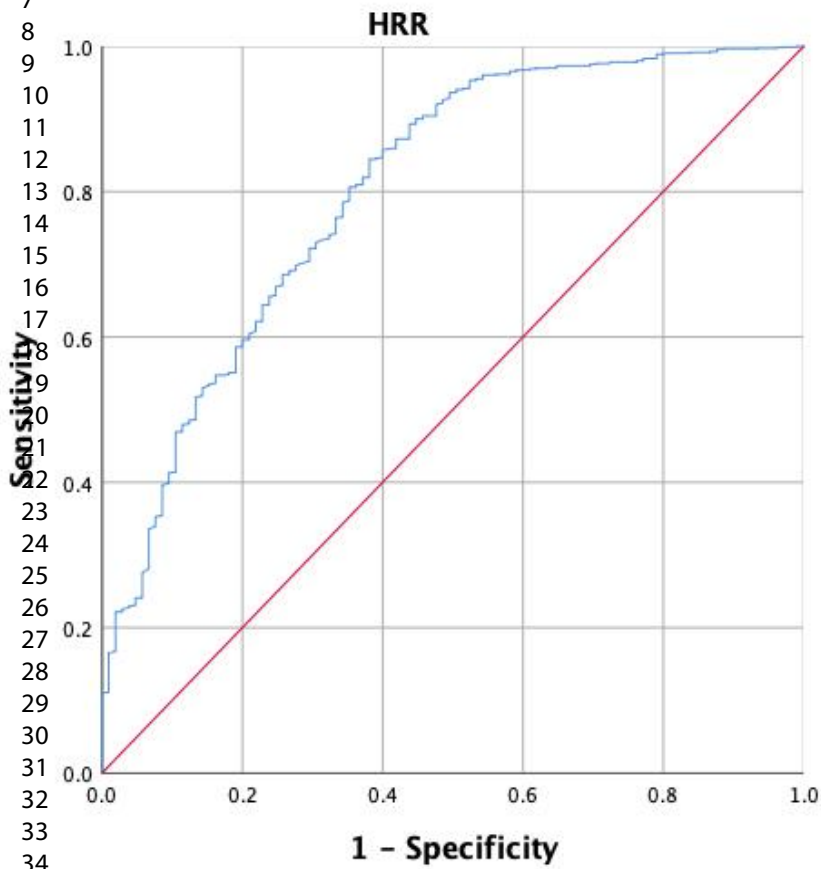
425 B. ROC curve indicated that the best intercept value for Hb was 131.5 (sensitivity 81% ,
426 specificity 57.1%, AUC = 0.742, P<0.001)

427 C. ROC curve indicated that the best intercept value for RDW was 13.45 (sensitivity 56.2% ,
428 specificity 81.1%, AUC = 0.712, P<0.001)

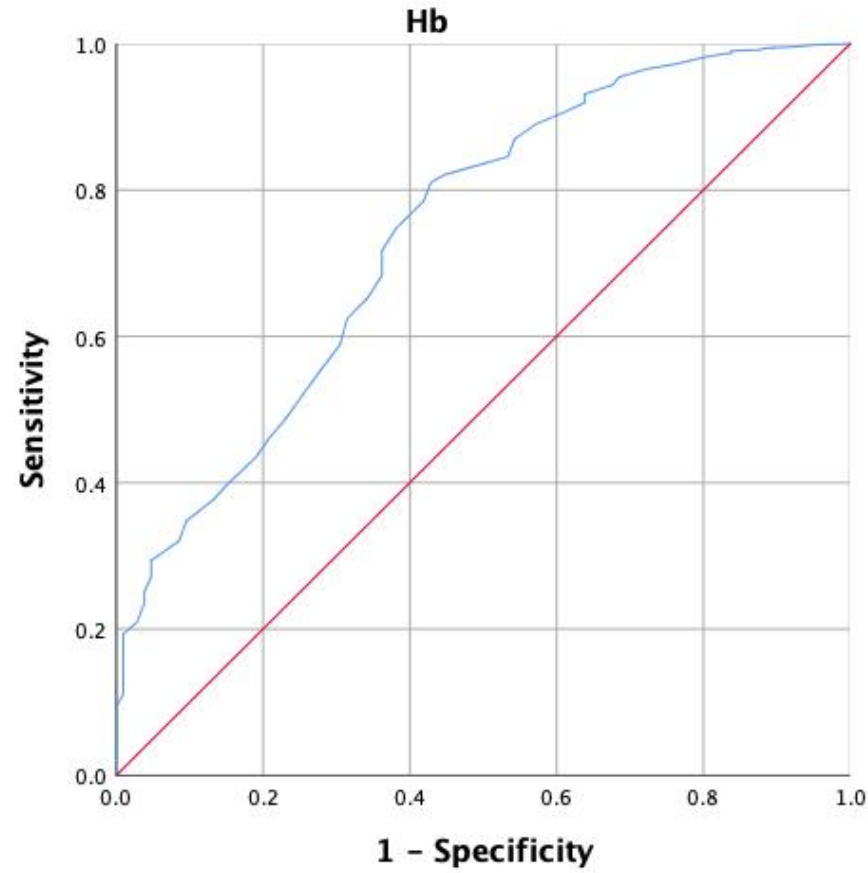
For peer review only

Figure 1.

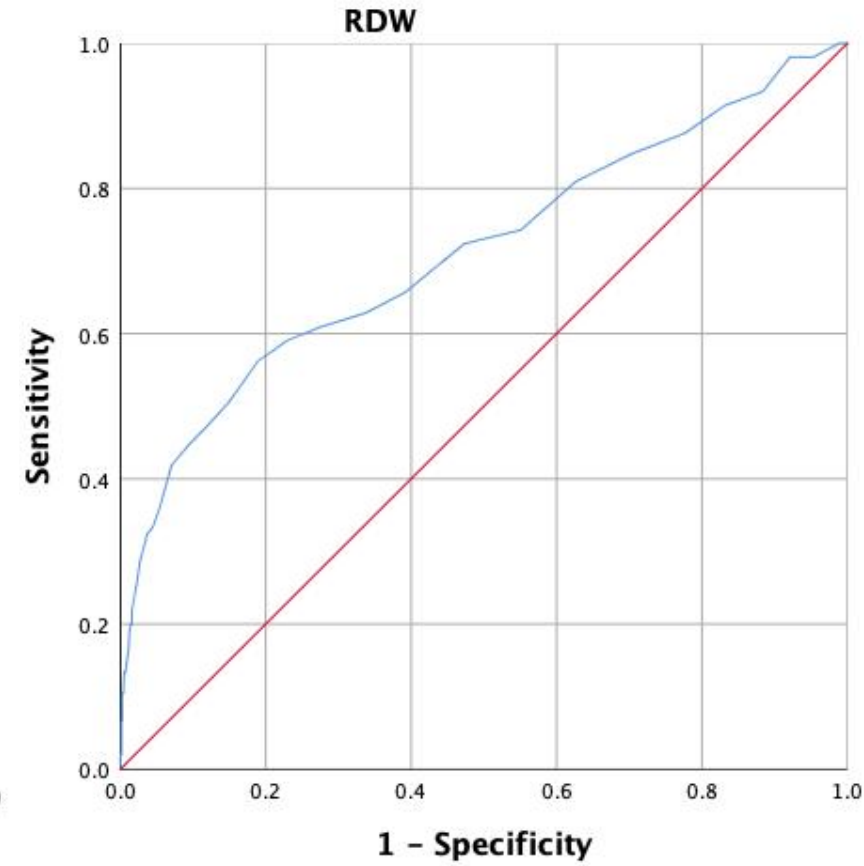
A



B



C



STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cross-sectional studies

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	5

Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7
		(b) Describe any methods used to examine subgroups and interactions	7
		(c) Explain how missing data were addressed	7
		(d) If applicable, describe analytical methods taking account of sampling strategy	7
		(e) Describe any sensitivity analyses	7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7-8
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	8
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-11
		(b) Report category boundaries when continuous variables were categorized	9-11

