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## The prognostic value of interaction between mean corpuscular volume and red cell distribution width in mortality in chronic kidney disease

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Recently, both red cell distribution width (RDW) and mean corpuscular volume (MCV) have been associated with unfavorable outcomes in several medical conditions. Therefore, we conducted this retrospective study of 1075 patients with stage 3–5 chronic kidney disease to investigate whether interactions between RDW and MCV influence the risk of mortality. These patients were divided into four groups: group A (n = 415), RDW ≤ 14.9% and MCV ≤ 91.6 fL; group B (n = 232), RDW > 14.9% and MCV ≤ 91.6 fL; group C (n = 307), RDW ≤ 14.9% and MCV > 91.6 fL; and group D (n = 121), RDW > 14.9% and MCV > 91.6 fL. The adjusted hazard ratio (HR) of all-cause mortality for group B versus group A was 1.44 (95% confidence interval [CI], 1.14–2.12, p = 0.02), group C versus group A 2.14 (95% CI, 1.31–3.48, p = 0.002), and group D versus group A 5.06 (95% CI, 3.06–8.37, p < 0.001). There was a multiplicative interaction between MCV and RDW in predicting patient mortality. The use of RDW in conjunction with MCV may improve healthcare by identifying those at an increased risk for mortality compared with the use of either RDW or MCV alone.

Anemia is a common condition encountered in many clinical settings, and is associated with adverse clinical outcomes. Mean corpuscular volume (MCV) is the measure of the average volume of circulatory red blood cells. Based on the value of MCV, anemia can be classified as microcytic, normocytic or macrocytic, which can aid in making a differential diagnosis. Normocytic anemia is the most common form in patients with chronic kidney disease (CKD)<sup>1</sup>. Red cell distribution width (RDW) is an index of erythrocyte size heterogeneity, and the combination of RDW and MCV has been shown to aid in identifying the cause of anemia.

In addition to its role in anemia, there has been a great increase in the amount of research investigating the role of RDW as an independent and significant predictor for mortality in many medical conditions in the last decade. For example, RDW has been demonstrated to be associated with unfavorable outcomes in heart failure, coronary artery disease, acute pulmonary embolism, septic shock, acute cerebral infarction, acute kidney injury treated with continuous renal replacement therapy and end-stage renal disease<sup>2–10</sup>. MCV has also been reported to be associated with adverse outcomes, however only a few studies have reported on the association between MCV and mortality. The majority of these studies have focused on cancer patients; however MCV has also been reported to be a predictor for mortality in patients undergoing primary coronary interventions with acute decompensated heart failure and CKD<sup>11–14</sup>.

Both RDW and MCV have therefore emerged as novel risk factors for negative outcomes, and both are now routinely reported as part of a complete blood cell count. We were interested in whether the concurrent use of these two hematological parameters can improve the prediction of patient mortality, and thus we conducted this study to investigate whether interactions between RDW and MCV influence the risk of mortality in patients with stage 3–5 CKD.

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## Results

**Baseline characteristics of the study cohort.** Of the 1075 participants, 470 (43.72%) were women. The mean age of the whole study cohort was  $64.2 \pm 12.35$  years with group D being the oldest, and the mean BMI was  $25.17 \pm 4.15$  kg/m<sup>2</sup>. The mean duration of follow-up was  $2.35 \pm 1.65$  years, and the three leading underlying etiologies of CKD were diabetes mellitus (39.35%), hypertension (23.81%) and chronic glomerulonephritis (10.23%). Seven hundred and ninety eight patients (74.23%) were not current smokers, and 905 (84.19%) were never drinkers. The relevant information of the four groups is shown in Table 1. Group A had the highest BMI ( $25.69 \pm 4.18$  kg/m<sup>2</sup>) while group D had the lowest BMI ( $24.72 \pm 3.98$  kg/m<sup>2</sup>). Concerning the prevalence of medical comorbidities, there were significant differences in malignancy, congestive heart failure, diabetes mellitus, hyperlipidemia and liver cirrhosis among the four groups. However, there were no significant differences in the prevalence of cerebrovascular disease, chronic lung disease, coronary artery disease, dementia, hypertension and peripheral artery disease. With regards to the laboratory parameters, there were significant differences in all measurements among the four groups except for cholesterol level. Regarding the medications use, there were significant differences in the prescriptions for iron preparations, folic acid supplements, and erythropoiesis stimulating agents among the four groups.

**The interaction between MCV and RDW in predicting all-cause mortality.** Overall, 158 patients died during a mean follow-up period of  $2.35 \pm 1.65$  years, including 28 (6.75%) in group A, 31 (13.36%) in group B, 51 (16.61%) in group C and 48 (39.67%) in group D. There was a significant difference in the crude mortality rate for the four groups ( $p < 0.001$ ), and also a significant difference in Kaplan-Meier survival curves (log-rank test,  $p < 0.001$ ; Fig. 1). Table 2 lists the unadjusted and adjusted HRs of Cox proportional hazard analysis for the outcomes among the four groups. There was a stepwise increase in the risk of all-cause mortality from group A to group D in the unadjusted and adjusted models (both  $p$  for trend  $< 0.001$ ). The adjusted HR for group B versus group A was 1.44 (95% CI, 1.14–2.12,  $p = 0.02$ ), group C versus group A 2.14 (95% CI, 1.31–3.48,  $p = 0.002$ ), and group D versus group A 5.06 (95% CI, 3.06–8.37,  $p < 0.001$ ). The adjusted HR for group D was higher than the expected value determined by the addition of adjusted HRs for group B and C, suggesting a multiplicative interaction between MCV and RDW in predicting overall mortality for patients with stage 3–5 CKD.

