

# 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](mailto:info.bmjopen@bmj.com)

# BMJ Open

## HAEMATOLOGICAL PROFILE OF CHRONIC KIDNEY DISEASE IN A MIXED-ANCESTRY SOUTH AFRICAN POPULATION: A CROSS-SECTIONAL STUDY

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-025694
Article Type:	Research
Date Submitted by the Author:	26-Jul-2018
Complete List of Authors:	George, Cindy; South African Medical Research Council, Non-Communicable Diseases Research Unit Matsha, Tandi; Cape Peninsula University of Technology, Department of Biomedical Sciences Erasmus, Rajiv; University of Stellenbosch, Chemical Pathology Kengne, AP; South African Medical Research Council, Non-Communicable Diseases Research Unit; University of Cape Town
Keywords:	Africa, HAEMATOLOGY, Chronic Kidney Disease

SCHOLARONE™  
Manuscripts

1  
2  
3 1 **HAEMATOLOGICAL PROFILE OF CHRONIC KIDNEY DISEASE IN A MIXED-**  
4 **ANCESTRY SOUTH AFRICAN POPULATION: A CROSS-SECTIONAL STUDY**  
5 2  
6  
7 3  
8 4  
9

10 5 Cindy George<sup>1</sup>; Tandi E. Matsha<sup>2</sup>, Rajiv T. Erasmus<sup>3</sup>, Andre P. Kengne<sup>1,4</sup>  
11  
12 6  
13 7  
14

15 8 <sup>1</sup>Non-Communicable Disease Research Unit, South African Medical Research Council, Cape  
16 Town, South Africa; <sup>2</sup>Department of Biomedical Sciences, Faculty of Health and Wellness  
17 9 Science, Cape Peninsula University of Technology, Bellville, Cape Town, South Africa;  
18 10 <sup>3</sup>Division of Chemical Pathology, Faculty of Medicine and Health Sciences, National Health  
19 11 Laboratory Service (NHLS) and University of Stellenbosch, Cape Town, South Africa,  
20 12 <sup>4</sup>Department of Medicine, University of Cape Town, Cape Town, South Africa.  
21  
22  
23  
24  
25  
26  
27  
28

29 16 **Corresponding author:** Cindy George; South African Medical Research Council, Non-  
30 17 Communicable Disease Research Unit, Francie van Zijl Drive, Parow Valley, Cape Town, PO  
31 18 Box 19070, South Africa; +27 21 9380482; [cindy.george@mrc.ac.za](mailto:cindy.george@mrc.ac.za)  
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

21 Word count: 3085

22 Abstract word count: 294

23 References: 50

24 Tables: 3

## 25 ABSTRACT

26 **Objectives:** The objectives were to characterise the haematological profile of screen-detected  
27 chronic kidney disease (CKD) participants and to correlate the complete blood count measures  
28 with the commonly advocated kidney function estimators. **Methods:** The current cross-sectional  
29 study utilized data, collected between February 2015 and November 2016, of 1564 adults of  
30 mixed-ancestry, who participated in the Cape Town Vascular and Metabolic Health study.  
31 Kidney function was estimated using the Modification of Diet in Renal Disease (MDRD) and  
32 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. CKD was defined  
33 as eGFR  $<60\text{ml/min}/1.73\text{m}^2$ , and anaemia as haemoglobin level  $<13.5\text{g/dL}$  (men) and  $<12\text{g/dL}$   
34 (women). **Results:** Based on the MDRD and CKD-EPI equations, the crude prevalence of CKD  
35 was 6% and 3%. Irrespective of the equation used, median red blood cell (RBC) indices were  
36 consistently lower in those with CKD compared to those without CKD (all  $p<0.0001$ ). Despite  
37 not showing any significant difference in total white blood cell (WBC) count between the two  
38 groups, the number of lymphocytes were lower ( $p=0.0001$  and  $p<0.0001$  for MDRD and CKD-  
39 EPI, respectively) and neutrophil count (both  $p\leq 0.0297$ ) and the ratio of lymphocytes to  
40 neutrophil (both  $p<0.0001$ ) higher in the CKD group compared to those without CKD; with the  
41 remaining WBC indices similar in the two groups. The platelet count was similar in both groups.  
42 Of the screen-detected CKD participants, 45.5% (MDRD) and 57.8% (CKD-EPI) were anaemic,  
43 with the prevalence increasing with increasing severity of CKD, from 37.2% (stage 3) to 82.4%  
44 (stages 4-5). Furthermore, CKD-EPI-estimated kidney function, but not MDRD, was positively  
45 associated with RBC indices. **Conclusion:** Though it remains unclear whether common kidney  
46 function estimators provide accurate estimates of CKD in Africans, the correlation of their  
47 estimates with deteriorating RBC profile, suggests that advocated estimators, to some extent  
48 approximate kidney function in African populations.

49  
50 **Key words:** chronic kidney disease; haematology; Africa

### 51 **Strengths and limitations of the study**

- 52 • The first study to characterize the haematological profile of individuals with reduced  
53 kidney function in a population-based setting in Africa, even more specific, individuals of  
54 mixed-ancestry
- 55 • We studied a community with a high burden of obesity, hypertension and diabetes,  
56 reflective of the current burden in Africa.
- 57 • This study was conducted in only one geographical area, which may not adequately  
58 reflect all the mixed ancestry population groups in Sub-Saharan Africa.
- 59 • Our study was based on a single serum creatinine measure to determine CKD and did not  
60 include estimates of albuminuria. Albuminuria, which are required for clinical and  
61 aetiological diagnosis of CKD, as this information is important particularly in the  
62 interpretation of eGFR greater than 60ml/min/1.73m<sup>2</sup> where inaccuracies of the eGFR  
63 equations are greatest

## 82 BACKGROUND

83 Chronic kidney disease (CKD) is a major global public health problem <sup>1</sup>, estimated to affect  
84 more than 10% of the general adult population and up to 50% of some high-risk subpopulations,  
85 such as the elderly <sup>2</sup>, those with non-communicable diseases (NCD), including type 2 diabetes  
86 mellitus (T2D) and hypertension, and communicable diseases (CD), including human  
87 immunodeficiency virus (HIV)/ acquired immunodeficiency syndrome (AIDS) <sup>3 4</sup>. Africa is  
88 currently experiencing the double burden of NCDs and CDs, which are all driving the increasing  
89 burden of CKD on the continent <sup>5</sup>. However, the exact burden of CKD in Africa has yet to be  
90 fully elucidated <sup>6-9</sup>, in part due to the absence of appropriate estimates for predicting reduced  
91 kidney function in individuals from African ancestry <sup>9 10</sup>.

92  
93 CKD encompasses a wide range of physiological processes altered by the progressive decline in  
94 glomerular filtration rate (GFR) <sup>11 12</sup>. Haematological parameters, particularly red blood cell  
95 (RBC) indices, are most commonly affected <sup>13</sup>, giving rise to anaemia. Anaemia is the most  
96 common, consistent and severe of the various haematological abnormalities, and has been shown  
97 to be a very common condition in black Africans <sup>14</sup>. Although anaemia may be found at any  
98 stage of CKD, the severity of anaemia increases with CKD progression <sup>15</sup>, resultantly affecting  
99 nearly all patients with end-stage renal disease (CKD stage 5) <sup>13</sup>. The predominant cause of  
100 anaemia in CKD is failure of the kidneys to produce enough endogenous erythropoietin, which  
101 accompanies the fall in GFR <sup>16 17</sup>. Untreated, prolonged anaemia is strongly predictive of all-  
102 cause and cardiovascular mortality, as well as reduced quality of life and increased morbidity in  
103 patients with CKD <sup>13 18</sup>. Untreated anaemia can also accelerate the decline in renal function by  
104 causing renal haemodynamic alterations and tissue hypoxia <sup>15</sup>. Other potentially affected  
105 haematological parameters in CKD, of which the association with CKD is not yet fully  
106 characterized, include total and differential white blood cell (WBC) counts. Persistent, low-grade  
107 inflammation is an essential part of the aetiology of CKD and has been recognized as such since  
108 the late 1990s, when it was linked to cardiovascular disease (CVD) and mortality <sup>19</sup>. Recently,  
109 the ratio of neutrophil-to-lymphocyte count (N/L) has been proposed as a novel measure of  
110 inflammation in distinct populations and has been shown to have prognostic value <sup>20</sup>; particularly  
111 for mortality risk in patients with myocardial infarction and heart failure <sup>21 22</sup>. However, studies  
112 on the relationship of N/L ratio with reduced eGFR are limited <sup>23</sup>. Thus, despite recent advances

1  
2  
3 113 in the aetiology governing the development and progression of CKD, population-based data on  
4 114 the haematological profile of people with CKD in Africa, are scanty.

5  
6 115  
7  
8 116 We therefore aimed to characterise the haematological profile of screen-detected CKD  
9 117 participants in a community-based sample, and to correlate the complete blood count measures  
10 118 with two commonly advocated kidney function estimators of CKD in urban South Africans of  
11 119 mixed-ancestry.  
12  
13  
14

15 120

## 16 121 **METHODS**

### 17 122 **Study setting and population**

18  
19 123 The current study utilized data from the ongoing Cape Town Vascular and Metabolic Health  
20 124 (VMH) study, an extension of the Cape Town Bellville-South study, which has been described in  
21 125 detail previously<sup>24</sup>. Bellville-South, with a population of approximately 29,301, is a township  
22 126 formed in the late 1950s, located in the metropolitan city of Cape Town, South Africa. The  
23 127 population consists predominantly of individuals of mixed-ancestry (coloured) (76%), followed  
24 128 by black Africans (18.5%), with only 1.5% of the population being of Caucasian and Asians  
25 129 ancestry. The data collection for the current analysis took place between February 2015 and  
26 130 November 2016 during a community-based survey involving only mixed-ancestry South  
27 131 Africans. The study was approved by the Research Ethics Committees of the Cape Peninsula  
28 132 University of Technology and Stellenbosch University (NHREC: REC—230 408–014 and  
29 133 N14/01/003, respectively). The study was conducted in accordance with the Declaration of  
30 134 Helsinki. All participants voluntarily signed written informed consent after all the procedures were  
31 135 fully explained in the language of their choice.  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42

43 136

### 44 137 **Participant involvement**

45 138 The participants were not involved in the design or recruitment process of this study. However,  
46 139 permission to conduct the study was obtained from relevant authorities including the city and  
47 140 community authorities.  
48  
49  
50

51 141

### 52 142 **Questionnaires and physical examination**

53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 143 All interviews and physical examinations took place at a research clinic on the campus of Cape  
4 Peninsula University of Technology, located within the study suburb. All consenting participants  
5 144 received a standardized interview, explained in great detail elsewhere <sup>25</sup>. Physical examination  
6 145 involved blood pressure (BP) determination, measured according to the World Health  
7 146 Organisation (WHO) guidelines <sup>26</sup>, using a semi-automatic digital blood pressure monitor  
8 147 (Omron M6 comfort-preformed cuff BP Monitor), placed on the right arm in sitting position and  
9 148 at rest for at least 10 min. Three measures were taken of which the average of the lowest two was  
10 149 used in all analyses. Body weight (to the nearest 0.1 kg) was measured with the participant in  
11 150 light clothing and without shoes, using an Omron body fat meter HBF-511 digital bathroom  
12 151 scale, which was calibrated and standardized using a weight of known mass. Height (to the  
13 152 nearest centimetre) was measured with a stadiometer, with subjects standing on a flat surface.  
14 153 Body mass index (BMI) was calculated as weight per square meter (kg/m<sup>2</sup>). Waist circumference  
15 154 (WC) was measured with a non-elastic tape measure at the level of the narrowest part of the  
16 155 torso, as seen from the anterior view. Anthropometric measurements were performed three times  
17 156 and the average used for analysis.  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30

### 31 159 **Biochemical analysis and calculations**

32 160 All biochemical analyses took place at an ISO 15189 accredited Pathology practice (Path-Care,  
33 161 Reference Laboratory, Cape Town, South Africa). Blood samples were collected from all  
34 162 participants after an overnight fast, and two hours after a 75g oral glucose tolerance test (OGTT)  
35 163 following the WHO recommendations <sup>27</sup>. Plasma glucose levels and haemoglobin A1c (HbA1c)  
36 164 were measured by enzymatic hexokinase method (Beckman AU, Beckman Coulter, South  
37 165 Africa) and high performance liquid chromatography (Biorad Variant Turbo, BioRad, South  
38 166 Africa), respectively. Insulin was determined by a paramagnetic particle chemiluminescence  
39 167 assay (Beckman DXI, Beckman Coulter, South Africa). Triglycerides (TG), total cholesterol  
40 168 (TC), and high-density lipoproteins (HDL-C) were analysed using the Roche Modular auto  
41 169 analyser and enzymatic colorimetric assays, and low-density lipoproteins (LDL-C) were  
42 170 calculated using the Friedewald formula <sup>28</sup>. The homeostatic model assessment of insulin  
43 171 resistance (HOMA-IR) was calculated according to the formula: HOMA-IR = [fasting insulin  
44 172 concentration (mIU/l) × fasting plasma glucose (mmol/l)/22.5. Serum concentration of high  
45 173 sensitivity C-reactive protein (hsCRP) (Immun Diagnostik AG, Bensheim, Germany) was  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 174 analysed using commercially available ELISA kits according to the manufacturer's protocols.  
4  
5 175 Serum creatinine was measured by the modified Jaffe-Kinetic method (Beckman AU, Beckman  
6  
7 176 Coulter, South Africa). Creatinine assays at our Partner pathology service are standardized to the  
8  
9 177 internationally accepted reference method (isotope dilution mass spectrophotometry [IDMS])  
10  
11 178 since 2009 and eGFR estimators applicable to standardised creatinine values were used. Kidney  
12  
13 179 function was assessed using serum creatinine-based estimators of glomerular filtration rate  
14  
15 180 (eGFR), namely, the 4-variable Modification of Diet in Renal Disease (MDRD) equation<sup>29</sup> and  
16  
17 181 the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation<sup>30</sup>. The African-  
18  
19 182 American ethnicity correction factor was omitted from the eGFR calculation, as the South  
20  
21 183 African Renal Society CKD guidelines promotes the exclusion of the correction factor, except in  
22  
23 184 the case of black Africans. Full blood counts, including total RBC, total WBC, lymphocytes  
24  
25 185 count and percentage, monocyte count and percentage, neutrophil count and percentage, basophil  
26  
27 186 count and percentage, eosinophil count and percentage, haemoglobin (Hb), haematocrit, mean  
28  
29 187 corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular  
30  
31 188 haemoglobin concentration (MCHC), red cell distribution width, and platelets, were measured on  
32  
33 189 a Coulter LH 750 hematology analyzer (Beckman Coulter, South Africa).