		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Lower Hemoglobin-to-Red Blood Cell Distribution Width Ratio Is Independently Associated With Frailty in Community-dwelling Older Adults: a cross-sectional study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-069141.R2
Article Type:	Original research
Date Submitted by the Author:	29-May-2023
Complete List of Authors:	Zhu, Mengpei; Institute of Geriatric Medicine, Liyuan Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wei, Chao; Wuhan Geriatric Hospital Yang, Xiongjun; Huazhong University of Science and Technology Huang, Yumei; Huazhong University of Science and Technology Xu, Yushuang; Liyuan Hospital of Tongji Medical College of Huazhong University of Science and Technology Xiong, Zhifan; Huazhong University of Science and Technology; Huazhong University of Science and Technology
Primary Subject Heading:	Geriatric medicine
Secondary Subject Heading:	Global health
Keywords:	GERIATRIC MEDICINE, EPIDEMIOLOGY, GENERAL MEDICINE (see Internal Medicine)

SCHOLARONE™
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1
2
3
4
5 1 **Lower Hemoglobin-to-Red Blood Cell Distribution**
6
7
8 2 **Width Ratio Is Independently Associated With Frailty**
9
10 3 **in Community-dwelling Older Adults: a cross-sectional**
11
12
13 4 **study**
14
15
16 5

17
18 6 Authors Name : Mengpei Zhu^{1,2}, Chao Wei³, Xiongjun Yang², Yumei Huang², Yushuang Xu^{1,2},
19
20
21 7 Zhifan Xiong^{1,2}

22
23 8 Affiliations: 1 Institute of Geriatric Medicine, Liyuan Hospital, Tongji Medical College,
24
25
26 9 Huazhong University of Science and Technology, Wuhan, China.

27
28 10 2 Division of Gastroenterology, Liyuan Hospital, Tongji Medical College, Huazhong University
29
30
31 11 of Science and Technology, Wuhan, China.

32
33 12 3 Physical Examination Center, East Lake Hospital & Wuhan Geriatric Hospital, Wuhan,
34
35
36 13 China.

37
38
39 14
40
41 15 Correspondence: Zhifan Xiong

42
43
44 16 Address: Division of Gastroenterology, Institute of Geriatric Medicine, Liyuan Hospital, Tongji
45
46
47 17 Medical College, Huazhong University of Science and Technology, 39 Lake Road, East Lake
48
49
50 18 Ecological Scenic, Wuhan 430077, Hubei, China

51
52 19 Email: xiongzhiban@126.com
53
54
55 20
56
57 21
58
59
60 22

1
2
3
4 23 **Abstract**

5
6 24 **Objectives:** The importance of blood cell markers in frailty has been studied. However,
7
8 25 research on hemoglobin-to-red blood cell distribution width ratio (HRR) and frailty in older
9
10 26 persons is still limited. We investigated the association between HRR and frailty in older
11
12 27 adults.

13
14 28 **Design:** Cross-sectional population-based study.

15
16
17 29 **Setting:** Community-dwelling older adults older than 65 years old were recruited from
18
19 30 September 2021 to December 2021.

20
21
22 31 **Participants:** A total of 1296 community-dwelling older adults (age \geq 65 years) in Wuhan
23
24 32 were included in the study.

25
26
27 33 **Main outcome measures:** The main outcome was the presence of frailty. The Fried Frailty
28
29 34 Phenotype Scale was used to evaluate the frailty status of the participants. Multivariable
30
31 35 logistic regression analysis was performed to determine the relationship between HRR and
32
33 36 frailty.

34
35
36
37 37 **Results:** A total of 1296 (564 men) older adults were included in this cross-sectional study.
38
39 38 Their mean age was 70.89 ± 4.85 years. ROC analysis showed that HRR is a good predictor
40
41 39 of frailty in older people, the area under the curve (AUC) was 0.802 (95% CI: 0.755 to 0.849),
42
43 40 and the highest sensitivity was 84.5% and the specificity was 61.9% with the optimal critical
44
45 41 values 9.97 ($P < 0.001$). Multiple logistic regression analysis indicated that lower HRR (< 9.97)
46
47 42 (OR:3.419, 1.679-6.964, $P = 0.001$) is independently associated with frailty in older people,
48
49 43 even after adjusting confounding factors.

50
51 44 **Conclusion:** Lower HRR is closely associated with an increased risk of frailty in older people.
52
53 45 Lower HRR may be an independent risk factor for frailty in community-dwelling older adults.
54
55
56
57
58
59
60

1
2
3
4 46 **Keywords:** Older people, Frailty, Healthy aging, Risk factor, HRR
5
6
7 47

8
9 48 **Strengths and limitations of this study**
10

11 49 Frailty was diagnosed by following Fried's frailty phenotype.

12
13 50 This cross-sectional analysis was performed in a medium-volume population.
14

15
16 51 Some variables were self-reported, but the best available measures were used.
17

18
19 52 The study reflects the situation of older adults in Wuhan, China, and the generalizability needs
20
21 53 to be further verified.
22

23
24 54 This was a cross-sectional study that cannot assess the cause-effect relationship.
25
26
27 55

28
29
30 56 **Introduction**
31

32 57 As life expectancy increases, human societies are aging globally, in both developed and
33
34 58 developing countries.¹ By 2050, the proportion of people aged over 60 years is projected to
35
36 59 increase from 11% to 22%, and the number of people aged over 60 years will increase from
37
38 60 605 million to 2.1 billion, including 425 million people aged over 80 in the world.² Frailty
39
40 61 becomes an emerging global public health burden, with the rapid growth of the global aging
41
42 62 population. Frailty is considered to be a complex age-related clinical condition characterized
43
44 63 by a decline in the physiological function of multiple organs, with a resultant increased
45
46 64 vulnerability to stressors.³ It is related to adverse health-related events, including increased
47
48 65 mortality, hospitalization, falls and fractures, cognitive decline, disability, and admission to
49
50 66 long-term care.⁴ Therefore, early identifying modifiable risk factors of frailty is becoming
51
52 67 increasingly crucial for delaying and reversing frailty and its associated adverse events in older
53
54
55
56
57
58
59
60

1
2
3
4 68 persons.⁵
5

6 69 As an important part of CBC, hemoglobin (Hb) is usually used as an indicator of the
7
8
9 70 degree of anemia. However, previous studies showed that low Hb reflects to a decline in
10
11
12 71 physiological function including decreased immune response, malnutrition, and low resistance
13
14
15 72 to external invasion.⁶ Meanwhile, there are several studies indicating that Hb is related to
16
17 73 frailty in older persons.⁷⁻¹⁰ Red blood cell distribution width (RDW) is a simple parameter of
18
19
20 74 CBC, which reflects the degree of heterogeneity of the erythrocyte volume, and is traditionally
21
22 75 used for the differential diagnosis of anemia.¹¹ However, with the deepening of the study, it
23
24
25 76 was found to be related to the prognosis of many diseases. Increased RDW reflects
26
27 77 dysregulation of erythrocyte homeostasis, which may be attributed to various underlying
28
29
30 78 metabolic abnormalities such as shortened telomere length, oxidative stress, inflammation,
31
32
33 79 malnutrition, dyslipidemia, hypertension, erythrocyte fragmentation, and altered erythropoietin
34
35 80 function.¹¹
36