**The interaction between MCV and RDW in predicting CVD-related mortality.** Overall, 35 patients died of CVD during a mean follow-up period of  $2.35 \pm 1.65$  years, including six (1.25%) in group A, three (1.23%) in group B, 11 (3.58%) in group C and 15 (12.4%) in group D. There was a significant difference in the crude CVD-related mortality rate for the four groups ( $p < 0.001$ ), and also a significant difference in Kaplan-Meier survival curves (log-rank test,  $p < 0.001$ ; Fig. 2). As shown in Table 2, there was a stepwise increase in the risk of CVD-related mortality from group A to group D in the unadjusted and adjusted models (both  $p$  for trend  $< 0.001$ ). The adjusted HR for group B versus group A was 0.5 (95% CI, 0.11–2.25,  $p = 0.37$ ), group C versus group A 2.62 (95% CI, 0.87–7.88,  $p = 0.08$ ), and group D versus group A 10.76 (95% CI, 3.48–33.21,  $p < 0.001$ ).

**The interaction between MCV and RDW in predicting infection-related mortality.** Overall, 75 patients died of infection during a mean follow-up period of  $2.35 \pm 1.65$  years, including 11 (2.65%) in group A, 19 (8.19%) in group B, 26 (8.47%) in group C and 19 (15.7%) in group D. There was a significant difference in the crude infection-related mortality rate for the four groups ( $p < 0.001$ ), and also a significant difference in Kaplan-Meier survival curves (log-rank test,  $p < 0.001$ ; Fig. 3). There was a stepwise increase in the risk of infection-related mortality from group A to group D in the unadjusted and adjusted models of Cox regression analyses (both  $p$  for trend  $< 0.001$ ; Table 2). The adjusted HR for group B versus group A was 2.15 (95% CI, 0.95–4.88,  $p = 0.06$ ), group C versus group A 2.96 (95% CI, 1.41–6.22,  $p = 0.004$ ), and group D versus group A 4.7 (95% CI, 2.12–10.4,  $p < 0.001$ ).

## Discussion

In this study, we assessed interactions between MCV and RDW in predicting mortality among patients with stage 3–5 CKD, and found that the combination of both parameters improved the risk prediction for clinical outcomes, including all-cause mortality, and cardiovascular disease and infection-related mortality.

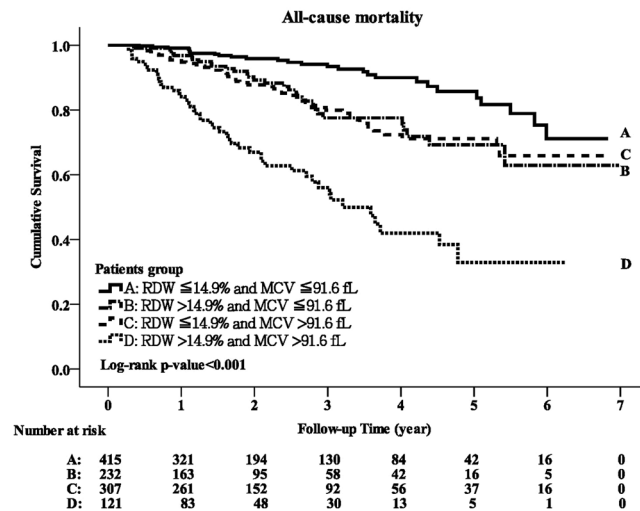
With respect to all-cause mortality using group A as the reference, the adjusted HRs were 1.44 and 2.14 for group B (higher RDW) and C (higher MCV), respectively. Mathematically, the adjusted HR for group D (both higher level of RDW and MCV) was expected to be 3.58 by adding 1.44 to 2.14. Actually, the adjusted HR was 5.06, which was higher than the expected value by the simple addition. Therefore, there was a multiplicative interaction between MCV and RDW in predicting overall mortality for patients with stage 3–5 CKD. The influences of RDW and MCV on the survival of the CKD patients were independent in the presence of other powerful predictors such as hemoglobin, albumin, renal function status and mineral disorders. Concerning the cause-specific mortality, there was also a trend of increasing mortality of CVD and infection from group A to D ( $P$  for trend  $< 0.001$  for both). Thus, patients with higher MCV and RDW levels were associated with the greatest risk of negative clinical outcomes.