### 34 191 **Classification of renal function and co-morbidities**

35 192 Staging of kidney function was based on the National Kidney Foundation Disease Outcomes  
36  
37 193 Quality Initiative (NKF-KDOQI) classification<sup>31</sup>. An eGFR<60 ml/min/1.73 m<sup>2</sup> was used to  
38  
39 194 define CKD (or CKD stage 3–5). Anaemia was defined using the National Kidney Foundation  
40  
41 195 Kidney Disease Outcome Quality Initiative (K/DOQI) guidelines (haemoglobin level <13.5g/dL  
42  
43 196 for men and <12g/dL for women)<sup>32</sup> and further classified into micro-, normo- and macrocytic  
44  
45 197 based on the MCV. Microcytic anaemia was defined as an MCV of <80fL, normocytic as 100-80  
46  
47 198 fL, and macrocytic as >100fL<sup>33</sup>. Hypertension was based on either a history of diagnosed  
48  
49 199 hypertension (receiving medications for hypertension) or screen-detected hypertension. The  
50  
51 200 latter being classified if they had a SBP≥140mmHg and/or DBP≥90 mmHg<sup>34</sup>. Diabetes status  
52  
53 201 was based on a history of diagnosed diabetes or screen-detected diabetes. OGTT glucose values  
54  
55 202 were used to classify the glucose tolerance status of participants as recommended by WHO<sup>35</sup> as:  
56  
57 203 (1) normal glucose tolerance (fasting plasma glucose (FPG) <6.1 mmol/l and 2-h glucose <7.8  
58  
59 204 mmol/l); (2) pre-diabetes including impaired fasting glycaemia (IGT, 6.1≤FPG< 7.0 mmol/l),

1  
2  
3 205 impaired glucose tolerance (IGT,  $7.8 < 2\text{-h glucose} < 11.1$  mmol/l) and the combination of both;  
4 206 and (3) diabetes ( $\text{FPG} \geq 7.0$  mmol/l and/or  $2\text{-h glucose} \geq 11.1$  mmol/l). A  $\text{BMI} \geq 25 \text{ kg/m}^2$  and  
5 207  $\text{BMI} \geq 30 \text{ kg/m}^2$  was classified as overweight obese, respectively.  
6  
7  
8  
9

## 10 209 **Statistical analysis**

11 210 All statistical analyses were performed using STATA version 13 (Statcorp, College Station, TX)  
12 211 and statistical significance was based on a p-value  $< 0.05$ . General characteristics of the  
13 212 participants are summarized as count and percentage for qualitative variables and median and  
14 213 25th-75th percentiles for quantitative variables. Group comparisons used chi-squared test for  
15 214 qualitative variables, and Wilcoxon rank-sum test for quantitative variables, respectively.  
16 215 Multiple linear regression models were used to assess the independent association between eGFR  
17 216 and haematological indices, while adjusting for age and gender.  
18  
19  
20  
21  
22  
23  
24

## 25 218 **RESULTS**

### 26 219 **Participant characteristics**

27 220 The initial study sample comprised 1,647 participants. Of those, 83 were excluded due to  
28 221 missing data on serum creatinine or any of the variables required to estimate kidney function,  
29 222 including age and gender. The general characteristics and the haematological profile of the study  
30 223 population are summarised in Tables 1 and 2, respectively. The final sample included 1,564  
31 224 participants, of which 24.9% were male, with a group median age of 50 years. The crude  
32 225 prevalence of CKD was 6% and 3%, based on the MDRD and CKD-EPI equations respectively.  
33 226 Of those participants with MDRD-diagnosed CKD, 80.7%, 14.8% and 4.5% were in stages 3, 4  
34 227 and 5, respectively. Similarly, of those diagnosed by means of the CKD-EPI equation, 68.9%,  
35 228 24.4% and 6.7% were in stages 3, 4 and 5, respectively. MDRD-diagnosed CKD participants  
36 229 had higher creatinine levels (111.5 vs. 59  $\mu\text{mol/l}$ ;  $p < 0.0001$ ) and lower eGFR (48.2 vs. 104  
37 230  $\text{ml/min/1.73m}^2$ ;  $p < 0.0001$ ), were on average older (68 vs. 49 years;  $p < 0.0001$ ), with a higher WC  
38 231 (97.7 vs. 91.2 cm;  $p = 0.0001$ ), BMI (30.3 vs. 28.3  $\text{kg/m}^2$ ;  $p = 0.0096$ ), and SBP (142 vs. 125  
39 232 mmHg;  $p < 0.0001$ ), compared to participants with normal kidney function. Furthermore, MDRD-  
40 233 diagnosed CKD participants had higher fasting and 2-hour blood glucose (5.3 vs. 5.0 mmol/l;  
41 234  $p < 0.0001$  and 7.2 vs. 6.0 mmol/l;  $p < 0.0001$ , respectively), HbA1c levels (6.2 vs. 5.7%;  
42 235  $p < 0.0001$ ), fasting and 2-hour insulin levels (8.4 vs. 6.7 IU/l;  $p = 0.0089$  and 62.0 vs. 37.5 IU/l;  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 236 p=0.0002, respectively), higher HOMA-IR index (2.1 vs. 1.6; p=0.0004), hsCRP (4.7 vs. 4.0  
4 237 µg/ml; p=0.0492), TG (1.6 vs. 1.2 mmol/l; p<0.0001) and TC (5.4 vs. 5.1 mmol/l; p=0.024);  
5 238 with similar LDL-C (3.2 vs. 3.1 mmol/l; p=0.0668) and HDL-C levels (1.3 vs. 1.3 mmol/l;  
6 239 p=0.7106) compared to those without CKD. When sub-dividing the groups based on CKD  
7 240 diagnosed by the CKD-EPI equation, similar differences were observed, with the exception of  
8 241 BMI, hsCRP and TC, which showed no difference between the groups (28.3 vs. 28.4 kg/m<sup>2</sup>;  
9 242 p=0.384, 4.8 vs. 4.0 µg/ml; p=0.4268, 5.3 vs. 5.1 mmol/l; p=0.2226, respectively). Participants  
10 243 with reduced kidney function, both MDRD and CKD-EPI-diagnosed, had a similar prevalence of  
11 244 overweight and obesity, however had a higher prevalence of hypertension and T2D, despite  
12 245 similar prevalence of pre-diabetes (IFG and IGT) between the two groups.  
13  
14  
15  
16  
17  
18  
19  
20  
21

22 247 The red blood cell indices, including RBC count, haematocrit and haemoglobin levels were  
23 248 consistently lower in CKD participants compared to the group with normal kidney function (all  
24 249 p<0.0001), irrespective of the eGFR equation used. Conversely, the morphology of the RBC's  
25 250 were not different, as similar values for MCV, MCH, MCHC and RDW were observed between  
26 251 CKD participants and the participants with normal kidney function. Despite not showing any  
27 252 significant difference in total WBC count between the two groups, the number of lymphocytes  
28 253 were lower and neutrophil count and the ratio of lymphocytes to neutrophil higher in the CKD  
29 254 group compared to those individuals with normal kidney function; with the remaining WBC  
30 255 indices similar in the two groups. The platelet count was similar in both groups. Furthermore,  
31 256 based on the K/DOQI guidelines, 45.5% (MDRD) and 57.8% (CKD-EPI) of the CKD  
32 257 participants had anaemia, with the majority of cases being normocytic. Moreover, the prevalence  
33 258 of anaemia increased with increasing severity of CKD, from 37.2% at stage 3 to 82.4%  
34 259 at stage 4-5.  
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

267 **Table 1:** Clinical characteristics of the study population overall and by CKD (MDRD and CKD-EPI) status

Variables	Total (n=1564)	MDRD			CKD-EPI		
		Without CKD (n=1470)	CKD (n=94)	p-value	Without CKD (n=1517)	CKD (n=47)	p-value
Age (years)	50 (37-61)	49 (36-59)	68 (62-74)	<0.0001	50 (36-60)	69 (63-77)	<0.0001
Gender (n,% male)	389 (24.9)	372 (25.3)	17 (18.1)	0.215	373 (24.6)	16 (34.0)	0.093
<b>Anthropometry</b>							
Weight (kg)	72.0 (59.2-85.5)	71.9 (59.0-85.5)	74.0 (64.6-85.8)	0.2058	72.0 (59.2-85.5)	73.5 (64.1-85.7)	0.6903
WC (cm)	91.8 (78.5-103.5)	91.2 (77.8-103.0)	97.7 (89.0-105.8)	0.0001	91.5 (78.1-103.5)	96.0 (87.8-106.5)	0.0225
HC (cm)	102.8 (92.5-113.5)	102.5 (92.1-113.5)	104.3 (96.5-114.2)	0.1138	102.8 (92.5-113.8)	101.5 (95.8-111.5)	0.9439
BMI (kg/m <sup>2</sup> )	28.4 (22.9-34.2)	28.3 (22.7-34.1)	30.3 (26.1-35.1)	0.0096	28.4 (22.9-34.2)	28.3 (24.7-34.4)	0.3836
<b>Biochemical analysis</b>							
Fasting blood glucose (mmol/l)	5.0 (4.6-5.7)	5.0 (4.6-5.6)	5.3 (5.0-6.9)	<0.0001	5.0 (4.6-5.6)	5.3 (5.0-7.7)	0.0014
2-hour glucose (mmol/l)	6.0 (4.9-7.6)	6.0 (4.8-7.5)	7.2 (5.8-9.2)	<0.0001	6.0 (4.8-7.5)	7.5 (5.7-9.2)	0.0034
HbA1c (%)	5.8 (5.4-6.3)	5.7 (5.4-6.2)	6.2 (5.9-7.1)	<0.0001	5.8 (5.4-6.2)	6.4 (5.9-7.3)	<0.0001
Fasting insulin (IU/l)	6.7 (4.3-11.1)	6.7 (4.2-10.9)	8.4 (5.3-12.4)	0.0089	6.7 (4.2-10.9)	9.0 (5.3-12.4)	0.0323
2-hour insulin (IU/l)	38 (20.6-71.8)	37.5 (19.8-69.8)	62.0 (30.3-105.6)	0.0002	37.8 (20.3-70.5)	63.5 (32.6-105.2)	0.0072
HOMA-IR (MU)	1.6 (0.9-2.9)	1.6 (0.9-2.8)	2.1 (1.2-3.9)	0.0004	1.6 (0.9-2.8)	2.4 (1.3-3.8)	0.0026
hsCRP (µg/ml)	4.0 (1.6-8.8)	4.0 (1.6-8.8)	4.7 (2.7-9.3)	0.0492	4.0 (1.6-8.8)	4.8 (2.4-7.5)	0.4268
TG (mmol/l)	1.2 (0.9-1.7)	1.2 (0.9-1.7)	1.6 (1.2-2.3)	<0.0001	1.2 (0.9-1.7)	1.8 (1.1-2.4)	0.0001
TC (mmol/l)	5.1 (4.4-5.9)	5.1 (4.3-5.9)	5.4 (4.8-6.4)	0.0024	5.1 (4.4-5.9)	5.3 (4.4-6.0)	0.2226
LDL-C (mmol/l)	3.1 (2.5-3.8)	3.1 (2.5-3.8)	3.2 (2.7-4.3)	0.0668	3.1 (2.5-3.8)	3.1 (2.5-3.9)	0.9444
HDL-C (mmol/l)	1.3 (1.1-1.5)	1.3 (1.1-1.5)	1.3 (1.1-1.5)	0.7106	1.3 (1.1-1.5)	1.3 (1.1-1.4)	0.5132
Creatinine (µmol/l)	60 (52-70)	59 (51-68)	111.5 (89.0-140.5)	<0.0001	59 (51-69)	140 (124-209)	<0.0001
eGFR (ml/min/1.73m <sup>2</sup> )	-	104.0 (88.0-121.0)	48.2 (33.7-55.4)	<0.0001	113.9 (101.4-126.5)	44.7 (26.4-49.6)	<0.0001

<b>Blood pressure measures</b>							
Mean SBP (mmHg)	125 (111-141)	125 (110-140)	142 (121-162)	<0.0001	125 (111-140)	150 (128-181)	<0.0001
Mean DBP (mmHg)	81 (72-90)	81 (72-90)	81 (74-95)	0.2114	81 (72-90)	85 (73-95)	0.2185
Pulse pressure (BPM)	70 (62-79)	70 (62-79)	70 (60-81)	0.9932	70 (62-79)	73 (62-82)	0.3861
<b>Co-morbidities</b>							
Overweight (BMI $\geq$ 25kg/m <sup>2</sup> ; n,%)	361 (23.2)	335 (22.9)	26 (29.5)	0.139	348 (23.1)	13 (28.9)	0.348
Obese (BMI $\geq$ 30kg/m <sup>2</sup> ; n,%)	662 (42.6)	617 (42.1)	45 (51.1)	0.085	642 (42.5)	20 (44.4)	0.771
Pre-diabetes (n, %)	238 (15.2)	226 (15.4)	12 (12.8)	0.671	233 (15.4)	5 (10.6)	0.436
T2D (n, %)	297 (19.0)	259 (17.6)	38 (40.4)	<0.0001	272 (17.9)	25 (53.2)	<0.0001
Hypertension (n, %)	567 (36.3)	517 (35.2)	50 (53.2)	<0.0001	537 (35.4)	30 (63.3)	<0.0001

268

269 Data is presented as median (25th-75<sup>th</sup> percentiles) and percentages. WC, waist circumference; HC, hip circumference; BMI, body  
 270 mass index; HbA1c, Glycated haemoglobin; HOMA-IR, Homeostatic model assessment-insulin resistance; MU, mass units; hsCRP,  
 271 high sensitivity C-reactive protein; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoproteins; HDL-C, high-density  
 272 lipoproteins; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; IFG/IGT,  
 273 impaired fasting glucose and impaired glucose tolerance; T2D, type 2 diabetes mellitus; MDRD, Modification of Diet in Renal  
 274 Disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CKD, chronic kidney disease.