37
38 81 Inflammation has been identified as a potential cause of frailty.¹² Inflammation in
39
40
41 82 response to elevated RDW may be highly correlated with frailty. Hou et al. indicated that RDW
42
43
44 83 is significantly associated with the risk of frailty in older patients with coronary heart disease
45
46
47 84 (CHD).¹³ In addition, studies showed that increased RDW is associated with frailty both in
48
49
50 85 older inpatients and community-dwelling older people.^{14,15} However, it is still controversial
51
52
53 86 whether RDW alone can predict frailty.^{16,17} The hemoglobin-to-RDW ratio (HRR) is a cheap,
54
55
56 87 rapid, and readily available novel prognostic, which combines the prognostic information of Hb
57
58
59 88 and RDW and reflects a more comprehensive health status.^{16,18} Recently, Qu et al. found that
60
61
62 89 lower HRR is independently related to the risk of frailty in older patients with CHD.¹⁶ They

1
2
3
4 90 verified that HRR may be a more useful biomarker compared with RDW or Hb alone.¹⁶
5
6

7 91 Studies have shown a significant association of HRR with frailty in specific populations
8
9 92 (patients with coronary heart disease).¹⁶ However, research on HRR and frailty in general
10
11 93 older persons is still limited, and the significance of evaluating frailty is not yet clear. In the
12
13 94 present study, we investigated the relationship between HRR and frailty in community-dwelling
14
15 95 older adults.
16
17
18
19
20
21

22 97 **Material & Methods**

23 98 ***Patient and public involvement***

24
25
26
27 99 The source population was the community-dwelling adults older than 65 living in communities
28
29
30 100 in Wuhan. The study population consisted of a random sample of older people from each
31
32 101 community. Inclusion criteria were the community-dwelling adults older than 65 living in
33
34 102 communities in Wuhan. Exclusion criteria were malignant disease or advanced organic
35
36 103 diseases, hematologic diseases, acute stage of disease, and participants with missing the key
37
38 104 parameters.
39
40
41
42

43 105 ***Participants and Sociodemographic Characteristics***

44
45 106 In this present study, we recruited 1,296 community-dwelling adults older than 65 living in
46
47
48 107 communities in Wuhan between September 2021 and December 2021. Sociodemographic
49
50 108 characteristics, including age, gender, education years, marital status, smoking history,
51
52 109 alcohol consumption, and comorbidities, including hypertension, diabetes, CHD,
53
54 110 hyperlipidemia, and cerebrovascular disease were recorded. The body mass index (BMI),
55
56 111 waistline, blood pressure, and pulse rate were measured by two professional clinicians.
57
58
59
60

112 ***Peripheral Blood Parameters***

113 Blood samples were collected, and full blood count was measured by an automated
114 hematology analyzer (Mindray, BC7500, China). Other related biochemical indicators were
115 detected by an automatic biochemical analyzer (Beckman, AU680, American). HRR = Hb
116 (g/L)/RDW (%).

117 ***Fried's frailty phenotype***

118 According to Fried's frailty phenotype, we evaluated frailty in five criteria, as follows (1) Weight
119 loss: unintentional weight loss >5% or a loss of more than 4.5 kg in the past 1 year. (2)
120 Physical weakness: a dynamometer was used for participants for three trials, and the
121 maximum value was recorded. Low grip strength was defined according to the standards
122 proposed by Fried et al.¹⁹ (3) Slowness: slowness was defined as when the time required to
123 walk 4.6 meters was more than 7 seconds for males (height ≤ 173 cm) and females (height ≤
124 159 cm) or more than 6 seconds for males (height >173 cm) and females (height >159 cm). (4)
125 Physical activity: low physical activity was defined as less than 383 kcal/week for males and
126 less than 270 kcal/week for females. (5) Exhaustion: exhaustion was assessed by the
127 following two questions from the CES-D. "In the last week, I felt that everything I did was an
128 effort" and "Could not get going in the last week." If the participant responded "yes" to either of
129 these questions, the participant was considered exhausted. Participants with >3 indicators
130 were defined as frail, 1-2 as prefrail, and none as robust.

131 ***Patient and public involvement statement***

132 Patients and/or the public were not involved in the design, conduct, reporting, or dissemination
133 of this research.

134 ***Statistical analysis***

135 Continuous and categorical variables were expressed as the mean standard deviation and
136 numbers with percentages, respectively. The baseline characteristics of the groups were
137 compared using a one-way analysis of variance and chi-squared test. The predictive value of
138 HRR for frailty was assessed by receiver operating curve (ROC) analysis. The prognostic
139 value of lower HRR on frailty was assessed using the logistic regression model. Variables
140 were selected as candidates for the multivariate analysis when $p < 0.1$ in the univariate
141 analysis. After adjustment for confounding factors including age, gender, marital status,
142 education years, alone living, BMI, diabetes, RBC, albumin, triglyceride, HDL-C, and LDL-C,
143 assessing the independent risk factors for frailty in older adults. Kendall's tau-b correlation
144 analysis was used to assessing the correlation between lower Hb, lower RDW, lower HRR,
145 and frailty in older adults. The p -values < 0.05 was considered statistically significant. All
146 statistical analyses were conducted using SPSS (ver. 26.0, IBM Corporation, Armonk, NY,
147 USA).

148

149 **Result**

150 ***Characteristics of the study population***

151 A total of 1296 (564 men) older adults were included in our study. Their mean age was $70.89 \pm$
152 4.85 years. Of the 1296 participants, 582 (44.9%) has hypertension, 183 (14.1%) were
153 diabetes patients, 98 (7.6%) had cardiac diseases, and 43 (3.3%) had cranial vascular
154 disease. The proportions of individuals with a habit of smoking and drinking were 11.1% and
155 11.7%, respectively. And 149 (11.5%) older adults were living alone. According to Fried's

1
2
3
4 156 frailty phenotype, there were 55.09% (714) in the robust group, 36.81% (477) in the pre-frail
5
6
7 157 group, and 8.10% (105) in the frail group. The baseline characteristics of the three groups
8
9 158 were shown in Table 1.

10
11 159 Table1. Baseline characteristics of the study population stratified by frailty