RDW is an automatically calculated index in routine complete blood cell count reports. It is a numerical index that reflects variations in the size of red blood cells, and it is calculated by the following equation: (standard deviation of MCV  $\div$  mean MCV)  $\times 100$ <sup>15</sup>. RDW has emerged as an independent prognostic factor in several medical conditions. However, little is known about the underlying mechanism by which an elevated level of RDW is associated with a negative impact on survival. RDW is commonly known to be elevated when there is an increase in the destruction of erythrocytes, and with increased and impaired erythropoiesis. An elevated RDW level has also been reported to reflect the presence of malnutrition, chronic inflammation and bone marrow dysfunction<sup>9,16,17</sup>. In addition, the prognostic implication of RDW has been reported to be similar in critically ill patients with a low

| Variable                               | Group A n = 415              | Group B n = 232              | Group C n = 307              | Group D n = 121              | p-value |
|--|------------------------------|------------------------------|------------------------------|------------------------------|---------|
|  | RDW ≤ 14.9%<br>MCV ≤ 91.6 fL | RDW > 14.9%<br>MCV > 91.6 fL | RDW ≤ 14.9%<br>MCV > 91.6 fL | RDW > 14.9%<br>MCV > 91.6 fL |         |
| <b>Sex</b>                             |                              |                              |                              |                              | 0.014*  |
| Female                                 | 158(48.02%)                  | 101(48.33%)                  | 147(37.4%)                   | 64(44.44%)                   |         |
| <b>Age (years)</b>                     | 59.07 ± 13.47                | 63.39 ± 12.17                | 67.1 ± 10.76                 | 69.23 ± 9.32                 | <0.001* |
| <b>BMI (kg/m<sup>2</sup>)</b>          | 25.69 ± 4.18                 | 25.12 ± 4.15                 | 24.91 ± 4.16                 | 24.72 ± 3.98                 | 0.037*  |
| <b>Smoker</b>                          |                              |                              |                              |                              | 0.094   |
| Non-current                            | 259(78.72%)                  | 152(72.73%)                  | 278(70.74%)                  | 109(75.69%)                  |         |
| Current                                | 70(21.28%)                   | 57(27.27%)                   | 115(29.26%)                  | 35(24.31%)                   |         |
| <b>Drinker</b>                         |                              |                              |                              |                              | 0.167   |
| Never                                  | 283(86.02%)                  | 183(87.56%)                  | 315(80.15%)                  | 124(86.11%)                  |         |
| Current                                | 21(6.38%)                    | 11(5.26%)                    | 31(7.89%)                    | 6(4.17%)                     |         |
| Former                                 | 25(7.6%)                     | 15(7.18%)                    | 47(11.96%)                   | 14(9.72%)                    |         |
| <b>Comorbidity</b>                     |                              |                              |                              |                              |         |
| Cancer                                 | 18(5.47%)                    | 14(6.7%)                     | 42(10.69%)                   | 28(19.44%)                   | <0.001* |
| Cerebrovascular disease                | 53(16.11%)                   | 34(16.27%)                   | 50(12.72%)                   | 22(15.28%)                   | 0.537   |
| Chronic lung disease                   | 36(10.94%)                   | 38(18.18%)                   | 63(16.03%)                   | 21(14.58%)                   | 0.099   |
| Congestive heart failure               | 36(10.94%)                   | 34(16.27%)                   | 29(7.38%)                    | 23(15.97%)                   | 0.003*  |
| Coronary artery disease                | 72(21.88%)                   | 60(28.71%)                   | 94(23.92%)                   | 43(29.86%)                   | 0.152   |
| Dementia                               | 6(1.82%)                     | 3(1.44%)                     | 14(3.56%)                    | 6(4.17%)                     | 0.21    |
| Diabetes mellitus                      | 183(55.62%)                  | 122(58.37%)                  | 159(40.46%)                  | 67(46.53%)                   | <0.001* |
| Hyperlipidemia                         | 148(44.98%)                  | 84(40.19%)                   | 138(35.11%)                  | 51(35.42%)                   | 0.04*   |
| Hypertension                           | 249(75.68%)                  | 156(74.64%)                  | 285(72.52%)                  | 92(63.89%)                   | 0.057   |
| Liver cirrhosis                        | 2(0.61%)                     | 2(0.96%)                     | 6(1.53%)                     | 7(4.86%)                     | 0.006*  |
| Peripheral artery disease              | 6(1.82%)                     | 2(0.96%)                     | 6(1.53%)                     | 1(0.69%)                     | 0.731   |
| <b>Medication prescription</b>         |                              |                              |                              |                              |         |
| ACE inhibitor/ARB                      | 226(68.69%)                  | 147(70.33%)                  | 241(61.32%)                  | 90(62.5%)                    | 0.064   |
| Vitamin B12 (cyanocobalamin)           | 57(17.33%)                   | 37(17.7%)                    | 93(23.66%)                   | 37(25.69%)                   | 0.053   |
| Iron preparations                      | 44(13.37%)                   | 62(29.67%)                   | 38(9.67%)                    | 25(17.36%)                   | <0.001* |
| Folic acid                             | 43(13.07%)                   | 50(23.92%)                   | 81(20.61%)                   | 40(27.78%)                   | 0.001*  |
| Erythropoiesis stimulating agents      | 56(17.02%)                   | 59(28.23%)                   | 58(14.76%)                   | 36(25%)                      | <0.001* |
| Vitamin D                              | 9(2.74%)                     | 4(1.91%)                     | 11(2.8%)                     | 7(4.86%)                     | 0.432   |
| <b>Laboratory data</b>                 |                              |                              |                              |                              |         |
| Albumin (g/dL)                         | 3.79 ± 0.6                   | 3.38 ± 0.72                  | 3.82 ± 0.62                  | 3.57 ± 0.62                  | <0.001* |
| BUN (mg/dL)                            | 46.02 ± 23.6                 | 52.61 ± 23.77                | 41.01 ± 20.35                | 50.89 ± 24.89                | <0.001* |
| Creatinine (mg/dL)                     | 3.58 ± 2.34                  | 3.91 ± 2.44                  | 3.24 ± 2.08                  | 3.76 ± 2.18                  | 0.003*  |
| eGFR (ml/min per 1.73 m <sup>2</sup> ) | 23.07 ± 13.23                | 20.58 ± 12.13                | 24.63 ± 12.63                | 20.72 ± 11.68                | <0.001* |
| Ca (mg/dL)                             | 8.82 ± 0.68                  | 8.61 ± 0.67                  | 8.85 ± 0.59                  | 8.81 ± 0.85                  | <0.001* |
| Phosphorus (mg/dL)                     | 4.27 ± 1.09                  | 4.45 ± 1.24                  | 4.01 ± 1.05                  | 4.16 ± 1.03                  | <0.001* |
| Cholesterol (mg/dL)                    | 191.25 ± 48.9                | 183.7 ± 66.21                | 182.77 ± 47.74               | 179.94 ± 58.92               | 0.089   |
| Triglyceride (mg/dL)                   | 169.92 ± 115.9               | 161.37 ± 123.95              | 140.04 ± 82.97               | 121.64 ± 74.94               | <0.001* |
| GPT (U/L)                              | 19.71 ± 16.84                | 22.65 ± 18.26                | 25.4 ± 39.57                 | 28.74 ± 29.76                | 0.007*  |
| WBC count (/μL)                        | 7624.49 ± 2369.9             | 8000.21 ± 2658.42            | 7129.99 ± 2398.77            | 7436.34 ± 2746.14            | <0.001* |
| Hemoglobin (g/dL)                      | 10.96 ± 2.04                 | 9.57 ± 1.96                  | 11.09 ± 2.12                 | 10.21 ± 2.11                 | <0.001* |
| Uric acid (mg/dL)                      | 8.17 ± 1.83                  | 8.2 ± 1.88                   | 7.68 ± 1.73                  | 8 ± 1.86                     | 0.001*  |
| 24-hour proteinuria (mg)               | 1242.9 ± 1967.1              | 1715.2 ± 2298.2              | 2044.4 ± 2795.7              | 2501.9 ± 3201.5              | <0.001* |