275

276

277

278

279

280

281

282 **Table 2:** Haematological profile of study population overall and by CKD (MDRD and CKD-EPI) status

283

Variables	Total (n=1564)	MDRD			CKD-EPI		
		Without CKD (n=1470)	CKD (n=94)	p-value	Without CKD (n=1517)	CKD (n=47)	p-value
RBC (x10 <sup>6</sup> /μl)	4.7 (4.3-5.0)	4.7 (4.4-5.0)	4.3 (3.9-4.7)	<0.0001	4.7 (4.4-5.0)	4.2 (3.8-4.7)	<0.0001
WBC (x10 <sup>6</sup> /μl)	7.5 (6.2-9.1)	7.4 (6.2-9.1)	7.7 (6.5-9.2)	0.5704	7.4 (6.2-9.1)	7.9 (6.3-9.3)	0.5458
N/L (ratio)	2.0 (1.5-2.6)	1.9 (1.5-2.5)	2.5 (1.7-3.5)	<0.0001	1.9 (1.5-2.5)	2.7 (2.0-3.7)	<0.0001
Lymphocyte count (x10 <sup>9</sup> /l)	2.2 (1.8-2.80)	2.2 (1.8-2.8)	1.9 (1.4-2.5)	0.0001	2.2 (1.8-2.8)	1.8 (1.4-2.4)	<0.0001
Monocyte count (x10 <sup>9</sup> /l)	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.4 (0.4-0.6)	0.1389	0.5 (0.4-0.6)	0.4 (0.4-0.6)	0.9446
Neutrophil count (x10 <sup>9</sup> /l)	4.5 (3.4-5.7)	4.5 (3.3-5.6)	5.0 (3.7-5.9)	0.0255	4.5 (3.4-5.6)	5.1 (4.3-6.1)	0.0297
Basophil count (x10 <sup>9</sup> /l)	0.1 (0.1-0.2)	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.283	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.1366
Eosinophil count (x10 <sup>9</sup> /l)	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.1579	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.1223
Platelet count (x10 <sup>9</sup> /l)	271 (227-322)	271 (228-322)	277 (214-324)	0.9417	271 (228-322)	266 (197-313)	0.2211
Haematocrit (volume %)	41 (39-44)	41 (39-44)	38 (35-41)	<0.0001	41 (39-44)	37 (34-41)	<0.0001
MCV (fl/cell)	89 (85-93)	89 (85-93)	89 (86-92)	0.8150	89 (85-93)	89 (86-91)	0.4748
MCH (pg/cell)	29 (28-31)	29 (28-31)	29 (28-30)	0.1399	29 (28-31)	29 (28-30)	0.057
MCHC (g/dl)	33 (32-33)	33 (32-33)	33 (32-33)	0.1471	33 (32-33)	32 (32-33)	0.1156
RDW (%)	14.2 (13.5-15.0)	14.1 (13.4-15.0)	14.5 (13.7-15.6)	0.0601	14.1 (13.4-15.0)	14.3 (13.8-15.5)	0.0673
Hb (g/dl)	13.5 (12.6-14.4)	13.5 (12.7-14.5)	12.2 (11.2-13.3)	<0.0001	13.5 (12.6-14.4)	11.9 (11.1-13.2)	<0.0001
Anaemia (n, %)	289 (18.48)	249 (16.9)	40 (45.5)	<0.0001	263 (17.3)	26 (57.8)	<0.0001
Microcytic	83 (28.7)	83 (33.3)	0 (0.0)	-	83 (31.6)	0 (0.0)	-
Normocytic	180 (62.3)	141 (56.6)	39 (97.5)	-	155 (58.9)	25 (96.2)	-
Macrocytic	26 (9.0)	25 (10.0)	1 (2.5)	-	25 (9.5)	1 (3.8)	-

284

285 Data are presented as median (25th-75<sup>th</sup> percentiles) and percentages. RBC, red blood cells; WBC, white blood cells; N/L ratio,  
 286 lymphocyte to neutrophil ratio; MCV, mean corpuscular volume, MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular

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

287 haemoglobin concentration; RDW, red cell distribution width; Hb, haemoglobin. MDRD, Modification of Diet in Renal Disease;  
288 CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

For peer review only

### 307 **Association between the different haematological indices and eGFR**

308 The age and gender-adjusted associations between the different haematological indices and  
309 eGFR, estimated by means of the MDRD and CKD-EPI equations, are presented in Table 3.  
310 Based on the CKD-EPI, however not the MDRD equation, eGFR was positively associated with  
311 all the RBC indices, including total RBC count, haemoglobin and haematocrit levels. eGFR was  
312 not associated with total WBC count, however a lower lymphocyte count was associated with a  
313 lower eGFR and N/L ratio was inversely associated with eGFR. Furthermore, male gender was  
314 significantly associated with all haematological measures, except basophil count and eosinophil  
315 count, and age was inversely associated with all RBC indices, lymphocytes, neutrophils, platelet  
316 count, MCHC and positively associated with RDW.

317  
318  
319  
320  
321  
322  
323  
324  
325  
326  
327  
328  
329  
330  
331  
332  
333  
334



**Table 3:** Linear regression coefficients, adjusted for age, gender (Model 1) and eGFR (MDRD and CKD-EPI-derived) (Models 2) for the prediction of haematological-derived measures

Haematological-derived measures	MODEL 1							MODEL 2.1				MODEL 2.2			
	Age			Gender			R <sup>2</sup>	eGFR (MDRD)			R <sup>2</sup>	eGFR (CKD-EPI)			R <sup>2</sup>
	β	95% CI	p	β	95% CI	p		β	95% CI	p		β	95% CI	p	
RBC (x10 <sup>3</sup> /μl)	-2.8	-4.5 to -1.2	0.001	327.4	269.6 to 385.3	<0.0001	0.08	0.3	-0.7 to 1.3	0.541	0.08	3.2	1.5 to 5.0	<0.0001	0.09
Haematocrit (%)	-0.2	-0.3 to -0.0	0.018	40.2	35.3 to 45.1	<0.0001	0.15	0.0	-0.1 to 0.1	0.709	0.15	0.3	0.1 to 0.4	<0.0001	0.16
Hb (g/l)	-0.1	-0.1 to -0.0	0.002	14.2	12.5 to 15.9	<0.0001	0.16	0.0	-0.0 to 0.0	0.907	0.16	0.1	0.0 to 0.1	<0.0001	0.16
WBC (x10 <sup>3</sup> /μl)	-15.1	-22.3 to -7.8	<0.0001	-431.9	-690.8 to -173.0	0.001	0.01	-0.5	-4.8 to 3.9	0.834	0.01	-1.7	-9.7 to 6.3	0.678	0.01
N/L (%)	-0.1	-3.8 to 3.5	0.941	136.2	5.6 to 266.7	0.041	0.00	-0.1	-0.4 to 0.1	0.214	0.00	-6.3	-10.3 to -2.3	0.002	0.01
Lymphocyte count (x10 <sup>6</sup> /l)	-2.9	-5.2 to -0.5	0.017	-257.10	-341.0 to -173.2	<0.0001	0.02	0.7	-0.8 to 2.1	0.364	0.02	3.0	0.4 to 5.6	0.022	0.03
Monocyte count (x10 <sup>6</sup> /l)	-0.8	-1.4 to -0.2	0.005	91.6	71.2 to 112.0	<0.0001	0.05	0.3	-0.1 to 0.6	0.114	0.05	0.5	-0.1 to 1.1	0.122	0.05
Neutrophil count (x10 <sup>6</sup> /l)	-10.9	-16.8 to -5.1	<0.0001	-291.8	-500 to -82.8	0.006	0.01	-1.1	-4.6 to 2.4	0.542	0.01	-4.7	-11.1 to 1.7	0.150	0.01
Basophil count (x10 <sup>6</sup> /l)	1.6	-8.4 to 11.5	0.759	-187.9	-541.9 to 166.1	0.298	0.00	0.7	-5.3 to 6.6	0.822	0.00	-8.3	-19.2 to 2.6	0.136	0.00
Eosinophil count (x10 <sup>6</sup> /l)	-0.5	-1.1 to 0.0	0.067	15.9	-4.9 to 36.7	0.135	0.00	-0.4	-0.7 to 0.0	0.071	0.00	-0.6	-1.2 to 0.1	0.074	0.00
Platelet count (x10 <sup>9</sup> /l)	-0.4	-0.6 to -0.1	0.003	-33.0	-42.0 to -24.0	<0.0001	0.03	0.1	-0.0 to 0.3	0.088	0.04	0.1	-0.0 to 0.3	0.088	0.04
MCV (fL/100cell)	1.4	-1.0 to 3.7	0.255	232.2	148.1 to 316.2	<0.0001	0.02	-0.2	-1.6 to 1.2	0.761	0.02	0.1	-2.5 to 2.7	0.946	0.02
MCH (pg/100cell)	-0.2	-1.1 to 0.7	0.698	95.3	63.3 to 127.4	<0.0001	0.02	-0.1	-0.7 to 0.4	0.646	0.02	0.1	-0.9 to 1.1	0.881	0.02
MCHC (g/l)	-0.1	-0.01 to -0.0	<0.0001	2.3	0.9 to 3.8	0.002	0.02	-0.0	-0.0 to 0.0	0.227	0.02	-0.0	-0.1 to 0.0	0.664	0.01
RDW (%)	0.1	0.0 to 0.1	0.004	-1.9	-3.7 to -0.0	0.05	0.01	0.1	0.0 to 0.1	<0.0001	0.02	0.1	0.0 to 0.1	0.025	0.01

337  
 338 Data presented as β-coefficient, 95% confidence interval and adjusted-R<sup>2</sup>. Analysis are adjusted for age and gender. RBC, red blood  
 339 cells; WBC, white blood cells; MCV, L/N ratio, lymphocyte to neutrophil ratio; mean corpuscular volume, MCH, mean corpuscular  
 340 haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width; Hb, haemoglobin. MDRD,  
 341 Modification of Diet in Renal Disease. Model 1 = age + gender; Model 2.1 = age + gender + eGFR (MDRD); Model 2.2 = age +  
 342 gender + eGFR (CKD-EPI)

## DISCUSSION

In this community-based sample of mixed-ancestry South Africans, we have shown that the haematological profile of individuals with reduced eGFR ( $<60\text{ml}/\text{min}/1.73\text{m}^2$ ) are substantially impaired compared to those with normal kidney function, giving rise to the high prevalence of anaemia in this screen-detected CKD population. Furthermore, despite eGFR being positively associated with RBC indices, indicative of the severity of kidney function impairment, the disease state had no effect on the morphology of the RBC. Lastly, we confirmed that a chronic pro-inflammatory state exists in participants with CKD.

This study, which is in accordance with other studies in Africa and other developing countries<sup>36-42</sup>, has shown that CKD is associated with significant impairment in RBC indices. Indeed, we have shown that total RBC count, haemoglobin concentration and percentage haematocrit were substantially reduced in participants with eGFR below  $60\text{ml}/\text{min}/1.73\text{m}^2$ , compared to those with normal kidney function, independent of age and gender. Since erythropoietin is produced mainly by the proximal tubule of the nephron, kidney function decline will result in a decline in erythropoietin production and as a consequence result in decreased haemoglobin synthesis, leading to a fall in total RBC count<sup>17</sup>. This significant reduction in RBC, inevitably gives rise to anaemia<sup>14</sup>. Indeed, our study and numerous other studies have shown that the severity of anaemia increases with disease progression; with most of these studies showing anaemia at least twice as prevalent in participants with CKD, compared to the general adult population<sup>37</sup>. Furthermore, we found that 17% of the sample population with normal kidney function had haemoglobin levels  $<13.5\text{g}/\text{dL}$  and  $<12\text{g}/\text{dL}$  for men and women, respectively. However, this is not uncommon in Africa as previous studies have found that Africa has a high prevalence of anaemia caused by iron deficiency. In South Africa in particular, the South African National Health and Nutrition Examination Survey (SANHANES-1)<sup>43</sup> showed that 22% and 12.2% of adult females and males have anaemia.

The activation of the immune system, caused by inflammation, increases white blood cell counts<sup>23</sup>; emphasising the potential of WBC indices as a surrogate marker of inflammation in CKD<sup>20</sup>. Our study showed that despite no correlation between total WBC and reduced kidney function, CKD was associated with higher neutrophil and lower lymphocyte counts; both of which are

1  
2  
3 374 independently associated with the promotion of atherosclerosis<sup>44 45</sup> and poor cardiovascular  
4 375 outcomes<sup>46</sup>. N/L ratio, which combines the predictive power of both increased neutrophil count  
5 376 and decreased lymphocyte count<sup>47</sup>, was associated with reduced eGFR in our study, as also  
6 377 found in other studies<sup>23 48 49</sup>. Indeed previous studies, which included CKD patients on  
7 378 haemodialysis<sup>23 48</sup> and pre-dialysis<sup>49</sup>, showed that an increased N/L ratio was associated with  
8 379 known inflammatory markers such as tumor necrosis factor (TNF)- $\alpha$ <sup>23</sup>, interleukin 6 (IL-6) and  
9 380 high-sensitivity C-reactive protein (hs-CRP) levels<sup>49</sup>. These studies demonstrated that these  
10 381 well-established markers of inflammation were independent factors for predicting N/L ratio, thus  
11 382 presenting N/L ratio as an inflammatory biomarker for CKD patients. Since full blood count  
12 383 analysis are done routinely and a relatively affordable and easy measure to acquire, these  
13 384 findings are especially valuable taking into account the severely resource limited setting found in  
14 385 Africa and other low and middle-income countries.

15 386  
16  
17 387 Our study has a few limitations. This study was conducted in only one geographical area, which  
18 388 may not adequately reflect all the mixed ancestry population groups in Sub-Saharan Africa.  
19 389 Furthermore, this was a community-based sample with high female to male participation,  
20 390 however the latter being a common trend in South African population studies. Our study also  
21 391 used a single serum creatinine measure to determine the grade of kidney function and did not  
22 392 include estimates of albuminuria. Albuminuria, in particular, is required for clinical and  
23 393 aetiological diagnosis of CKD, as this information is important particularly in the interpretation  
24 394 of eGFR greater than 60ml/min/1.73m<sup>2</sup> where inaccuracies of the eGFR equations are greatest. It  
25 395 is however a common practice in community-based studies to diagnose CKD using a single  
26 396 measurement of serum creatinine. Furthermore, we did not investigate other haematologic  
27 397 deficiencies, such as vitamin B12 and iron deficiencies, which if present however, are less likely  
28 398 to affect haematological profile in a differential way in people with and without CKD. However,  
29 399 despite these limitations, we are not aware of other studies that have assessed the haematological  
30 400 profile of individuals with reduced kidney function in a population-based setting in Africa, even  
31 401 more specific, individuals of mixed-ancestry. Furthermore, we studied a community with a high  
32 402 burden of obesity, hypertension and diabetes, reflective of the current burden in Africa. This  
33 403 study provides much needed evidence for the association between the haematological profile and

1  
2  
3 404 CKD as population-based data on the haematological profile of people with CKD in Africa, are  
4  
5 405 very limited.  
6  
7 406

8 407 In conclusion, the findings from our study are valuable as full blood count analyses are done  
9  
10 408 routinely and are relatively affordable, taking into account the severely resource limited setting  
11  
12 409 found in Africa and other low and middle-income countries. Furthermore, though it still remains  
13  
14 410 unclear whether the advocated kidney function estimators provide accurate estimates of CKD  
15  
16 411 burden in African populations<sup>50</sup>, the correlation of these estimates, with deteriorating profile of  
17  
18 412 blood cell counts, suggests that these advocated GFR estimates, particularly the CKD-EPI  
19  
20 413 equation, to some extent, measure kidney function in African populations.  
21  
22 414

## 22 415 **DECLARATIONS**

### 24 416 **Ethics approval and consent to participate**

25 417 The study was approved by the Research Ethics Committees of the Cape Peninsula University of  
26  
27 418 Technology and Stellenbosch University (NHREC: REC—230 408–014 and N14/01/003,  
28  
29 419 respectively). The study was conducted in accordance with the Declaration of Helsinki. All  
30  
31 420 participants voluntarily signed written informed consent after all the procedures were fully  
32  
33 421 explained in the language of their choice. Permission to conduct the study was also obtained  
34  
35 422 from relevant authorities including the city and community authorities.  
36  
37 423