Characteristics	Robust (n=714)	Pre-frail (n=477)	Frailty (n=105)	p-value
Age, years (SD)	69.81 ± 3.89	71.71 ± 5.34	70.89 ± 4.85	<0.001
Male gender, n (%)	347 (48.60%)	184 (38.57%)	33 (31.43%)	<0.001
Marital status Married, n (%)	622 (87.11%)	408 (85.53)	77 (73.33%)	0.001
Other*, n (%)	92 (12.89%)	69 (14.47%)	28 (26.67%)	
Education years 0-12, n (%)	466(65.27%)	337(70.65%)	85(80.95%)	0.002
>12, n (%)	248(24.73%)	140(29.35%)	20(19.05%)	
Alone living, n (%)	74 (10.36%)	53 (11.11%)	22 (20.95%)	0.006
Smoking, n (%)	90(12.61%)	44(9.22%)	10(9.52%)	0.165
Drinking, n (%)	92(12.89%)	50(10.48%)	10(9.52%)	0.345
Hypertension, n (%)	314(43.98%)	212(44.44%)	56(53.33%)	0.192
Diabetes mellitus, n (%)	81(11.34%)	82(17.19%)	20(19.05%)	0.006
Cardiac diseases, n (%)	46(6.44%)	41(8.60%)	11(10.48%)	0.194
Cranial vascular disease, n (%)	24(2.94%)	15(3.14%)	4(3.80%)	0.938
BMI, kg/m ² (SD)	24.69 ± 3.05	24.32 ± 3.14	23.83 ± 3.51	0.011
Waist circumference, cm (SD)	86.99 ± 8.66	86.32 ± 8.57	85.42 ± 9.46	0.143
SBP, mmHg (SD)	141.61 ± 18.53	142.73 ± 17.74	143.01 ± 19.37	0.516
DBP, mmHg (SD)	83.77 ± 10.44	82.87 ± 9.75	80.44 ± 10.91	0.006
Heart rate, beats/min (SD)	79.9 ± 13.4	79.8 ± 13.2	81.1 ± 13.0	0.659
WBC, 10 ⁹ /L (SD)	6.39 ± 1.51	6.36 ± 1.56	6.29 ± 1.69	0.781
Neutrophils, 10 ⁹ /L (SD)	3.89 ± 1.22	3.86 ± 1.26	3.96 ± 1.29	0.699
Lymphocytes, 10 ⁹ /L (SD)	1.95 ± 0.58	1.96 ± 0.64	1.76 ± 0.59	0.007
Eosinophils, 10 ⁹ /L (SD)	0.14 ± 0.12	0.14 ± 0.12	0.15 ± 0.18	0.628
PLT, 10 ⁹ /L (SD)	212.4 ± 52.2	216.3 ± 52.0	207.9 ± 66.1	0.251
RBC, 10 ⁹ /L (SD)	4.73 ± 0.41	4.51 ± 0.42	4.41 ± 0.65	<0.001
Hemoglobin, g/L (SD)	145.51 ± 12.15	137.29 ± 11.79	129.55 ± 13.73	<0.001
Anemia, n (%)	6 (0.84%)	17 (3.56%)	21 (20%)	<0.001
RDW, % (SD)	12.89 ± 0.55	13.15 ± 0.69	13.88 ± 1.39	<0.001
HRR	11.31 ± 1.02	10.47 ± 1.05	9.43 ± 1.41	<0.001
FBG, mmol/L (SD)	6.28 ± 1.59	6.37 ± 1.90	6.56 ± 2.13	0.278
Albumin, g/dL (SD)	46.23 ± 2.19	45.86 ± 2.47	45.13 ± 2.90	<0.001
Globulin, g/dL (SD)	30.26 ± 3.44	30.64 ± 3.65	30.55 ± 5.25	0.194
Triglyceride, mmol/L (SD)	1.49 ± 0.85	1.46 ± 0.90	1.44 ± 0.85	0.883
Total Cholesterol, mmol/L (SD)	5.17 ± 1.04	5.19 ± 1.15	4.96 ± 1.05	0.122
HDL-C, mmol/L (SD)	1.50 ± 0.39	1.54 ± 0.42	1.54 ± 0.38	0.333
LDL-C, mmol/L (SD)	3.03 ± 0.82	3.02 ± 0.90	2.82 ± 0.88	0.060

1
2
3 160 SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, FBG=fasting blood-glucose,
4 161 WBC=white blood cell, PLT=platelets count, RBC=red blood cell, RDW=red blood cell distribution width,
5 162 HRR=hemoglobin-to-RDW ratio.
6
7 163 Other* including separated, divorced, never married or widowed
8

9 164 ***ROC curve analysis***

10
11 165 The predictive value of HRR for frailty in older adults was assessed by ROC analysis. The
12
13 166 AUC for HRR in the frailty older adults was 0.802 (95% CI: 0.755 to 0.849), the highest
14
15 167 sensitivity was 84.5% and the specificity was 61.9% with the optimal critical value of 9.97
16
17 168 (Figure 1). The AUC for Hb was 0.742 (95% CI: 0.691 to 0.793), the highest sensitivity was
18
19 169 81% and the specificity was 57.1% with the optimal critical value of 131.5 (Figure 1). The AUC
20
21 170 for RDW was 0.712 (95% CI: 0.651 to 0.772), the highest sensitivity was 56.2% and the
22
23 171 specificity was 81.1% with the optimal critical values 13.45 (Figure 1). Compared with Hb and
24
25 172 RDW alone, HRR was a more strong prognostic biomarker for frailty.
26
27
28
29
30

31 173 ***Differences in clinical characteristics of the study population*** 32 33 174 ***stratified by HRR*** 34 35 36

37 175 According to ROC analysis, the optimal critical value of HRR was 9.97. Participants were
38
39 176 grouped according to the optimal critical values of HRR, as follows: 1046 (80.71%) in the
40
41 177 normal HRR group and 250(19.29%) in the lower HRR group (Table 2). Compared with the
42
43 178 normal HRR group, the lower HRR group had a higher lymphocytes count (P=0.002), and
44
45 179 RDW (P<0.001), but lower hemoglobin (P<0.001), RBC (P<0.001), and albumin (P<0.001).
46
47 180 Compared with the normal HRR group, the lower HRR group was more likely to have frailty
48
49 181 (P<0.001).
50
51
52
53
54
55

56 182

57
58 183
59
60

184

185

186 Table2. Baseline characteristics of the study population stratified by HRR

Characteristics	Normal HRR (n=1046)	Lower HRR (n=250)	P-value
Age, years (SD)	70.50 ± 4.64	72.50 ± 5.40	<0.001
Male gender, n (%)	501 (47.9%)	63 (25.2%)	<0.001
Marital status Married, n (%)	901 (86.14%)	206 (82.4%)	0.133
Other*, n (%)	145 (13.86%)	44 (17.6%)	
Education years 0-12, n (%)	691 (66.06%)	197 (78.8%)	<0.001
>12, n (%)	355 (33.94%)	53 (21.2%)	
Alone living, n (%)	114 (10.90%)	35 (14.0%)	0.167
Smoking, n (%)	129 (12.33%)	15 (6.0%)	0.004
Drinking, n (%)	136 (13.0%)	16 (6.4%)	0.004
Hypertension, n (%)	456 (43.59%)	126 (50.4%)	0.052
Diabetes mellitus, n (%)	140 (13.38%)	43 (17.2%)	0.120
Cardiac diseases, n (%)	82 (7.84%)	16 (6.4%)	0.439
Cranial vascular disease, n (%)	32 (3.06%)	11 (4.4%)	0.288
BMI, kg/m ² (SD)	24.56 ± 3.07	24.15 ± 3.36	0.026
Waist circumference, cm (SD)	86.90 ± 8.51	85.45 ± 9.37	0.143
SBP, mmHg (SD)	142.3 ± 18.3	141.3 ± 18.5	0.611
DBP, mmHg (SD)	83.7 ± 10.2	80.9 ± 10.1	0.004
Heart rate, beats/min (SD)	80.2 ± 13.4	79.0 ± 12.7	0.362
WBC, 10 ⁹ /L (SD)	6.39 ± 1.49	6.28 ± 1.75	0.556
Neutrophils, 10 ⁹ /L (SD)	3.89 ± 1.19	3.86 ± 1.43	0.441
Lymphocytes, 10 ⁹ /L (SD)	1.96 ± 0.59	1.86 ± 0.69	0.002
Eosinophils, 10 ⁹ /L (SD)	0.14 ± 0.11	0.15 ± 0.17	0.621
PLT, 10 ⁹ /L (SD)	212.3 ± 50.8	218.3 ± 62.8	0.264
RBC, 10 ⁹ /L (SD)	4.71 ± 0.38	4.27 ± 0.56	<0.001
Hemoglobin, g/L (SD)	145.08 ± 10.98	124.90 ± 8.46	<0.001
RDW, % (IQR)	12.89 ± 0.51	13.79 ± 1.09	<0.001
FBG, mmol/L (SD)	6.36 ± 1.77	6.24 ± 1.70	0.166
Albumin, g/dL (SD)	46.18 ± 2.27	45.30 ± 2.69	<0.001
Globulin, g/dL (SD)	30.35 ± 3.45	30.72 ± 4.58	0.708
Triglyceride, mmol/L (SD)	1.48 ± 0.87	1.42 ± 0.83	0.755
Total Cholesterol, mmol/L (SD)	5.17 ± 1.07	5.10 ± 1.27	0.041
HDL-C, mmol/L (SD)	1.50 ± 0.39	1.58 ± 0.42	0.539
LDL-C, mmol/L (SD)	3.03 ± 0.85	2.91 ± 0.87	0.019
Frailty status Robust, n (%)	669 (63.96%)	45 (18.0%)	<0.001
Pre-frail, n (%)	337 (32.22%)	140 (56.0%)	
Frailty, n (%)	40 (3.82%)	65 (26.0%)	