**Table 1.** Baseline characteristics of the study population by MCV and RDW. Values are expressed as mean ± SD or number (percentage). CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; BMI, body mass index; ACE inhibitor, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker. \*p < 0.05.

CRP level compared to those with a high CRP level<sup>18</sup>. Therefore, increased inflammation may not fully explain the relationship between RDW and unfavorable outcomes. Another possible explanation is oxidative stress. It has been shown that high oxidative stress can impair erythropoiesis, shorten erythrocyte half-life and affect deformities in red blood cells which may ultimately result in increased RDW due to a mixed population of small and large erythrocytes in the circulation (anisocytosis)<sup>19,20</sup>. The release of heterogeneous erythrocytes with poor oxygen-carrying capacity into the peripheral circulation has been reported to disrupt the micro-circulation with



**Figure 1.** Kaplan-Meier curve of overall patient survival according to the groups stratified by MCV and RDW (log-rank test,  $p < 0.001$ ).

local tissue hypoxia<sup>21</sup>. Although further studies are required to elucidate this hypothesis, it seems to be one of the most plausible mechanisms responsible for the association between RDW and negative clinical outcomes.

Erythrocytes are non-nucleated blood cells typically with an oval biconcave shape. The typical trait of anemia related to CKD is normocytic and normochromic in nature. In the general population, the causes of macrocytosis include vitamin B12 or folic acid deficiency, alcohol abuse, HIV medications, chemotherapeutics, reticulocytosis due to hemolysis or hemorrhage, hypothyroidism or liver disease<sup>22</sup>. Furthermore, bone marrow dysfunction has been reported to be a more common reason for macrocytic anemia in older patients than in younger patients<sup>22</sup>. The majority of clinical investigations on MCV in the prediction of mortality have focused on patients with malignancy. For example, the development of macrocytosis after capecitabine treatment has been shown to be a prognostic factor for a clinical response and overall survival in patients with advanced gastric cancer and breast cancer<sup>23,24</sup>. In addition, an elevation in MCV may be the result of impaired DNA biosynthesis caused by capecitabine-induced thymidylate synthetase inhibition<sup>25,26</sup>. Wenzel *et al.* demonstrated that a higher MCV value was more commonly noted in patients with a complete or partial response than in those with tumor progression<sup>27</sup>. Zheng *et al.* also concluded that a high preoperative MCV value was a negative prognostic predictor in esophageal squamous cell carcinoma independently of other risk factors<sup>28</sup>.