### 38 424 **Consent for publication**

39 425 Not applicable  
40  
41 426

### 43 427 **Availability of data and material**

44 428 The datasets used and/or analyzed during the current study are available from the corresponding  
45  
46 429 author on reasonable request.  
47  
48 430

### 50 431 **Competing interest**

51 432 The authors declare that they have no competing interests  
52  
53 433

### 55 434 **Funding**

56  
57  
58  
59  
60

1  
2  
3 435 The South African Medical Research Council (SAMRC) funded this research project with funds  
4  
5 436 from National Treasury under its Economic Competitiveness and Support Package (MRC-RFA-  
6  
7 437 UFSP-01-2013/VMH Study).  
8  
9 438

### 10 439 **Authors' contribution**

11 440 Study conception and funding acquisition (TEM, APK, RTE), operationalization and supervision  
12  
13 441 of the data collection (TEM), data analysis and interpretation (CG, APK), drafting the  
14  
15 442 manuscript (CG, APK), critical revision of the manuscript and approval of the final version (all  
16  
17 443 co-authors).  
18  
19 444

### 20 445 **Acknowledgements**

21  
22 446 We are grateful to the Cape Town VMH study investigation team and population of Bellville-  
23  
24 447 South for their participation.  
25  
26 448

### 27 449 **REFERENCES**

- 28  
29 450 1. Bolton K, Culleton B, Harvey K. K/DOQI clinical practice guidelines for chronic kidney disease:  
30 451 evaluation, classification, and stratification. *Kidney Disease Outcome Quality Initiative. Am*  
31 452 *JKidney Dis* 2002;39(2 Suppl 1):S1-246.  
32 453 2. Nitta K, Okada K, Yanai M, et al. Aging and chronic kidney disease. *Kidney & blood pressure*  
33 454 *research* 2013;38(1):109-20. doi: 10.1159/000355760 [published Online First: 2014/03/20]  
34 455 3. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20  
35 456 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010.  
36 457 *Lancet* 2012;380(9859):2095-128. doi: 10.1016/S0140-6736(12)61728-0  
37 458 4. Eckardt KU, Coresh J, Devuyst O, et al. Evolving importance of kidney disease: from subspecialty to  
38 459 global health burden. *Lancet* 2013;382(9887):158-69. doi: 10.1016/s0140-6736(13)60439-0  
39 460 5. Ayodele OE, Alebiosu CO. Burden of chronic kidney disease: an international perspective. *Adv*  
40 461 *Chronic Kidney Dis* 2010;17(3):215-24. doi: 10.1053/j.ackd.2010.02.001  
41 462 6. Naicker S. End-stage renal disease in sub-Saharan Africa. *Ethn Dis* 2009;19(1 Suppl 1):S1-13-5.  
42 463 [published Online First: 2009/06/02]  
43 464 7. Peralta CA, Risch N, Lin F, et al. The Association of African Ancestry and elevated creatinine in the  
44 465 Coronary Artery Risk Development in Young Adults (CARDIA) Study. *American journal of*  
45 466 *nephrology* 2010;31(3):202-8. doi: 10.1159/000268955 [published Online First: 2009/12/24]  
46 467 8. Kiberd BA, Clase CM. Cumulative risk for developing end-stage renal disease in the US population.  
47 468 *Journal of the American Society of Nephrology : JASN* 2002;13(6):1635-44. [published Online  
48 469 First: 2002/06/01]  
49 470 9. Stanifer JW, Jing B, Tolan S, et al. The epidemiology of chronic kidney disease in sub-Saharan Africa:  
50 471 a systematic review and meta-analysis. *Lancet Glob Health* 2014;2(3):e174-81. doi:  
51 472 10.1016/S2214-109X(14)70002-6  
52 473 10. Stanifer JW, Muiru A, Jafar TH, et al. Chronic kidney disease in low- and middle-income countries.  
53 474 *Nephrol Dial Transplant* 2016;31(6):868-74. doi: 10.1093/ndt/gfv466  
54  
55  
56  
57  
58  
59

- 1  
2  
3 475 11. Hamer RA, El Nahas AM. The burden of chronic kidney disease. *BMJ* 2006;332(7541):563-4. doi:  
4 476 10.1136/bmj.332.7541.563
- 5 477 12. Jha V, Garcia-Garcia G, Iseki K, et al. Chronic kidney disease: global dimension and perspectives.  
6 478 *Lancet* 2013;382(9888):260-72. doi: 10.1016/S0140-6736(13)60687-X
- 7 479 13. Babitt JL, Lin HY. Mechanisms of anemia in CKD. *Journal of the American Society of Nephrology* :  
8 480 *JASN* 2012;23(10):1631-4. doi: 10.1681/asn.2011111078 [published Online First: 2012/09/01]
- 9 481 14. Astor BC, Muntner P, Levin A, et al. Association of kidney function with anemia: The third national  
10 482 health and nutrition examination survey (1988-1994). *Archives of Internal Medicine*  
11 483 2002;162(12):1401-08. doi: 10.1001/archinte.162.12.1401
- 12 484 15. Webster AC, Nagler EV, Morton RL, et al. Chronic Kidney Disease. *Lancet* 2017;389(10075):1238-  
13 485 52. doi: 10.1016/s0140-6736(16)32064-5 [published Online First: 2016/11/27]
- 14 486 16. Kazmi WH, Kausz AT, Khan S, et al. Anemia: An early complication of chronic renal insufficiency.  
15 487 *American Journal of Kidney Diseases* 2001;38(4):803-12. doi: 10.1053/ajkd.2001.27699
- 16 488 17. Kutuby F, Wang S, Desai C, et al. Anemia of chronic kidney disease. *Disease-a-month : DM*  
17 489 2015;61(10):421-4. doi: 10.1016/j.disamonth.2015.08.002 [published Online First: 2015/09/15]
- 18 490 18. Levey AS, Coresh J. Chronic kidney disease. *Lancet* 2012;379(9811):165-80. doi: 10.1016/S0140-  
19 491 6736(11)60178-5
- 20 492 19. Stenvinkel P, Heimbürger O, Paulter F, et al. Strong association between malnutrition, inflammation,  
21 493 and atherosclerosis in chronic renal failure. *Kidney Int* 1999;55(5):1899-911. doi: 10.1046/j.1523-  
22 494 1755.1999.00422.x [published Online First: 1999/05/07]
- 23 495 20. Okyay GU, İnal S, Öneç K, et al. Neutrophil to Lymphocyte Ratio in Evaluation of Inflammation in  
24 496 Patients with Chronic Kidney Disease. *Renal Fail* 2013;35(1):29-36. doi:  
25 497 10.3109/0886022X.2012.734429
- 26 498 21. Azab B, Zaher M, Weiserbs KF, et al. Usefulness of neutrophil to lymphocyte ratio in predicting  
27 499 short- and long-term mortality after non-ST-elevation myocardial infarction. *Am J Cardiol*  
28 500 2010;106(4):470-6. doi: 10.1016/j.amjcard.2010.03.062 [published Online First: 2010/08/10]
- 29 501 22. Uthamalingam S, Patvardhan EA, Subramanian S, et al. Utility of the neutrophil to lymphocyte ratio  
30 502 in predicting long-term outcomes in acute decompensated heart failure. *Am J Cardiol*  
31 503 2011;107(3):433-8. doi: 10.1016/j.amjcard.2010.09.039 [published Online First: 2011/01/25]
- 32 504 23. Turkmen K, Guney I, Yerlikaya FH, et al. The relationship between neutrophil-to-lymphocyte ratio  
33 505 and inflammation in end-stage renal disease patients. *Ren Fail* 2012;34(2):155-9. doi:  
34 506 10.3109/0886022x.2011.641514 [published Online First: 2011/12/17]
- 35 507 24. Masconi K, Matsha TE, Erasmus RT, et al. Independent external validation and comparison of  
36 508 prevalent diabetes risk prediction models in a mixed-ancestry population of South Africa.  
37 509 *Diabetol Metab Syndr* 2015;7:42. doi: 10.1186/s13098-015-0039-y [published Online First:  
38 510 2015/05/20]
- 39 511 25. Kengne AP, Erasmus RT, Levitt NS, et al. Alternative indices of glucose homeostasis as biochemical  
40 512 diagnostic tests for abnormal glucose tolerance in an African setting. *Prim Care Diabetes*  
41 513 2017;11(2):119-31. doi: 10.1016/j.pcd.2017.01.004 [published Online First: 2017/01/31]
- 42 514 26. Chalmers J, MacMahon S, Mancia G, et al. 1999 World Health Organization-International Society of  
43 515 Hypertension Guidelines for the management of hypertension. Guidelines sub-committee of the  
44 516 World Health Organization. *Clinical and experimental hypertension (New York, NY : 1993)*  
45 517 1999;21(5-6):1009-60. doi: 10.3109/10641969909061028 [published Online First: 1999/07/28]
- 46 518 27. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its  
47 519 complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a  
48 520 WHO consultation. *Diabet Med* 1998;15(7):539-53. doi: 10.1002/(sici)1096-  
49 521 9136(199807)15:7<539::aid-dia668>3.0.co;2-s [published Online First: 1998/08/01]
- 50 522 28. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein  
51 523 cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18(6):499-  
52 524 502.



- 1  
2  
3 525 29. Levey AS, Bosch JP, Lewis JB, et al. A more accurate method to estimate glomerular filtration rate  
4 526 from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study  
5 527 Group. *Ann Intern Med* 1999;130(6):461-70.  
6 528 30. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann*  
7 529 *Intern Med* 2009;150(9):604-12.  
8 530 31. Levey AS, Coresh J, Balk E, et al. National Kidney Foundation practice guidelines for chronic kidney  
9 531 disease: evaluation, classification, and stratification. *Ann Intern Med* 2003;139(2):137-47.  
10 532 [published Online First: 2003/07/16]  
11 533 32. National Kidney Foundation. KDOQI Clinical Practice Guidelines and Clinical Practice  
12 534 Recommendations for Anemia in Chronic Kidney Disease. *Am J Kidney Dis* 2006;47(5 Suppl  
13 535 3):S11-145. doi: 10.1053/j.ajkd.2006.03.010 [published Online First: 2006/05/09]  
14 536 33. Bessman JD, Johnson RK. Erythrocyte volume distribution in normal and abnormal subjects. *Blood*  
15 537 1975;46(3):369-79. [published Online First: 1975/09/01]  
16 538 34. World Health Organization. A global brief on Hypertension: Silent killer, global public health crisis,  
17 539 2013.  
18 540 35. World Health Organisation, International Diabetes Federation. Definition and diagnosis of diabetes  
19 541 and intermediate hyperglycemia. In: consultation WI, ed. Geneva, 2006.  
20 542 36. Afshar R, Sanavi S, Salimi J, et al. Hematological profile of chronic kidney disease (CKD) patients in  
21 543 Iran, in pre-dialysis stages and after initiation of hemodialysis. *Saudi Journal of Kidney Diseases*  
22 544 *and Transplantation* 2010;21(2):368-71.  
23 545 37. Akinsola A, Durosinmi MO, Akinola NO. The haematological profile of Nigerians with chronic renal  
24 546 failure. *Afr J Med Med Sci* 2000;29(1):13-6. [published Online First: 2001/05/31]  
25 547 38. Asif N, Hasan S, Hassan K. Hematological Changes in Patients of Chronic Renal Disease and Their  
26 548 Response to Treatment with Erythropoietin. *Int J Pathol* 2015;13(1):14-19.  
27 549 39. Bhattacharjee K, Das D, Rabha P, et al. A study on hematological profile in patients of chronic renal  
28 550 failure with special reference to serum iron profile. *Journal of Evidence based Medicine and*  
29 551 *Healthcare* 2015;2(46):8212-19.  
30 552 40. Dabrowska MM, Mikula T, Wiercinska-Drapalo A. The anemia prevalence and the association  
31 553 between complete blood count analysis and renal function parameters in HIV-1-infected patients.  
32 554 *Current HIV research* 2012;10(3):247-51. [published Online First: 2012/03/06]  
33 555 41. Islam MN, Ferdous A, Zahid AZ, et al. Haematological Profile of Patients with Chronic Kidney  
34 556 Disease in Northern Bangladesh. *Dinajpur Med Col J* 2015;8(1):21-27.  
35 557 42. Latiweshob OB, Elwerfaly HH, Sheriff DS, et al. Haematological Changes in Predialyzed and  
36 558 Hemodialyzed Chronic Kidney Disease patients in Libya. *IOSR Journal of Dental and Medical*  
37 559 *Sciences* 2017;16(2):106-12.  
38 560 43. Shisana O, Labadarios D, Rehle T, et al. The South African National Health and Nutrition  
39 561 Examination Survey (SANHANES-1), 2013.  
40 562 44. Drechsler M, Doring Y, Megens RT, et al. Neutrophilic granulocytes - promiscuous accelerators of  
41 563 atherosclerosis. *Thrombosis and haemostasis* 2011;106(5):839-48. doi: 10.1160/th11-07-0501  
42 564 [published Online First: 2011/10/21]  
43 565 45. Nunez J, Minana G, Bodi V, et al. Low lymphocyte count and cardiovascular diseases. *Curr Med*  
44 566 *Chem* 2011;18(21):3226-33. [published Online First: 2011/06/16]  
45 567 46. Reddan DN, Klassen PS, Szczech LA, et al. White blood cells as a novel mortality predictor in  
46 568 haemodialysis patients. *Nephrol Dial Transplant* 2003;18(6):1167-73. [published Online First:  
47 569 2003/05/16]  
48 570 47. Solak Y, Yilmaz MI, Sonmez A, et al. Neutrophil to lymphocyte ratio independently predicts  
49 571 cardiovascular events in patients with chronic kidney disease. *Clin Exp Nephrol* 2013;17(4):532-  
50 572 40. doi: 10.1007/s10157-012-0728-x [published Online First: 2012/11/28]  
51 573 48. An X, Mao HP, Wei X, et al. Elevated neutrophil to lymphocyte ratio predicts overall and  
52 574 cardiovascular mortality in maintenance peritoneal dialysis patients. *Int Urol Nephrol*  
53 575 2012;44(5):1521-8. doi: 10.1007/s11255-012-0130-3 [published Online First: 2012/02/01]  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 576 49. Okyay GU, Inal S, Onec K, et al. Neutrophil to lymphocyte ratio in evaluation of inflammation in  
4 577 patients with chronic kidney disease. *Ren Fail* 2013;35(1):29-36. doi:  
5 578 10.3109/0886022x.2012.734429 [published Online First: 2012/11/02]  
6 579 50. Agoons DD, Balti EV, Kaze FF, et al. Performance of three glomerular filtration rate estimation  
7 580 equations in a population of sub-Saharan Africans with Type 2 diabetes. *Diabet Med*  
8 581 2016;33(9):1291-8. doi: 10.1111/dme.12996 [published Online First: 2015/10/21]  
9