187 SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, FBG=fasting blood-glucose,

188 WBC=white blood cell, PLT=platelets count, RBC=red blood cell, RDW=red blood cell distribution width,

189 HRR=hemoglobin-to-RDW ratio.
 190 Normal HRR=HRR above 9.97, Lower HRR: HRR below 9.97 (Optimal cut-off value of the ROC curve)
 191 Other* including separated, divorced, never married or widowed

193 **Logistic regression analysis**

194 Among 1296 older adults, 105 (8.10%) were considered frail. Multiple logistic regression
 195 analysis was conducted to assess the associations of the lower HRR with frailty.
 196 Unadjusted model1 showed that lower Hb (OR:2.129,1.133-4.001, p=0.019) and lower
 197 HRR (OR:3.285,1.676-6.440, p=0.001) were risk factors related to frailty, whereas lower
 198 RDW (OR:0.310, 0.193-0.497, p<0.001) was a protective factor. After adjustment for
 199 confounding factors (including age, gender, marital status, education years, living alone,
 200 BMI, diabetes, RBC, albumin, triglyceride, HDL-C, and LDL-C), there was a significant
 201 association of lower HRR (OR:3.419, 1.679-6.964, p=0.001) and lower RDW (OR:0.285,
 202 0.170-0.477, p< 0.001) with frailty (Table 3). Lower HRR was independently related to
 203 frailty in older adults.

205 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

206 Hb=hemoglobin, RDW=red blood cell distribution width, HRR=hemoglobin-to-RDW ratio.
 207 Lower Hb: <131.5g/L, Lower RDW: <13.45%, Lower HRR: <9.97. (Optimal cut-off value of the ROC
 208 curve)

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

211

212 **Correlation Analysis**

213 Correlation analysis indicated that there was an obvious positive correlation between
 214 RDW (Kendall's tau-b=0.173, P<0.001) and frailty. Nevertheless, HRR (Kendall's
 215 tau-b=-0.239, P<0.001) and Hb (Kendall's tau-b=-0.194, P<0.001) were a negative
 216 correlation with frailty (Table 4).

217

218 Table4. Correlation analysis of Hb, RDW, HRR, and frailty in older adults

219

	Kendall's tau-b	p
Hb	-0.194	<0.001
RDW	0.173	<0.001
HRR	-0.239	<0.001

220 **. Correlation is significant at the 0.01 level (2-tailed).

221

222 **Discussion**

223 Frailty, as a geriatric syndrome, has attracted more and more scientific attention in the
 224 background of continuously increasing global population aging.²⁰ In this cross-sectional study
 225 including 1,296 community-dwelling older adults, we found that lower HRR is independently
 226 related to frailty in older people, even after adjusting confounding factors (P=0.001). Multiple
 227 logistic regression analysis showed that lower HRR is associated with a 3-fold more likelihood
 228 or odds of frailty (OR = 3.419, 95%CI 1.679-6.964). ROC analysis showed that the AUC for
 229 HRR in the frailty older adults was 0.802, the highest sensitivity was 84.5% and the specificity

1
2
3
4 230 was 61.9% with the optimal critical value of 9.97. The results of the present study confirmed
5
6 231 that HRR was also significantly associated with frailty in general older people, not only in
7
8
9 232 patients with coronary heart disease in previous studies.
10

11 233 HRR is a cost-effective, common, and accessible laboratory parameter for clinicians.
12
13
14 234 As a novel inflammatory factor, Qu et.al found that HRR is significant associated with frailty in
15
16
17 235 older patients with CHD.¹⁶ In their study the AUC for HRR in the frailty patients was exceed Hb
18
19
20 236 and RDW, and after adjusting confounding factors lower HRR was a risk factor for frailty in
21
22 237 older patients with CHD. These findings are consistent with our results. Now the
23
24
25 238 pathophysiological mechanism has not been fully understood. We try to provide a possible
26
27
28 239 explanation for the association between HRR and frailty in older adults.
29

30 240 A decrease in HRR may be due to low Hb, high RDW, or both. As we all know, low Hb
31
32
33 241 indicates a condition of anemia, which is one of the acknowledged risk factors for
34
35
36 242 hospitalization, morbidity, and mortality in older people.²¹ Anemia decreases the
37
38
39 243 oxygen-carrying capacity, leading to tissue hypoxia and even organ failure, especially in older
40
41
42 244 patients, increasing the risk of frailty.²² Besides, anemia can cut down submaximal and
43
44
45 245 maximal aerobic capacity, leading to several adverse outcomes including loss of muscle
46
47
48 246 strength, cognitive decline, and development of frailty.¹⁰ In addition, chronic conditions and
49
50
51 247 comorbidities leading to a low grade of inflammation-reducing hemoglobin level,²³ also known
52
53
54 248 as chronic diseases anemia, which is the most common type of anemia in older adults.²² And a
55
56
57 249 state of chronic inflammation has been suggested as contributor to frailty.²⁴ Furthermore,
58
59
60 250 anemia caused by malnutrition is also a significant health-affecting factor among older
251 adults.²²

1
2
3
4 252 Anemia reaching a prevalence of 17% in older people, is a threat to healthy aging.²⁵
5
6
7 253 Corona et.al found that lower hemoglobin and anemia were related to frailty in Brazilian older
8
9 254 adults.⁸ In their study, anemia was related to low physical activity, weakness(weaker), and
10
11 255 walking more slowly. Another study from Spain indicated that anemia is independently
12
13
14 256 associated with frailty in older people.²⁶ Moreover, Xu et.al found that Hb is closely associated
15
16
17 257 with frailty in elder patients in the hospital.⁹ A systematic review and meta-analysis including
18
19 258 19 studies indicated that older adults with anemia have more than a two-fold increased odds of
20
21
22 259 frailty.²⁷ Silva et.al suggested that lower Hb levels should be considered a significant
23
24
25 260 component of frailty in older persons.²⁸ Similarly, another meta-analysis including 32,934
26
27 261 robust participants and 6864 frail participants found that Hb is a useful biomarker of frailty.⁷
28
29
30 262 However, there were no significant association between lower Hb and frailty after adjusting
31
32 263 confounding factors in our study. The discrepancy in results may be due to the definition of
33
34
35 264 lower Hb being determined by the optimal cut-off value of the ROC curve in this research.
36
37
38 265 Therefore, further studies are needed to confirm the relationship between Hb and frailty in
39
40 266 older people.