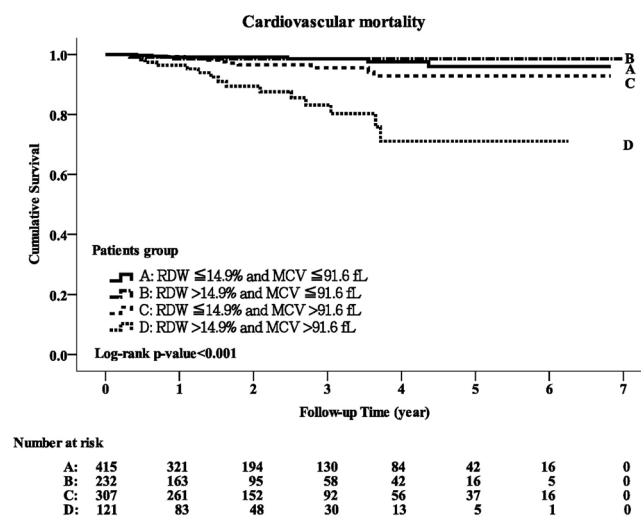
The clinical implications of MCV have also been addressed in patients with cardiovascular disease. For example, it has been shown to be positively correlated with atrial fibrillation (AF)<sup>29,30</sup>, and also to be significantly elevated in patients with chronic AF both before and after onset, suggesting that larger erythrocytes are already present before AF develops<sup>29</sup>. Likewise, MCV has been reported to be a potential risk factor for peripheral artery disease and to be associated with the severity<sup>31,32</sup>. Furthermore, Hatamian *et al.* studied 98 patients with ischemic stroke in middle cerebral artery territory and observed that MCV was a significant predictor for 1-week and 1-month mortality after ischemic stroke<sup>33</sup>. In addition, macrocytosis has been reported to be a predictor of survival in patients undergoing chronic hemodialysis<sup>13</sup>. Interestingly, a recent prospective study of 309 CKD patients showed that MCV was correlated with endothelial dysfunction and that it was an independent predictor for composite cardiovascular events<sup>14</sup>.

The mechanism responsible for the association between MCV and mortality is unknown, although several hypotheses have been proposed. One hypothesis involves oxidative stress, in that it has been implicated in a variety of chronic diseases, and the total antioxidant capacity of the body has been reported to be closely related to the size of circulating red blood cells<sup>34</sup>. As an elevated MCV level may reflect functional or structural disorders of the erythrocytes, the unfavorable impact of macrocytosis on survival can be explained by a deranged antioxidant capacity. In addition, impaired deformability of erythrocytes owing to high oxidative stress can further damage the microcirculation and oxygen delivery to tissues<sup>35,36</sup>. A second hypothesis is that macrocytosis may be a manifestation of deranged hematopoiesis due to bone marrow dysfunction. Bone marrow-derived mesenchymal stem cells have been reported to play a critical role in the repair of many damaged vital organs<sup>37</sup>. Another hypothesis is that nutrition may in part explain the association between MCV and patient survival. Crystal osmotic pressure, mainly determined by the serum concentrations of glucose, amino acids and electrolytes, is a key regulator of erythrocyte size<sup>38</sup>. Therefore, it is reasonable to speculate that malnourished patients are prone to higher MCV levels as a result of reduced serum osmotic pressure.

There are several potential limitations to this study associated its retrospective nature. First, we only took single measurements of MCV and RDW as the predictors for analysis, and thus the effect of variations in laboratory data over time was not evaluated which may have led to under-estimation of the association. Second, we did not assess the factors that can cause increased levels of RDW and MCV, such as acute bleeding (reticulocytosis), depletion of vitamin B12 or folic acid, and iron status. Because of the lack of laboratory data on blood levels of folic acid, vitamin B 12 and iron profile, the statistical analyses were instead adjusted for the use

| Group                                      | All-cause mortality |            |                      |            | CVD mortality     |            |                      |            | Infection related mortality |            |                      |            |
|--|---------------------|------------|----------------------|------------|-------------------|------------|----------------------|------------|-----------------------------|------------|----------------------|------------|
|  | crude HR (95% CI)   | p-value    | adjusted HR (95% CI) | p-value    | crude HR (95% CI) | p-value    | adjusted HR (95% CI) | p-value    | crude HR (95% CI)           | p-value    | adjusted HR (95% CI) | p-value    |
| A: RDW $\leq$ 14.9% and MCV $\leq$ 91.6 fL | 1.000               | —          | 1.000                | —          | 1.000             | —          | 1.000                | —          | 1.000                       | —          | 1.000                | —          |
| B: RDW $>$ 14.9% and MCV $\leq$ 91.6 fL    | 2.25(1.35,3.75)     | 0.002*     | 1.44(1.14,2.12)      | 0.02*      | 0.98(0.25,3.93)   | 0.989      | 0.51(0.11,2.25)      | 0.37       | 3.56(1.69,7.49)             | 0.001*     | 2.15(0.95,4.88)      | 0.06       |
| C: RDW $\leq$ 14.9% and MCV $>$ 91.6 fL    | 2.36(1.49,3.75)     | $<$ 0.001* | 2.14(1.31,3.48)      | 0.002*     | 2.40(0.89,6.50)   | 0.08       | 2.62(0.87,7.88)      | 0.08       | 3.08(1.52,6.23)             | 0.002*     | 2.96(1.41,6.22)      | 0.004*     |
| D: RDW $>$ 14.9% and MCV $>$ 91.6 fL       | 7.26(4.54,11.59)    | $<$ 0.001* | 5.06(3.06,8.37)      | $<$ 0.001* | 9.98(3.87,25.75)  | $<$ 0.001* | 10.76(3.48,33.21)    | $<$ 0.001* | 7.56(3.59,15.93)            | $<$ 0.001* | 4.7(2.12,10.4)       | $<$ 0.001* |
| <sup>†</sup> p-value for trend             | $<$ 0.001           |            | $<$ 0.001            |            | $<$ 0.001         |            | $<$ 0.001            |            | $<$ 0.001                   |            | $<$ 0.001            |            |

**Table 2.** Cox proportional hazard models of clinical outcomes for the groups stratified by RDW and MCV. <sup>†</sup>A test for trend was conducted by treating group A to D as a continuous variable. \* $p < 0.05$ .