10 582  
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



# BMJ Open

## HAEMATOLOGICAL PROFILE OF CHRONIC KIDNEY DISEASE IN A MIXED-ANCESTRY SOUTH AFRICAN POPULATION: A CROSS-SECTIONAL STUDY

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-025694.R1
Article Type:	Research
Date Submitted by the Author:	10-Sep-2018
Complete List of Authors:	George, Cindy; South African Medical Research Council, Non-Communicable Diseases Research Unit Matsha, Tandi; Cape Peninsula University of Technology, Department of Biomedical Sciences Erasmus, Rajiv; University of Stellenbosch, Chemical Pathology Kengne , AP; South African Medical Research Council, Non-Communicable Diseases Research Unit; University of Cape Town
<b>Primary Subject Heading</b>:	Haematology (incl blood transfusion)
Secondary Subject Heading:	Renal medicine
Keywords:	Africa, HAEMATOLOGY, Chronic Kidney Disease

SCHOLARONE™  
Manuscripts

1  
2  
3 1 **HAEMATOLOGICAL PROFILE OF CHRONIC KIDNEY DISEASE IN A MIXED-**  
4 **ANCESTRY SOUTH AFRICAN POPULATION: A CROSS-SECTIONAL STUDY**  
5 2  
6  
7 3  
8 4  
9

10 5 Cindy George<sup>1</sup>; Tandi E. Matsha<sup>2</sup>, Rajiv T. Erasmus<sup>3</sup>, Andre P. Kengne<sup>1,4</sup>  
11  
12 6  
13 7  
14

15 8 <sup>1</sup>Non-Communicable Disease Research Unit, South African Medical Research Council, Cape  
16 9 Town, South Africa; <sup>2</sup>Department of Biomedical Sciences, Faculty of Health and Wellness  
17 10 Science, Cape Peninsula University of Technology, Bellville, Cape Town, South Africa; <sup>3</sup>Division  
18 11 of Chemical Pathology, Faculty of Medicine and Health Sciences, National Health Laboratory  
19 12 Service (NHLS) and University of Stellenbosch, Cape Town, South Africa, <sup>4</sup>Department of  
20 13 Medicine, University of Cape Town, Cape Town, South Africa.  
21  
22  
23  
24  
25  
26  
27  
28

29 16 **Corresponding author:** Cindy George; South African Medical Research Council, Non-  
30 17 Communicable Disease Research Unit, Francie van Zijl Drive, Parow Valley, Cape Town, PO Box  
31 18 19070, South Africa; +27 21 9380482; [cindy.george@mrc.ac.za](mailto:cindy.george@mrc.ac.za)  
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

21 Word count: 3085

22 Abstract word count: 294

23 References: 50

24 Tables: 3

## 25 ABSTRACT

26 **Objectives:** The objectives were to characterise the haematological profile of screen-detected  
27 chronic kidney disease (CKD) participants and to correlate the complete blood count measures  
28 with the commonly advocated kidney function estimators. **Methods:** The current cross-sectional  
29 study utilized data, collected between February 2015 and November 2016, of 1564 adults of  
30 mixed-ancestry, who participated in the Cape Town Vascular and Metabolic Health study. Kidney  
31 function was estimated using the Modification of Diet in Renal Disease (MDRD) and Chronic  
32 Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. CKD was defined as eGFR  
33  $<60\text{ml/min/1.73m}^2$ , and anaemia as haemoglobin level  $<13.5\text{g/dL}$  (men) and  $<12\text{g/dL}$  (women).  
34 **Results:** Based on the MDRD and CKD-EPI equations, the crude prevalence of CKD was 6% and  
35 3%. Irrespective of the equation used, median red blood cell (RBC) indices were consistently lower  
36 in those with CKD compared to those without CKD (all  $p<0.0001$ ). Despite not showing any  
37 significant difference in total white blood cell (WBC) count between the two groups, the number  
38 of lymphocytes were lower ( $p=0.0001$  and  $p<0.0001$  for MDRD and CKD-EPI, respectively) and  
39 neutrophil count (both  $p\leq 0.0297$ ) and the ratio of lymphocytes to neutrophil (both  $p<0.0001$ )  
40 higher in the CKD group compared to those without CKD; with the remaining WBC indices  
41 similar in the two groups. The platelet count was similar in both groups. Of the screen-detected  
42 CKD participants, 45.5% (MDRD) and 57.8% (CKD-EPI) were anaemic, with the prevalence  
43 increasing with increasing severity of CKD, from 37.2% (stage 3) to 82.4% (stages 4-5).  
44 Furthermore, CKD-EPI-estimated kidney function, but not MDRD, was positively associated with  
45 RBC indices. **Conclusion:** Though it remains unclear whether common kidney function estimators  
46 provide accurate estimates of CKD in Africans, the correlation of their estimates with deteriorating  
47 RBC profile, suggests that advocated estimators, to some extent approximate kidney function in  
48 African populations.

49  
50 **Key words:** chronic kidney disease; haematology; Africa

### 51 **Strengths and limitations of the study**

- 52 • The first study to characterize the haematological profile of individuals with reduced  
53 kidney function in a population-based setting in Africa, even more specific, individuals of  
54 mixed-ancestry
- 55 • We studied a community with a high burden of obesity, hypertension and diabetes,  
56 reflective of the current burden in Africa.
- 57 • This study was conducted in only one geographical area, which may not adequately reflect  
58 all the mixed ancestry population groups in Sub-Saharan Africa.
- 59 • Our study was based on a single serum creatinine measure to determine CKD and did not  
60 include estimates of albuminuria. Albuminuria, which are required for clinical and  
61 aetiological diagnosis of CKD, as this information is important particularly in the  
62 interpretation of eGFR greater than 60ml/min/1.73m<sup>2</sup> where inaccuracies of the eGFR  
63 equations are greatest

## 82 BACKGROUND

83 Chronic kidney disease (CKD) is a major global public health problem <sup>1</sup>, estimated to affect more  
84 than 10% of the general adult population and up to 50% of some high-risk subpopulations, such  
85 as the elderly <sup>2</sup>, those with non-communicable diseases (NCD), including type 2 diabetes mellitus  
86 (T2D) and hypertension, and communicable diseases (CD), including human immunodeficiency  
87 virus (HIV)/ acquired immunodeficiency syndrome (AIDS) <sup>3 4</sup>. Africa is currently experiencing  
88 the double burden of NCDs and CDs, which are all driving the increasing burden of CKD on the  
89 continent <sup>5</sup>. However, the exact burden of CKD in Africa has yet to be fully elucidated <sup>6-9</sup>, in part  
90 due to the absence of appropriate estimates for predicting reduced kidney function in individuals  
91 from African ancestry <sup>9 10</sup>.

92  
93 CKD encompasses a wide range of physiological processes altered by the progressive decline in  
94 glomerular filtration rate (GFR) <sup>11 12</sup>. Haematological parameters, particularly red blood cell  
95 (RBC) indices, are most commonly affected <sup>13</sup>, giving rise to anaemia. Anaemia is the most  
96 common, consistent and severe of the various haematological abnormalities, and has been shown  
97 to be a very common condition in black Africans <sup>14</sup>. Although anaemia may be found at any stage  
98 of CKD, the severity of anaemia increases with CKD progression <sup>15</sup>, resultantly affecting nearly  
99 all patients with end-stage renal disease (CKD stage 5) <sup>13</sup>. The predominant cause of anaemia in  
100 CKD is failure of the kidneys to produce enough endogenous erythropoietin, which accompanies  
101 the fall in GFR <sup>16 17</sup>. Untreated, prolonged anaemia is strongly predictive of all-cause and  
102 cardiovascular mortality, as well as reduced quality of life and increased morbidity in patients with  
103 CKD <sup>13 18</sup>. Untreated anaemia can also accelerate the decline in renal function by causing renal  
104 haemodynamic alterations and tissue hypoxia <sup>15</sup>. Other potentially affected haematological  
105 parameters in CKD, of which the association with CKD is not yet fully characterized, include total  
106 and differential white blood cell (WBC) counts. Persistent, low-grade inflammation is an essential  
107 part of the aetiology of CKD and has been recognized as such since the late 1990s, when it was  
108 linked to cardiovascular disease (CVD) and mortality <sup>19</sup>. Recently, the ratio of neutrophil-to-  
109 lymphocyte count (N/L) has been proposed as a novel measure of inflammation in distinct  
110 populations and has been shown to have prognostic value <sup>20</sup>; particularly for mortality risk in  
111 patients with myocardial infarction and heart failure <sup>21 22</sup>. However, studies on the relationship of  
112 N/L ratio with reduced eGFR are limited <sup>23</sup>. Thus, despite recent advances in the aetiology

1  
2  
3 113 governing the development and progression of CKD, population-based data on the haematological  
4 114 profile of people with CKD in Africa, are scanty.

5  
6 115  
7  
8 116 We therefore aimed to characterise the haematological profile of screen-detected CKD participants  
9 117 in a community-based sample, and to correlate the complete blood count measures with two  
10 118 commonly advocated kidney function estimators of CKD in urban South Africans of mixed-  
11 119 ancestry.

12  
13  
14  
15 120

## 16 17 121 **METHODS**

### 18 19 122 **Study setting and population**

20 123 The current study utilized data from the ongoing Cape Town Vascular and Metabolic Health  
21 124 (VMH) study, an extension of the Cape Town Bellville-South study, which has been described in  
22 125 detail previously<sup>24</sup>. Bellville-South, with a population of approximately 29,301, is a township  
23 126 formed in the late 1950s, located in the metropolitan city of Cape Town, South Africa. The  
24 127 population consists predominantly of individuals of mixed-ancestry (coloured) (76%), followed  
25 128 by black Africans (18.5%), with only 1.5% of the population being of Caucasian and Asians  
26 129 ancestry. The data collection for the current analysis took place between February 2015 and  
27 130 November 2016 during a community-based survey involving only mixed-ancestry South Africans.  
28 131 The study was approved by the Research Ethics Committees of the Cape Peninsula University of  
29 132 Technology and Stellenbosch University (NHREC: REC—230 408–014 and N14/01/003,  
30 133 respectively). The study was conducted in accordance with the Declaration of Helsinki. All  
31 134 participants voluntarily signed written informed consent after all the procedures were fully  
32 135 explained in the language of their choice.

33  
34  
35  
36 136

### 37 38 137 **Participant involvement**

39 138 The participants were not involved in the design or recruitment process of this study. However,  
40 139 permission to conduct the study was obtained from relevant authorities including the city and  
41 140 community authorities.

42  
43  
44  
45 141

### 46 47 142 **Questionnaires and physical examination**

1  
2  
3 143 All interviews and physical examinations took place at a research clinic on the campus of Cape  
4 Peninsula University of Technology, located within the study suburb. All consenting participants  
5 144 received a standardized interview, explained in great detail elsewhere <sup>25</sup>. Physical examination  
6 145 involved blood pressure (BP) determination, measured according to the World Health  
7 146 Organisation (WHO) guidelines <sup>26</sup>, using a semi-automatic digital blood pressure monitor (Omron  
8 147 M6 comfort-preformed cuff BP Monitor), placed on the right arm in sitting position and at rest for  
9 148 at least 10 min. Three measures were taken of which the average of the lowest two was used in all  
10 149 analyses. Body weight (to the nearest 0.1 kg) was measured with the participant in light clothing  
11 150 and without shoes, using an Omron body fat meter HBF-511 digital bathroom scale, which was  
12 151 calibrated and standardized using a weight of known mass. Height (to the nearest centimetre) was  
13 152 measured with a stadiometer, with subjects standing on a flat surface. Body mass index (BMI) was  
14 153 calculated as weight per square meter (kg/m<sup>2</sup>). Waist circumference (WC) was measured with a  
15 154 non-elastic tape measure at the level of the narrowest part of the torso, as seen from the anterior  
16 155 view. Anthropometric measurements were performed three times and the average used for  
17 156 analysis.  
18 157  
19 158

### 31 159 **Biochemical analysis and calculations**

32 160 All biochemical analyses took place at an ISO 15189 accredited Pathology practice (Path-Care,  
33 161 Reference Laboratory, Cape Town, South Africa). Blood samples were collected from all  
34 162 participants after an overnight fast, and two hours after a 75g oral glucose tolerance test (OGTT)  
35 163 following the WHO recommendations <sup>27</sup>. Plasma glucose levels and haemoglobin A1c (HbA1c)  
36 164 were measured by enzymatic hexokinase method (Beckman AU, Beckman Coulter, South Africa)  
37 165 and high performance liquid chromatography (Biorad Variant Turbo, BioRad, South Africa),  
38 166 respectively. Insulin was determined by a paramagnetic particle chemiluminescence assay  
39 167 (Beckman DXI, Beckman Coulter, South Africa). Triglycerides (TG), total cholesterol (TC), and  
40 168 high-density lipoproteins (HDL-C) were analysed using the Roche Modular auto analyser and  
41 169 enzymatic colorimetric assays, and low-density lipoproteins (LDL-C) were calculated using the  
42 170 Friedewald formula <sup>28</sup>. The homeostatic model assessment of insulin resistance (HOMA-IR) was  
43 171 calculated according to the formula:  $HOMA-IR = [\text{fasting insulin concentration (mIU/l)} \times \text{fasting}$   
44 172  $\text{plasma glucose (mmol/l)}] / 22.5$ . Serum concentration of high sensitivity C-reactive protein (hsCRP)  
45 173 (Immun Diagnostik AG, Bensheim, Germany) was analysed using commercially available ELISA

174 kits according to the manufacturer's protocols. Serum creatinine was measured by the modified  
175 Jaffe-Kinetic method (Beckman AU, Beckman Coulter, South Africa). Creatinine assays at our  
176 Partner pathology service are standardized to the internationally accepted reference method  
177 (isotope dilution mass spectrophotometry [IDMS]) since 2009 and eGFR estimators applicable to  
178 standardised creatinine values were used. Kidney function was assessed using serum creatinine-  
179 based estimators of glomerular filtration rate (eGFR), namely, the 4-variable Modification of Diet  
180 in Renal Disease (MDRD) equation <sup>29</sup> and the Chronic Kidney Disease Epidemiology  
181 Collaboration (CKD-EPI) equation <sup>30</sup>. The African-American ethnicity correction factor was  
182 omitted from the eGFR calculation, as the South African Renal Society CKD guidelines promotes  
183 the exclusion of the correction factor, except in the case of black Africans. Full blood counts,  
184 including total RBC, total WBC, lymphocytes count and percentage, monocyte count and  
185 percentage, neutrophil count and percentage, basophil count and percentage, eosinophil count and  
186 percentage, haemoglobin (Hb), haematocrit, mean corpuscular volume (MCV), mean corpuscular  
187 haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution  
188 width, and platelets, were measured on a Coulter LH 750 hematology analyzer (Beckman Coulter,  
189 South Africa).