41
42
43 267 An increasing RDW also can lead to low HRR. Studies have proved that increased
44
45 268 RDW is related to inflammation. Inflammatory cytokines reduce erythropoietin gene
46
47
48 269 expression and erythropoietin receptor expression, which leads to the release of immature
49
50
51 270 erythrocytes and the heterogeneity of the erythrocyte volume increasing.^{29,30} In addition,
52
53 271 metabolic abnormalities including shortened telomere length, oxidative stress, and malnutrition
54
55
56 272 may also contribute to increased RDW.^{11,31} What's more, others have suggested that RDW
57
58
59 273 may be a potential biomarker for biological aging.³⁰ Study has indicated that a high RDW was
60

1
2
3
4 274 related to a high sarcopenia risk.³² Sarcopenia, which plays a key role in frailty, is a
5
6 275 progressive loss of skeletal muscle mass and strength.²⁸
7
8

9 276 RDW is a biomarker of inflammation, oxidative stress, poor nutritional status, aging,
10
11 277 and sarcopenia, and all of these could be underlying reasons for the development of frailty. A
12
13 278 study that enrolled 3,635 community-dwelling older men indicated that participants with a high
14
15 279 RDW are more likely to have functional limitations and frailty.³⁰ Li et.al indicated that increased
16
17 280 RDW may be closely related to frailty through inflammation.¹⁵ Hou et.al proved that frailty is
18
19 281 closely associated with RDW in elder patients with CHD.¹³ Another study including 2,932
20
21 282 community-dwelling older adults found RDW is independently associated with high frailty risk
22
23 283 even after adjusting for potential confounding factors.¹⁴
24
25
26
27
28
29

30 284 Increased RDW combined with anemia is more likely to lead to decreased HRR.
31
32 285 Elevated RDW suggests chronic inflammation, malnutrition, and aging.¹¹ Anemia is the cause
33
34 286 of reduced tissue oxygenation and the consequent increase in fatigue, weakness, and
35
36 287 functional impairment.³³ Also anemia may affect muscle mass and strength loss through
37
38 288 inflammatory pathways.³³ Therefore, decreased HRR may be associated with sarcopenia,
39
40 289 slowness, weakness, inflammation, malnutrition, and weight loss in frailty patients.
41
42
43
44

45 290 To sum up, a large number of researchers have verified the association between frailty
46
47 291 and a low Hb and a high RDW among older persons. However, both RDW and Hb are
48
49 292 susceptible to many other disease conditions and sub-health states, HRR may provide a more
50
51 293 powerful parameter than a single parameter alone. Moreover, ROC analysis showed that the
52
53 294 AUC and highest sensitivity of HRR are higher than RDW and Hb. It may be a more reliable
54
55 295 and effective marker than Hb and RDW alone.
56
57
58
59
60

1
2
3
4 296 This inexpensive and common laboratory parameter may provide useful information to
5
6 297 identify the risk of frailty in older adults. Furthermore, the use of the HRR may help clinicians to
7
8
9 298 identify people at high risk of frailty and take effective measures to reduce the occurrence and
10
11 299 development of frailty, reduce the rate of disability and mortality related to frailty in the elderly,
12
13
14 300 and reduce the waste of medical resources, and promote healthy aging.

15
16
17 301 There were also some limitations of our present study. Firstly, because cross-sectional
18
19 302 studies measure the outcome and the exposures in the study participants at the same time, it
20
21
22 303 is difficult to assess the cause-effect relationship. Secondly, our participants are limited to local
23
24 304 participants, these findings need to be validated in different populations around the world.
25
26
27 305 What is more, we are unable to investigate the temporal relation between outcomes and risk
28
29
30 306 factors. In addition, despite the inevitable selection bias and information bias in cross-sectional
31
32 307 studies, we improved this problem through more rational statistical methods and interviewer
33
34
35 308 training. Finally, we did not assess iron, folic, and vitamin B12, which may affect RDW and Hb
36
37
38 309 levels.

39
40 310

41 42 43 311 **Conclusion**

44
45 312 In conclusion, a low HRR is independently associated with higher frailty risk in
46
47
48 313 community-dwelling older adults. And this relationship is not affected by confounding factors.
49
50
51 314 However, the causal relationship and the specific mechanism between HRR and frailty are
52
53 315 unclear. Evidence is needed from prospective studies to verify these conclusions in the future.

54
55
56 316

57
58 317
59
60

1
2
3
4 318
5
6
7

8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

319 320 **Author contributions**

321 Conceptualization: Zhifan Xiong, Mengpei Zhu

322 Data curation: Mengpei Zhu, Chao Wei, Yushuang Xu

323 Formal analysis: Mengpei Zhu, Chao Wei

324 Visualization: Xiongjun Yang, Yumei Huang

325 Writing – original draft: Mengpei Zhu

326 Writing – review and editing: Zhifan Xiong, Mengpei Zhu, Chao Wei, Xiongjun Yang, Yumei

327 Huang, Yushuang Xu. All authors read and approved the final manuscript.

328

329 **Acknowledgements**

330 The authors thank all the volunteers that participated in this study.

331

332 **Funding**

333 This study was supported by the National Key Research and Development Program of China

334 (2018YFC2002000).

335

336 **Data availability**

337 Data for this study are available from the corresponding author.

338

339 **Declaration**

1
2
3
4 340 The authors report no conflicts of interest in this work.
5
6
7 341

8 9 342 **Ethics statements**

10
11 343 The research protocol was approved by the Medical Ethics Committee of Liyuan Hospital,
12
13
14 344 Tongji Medical College, Huazhong University of Science and Technology (IRB ID: [2020] IEC
15
16
17 345 (A016)). All the participants gave written informed consent. The study was conducted in
18
19
20 346 accordance with the tenets of the Declaration of Helsinki.
21
22 347

23
24
25 348

26
27 349

28
29
30 350

31 32 33 351 **References**

- 34
35 352 1. Kameda M, Teruya T, Yanagida M, Kondoh H. Frailty markers comprise blood metabolites
36 353 involved in antioxidation, cognition, and mobility. *Proc Natl Acad Sci U S A*.
37 354 2020;117(17):9483-9489.
38
39 355 2. Travers J, Romero-Ortuno R, Bailey J, Cooney M-T. Delaying and reversing frailty: a
40 356 systematic review of primary care interventions. *Br J Gen Pract*. 2019;69(678):e61-e69.
41
42 357 3. Dent E, Martin FC, Bergman H, Woo J, Romero-Ortuno R, Walston JD. Management of frailty:
43 358 opportunities, challenges, and future directions. *Lancet*. 2019;394(10206):1376-1386.
44
45 359 4. Coelho-Junior HJ, Marzetti E, Picca A, Cesari M, Uchida MC, Calvani R. Protein Intake and
46 360 Frailty: A Matter of Quantity, Quality, and Timing. *Nutrients*. 2020;12(10).
47
48 361 5. Ward RE, Orkaby AR, Chen J, et al. Association between Diet Quality and Frailty Prevalence in
49 362 the Physicians' Health Study. *J Am Geriatr Soc*. 2020;68(4):770-776.
50
51 363 6. Zhai Z, Gao J, Zhu Z, et al. The Ratio of the Hemoglobin to Red Cell Distribution Width
52 364 Combined with the Ratio of Platelets to Lymphocytes Can Predict the Survival of Patients
53 365 with Gastric Cancer Liver Metastasis. *Biomed Res Int*. 2021;2021:8729869.
54
55 366 7. Mailliez A, Guilbaud A, Puisieux F, Dauchet L, Boulanger É. Circulating biomarkers
56 367 characterizing physical frailty: CRP, hemoglobin, albumin, 25OHD and free testosterone as
57 368 best biomarkers. Results of a meta-analysis. *Exp Gerontol*. 2020;139:111014.
58
59 369 8. Pires Corona L, Drumond Andrade FC, de Oliveira Duarte YA, Lebrao ML. The Relationship
60 370 between Anemia, Hemoglobin Concentration and Frailty in Brazilian Older Adults. *J Nutr
371 Health Aging*. 2015;19(9):935-940.