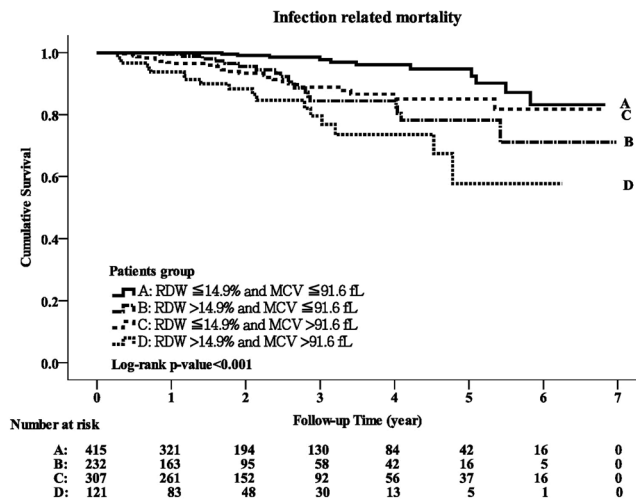


**Figure 2.** Kaplan-Meier curve of cardiovascular disease-related survival according to the groups stratified by MCV and RDW (log-rank test,  $p < 0.001$ ).

with folic acid, iron or vitamin B12. Although the utilization of certain medication does not guarantee appropriate replacement, some authors adopted the similar approaches. For example, the dietary intake of folate, vitamin B12 and iron was recorded for adjustments, rather than their respective serum levels<sup>39</sup>. Medication use of erythropoietin-stimulating agents, iron, folate and multivitamin was adjusted by others<sup>13,17</sup>. Furthermore, the study cohort was selected from the CKD care program in an outpatient setting, and was therefore relatively stable without acute events at the time of enrollment. Third, the lack of CRP for adjustment might bias our results. However, WBC was adjusted as a parameter for systemic inflammation. Finally, the most common cause of mortality was infection, followed by cardiovascular events in our study cohort. The discrepancy between ours and other studies might be attributable to the racial differences, diverse characteristics of study population or delivered healthcare. Nevertheless, the association between the interaction of MCV and RDW and overall mortality remained to be significant, irrespective of cause-specific mortality.

Consistent to our results, the multiplicative effect was also observed by a recent study of a large outpatient elderly population showing that the addition of MCV appears to improve the prognostic value of RDW as a predictor of overall survival<sup>40</sup>. Although the mechanism for our findings is unclear, the concurrent use of RDW and MCV may improve healthcare by identifying those at a greatest risk for mortality compared with the use of either RDW or MCV alone. The determination of MCV and RDW is not associated with any additional cost, and such data are widely available in complete blood cell count reports generated by hematological analyzers, whereas other novel prognostic parameters may be expensive and limited in a clinical setting. Therefore, we suggest that RDW and MCV may be novel risk factors that can be used in everyday clinical practice and should be considered to be additive parameters in risk stratification models for patients with CKD. Further large-scale prospective multi-center studies are required to validate our findings and elucidate the pathophysiologic mechanisms.

**Patients and methods.** This is a retrospective cohort study conducted at a single medical center including patients with moderate to severe CKD. All of the enrolled subjects were diagnosed with CKD based on the National Kidney Foundation K/DOQI guidelines. The severity of CKD was defined by CKD stage according to estimated glomerular filtration rate (eGFR) calculated using the simplified 4-variable Modification of Diet in



**Figure 3.** Kaplan-Meier curve of infection-related survival according to the groups stratified by MCV and RDW (log-rank test,  $p < 0.001$ ).

Renal Disease (MDRD) Study equation. Most of patients participating in the integrated CKD care program at our nephrology outpatient clinic were referred from primary care physicians or non-nephrology specialists for suspected or manifest renal failure. Enrollment criteria included patients with stage 3–5 CKD of at least 20 years old, less than 80 years, and with a follow-up period of at least 3 months. The final study cohort included 1075 patients with stage 3–5 CKD from January 1, 2006 to December 31, 2011. All of the patients were followed up from the index date, defined as the date when they joined the program, until death or the end of 2012. The Institutional Review Board of Changhua Christian Hospital approved the study protocol, and the investigation was carried out in compliance with the declaration of Helsinki. It is not necessary to obtain informed consent from study participants for a retrospective study in Taiwan. All of the data relevant to the patients were anonymized and de-identified before analysis.

Baseline variables at enrollment for each patient were obtained from medical records and a computerized electronic database, and included demographic data, medical history, body mass index (BMI), the underlying etiology of CKD, medical history, medication use, smoking and alcohol status, as well as laboratory data. The medical history consisted of diabetes mellitus (DM), hypertension, hyperlipidemia, coronary artery disease, congestive heart failure, cerebrovascular disease and peripheral artery disease, cancer, dementia, chronic lung disease, and liver cirrhosis. The history of medication use included angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARB), anti-anemia agents (iron preparation, folic acid, and vitamin B12), erythropoiesis stimulating agents (ESA), and vitamin D. The laboratory tests included blood levels of blood urea nitrogen (BUN), creatinine, albumin, white blood cell (WBC) counts, hemoglobin, RDW, MCV, cholesterol, triglyceride, glutamic-pyruvic transaminase (GPT), uric acid, calcium, phosphate, and 24-hour proteinuria. Smoking habit was recorded as current or non-current smokers. Alcohol consumption was classified as never, former or current drinkers.