### 191 **Classification of renal function and co-morbidities**

192 Staging of kidney function was based on the National Kidney Foundation Disease Outcomes  
193 Quality Initiative (NKF-KDOQI) classification <sup>31</sup>. An eGFR<60 ml/min/1.73 m<sup>2</sup> was used to  
194 define CKD (or CKD stage 3–5). Anaemia was defined using the National Kidney Foundation  
195 Kidney Disease Outcome Quality Initiative (K/DOQI) guidelines (haemoglobin level <13.5g/dL  
196 for men and <12g/dL for women) <sup>32</sup> and further classified into micro-, normo- and macrocytic  
197 based on the MCV. Microcytic anaemia was defined as an MCV of <80fL, normocytic as 100-80  
198 fL, and macrocytic as >100fL <sup>33</sup>. Hypertension was based on either a history of diagnosed  
199 hypertension (receiving medications for hypertension) or screen-detected hypertension. The latter  
200 being classified if they had a SBP≥140mmHg and/or DBP≥90 mmHg <sup>34</sup>. Diabetes status was based  
201 on a history of diagnosed diabetes or screen-detected diabetes. OGTT glucose values were used to  
202 classify the glucose tolerance status of participants as recommended by WHO <sup>35</sup> as: (1) normal  
203 glucose tolerance (fasting plasma glucose (FPG) <6.1 mmol/l and 2-h glucose <7.8 mmol/l); (2)  
204 pre-diabetes including impaired fasting glycaemia (IGT, 6.1≤FPG< 7.0 mmol/l), impaired glucose



1  
2  
3 205 tolerance (IGT,  $7.8 < 2\text{-h glucose} < 11.1$  mmol/l) and the combination of both; and (3) diabetes  
4 206 (FPG  $\geq 7.0$  mmol/l and/or 2-h glucose  $\geq 11.1$  mmol/l). A BMI  $\geq 25$  kg/m<sup>2</sup> and BMI  $\geq 30$  kg/m<sup>2</sup> was  
5  
6 207 classified as overweight obese, respectively.  
7  
8  
9

## 10 209 **Statistical analysis**

11  
12 210 All statistical analyses were performed using STATA version 13 (Statcorp, College Station, TX)  
13  
14 211 and statistical significance was based on a p-value  $< 0.05$ . General characteristics of the participants  
15  
16 212 are summarized as count and percentage for qualitative variables and median and 25th-75th  
17  
18 213 percentiles for quantitative variables. Group comparisons used chi-squared test for qualitative  
19  
20 214 variables, and Wilcoxon rank-sum test for quantitative variables, respectively. Multiple linear  
21  
22 215 regression models were used to assess the independent association between eGFR and  
23  
24 216 haematological indices, while adjusting for age and gender.  
25

## 26 218 **RESULTS**

### 27 219 **Participant characteristics**

28  
29 220 The initial study sample comprised 1,647 participants. Of those, 83 were excluded due to missing  
30  
31 221 data on serum creatinine or any of the variables required to estimate kidney function, including  
32  
33 222 age and gender. The general characteristics and the haematological profile of the study population  
34  
35 223 are summarised in Tables 1 and 2, respectively. The final sample included 1,564 participants, of  
36  
37 224 which 24.9% were male, with a group median age of 50 years. The crude prevalence of CKD was  
38  
39 225 6% and 3%, based on the MDRD and CKD-EPI equations respectively. Of those participants with  
40  
41 226 MDRD-diagnosed CKD, 80.7%, 14.8% and 4.5% were in stages 3, 4 and 5, respectively.  
42  
43 227 Similarly, of those diagnosed by means of the CKD-EPI equation, 68.9%, 24.4% and 6.7% were  
44  
45 228 in stages 3, 4 and 5, respectively. MDRD-diagnosed CKD participants had higher creatinine levels  
46  
47 229 (111.5 vs. 59  $\mu\text{mol/l}$ ;  $p < 0.0001$ ) and lower eGFR (48.2 vs. 104 ml/min/1.73m<sup>2</sup>;  $p < 0.0001$ ), were  
48  
49 230 on average older (68 vs. 49 years;  $p < 0.0001$ ), with a higher WC (97.7 vs. 91.2 cm;  $p = 0.0001$ ),  
50  
51 231 BMI (30.3 vs. 28.3 kg/m<sup>2</sup>;  $p = 0.0096$ ), and SBP (142 vs. 125 mmHg;  $p < 0.0001$ ), compared to  
52  
53 232 participants with normal kidney function. Furthermore, MDRD-diagnosed CKD participants had  
54  
55 233 higher fasting and 2-hour blood glucose (5.3 vs. 5.0 mmol/l;  $p < 0.0001$  and 7.2 vs. 6.0 mmol/l;  
56  
57 234  $p < 0.0001$ , respectively), HbA1c levels (6.2 vs. 5.7%;  $p < 0.0001$ ), fasting and 2-hour insulin levels  
58  
59 235 (8.4 vs. 6.7 IU/l;  $p = 0.0089$  and 62.0 vs. 37.5 IU/l;  $p = 0.0002$ , respectively), higher HOMA-IR index  
60

1  
2  
3 236 (2.1 vs. 1.6;  $p=0.0004$ ), hsCRP (4.7 vs. 4.0  $\mu\text{g/ml}$ ;  $p=0.0492$ ), TG (1.6 vs. 1.2  $\text{mmol/l}$ ;  $p<0.0001$ )  
4 237 and TC (5.4 vs. 5.1  $\text{mmol/l}$ ;  $p=0.024$ ); with similar LDL-C (3.2 vs. 3.1  $\text{mmol/l}$ ;  $p=0.0668$ ) and  
5 238 HDL-C levels (1.3 vs. 1.3  $\text{mmol/l}$ ;  $p=0.7106$ ) compared to those without CKD. When sub-dividing  
6 239 the groups based on CKD diagnosed by the CKD-EPI equation, similar differences were observed,  
7 240 with the exception of BMI, hsCRP and TC, which showed no difference between the groups (28.3  
8 241 vs. 28.4  $\text{kg/m}^2$ ;  $p=0.384$ , 4.8 vs. 4.0  $\mu\text{g/ml}$ ;  $p=0.4268$ , 5.3 vs. 5.1  $\text{mmol/l}$ ;  $p=0.2226$ , respectively).  
9 242 Participants with reduced kidney function, both MDRD and CKD-EPI-diagnosed, had a similar  
10 243 prevalence of overweight and obesity, however had a higher prevalence of hypertension and T2D,  
11 244 despite similar prevalence of pre-diabetes (IFG and IGT) between the two groups.  
12  
13  
14  
15  
16  
17  
18  
19

20 245  
21 246 The red blood cell indices, including RBC count, haematocrit and haemoglobin levels were  
22 247 consistently lower in CKD participants compared to the group with normal kidney function (all  
23 248  $p<0.0001$ ), irrespective of the eGFR equation used. Conversely, the morphology of the RBC's  
24 249 were not different, as similar values for MCV, MCH, MCHC and RDW were observed between  
25 250 CKD participants and the participants with normal kidney function. Despite not showing any  
26 251 significant difference in total WBC count between the two groups, the number of lymphocytes  
27 252 were lower and neutrophil count and the ratio of lymphocytes to neutrophil higher in the CKD  
28 253 group compared to those individuals with normal kidney function; with the remaining WBC  
29 254 indices similar in the two groups. The platelet count was similar in both groups. Furthermore,  
30 255 based on the K/DOQI guidelines, 45.5% (MDRD) and 57.8% (CKD-EPI) of the CKD participants  
31 256 had anaemia, with the majority of cases being normocytic. Moreover, the prevalence  
32 257 of anaemia increased with increasing severity of CKD, from 37.2% at stage 3 to 82.4% at stage 4-  
33 258 5.  
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

266 **Table 1:** Clinical characteristics of the study population overall and by CKD (MDRD and CKD-EPI) status

Variables	Total (n=1564)	MDRD			CKD-EPI		
		Without CKD (n=1470)	CKD (n=94)	p-value	Without CKD (n=1517)	CKD (n=47)	p-value
Age (years)	50 (37-61)	49 (36-59)	68 (62-74)	<0.0001	50 (36-60)	69 (63-77)	<0.0001
Gender (n,% male)	389 (24.9)	372 (25.3)	17 (18.1)	0.215	373 (24.6)	16 (34.0)	0.093
<b>Anthropometry</b>							
Weight (kg)	72.0 (59.2-85.5)	71.9 (59.0-85.5)	74.0 (64.6-85.8)	0.2058	72.0 (59.2-85.5)	73.5 (64.1-85.7)	0.6903
WC (cm)	91.8 (78.5-103.5)	91.2 (77.8-103.0)	97.7 (89.0-105.8)	0.0001	91.5 (78.1-103.5)	96.0 (87.8-106.5)	0.0225
HC (cm)	102.8 (92.5-113.5)	102.5 (92.1-113.5)	104.3 (96.5-114.2)	0.1138	102.8 (92.5-113.8)	101.5 (95.8-111.5)	0.9439
BMI (kg/m <sup>2</sup> )	28.4 (22.9-34.2)	28.3 (22.7-34.1)	30.3 (26.1-35.1)	0.0096	28.4 (22.9-34.2)	28.3 (24.7-34.4)	0.3836
<b>Biochemical analysis</b>							
Fasting blood glucose (mmol/l)	5.0 (4.6-5.7)	5.0 (4.6-5.6)	5.3 (5.0-6.9)	<0.0001	5.0 (4.6-5.6)	5.3 (5.0-7.7)	0.0014
2-hour glucose (mmol/l)	6.0 (4.9-7.6)	6.0 (4.8-7.5)	7.2 (5.8-9.2)	<0.0001	6.0 (4.8-7.5)	7.5 (5.7-9.2)	0.0034
HbA1c (%)	5.8 (5.4-6.3)	5.7 (5.4-6.2)	6.2 (5.9-7.1)	<0.0001	5.8 (5.4-6.2)	6.4 (5.9-7.3)	<0.0001
Fasting insulin (IU/l)	6.7 (4.3-11.1)	6.7 (4.2-10.9)	8.4 (5.3-12.4)	0.0089	6.7 (4.2-10.9)	9.0 (5.3-12.4)	0.0323
2-hour insulin (IU/l)	38 (20.6-71.8)	37.5 (19.8-69.8)	62.0 (30.3-105.6)	0.0002	37.8 (20.3-70.5)	63.5 (32.6-105.2)	0.0072
HOMA-IR (MU)	1.6 (0.9-2.9)	1.6 (0.9-2.8)	2.1 (1.2-3.9)	0.0004	1.6 (0.9-2.8)	2.4 (1.3-3.8)	0.0026
hsCRP (µg/ml)	4.0 (1.6-8.8)	4.0 (1.6-8.8)	4.7 (2.7-9.3)	0.0492	4.0 (1.6-8.8)	4.8 (2.4-7.5)	0.4268
TG (mmol/l)	1.2 (0.9-1.7)	1.2 (0.9-1.7)	1.6 (1.2-2.3)	<0.0001	1.2 (0.9-1.7)	1.8 (1.1-2.4)	0.0001
TC (mmol/l)	5.1 (4.4-5.9)	5.1 (4.3-5.9)	5.4 (4.8-6.4)	0.0024	5.1 (4.4-5.9)	5.3 (4.4-6.0)	0.2226
LDL-C (mmol/l)	3.1 (2.5-3.8)	3.1 (2.5-3.8)	3.2 (2.7-4.3)	0.0668	3.1 (2.5-3.8)	3.1 (2.5-3.9)	0.9444
HDL-C (mmol/l)	1.3 (1.1-1.5)	1.3 (1.1-1.5)	1.3 (1.1-1.5)	0.7106	1.3 (1.1-1.5)	1.3 (1.1-1.4)	0.5132
Creatinine (µmol/l)	60 (52-70)	59 (51-68)	111.5 (89.0-140.5)	<0.0001	59 (51-69)	140 (124-209)	<0.0001
eGFR (ml/min/1.73m <sup>2</sup> )	-	104.0 (88.0-121.0)	48.2 (33.7-55.4)	<0.0001	113.9 (101.4-126.5)	44.7 (26.4-49.6)	<0.0001

<b>Blood pressure measures</b>							
Mean SBP (mmHg)	125 (111-141)	125 (110-140)	142 (121-162)	<0.0001	125 (111-140)	150 (128-181)	<0.0001
Mean DBP (mmHg)	81 (72-90)	81 (72-90)	81 (74-95)	0.2114	81 (72-90)	85 (73-95)	0.2185
Pulse pressure (BPM)	70 (62-79)	70 (62-79)	70 (60-81)	0.9932	70 (62-79)	73 (62-82)	0.3861
<b>Co-morbidities</b>							
Overweight (BMI $\geq$ 25kg/m <sup>2</sup> ; n,%)	361 (23.2)	335 (22.9)	26 (29.5)	0.139	348 (23.1)	13 (28.9)	0.348
Obese (BMI $\geq$ 30kg/m <sup>2</sup> ; n,%)	662 (42.6)	617 (42.1)	45 (51.1)	0.085	642 (42.5)	20 (44.4)	0.771
Pre-diabetes (n, %)	238 (15.2)	226 (15.4)	12 (12.8)	0.671	233 (15.4)	5 (10.6)	0.436
T2D (n, %)	297 (19.0)	259 (17.6)	38 (40.4)	<0.0001	272 (17.9)	25 (53.2)	<0.0001
Hypertension (n, %)	567 (36.3)	517 (35.2)	50 (53.2)	<0.0001	537 (35.4)	30 (63.3)	<0.0001

267

268 Data is presented as median (25th-75<sup>th</sup> percentiles) and percentages. WC, waist circumference; HC, hip circumference; BMI, body mass  
 269 index; HbA1c, Glycated haemoglobin; HOMA-IR, Homeostatic model assessment-insulin resistance; MU, mass units; hsCRP, high  
 270 sensitivity C-reactive protein; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoproteins; HDL-C, high-density  
 271 lipoproteins; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; IFG/IGT, impaired  
 272 fasting glucose and impaired glucose tolerance; T2D, type 2 diabetes mellitus; MDRD, Modification of Diet in Renal Disease; CKD-  
 273 EPI, Chronic Kidney Disease Epidemiology Collaboration; CKD, chronic kidney disease.