- 1
2
3 372 9. Xu L, Zhang J, Shen S, et al. Clinical Frailty Scale and Biomarkers for Assessing Frailty in Elder
4 373 Inpatients in China. *J Nutr Health Aging*. 2021;25(1):77-83.
- 5 374 10. Ruan Y, Guo Y, Kowal P, et al. Association between anemia and frailty in 13,175
6 375 community-dwelling adults aged 50 years and older in China. *BMC Geriatr*. 2019;19(1):327.
- 7 376 11. Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: A simple
8 377 parameter with multiple clinical applications. *Crit Rev Clin Lab Sci*. 2015;52(2).
- 9 378 12. Dent E, Lien C, Lim WS, et al. The Asia-Pacific Clinical Practice Guidelines for the Management
10 379 of Frailty. *J Am Med Dir Assoc*. 2017;18(7):564-575.
- 11 380 13. Hou P, Xue H-P, Mao X-E, Li Y-N, Wu L-F, Liu Y-B. Inflammation markers are associated with
12 381 frailty in elderly patients with coronary heart disease. *Aging (Albany NY)*.
13 382 2018;10(10):2636-2645.
- 14 383 14. Li C-M, Chao C-T, Chen S-I, Han D-S, Huang K-C. Elevated Red Cell Distribution Width Is
15 384 Independently Associated With a Higher Frailty Risk Among 2,932 Community-Dwelling Older
16 385 Adults. *Front Med (Lausanne)*. 2020;7:470.
- 17 386 15. Li Q, Chen X, Han B. Red blood cell distribution width is associated with frailty in older
18 387 inpatients in China: Sex differences in a cross-sectional study. *Exp Gerontol*.
19 388 2021;150:111392.
- 20 389 16. Qu J, Zhou T, Xue M, et al. Correlation Analysis of Hemoglobin-to-Red Blood Cell Distribution
21 390 Width Ratio and Frailty in Elderly Patients With Coronary Heart Disease. *Front Cardiovasc*
22 391 *Med*. 2021;8:728800.
- 23 392 17. Su Y-C, Wen S-C, Li C-C, et al. Low Hemoglobin-to-Red Cell Distribution Width Ratio Is
24 393 Associated with Disease Progression and Poor Prognosis in Upper Tract Urothelial Carcinoma.
25 394 *Biomedicines*. 2021;9(6).
- 26 395 18. Sun P, Zhang F, Chen C, et al. The ratio of hemoglobin to red cell distribution width as a novel
27 396 prognostic parameter in esophageal squamous cell carcinoma: a retrospective study from
28 397 southern China. *Oncotarget*. 2016;7(27):42650-42660.
- 29 398 19. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. *J*
30 399 *Gerontol A Biol Sci Med Sci*. 2001;56(3):M146-M156.
- 31 400 20. Kojima G, Taniguchi Y, Iliffe S, Jivraj S, Walters K. Transitions between frailty states among
32 401 community-dwelling older people: A systematic review and meta-analysis. *Ageing Res Rev*.
33 402 2019;50:81-88.
- 34 403 21. Lee CT, Chen MZ, Yip CYC, Yap ES, Lee SY, Merchant RA. Prevalence of Anemia and Its
35 404 Association with Frailty, Physical Function and Cognition in Community-Dwelling Older
36 405 Adults: Findings from the HOPE Study. *J Nutr Health Aging*. 2021;25(5):679-687.
- 37 406 22. Röhrig G. Anemia in the frail, elderly patient. *Clin Interv Aging*. 2016;11:319-326.
- 38 407 23. Steinmeyer Z, Delpierre C, Soriano G, et al. Hemoglobin concentration; a pathway to frailty.
39 408 *BMC Geriatr*. 2020;20(1):202.
- 40 409 24. Wilson D, Jackson T, Sapey E, Lord JM. Frailty and sarcopenia: The potential role of an aged
41 410 immune system. *Ageing Res Rev*. 2017;36.
- 42 411 25. Stauder R, Valent P, Theurl I. Anemia at older age: etiologies, clinical implications, and
43 412 management. *Blood*. 2018;131(5):505-514.
- 44 413 26. Esquinas-Requena JL, García-Nogueras I, Hernández-Zegarra P, Atienzar-Núñez P, Sánchez-Jurado PM, Abizanda P. [Anemia and frailty in older adults from Spain. The FRADEA
45 414 Study]. *Rev Esp Geriatr Gerontol*. 2021;56(3):129-135.
46 415

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 416 27. Palmer K, Vetrano DL, Marengoni A, et al. The Relationship between Anaemia and Frailty: A
417 Systematic Review and Meta-Analysis of Observational Studies. *J Nutr Health Aging*.
418 2018;22(8):965-974.
- 419 28. Silva JC, Moraes ZVd, Silva C, et al. Understanding red blood cell parameters in the context of
420 the frailty phenotype: interpretations of the FIBRA (Frailty in Brazilian Seniors) study. *Arch*
421 *Gerontol Geriatr*. 2014;59(3):636-641.
- 422 29. Pierce CN, Larson DF. Inflammatory cytokine inhibition of erythropoiesis in patients
423 implanted with a mechanical circulatory assist device. *Perfusion*. 2005;20(2):83-90.
- 424 30. Kim KM, Lui L-Y, Browner WS, et al. Association Between Variation in Red Cell Size and
425 Multiple Aging-Related Outcomes. *J Gerontol A Biol Sci Med Sci*. 2021;76(7):1288-1294.
- 426 31. Ghaffari S. Oxidative stress in the regulation of normal and neoplastic hematopoiesis.
427 *Antioxid Redox Signal*. 2008;10(11):1923-1940.
- 428 32. Kim J, Im J-S, Choi CH, et al. The Association between Red Blood Cell Distribution Width and
429 Sarcopenia in U.S. Adults. *Sci Rep*. 2018;8(1):11484.
- 430 33. Picca A, Coelho-Junior HJ, Calvani R, Marzetti E, Vetrano DL. Biomarkers shared by frailty and
431 sarcopenia in older adults: A systematic review and meta-analysis. *Ageing Res Rev*.
432 2022;73:101530.