Complete blood cell counts were measured using an automatic hematology analyzer (DxH 800, Beckman Coulter). The upper normal limit of RDW is 14.9% at our laboratory. The optimal MCV cutoff value was determined to be 91.6 fL in receiver operator characteristic curve (ROC curve) analysis to best predict patient mortality, with a sensitivity of 0.627, specificity of 0.641 and area under the curve of 0.634 (95% CI, 0.587–0.681,  $p < 0.001$ ). In order to assess the influence of the interaction between RDW and MCV on patient survival, the study cohort was categorized into four groups: group A ( $n = 415$ ), RDW  $\leq 14.9\%$  and MCV  $\leq 91.6$  fL; group B ( $n = 232$ ), RDW  $> 14.9\%$  and MCV  $\leq 91.6$  fL; group C ( $n = 307$ ), RDW  $\leq 14.9\%$  and MCV  $> 91.6$  fL; and group D ( $n = 121$ ), RDW  $> 14.9\%$  and MCV  $> 91.6$  fL. Cardiovascular disease (CVD) and infection were the two leading causes of death. Three outcomes were assessed: all-cause mortality, CVD-related mortality, and infection-related mortality.

**Statistical analysis.** The whole study population was divided into four groups based on the optimal MCV value and the upper normal limit of RDW to facilitate the statistical analysis. The baseline characteristics of the study cohort stratified by MCV and RDW were expressed as mean  $\pm$  standard deviation (SD) for numerical variables or number with percentage for categorical data. Comparisons of differences in baseline characteristics between the four groups were assessed using the chi-square test or Fisher's exact test for categorical data, as appropriate. Analysis of variance (ANOVA) or the Kruskal-Wallis test was performed to assess differences in categorical covariates among the four groups.

For comparisons of survival status in the four groups, Kaplan-Meier survival curves were drawn and log-rank tests were performed to determine the statistical significance. Prognostic covariates for clinical outcomes were assessed using Cox proportional hazards models. An adjusted multivariate Cox proportional hazards model was used to determine the independent variables. The adjusted covariates included age, gender, BMI, smoking status,

alcohol status, the causes of CKD, laboratory parameters, medication use, and comorbid conditions. The results of Cox regression analysis were expressed as hazard ratios (HR) and 95% confidence intervals (CI). All statistical analyses were carried out using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY). The results were considered to be statistically significant at a two-tailed p value < 0.05.