274

275

276

277

278

279

280

281 **Table 2:** Haematological profile of study population overall and by CKD (MDRD and CKD-EPI) status

282

Variables	Total (n=1564)	MDRD			CKD-EPI		
		Without CKD (n=1470)	CKD (n=94)	p-value	Without CKD (n=1517)	CKD (n=47)	p-value
RBC (x10 <sup>6</sup> /μl)	4.7 (4.3-5.0)	4.7 (4.4-5.0)	4.3 (3.9-4.7)	<0.0001	4.7 (4.4-5.0)	4.2 (3.8-4.7)	<0.0001
WBC (x10 <sup>6</sup> /μl)	7.5 (6.2-9.1)	7.4 (6.2-9.1)	7.7 (6.5-9.2)	0.5704	7.4 (6.2-9.1)	7.9 (6.3-9.3)	0.5458
N/L (ratio)	2.0 (1.5-2.6)	1.9 (1.5-2.5)	2.5 (1.7-3.5)	<0.0001	1.9 (1.5-2.5)	2.7 (2.0-3.7)	<0.0001
Lymphocyte count (x10 <sup>9</sup> /l)	2.2 (1.8-2.80)	2.2 (1.8-2.8)	1.9 (1.4-2.5)	0.0001	2.2 (1.8-2.8)	1.8 (1.4-2.4)	<0.0001
Monocyte count (x10 <sup>9</sup> /l)	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.4 (0.4-0.6)	0.1389	0.5 (0.4-0.6)	0.4 (0.4-0.6)	0.9446
Neutrophil count (x10 <sup>9</sup> /l)	4.5 (3.4-5.7)	4.5 (3.3-5.6)	5.0 (3.7-5.9)	0.0255	4.5 (3.4-5.6)	5.1 (4.3-6.1)	0.0297
Basophil count (x10 <sup>9</sup> /l)	0.1 (0.1-0.2)	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.283	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.1366
Eosinophil count (x10 <sup>9</sup> /l)	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.1579	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.1223
Platelet count (x10 <sup>9</sup> /l)	271 (227-322)	271 (228-322)	277 (214-324)	0.9417	271 (228-322)	266 (197-313)	0.2211
Haematocrit (volume %)	41 (39-44)	41 (39-44)	38 (35-41)	<0.0001	41 (39-44)	37 (34-41)	<0.0001
MCV (fl/cell)	89 (85-93)	89 (85-93)	89 (86-92)	0.8150	89 (85-93)	89 (86-91)	0.4748
MCH (pg/cell)	29 (28-31)	29 (28-31)	29 (28-30)	0.1399	29 (28-31)	29 (28-30)	0.057
MCHC (g/dl)	33 (32-33)	33 (32-33)	33 (32-33)	0.1471	33 (32-33)	32 (32-33)	0.1156
RDW (%)	14.2 (13.5-15.0)	14.1 (13.4-15.0)	14.5 (13.7-15.6)	0.0601	14.1 (13.4-15.0)	14.3 (13.8-15.5)	0.0673
Hb (g/dl)	13.5 (12.6-14.4)	13.5 (12.7-14.5)	12.2 (11.2-13.3)	<0.0001	13.5 (12.6-14.4)	11.9 (11.1-13.2)	<0.0001
Anaemia (n, %)	289 (18.48)	249 (16.9)	40 (45.5)	<0.0001	263 (17.3)	26 (57.8)	<0.0001
Microcytic	83 (28.7)	83 (33.3)	0 (0.0)	-	83 (31.6)	0 (0.0)	-
Normocytic	180 (62.3)	141 (56.6)	39 (97.5)	-	155 (58.9)	25 (96.2)	-
Macrocytic	26 (9.0)	25 (10.0)	1 (2.5)	-	25 (9.5)	1 (3.8)	-

283

284 Data are presented as median (25th-75<sup>th</sup> percentiles) and percentages. RBC, red blood cells; WBC, white blood cells; N/L ratio,  
285 lymphocyte to neutrophil ratio; MCV, mean corpuscular volume, MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular

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

286 haemoglobin concentration; RDW, red cell distribution width; Hb, haemoglobin. MDRD, Modification of Diet in Renal Disease; CKD-  
287 EPI, Chronic Kidney Disease Epidemiology Collaboration.

For peer review only

### 306 **Association between the different haematological indices and eGFR**

307 The age and gender-adjusted associations between the different haematological indices and eGFR,  
308 estimated by means of the MDRD and CKD-EPI equations, are presented in Table 3. Based on the  
309 CKD-EPI, however not the MDRD equation, eGFR was positively associated with all the RBC  
310 indices, including total RBC count, haemoglobin and haematocrit levels. eGFR was not associated  
311 with total WBC count, however a lower lymphocyte count was associated with a lower eGFR and  
312 N/L ratio was inversely associated with eGFR. Furthermore, male gender was significantly  
313 associated with all haematological measures, except basophil count and eosinophil count, and age  
314 was inversely associated with all RBC indices, lymphocytes, neutrophils, platelet count, MCHC  
315 and positively associated with RDW.

316

317

318

319

320

321

322

323

324

325

326

327

328

329

330

331

332

333

**Table 3:** Linear regression coefficients, adjusted for age, gender (Model 1) and eGFR (MDRD and CKD-EPI-derived) (Models 2) for the prediction of haematological-derived measures

Haematological-derived measures	MODEL 1								MODEL 2.1				MODEL 2.2			
	Age			Gender			R <sup>2</sup>	eGFR (MDRD)			R <sup>2</sup>	eGFR (CKD-EPI)			R <sup>2</sup>	
	β	95% CI	p	β	95% CI	p		β	95% CI	p		β	95% CI	p		
RBC (x10 <sup>3</sup> /μl)	-2.8	-4.5 to -1.2	0.001	327.4	269.6 to 385.3	<0.0001	0.08	0.3	-0.7 to 1.3	0.541	0.08	3.2	1.5 to 5.0	<0.0001	0.09	
Haematocrit (%)	-0.2	-0.3 to -0.0	0.018	40.2	35.3 to 45.1	<0.0001	0.15	0.0	-0.1 to 0.1	0.709	0.15	0.3	0.1 to 0.4	<0.0001	0.16	
Hb (g/l)	-0.1	-0.1 to -0.0	0.002	14.2	12.5 to 15.9	<0.0001	0.16	0.0	-0.0 to 0.0	0.907	0.16	0.1	0.0 to 0.1	<0.0001	0.16	
WBC (x10 <sup>3</sup> /μl)	-15.1	-22.3 to -7.8	<0.0001	-431.9	-690.8 to -173.0	0.001	0.01	-0.5	-4.8 to 3.9	0.834	0.01	-1.7	-9.7 to 6.3	0.678	0.01	
N/L (%)	-0.1	-3.8 to 3.5	0.941	136.2	5.6 to 266.7	0.041	0.00	-0.1	-0.4 to 0.1	0.214	0.00	-6.3	-10.3 to -2.3	0.002	0.01	
Lymphocyte count (x10 <sup>6</sup> /l)	-2.9	-5.2 to -0.5	0.017	-257.10	-341.0 to -173.2	<0.0001	0.02	0.7	-0.8 to 2.1	0.364	0.02	3.0	0.4 to 5.6	0.022	0.03	
Monocyte count (x10 <sup>6</sup> /l)	-0.8	-1.4 to -0.2	0.005	91.6	71.2 to 112.0	<0.0001	0.05	0.3	-0.1 to 0.6	0.114	0.05	0.5	-0.1 to 1.1	0.122	0.05	
Neutrophil count (x10 <sup>6</sup> /l)	-10.9	-16.8 to -5.1	<0.0001	-291.8	-500 to -82.8	0.006	0.01	-1.1	-4.6 to 2.4	0.542	0.01	-4.7	-11.1 to 1.7	0.150	0.01	
Basophil count (x10 <sup>6</sup> /l)	1.6	-8.4 to 11.5	0.759	-187.9	-541.9 to 166.1	0.298	0.00	0.7	-5.3 to 6.6	0.822	0.00	-8.3	-19.2 to 2.6	0.136	0.00	
Eosinophil count (x10 <sup>6</sup> /l)	-0.5	-1.1 to 0.0	0.067	15.9	-4.9 to 36.7	0.135	0.00	-0.4	-0.7 to 0.0	0.071	0.00	-0.6	-1.2 to 0.1	0.074	0.00	
Platelet count (x10 <sup>9</sup> /l)	-0.4	-0.6 to -0.1	0.003	-33.0	-42.0 to -24.0	<0.0001	0.03	0.1	-0.0 to 0.3	0.088	0.04	0.1	-0.0 to 0.3	0.088	0.04	
MCV (fL/100cell)	1.4	-1.0 to 3.7	0.255	232.2	148.1 to 316.2	<0.0001	0.02	-0.2	-1.6 to 1.2	0.761	0.02	0.1	-2.5 to 2.7	0.946	0.02	
MCH (pg/100cell)	-0.2	-1.1 to 0.7	0.698	95.3	63.3 to 127.4	<0.0001	0.02	-0.1	-0.7 to 0.4	0.646	0.02	0.1	-0.9 to 1.1	0.881	0.02	
MCHC (g/l)	-0.1	-0.01 to -0.0	<0.0001	2.3	0.9 to 3.8	0.002	0.02	-0.0	-0.0 to 0.0	0.227	0.02	-0.0	-0.1 to 0.0	0.664	0.01	
RDW (%)	0.1	0.0 to 0.1	0.004	-1.9	-3.7 to -0.0	0.05	0.01	0.1	0.0 to 0.1	<0.0001	0.02	0.1	0.0 to 0.1	0.025	0.01	

Data presented as β-coefficient, 95% confidence interval and adjusted-R<sup>2</sup>. Analysis are adjusted for age and gender. RBC, red blood cells; WBC, white blood cells; MCV, L/N ratio, lymphocyte to neutrophil ratio; mean corpuscular volume, MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width; Hb, haemoglobin. MDRD, Modification of Diet in Renal Disease. Model 1 = age + gender; Model 2.1 = age + gender + eGFR (MDRD); Model 2.2 = age + gender + eGFR (CKD-EPI)



## DISCUSSION

In this community-based sample of mixed-ancestry South Africans, we have shown that the haematological profile of individuals with reduced eGFR ( $<60\text{ml}/\text{min}/1.73\text{m}^2$ ) are substantially impaired compared to those with normal kidney function, giving rise to the high prevalence of anaemia in this screen-detected CKD population. Furthermore, despite eGFR being positively associated with RBC indices, indicative of the severity of kidney function impairment, the disease state had no effect on the morphology of the RBC. Lastly, we confirmed that a chronic pro-inflammatory state exists in participants with CKD.

This study, which is in accordance with other studies in Africa and other developing countries<sup>36-42</sup>, has shown that CKD is associated with significant impairment in RBC indices. Indeed, we have shown that total RBC count, haemoglobin concentration and percentage haematocrit were substantially reduced in participants with eGFR below  $60\text{ml}/\text{min}/1.73\text{m}^2$ , compared to those with normal kidney function, independent of age and gender. Since erythropoietin is produced mainly by the proximal tubule of the nephron, kidney function decline will result in a decline in erythropoietin production and as a consequence result in decreased haemoglobin synthesis, leading to a fall in total RBC count<sup>17</sup>. This significant reduction in RBC, inevitably gives rise to anaemia<sup>14</sup>. Indeed, our study and numerous other studies have shown that the severity of anaemia increases with disease progression; with most of these studies showing anaemia at least twice as prevalent in participants with CKD, compared to the general adult population<sup>37</sup>. Furthermore, we found that 17% of the sample population with normal kidney function had haemoglobin levels  $<13.5\text{g}/\text{dL}$  and  $<12\text{g}/\text{dL}$  for men and women, respectively. However, this is not uncommon in Africa as previous studies have found that Africa has a high prevalence of anaemia caused by iron deficiency. In South Africa in particular, the South African National Health and Nutrition Examination Survey (SANHANES-1)<sup>43</sup> showed that 22% and 12.2% of adult females and males have anaemia.

The activation of the immune system, caused by inflammation, increases white blood cell counts<sup>23</sup>; emphasising the potential of WBC indices as a surrogate marker of inflammation in CKD<sup>20</sup>. Our study showed that despite no correlation between total WBC and reduced kidney function, CKD was associated with higher neutrophil and lower lymphocyte counts; both of which are independently associated with the promotion of atherosclerosis<sup>44 45</sup> and poor cardiovascular

1  
2  
3 373 outcomes<sup>46</sup>. N/L ratio, which combines the predictive power of both increased neutrophil count  
4 374 and decreased lymphocyte count<sup>47</sup>, was associated with reduced eGFR in our study, as also found  
5 375 in other studies<sup>23 48 49</sup>. Indeed previous studies, which included CKD patients on haemodialysis<sup>23</sup>  
6 376<sup>48</sup> and pre-dialysis<sup>49</sup>, showed that an increased N/L ratio was associated with known inflammatory  
7 377 markers such as tumor necrosis factor (TNF)- $\alpha$ <sup>23</sup>, interleukin 6 (IL-6) and high-sensitivity C-  
8 378 reactive protein (hs-CRP) levels<sup>49</sup>. These studies demonstrated that these well-established markers  
9 379 of inflammation were independent factors for predicting N/L ratio, thus presenting N/L ratio as an  
10 380 inflammatory biomarker for CKD patients. Since full blood count analysis are done routinely and  
11 381 a relatively affordable and easy measure to acquire, these findings are especially valuable taking  
12 382 into account the severely resource limited setting found in Africa and other low and middle-income  
13 383 countries.

14 384  
15 385 Our study has a few limitations. This study was conducted in only one geographical area, which  
16 386 may not adequately reflect all the mixed ancestry population groups in Sub-Saharan Africa.  
17 387 Furthermore, this was a community-based sample with high female to male participation, however  
18 388 the latter being a common trend in South African population studies. Our study also used a single  
19 389 serum creatinine measure to determine the grade of kidney function and did not include estimates  
20 390 of albuminuria. Albuminuria, in particular, is required for clinical and aetiological diagnosis of  
21 391 CKD, as this information is important particularly in the interpretation of eGFR greater than  
22 392 60ml/min/1.73m<sup>2</sup> where inaccuracies of the eGFR equations are greatest. It is however a common  
23 393 practice in community-based studies to diagnose CKD using a single measurement of serum  
24 394 creatinine. Furthermore, we did not investigate other haematinic deficiencies, such as vitamin B12  
25 395 and iron deficiencies, which if present however, are less likely to affect haematological profile in  
26 396 a differential way in people with and without CKD. However, despite these limitations, we are not  
27 397 aware of other studies that have assessed the haematological profile of individuals with reduced  
28 398 kidney function in a population-based setting in Africa, even more specific, individuals of mixed-  
29 399 ancestry. Furthermore, we studied a community with a high burden of obesity, hypertension and  
30 400 diabetes, reflective of the current burden in Africa. This study provides much needed evidence for  
31 401 the association between the haematological profile and CKD as population-based data on the  
32 402 haematological profile of people with CKD in Africa, are very limited.