433

434

435

436

437

438

439 **Figure legends**

440 **Figure1** ROC curve for HRR (A), Hb (B) and RDW (C)

441

442 **Notes:**

443 A. ROC curve indicated that the best intercept value for HRR was 9.97 (sensitivity 84.5%,
444 specificity 61.9%, AUC = 0.802, P<0.001)

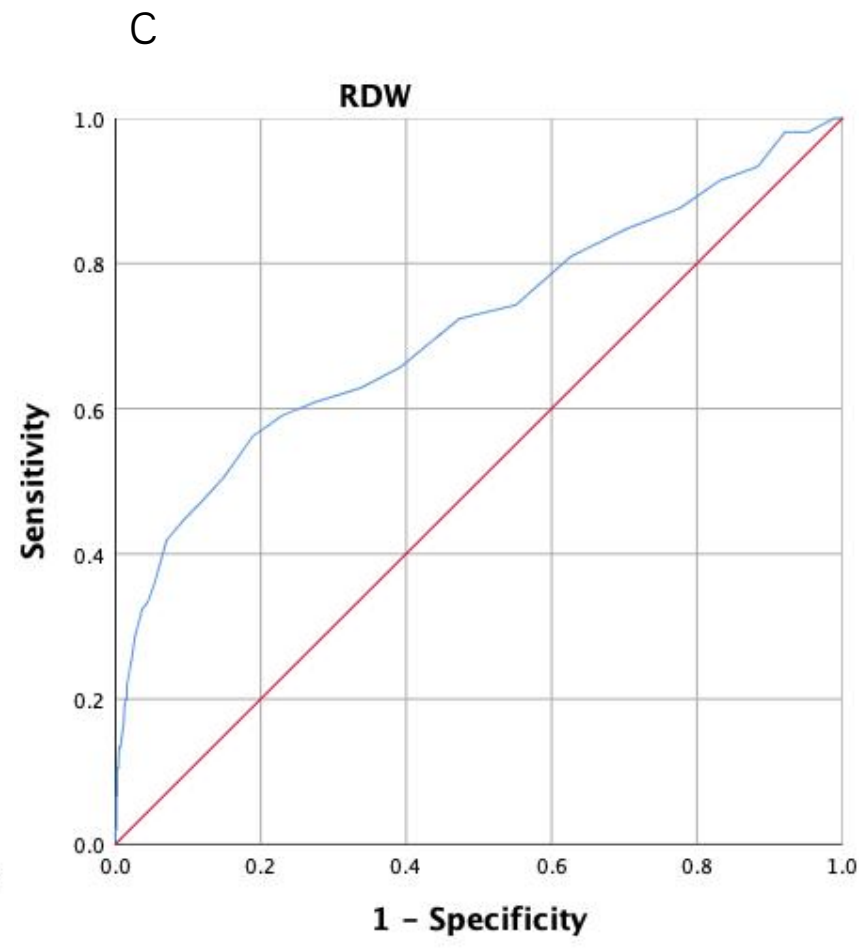
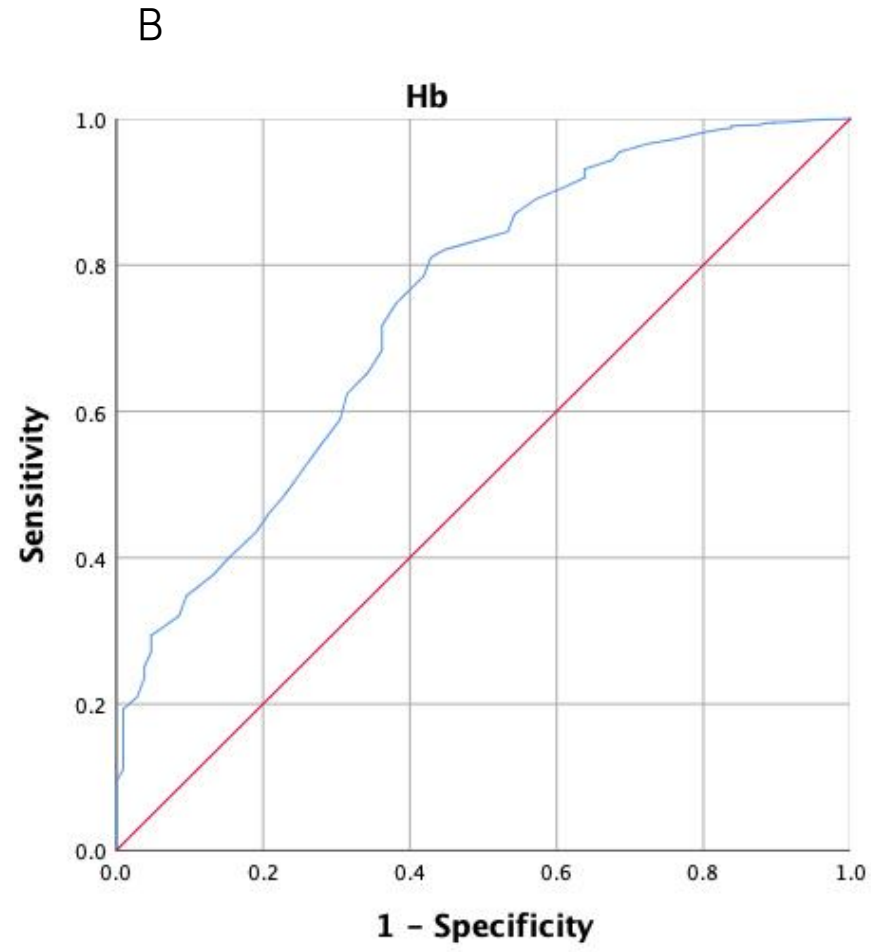
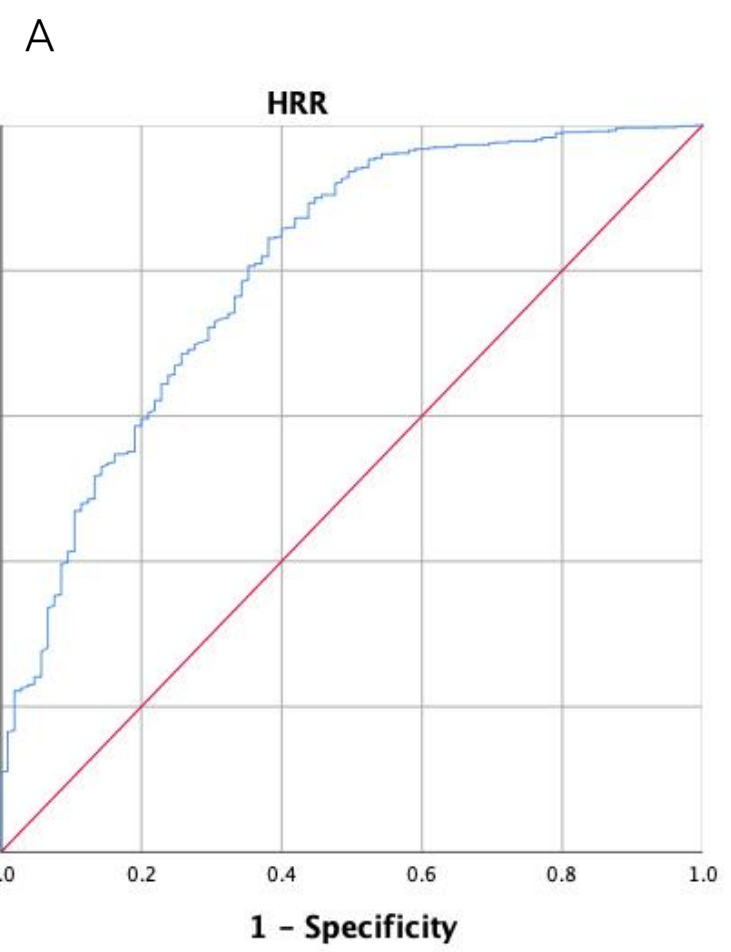
445 B. ROC curve indicated that the best intercept value for Hb was 131.5 (sensitivity 81% ,
446 specificity 57.1%, AUC = 0.742, P<0.001)

1
2
3
4 447 C. ROC curve indicated that the best intercept value for RDW was 13.45 (sensitivity 56.2% ,
5
6 448 specificity 81.1%, AUC = 0.712, P<0.001)
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

Figure 1.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41



STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7
		(b) Describe any methods used to examine subgroups and interactions	7
		(c) Explain how missing data were addressed	7
		(d) If applicable, describe analytical methods taking account of sampling strategy	7
		(e) Describe any sensitivity analyses	7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	7-8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest	8
Outcome data	15*	Report numbers of outcome events or summary measures	8
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9-11 9-11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.