## References

- Zachee, P., Vermeylen, J. & Boogaerts, M. A. Hematologic aspects of end-stage renal failure. *Ann. Hematol.* **69**, 33–40 (1994).
- Hammarsten, O., Jacobsson, S. & Fu, M. Red cell distribution width in chronic heart failure: A new independent marker for prognosis? *Eur J Heart Fail* **12**, 213–214 (2012).
- Zalawadiya, S. K. *et al.* Red cell distribution width and mortality in predominantly African-American population with decompensated heart failure. *J Card Fail* **17**, 292–298 (2011).
- Fatmeni, O. *et al.* Red cell distribution width is a predictor of mortality in patients undergoing percutaneous coronary intervention. *J Thromb Thrombolysis* **35**, 57–64 (2013).
- Cavusoglu, E. *et al.* Relation between red blood cell distribution width (RDW) and all-cause mortality at two years in an unselected population referred for coronary angiography. *Int J Cardiol* **141**, 141–146 (2010).
- Zorlu, A. *et al.* Usefulness of red cell distribution width as a predictor of early mortality in patients with acute pulmonary embolism. *Am J Cardiol* **109**, 128–134 (2012).
- Sadaka, F., O'Brien, J. & Prakash, S. Red cell distribution width and outcome in patients with septic shock. *J Intensive Care Med* **28**(5), 307–313 (2013).
- Kim, J. *et al.* Red blood cell distribution width is associated with poor clinical outcome in acute cerebral infarction. *Thromb Haemost* **108**, 349–356 (2012).
- Oh, H. J. *et al.* Red blood cell distribution width is an independent predictor of mortality in acute kidney injury patients treated with continuous renal replacement therapy. *Nephrol Dial Transplant* **27**, 589–594 (2012).
- Cantaro, S. & Piva, E. Hematological and iron parameters to predict mortality in ESRD. *G Ital Nefrol* **31**, S135–S139 (2005).
- Myojo, M. *et al.* Prognostic implication of macrocytosis on adverse outcomes after coronary intervention. *Atherosclerosis* **221**, 148–153 (2012).
- Ueda, T. *et al.* High mean corpuscular volume is a new indicator of prognosis in acute decompensated heart failure. *Circ J.* **77**(11), 2766–2771 (2013).
- Tennankore, K. K., Soroka, S. D., West, K. A. & Kiberd, B. A. Macrocytosis may be associated with mortality in chronic hemodialysis patients: A prospective study. *BMC Nephrol.* **12**, 19 (2011).
- Solak, Y. *et al.* Mean corpuscular volume is associated with endothelial dysfunction and predicts composite cardiovascular events in patients with chronic kidney disease. *Nephrology (Carlton)*. **18**(11), 728–735 (2013).
- Sićaja, M. *et al.* Red blood cell distribution width as a prognostic marker of mortality in patients on chronic dialysis: a single center, prospective longitudinal study. *Croat Med J.* **54**(1), 25–32 (2013).
- Förhercz, Z. *et al.* Red cell distribution width in heart failure: prediction of clinical events and relationship with markers of ineffective erythropoiesis, inflammation, renal function, and nutritional state. *Am Heart J* **158**, 659–666 (2009).
- Peng, F. *et al.* An increasing of red blood cell distribution width was associated with cardiovascular mortality in patients on peritoneal dialysis. *Int J Cardiol.* **176**(3), 1379–1381 (2014).
- Hunziker, S., Celi, L. A., Lee, J. & Howell, M. D. Red cell distribution width improves the simplified acute physiology score for risk prediction in unselected critically ill patients. *Crit Care.* **16**, R89 (2012).
- Kiefer, C. R. & Snyder, L. M. Oxidation and erythrocyte senescence. *Curr Opin Hematol* **7**, 113–116 (2000).
- Weiss, G. & Goodnough, L. T. Anemia of chronic disease. *N Engl J Med* **352**, 1011–1123 (2005).
- Patel, K. V. *et al.* Association of the red cell distribution width with red blood cell deformability. *Adv Exp Med. Biol* **765**, 211–216 (2013).
- Kaferle, J. & Strzoda, C. E. Evaluation of macrocytosis. *Am Fam Physician.* **79**(3), 203–208 (2009).
- Jung, H. A. *et al.* Changes in the mean corpuscular volume after capecitabine treatment are associated with clinical response and survival in patients with advanced gastric cancer. *Cancer Res Treat.* **47**(1), 72–77 (2015).
- Dellapasqua, S. *et al.* Increased mean corpuscular volume of red blood cells predicts response to metronomic capecitabine and cyclophosphamide in combination with bevacizumab. *Breast* **21**, 309–313 (2012).
- Scott, J. M. & Weir, D. G. Drug-induced megaloblastic change. *Clin Haematol* **9**, 587–606 (1980).
- Iacopetta, B., Grieco, F., Joseph, D. & Elsaleh, H. A polymorphism in the enhancer region of the thymidylate synthase promoter influences the survival of colorectal cancer patients treated with 5-fluorouracil. *Br J Cancer* **85**, 827–830 (2001).
- Wenzel, C. *et al.* Capecitabine treatment results in increased mean corpuscular volume of red blood cells in patients with advanced solid malignancies. *Anticancer Drugs.* **14**, 119–123 (2003).
- Zheng, Y. Z. *et al.* Prognostic value of preoperative mean corpuscular volume in esophageal squamous cell carcinoma. *World J Gastroenterol* **19**(18), 2811–2817 (2013).
- Takahashi, N. *et al.* Increase in erythrocyte volume in patients with chronic atrial fibrillation. *Jpn Heart J* **38**, 387–391 (1997).
- Peverill, R. E., Harper, R. W. & Smolich, J. J. Inverse relation of haematocrit to cardiac index in mitral stenosis and atrial fibrillation. *Int J Cardiol* **71**, 149–155 (1999).
- Haltmayer, M. *et al.* Erythrocyte mean corpuscular volume associated with severity of peripheral arterial disease: an angiographic evaluation. *Ann Vasc Surg* **16**, 474–479 (2002).
- Mueller, T. *et al.* Association between erythrocyte mean corpuscular volume and peripheral arterial disease in male subjects: a case control study. *Angiology* **52**, 605–613 (2001).
- Hatamian, H., Saberi, A. & Pourghasem, M. The relationship between stroke mortality and red blood cell parameters. *Iran J Neurol.* **13**(4), 237–40 (2014).
- Tsantes, A. E., Bonovas, S., Travlou, A. & Sitaras, N. M. Redox imbalance, macrocytosis, and RBC homeostasis. *Antioxid Redox Signal* **8**, 1205–1216 (2006).
- Skjelbakken, T. *et al.* Red cell distribution width is associated with incident myocardial infarction in a general population: the Tromsø Study. *J Am Heart Assoc* **3**, 1–10 (2014).
- Mohanty, J. G., Nagababu, E. & Rifkind, J. M. Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. *Front Physiol* **5**, 84 (2014).
- Li, M. & Ikehara, S. Bone-marrow-derived mesenchymal stem cells for organ repair. *Stem Cells Int* **2013**, 132642 (2013).
- Porath-Furedi, A. The mutual effect of hydrogen ion concentration and osmotic pressure on the shape of the human erythrocyte as determined by light scattering and by electronic cell volume measurement. *Cytometry* **4**, 263–267 (1983).
- Eryd, S. A. *et al.* Red blood cell distribution width is associated with incidence of atrial fibrillation. *J Intern Med.* **275**(1), 84–92 (2014).
- Lam, A. P. *et al.* Multiplicative interaction between mean corpuscular volume and red cell distribution width in predicting mortality of elderly patients with and without anemia. *Am J Hematol.* **88**(11), E245–9 (2013).

### Author Contributions

All authors listed have contributed sufficiently to the project to be included as authors, and all those who are qualified to be authors are listed in the author byline. Chia-Chu Chang conceived and designed the experiments. Ping-Fang Chiu performed the experiments. Chew-Teng Kor analyzed the data and wrote the paper. Yao-Peng Hsieh wrote the paper.

### Additional Information

**Competing Interests:** The authors declare that they have no competing interests.

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