33 403

1  
2  
3 404 In conclusion, the findings from our study are valuable as full blood count analyses are done  
4  
5 405 routinely and are relatively affordable, taking into account the severely resource limited setting  
6  
7 406 found in Africa and other low and middle-income countries. Furthermore, though it still remains  
8  
9 407 unclear whether the advocated kidney function estimators provide accurate estimates of CKD  
10  
11 408 burden in African populations <sup>50</sup>, the correlation of these estimates, with deteriorating profile of  
12  
13 409 blood cell counts, suggests that these advocated GFR estimates, particularly the CKD-EPI  
14  
15 410 equation, to some extent, measure kidney function in African populations.  
16

## 17 411 18 412 **DECLARATIONS**

### 19 413 **Ethics approval and consent to participate**

20 414 The study was approved by the Research Ethics Committees of the Cape Peninsula University of  
21  
22 415 Technology and Stellenbosch University (NHREC: REC—230 408–014 and N14/01/003,  
23  
24 416 respectively). The study was conducted in accordance with the Declaration of Helsinki. All  
25  
26 417 participants voluntary signed written informed consent after all the procedures were fully  
27  
28 418 explained in the language of their choice. Permission to conduct the study was also obtained from  
29  
30 419 relevant authorities including the city and community authorities.  
31

### 32 420 33 421 **Consent for publication**

34 422 Not applicable  
35

### 36 423 37 424 **Data sharing statement**

38 425 The datasets used and/or analyzed during the current study are available from the corresponding  
39  
40 426 author on reasonable request.  
41

### 42 427 43 428 **Competing interest**

44 429 The authors declare that they have no competing interests  
45  
46 430

### 47 431 **Funding**

48 432 The South African Medical Research Council (SAMRC) funded this research project with funds  
49  
50 433 from National Treasury under its Economic Competitiveness and Support Package (MRC-RFA-  
51  
52 434 UFSP-01-2013/VMH Study).  
53  
54  
55  
56  
57  
58  
59  
60

435

**436 Authors' contribution**

437 Study conception and funding acquisition (TEM, APK, RTE), operationalization and supervision  
438 of the data collection (TEM), data analysis and interpretation (CG, APK), drafting the manuscript  
439 (CG, APK), critical revision of the manuscript and approval of the final version (all co-authors).

440

**441 Acknowledgements**

442 Poster presented at the 28th European Meeting on Hypertension and Cardiovascular Protection,  
443 held in Barcelona, June 8-11, 2018. As such, results have been published as an abstract. We are  
444 also grateful to the Cape Town VMH study investigation team and population of Bellville-South  
445 for their participation.

446

**447 REFERENCES**

- 448 1. Bolton K, Culleton B, Harvey K. K/DOQI clinical practice guidelines for chronic kidney disease:  
449 evaluation, classification, and stratification. *Kidney Disease Outcome Quality Initiative. Am*  
450 *JKidney Dis* 2002;39(2 Suppl 1):S1-246.
- 451 2. Nitta K, Okada K, Yanai M, et al. Aging and chronic kidney disease. *Kidney & blood pressure research*  
452 2013;38(1):109-20. doi: 10.1159/000355760 [published Online First: 2014/03/20]
- 453 3. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20  
454 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010.  
455 *Lancet* 2012;380(9859):2095-128. doi: 10.1016/S0140-6736(12)61728-0
- 456 4. Eckardt KU, Coresh J, Devuyst O, et al. Evolving importance of kidney disease: from subspecialty to  
457 global health burden. *Lancet* 2013;382(9887):158-69. doi: 10.1016/s0140-6736(13)60439-0
- 458 5. Ayodele OE, Alebiosu CO. Burden of chronic kidney disease: an international perspective. *Adv Chronic*  
459 *Kidney Dis* 2010;17(3):215-24. doi: 10.1053/j.ackd.2010.02.001
- 460 6. Naicker S. End-stage renal disease in sub-Saharan Africa. *Ethn Dis* 2009;19(1 Suppl 1):S1-13-5.  
461 [published Online First: 2009/06/02]
- 462 7. Peralta CA, Risch N, Lin F, et al. The Association of African Ancestry and elevated creatinine in the  
463 Coronary Artery Risk Development in Young Adults (CARDIA) Study. *American journal of*  
464 *nephrology* 2010;31(3):202-8. doi: 10.1159/000268955 [published Online First: 2009/12/24]
- 465 8. Kiberd BA, Clase CM. Cumulative risk for developing end-stage renal disease in the US population.  
466 *Journal of the American Society of Nephrology : JASN* 2002;13(6):1635-44. [published Online  
467 First: 2002/06/01]
- 468 9. Stanifer JW, Jing B, Tolan S, et al. The epidemiology of chronic kidney disease in sub-Saharan Africa:  
469 a systematic review and meta-analysis. *Lancet Glob Health* 2014;2(3):e174-81. doi:  
470 10.1016/S2214-109X(14)70002-6
- 471 10. Stanifer JW, Muiru A, Jafar TH, et al. Chronic kidney disease in low- and middle-income countries.  
472 *Nephrol Dial Transplant* 2016;31(6):868-74. doi: 10.1093/ndt/gfv466
- 473 11. Hamer RA, El Nahas AM. The burden of chronic kidney disease. *BMJ* 2006;332(7541):563-4. doi:  
474 10.1136/bmj.332.7541.563
- 475 12. Jha V, Garcia-Garcia G, Iseki K, et al. Chronic kidney disease: global dimension and perspectives.  
476 *Lancet* 2013;382(9888):260-72. doi: 10.1016/S0140-6736(13)60687-X

- 1  
2  
3 477 13. Babitt JL, Lin HY. Mechanisms of anemia in CKD. *Journal of the American Society of Nephrology* :  
4 478 *JASN* 2012;23(10):1631-4. doi: 10.1681/asn.2011111078 [published Online First: 2012/09/01]  
5 479 14. Astor BC, Muntner P, Levin A, et al. Association of kidney function with anemia: The third national  
6 480 health and nutrition examination survey (1988-1994). *Archives of Internal Medicine*  
7 481 2002;162(12):1401-08. doi: 10.1001/archinte.162.12.1401  
8 482 15. Webster AC, Nagler EV, Morton RL, et al. Chronic Kidney Disease. *Lancet* 2017;389(10075):1238-  
9 483 52. doi: 10.1016/s0140-6736(16)32064-5 [published Online First: 2016/11/27]  
10 484 16. Kazmi WH, Kausz AT, Khan S, et al. Anemia: An early complication of chronic renal insufficiency.  
11 485 *American Journal of Kidney Diseases* 2001;38(4):803-12. doi: 10.1053/ajkd.2001.27699  
12 486 17. Kutuby F, Wang S, Desai C, et al. Anemia of chronic kidney disease. *Disease-a-month : DM*  
13 487 2015;61(10):421-4. doi: 10.1016/j.disamonth.2015.08.002 [published Online First: 2015/09/15]  
14 488 18. Levey AS, Coresh J. Chronic kidney disease. *Lancet* 2012;379(9811):165-80. doi: 10.1016/S0140-  
15 489 6736(11)60178-5  
16 490 19. Stenvinkel P, Heimbürger O, Paulter F, et al. Strong association between malnutrition, inflammation,  
17 491 and atherosclerosis in chronic renal failure. *Kidney Int* 1999;55(5):1899-911. doi: 10.1046/j.1523-  
18 492 1755.1999.00422.x [published Online First: 1999/05/07]  
19 493 20. Okyay GU, İnal S, Öneç K, et al. Neutrophil to Lymphocyte Ratio in Evaluation of Inflammation in  
20 494 Patients with Chronic Kidney Disease. *Renal Fail* 2013;35(1):29-36. doi:  
21 495 10.3109/0886022X.2012.734429  
22 496 21. Azab B, Zaher M, Weiserbs KF, et al. Usefulness of neutrophil to lymphocyte ratio in predicting short-  
23 497 and long-term mortality after non-ST-elevation myocardial infarction. *Am J Cardiol*  
24 498 2010;106(4):470-6. doi: 10.1016/j.amjcard.2010.03.062 [published Online First: 2010/08/10]  
25 499 22. Uthamalingam S, Patvardhan EA, Subramanian S, et al. Utility of the neutrophil to lymphocyte ratio in  
26 500 predicting long-term outcomes in acute decompensated heart failure. *Am J Cardiol*  
27 501 2011;107(3):433-8. doi: 10.1016/j.amjcard.2010.09.039 [published Online First: 2011/01/25]  
28 502 23. Turkmen K, Guney I, Yerlikaya FH, et al. The relationship between neutrophil-to-lymphocyte ratio and  
29 503 inflammation in end-stage renal disease patients. *Ren Fail* 2012;34(2):155-9. doi:  
30 504 10.3109/0886022x.2011.641514 [published Online First: 2011/12/17]  
31 505 24. Masconi K, Matsha TE, Erasmus RT, et al. Independent external validation and comparison of prevalent  
32 506 diabetes risk prediction models in a mixed-ancestry population of South Africa. *Diabetol Metab*  
33 507 *Syndr* 2015;7:42. doi: 10.1186/s13098-015-0039-y [published Online First: 2015/05/20]  
34 508 25. Kengne AP, Erasmus RT, Levitt NS, et al. Alternative indices of glucose homeostasis as biochemical  
35 509 diagnostic tests for abnormal glucose tolerance in an African setting. *Prim Care Diabetes*  
36 510 2017;11(2):119-31. doi: 10.1016/j.pcd.2017.01.004 [published Online First: 2017/01/31]  
37 511 26. Chalmers J, MacMahon S, Mancia G, et al. 1999 World Health Organization-International Society of  
38 512 Hypertension Guidelines for the management of hypertension. Guidelines sub-committee of the  
39 513 World Health Organization. *Clinical and experimental hypertension (New York, NY : 1993)*  
40 514 1999;21(5-6):1009-60. doi: 10.3109/10641969909061028 [published Online First: 1999/07/28]  
41 515 27. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its  
42 516 complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO  
43 517 consultation. *Diabet Med* 1998;15(7):539-53. doi: 10.1002/(sici)1096-  
44 518 9136(199807)15:7<539::aid-dia668>3.0.co;2-s [published Online First: 1998/08/01]  
45 519 28. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein  
46 520 cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18(6):499-  
47 521 502.  
48 522 29. Levey AS, Bosch JP, Lewis JB, et al. A more accurate method to estimate glomerular filtration rate  
49 523 from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study  
50 524 Group. *Ann Intern Med* 1999;130(6):461-70.  
51 525 30. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann*  
52 526 *Intern Med* 2009;150(9):604-12.



- 1  
2  
3 527 31. Levey AS, Coresh J, Balk E, et al. National Kidney Foundation practice guidelines for chronic kidney  
4 528 disease: evaluation, classification, and stratification. *Ann Intern Med* 2003;139(2):137-47.  
5 529 [published Online First: 2003/07/16]  
6 530 32. National Kidney Foundation. KDOQI Clinical Practice Guidelines and Clinical Practice  
7 531 Recommendations for Anemia in Chronic Kidney Disease. *Am J Kidney Dis* 2006;47(5 Suppl  
8 532 3):S11-145. doi: 10.1053/j.ajkd.2006.03.010 [published Online First: 2006/05/09]  
9 533 33. Bessman JD, Johnson RK. Erythrocyte volume distribution in normal and abnormal subjects. *Blood*  
10 534 1975;46(3):369-79. [published Online First: 1975/09/01]  
11 535 34. World Health Organization. A global brief on Hypertension: Silent killer, global public health crisis,  
12 536 2013.  
13 537 35. World Health Organisation, International Diabetes Federation. Definition and diagnosis of diabetes and  
14 538 intermediate hyperglycemia. In: consultation WI, ed. Geneva, 2006.  
15 539 36. Afshar R, Sanavi S, Salimi J, et al. Hematological profile of chronic kidney disease (CKD) patients in  
16 540 Iran, in pre-dialysis stages and after initiation of hemodialysis. *Saudi Journal of Kidney Diseases*  
17 541 *and Transplantation* 2010;21(2):368-71.  
18 542 37. Akinsola A, Durosinmi MO, Akinola NO. The haematological profile of Nigerians with chronic renal  
19 543 failure. *Afr J Med Med Sci* 2000;29(1):13-6. [published Online First: 2001/05/31]  
20 544 38. Asif N, Hasan S, Hassan K. Hematological Changes in Patients of Chronic Renal Disease and Their  
21 545 Response to Treatment with Erythropoietin. *Int J Pathol* 2015;13(1):14-19.  
22 546 39. Bhattacharjee K, Das D, Rabha P, et al. A study on hematological profile in patients of chronic renal  
23 547 failure with special reference to serum iron profile. *Journal of Evidence based Medicine and*  
24 548 *Healthcare* 2015;2(46):8212-19.  
25 549 40. Dabrowska MM, Mikula T, Wiercinska-Drapalo A. The anemia prevalence and the association between  
26 550 complete blood count analysis and renal function parameters in HIV-1-infected patients. *Current*  
27 551 *HIV research* 2012;10(3):247-51. [published Online First: 2012/03/06]  
28 552 41. Islam MN, Ferdous A, Zahid AZ, et al. Haematological Profile of Patients with Chronic Kidney Disease  
29 553 in Northern Bangladesh. *Dinajpur Med Col J* 2015;8(1):21-27.  
30 554 42. Latiweshob OB, Elwerfaly HH, Sherif DS, et al. Haematological Changes in Predialyzed and  
31 555 Hemodialyzed Chronic Kidney Disease patients in Libya. *IOSR Journal of Dental and Medical*  
32 556 *Sciences* 2017;16(2):106-12.  
33 557 43. Shisana O, Labadarios D, Rehle T, et al. The South African National Health and Nutrition Examination  
34 558 Survey (SANHANES-1), 2013.  
35 559 44. Drechsler M, Doring Y, Megens RT, et al. Neutrophilic granulocytes - promiscuous accelerators of  
36 560 atherosclerosis. *Thrombosis and haemostasis* 2011;106(5):839-48. doi: 10.1160/th11-07-0501  
37 561 [published Online First: 2011/10/21]  
38 562 45. Nunez J, Minana G, Bodi V, et al. Low lymphocyte count and cardiovascular diseases. *Curr Med Chem*  
39 563 2011;18(21):3226-33. [published Online First: 2011/06/16]  
40 564 46. Reddan DN, Klassen PS, Szczech LA, et al. White blood cells as a novel mortality predictor in  
41 565 haemodialysis patients. *Nephrol Dial Transplant* 2003;18(6):1167-73. [published Online First:  
42 566 2003/05/16]  
43 567 47. Solak Y, Yilmaz MI, Sonmez A, et al. Neutrophil to lymphocyte ratio independently predicts  
44 568 cardiovascular events in patients with chronic kidney disease. *Clin Exp Nephrol* 2013;17(4):532-  
45 569 40. doi: 10.1007/s10157-012-0728-x [published Online First: 2012/11/28]  
46 570 48. An X, Mao HP, Wei X, et al. Elevated neutrophil to lymphocyte ratio predicts overall and cardiovascular  
47 571 mortality in maintenance peritoneal dialysis patients. *Int Urol Nephrol* 2012;44(5):1521-8. doi:  
48 572 10.1007/s11255-012-0130-3 [published Online First: 2012/02/01]  
49 573 49. Okyay GU, Inal S, Onec K, et al. Neutrophil to lymphocyte ratio in evaluation of inflammation in  
50 574 patients with chronic kidney disease. *Ren Fail* 2013;35(1):29-36. doi:  
51 575 10.3109/0886022x.2012.734429 [published Online First: 2012/11/02]  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 576 50. Agoons DD, Balti EV, Kaze FF, et al. Performance of three glomerular filtration rate estimation  
4 577 equations in a population of sub-Saharan Africans with Type 2 diabetes. *Diabet Med*  
5 578 2016;33(9):1291-8. doi: 10.1111/dme.12996 [published Online First: 2015/10/21]  
6  
7 579  
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