PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	HAEMATOLOGICAL PROFILE OF CHRONIC KIDNEY DISEASE IN A MIXED-ANCESTRY SOUTH AFRICAN POPULATION: A CROSS-SECTIONAL STUDY
AUTHORS	George, Cindy; Matsha, Tandi; Erasmus, Rajiv; Kengne, AP

VERSION 1 – REVIEW

REVIEWER	Nomandla D. Madala Sefako Makgatho Health Sciences University, South Africa
REVIEW RETURNED	07-May-2018

GENERAL COMMENTS	Overall comments: The study sought to determine the haematological profile of screen-detected CKD participants and the association of various haematological parameters with eGFR. The authors make assumptions about CKD based on a single serum-creatinine level and provide no indication that the diagnosis is based on previous repeated measurements. This failure to fulfil the critical criteria for defining CKD using duration of disease has not been acknowledged hence conclusions on CKD in this study may be misleading. I have listed several concerns below that I believe need to be addressed to put more context in this contribution. Specific comments: 1. Abstract The statement that 'little is known about the association between CKD and white blood cells' seems incorrect and must be qualified. Rather existing data should be recognized with gaps in knowledge/ understanding appropriately explained in the literature review section. Lines 27-28, page 2/25 2. Background It is also unclear to what extent observed haematological abnormalities in this study population differ from the general population or other published studies. The effect of aging on both eGFR and haematological parameters is not discussed. 3. Methods a. Biochemical analysis and calculations Specify whether creatinine levels were standardized or calibrated to be isotope dilution mass spectrometry (IDMS)-compatible, and relevant IDMS eGFR formulae were used. Lines 170-174. b. Classification The NKF-KDOQI CKD definition requires that the kidney function abnormalities are present for at least 3 months. Clarify how this minimum duration was established in this study population. If only a single measurement was used (without any previous record), then

	that must be explicit in the manuscript and reference of reduced eGFR as equivalent to CKD re-considered. Lines 117-120, page 6/25 and 184-187, page 7/25. Line 192, page 7/25 should read: screen-detected hypertension, not hypertensive. 4. Results How have authors interpreted the prevalence of anaemia of 17% in those with normal kidney function? Table 2, Line 31, page 13/25. And could prevalence in the general population (whether high or low) influence the observations made in this study? Although aging has a significant effect on both eGFR as well as various haematological variables such as neutrophil: lymphocyte ratio, anaemia, etc. the associations with age have not been sufficiently explored. The eGFR models for each haematological parameter were adjusted for age but it is not clear whether interaction with age was evaluated in the observed relationships. 5. Discussion While there was no relationship with MCV/MCH/MCHC, is there any explanation for the positive trend observed with red cell distribution width (CKD-EPI-eGFR) as this has not been explained. Line 33, page 17/25 and lines 358-360, page 19/25. Was the association of obesity with N:L ratio evaluated as two-thirds of the population were classified overweight/obese? The role, in a resource-limited setting, of the increasing N:L ratio (while within the normal range) with falling eGFR has not been argued sufficiently. Lines 385-389, page 15/25. Conclusion It is unclear how the authors arrive at the conclusion that the association of haematological measures with eGFR, suggests that the GFR estimates used measure true kidney function since that was not what they sought to determine in this study. Furthermore, other confounders in such a relationship were not assessed, other than age, especially haematinic/ nutritional deficiencies. 6. Strengths and limitations Single versus repeat measurement, if applicable. Haematinic deficiencies were not evaluated as the most common causes of anaemia, particularly that almost 10% of the population had macrocytic anaemia while almost 30% had microcytosis. This is a shortcoming in this study, especially in the older population evaluated, thus should be acknowledged, as it limits the conclusions that can be made about anaemia and severity of kidney function in this study.

REVIEWER	SECK SIDY INTERNAL MEDICINE AND NEPHROLOGY DEPARTMENT - FACULTY OF HEALTH SCIENCES - UNIVERSITY GASTON BERGER OF SAINT-LOUIS, SENEGAL
REVIEW RETURNED	09-May-2018

GENERAL COMMENTS	This study tried to address an interesting research question but I think the methodology was not adequate and that gives serious limitations to its results. The relation should be eGFR explaining hematological profile but not the inverse. It is clinically not to try to predict CKD from only FBC analysis because there are many possible confounding factors that can modify hematologic profile ranging from common infections to hemoglobinopathies like sickle cell disease or beta-thalassemia.

	These diseases are known frequent in African patients but authors did not look at them in their study. So as long as you cannot have demonstrate that hematological abnormalities observed in some of the study participants are secondary to uremia-associated erythropoeisis disturbances, it will be very difficult to conclude that correlations found in this study confirmed in some extend validity of eGFR formulas in African populations. Moreover, like many other cross-studies looking at CKD, there is a high probability of "false positive cases" because one single Scr dosage can be erronously interpreted as it was nicely demonstrated in data from population-based cohort.

REVIEWER	Francois Folefack Kaze Faculty of Medicine and Biomedical Sciences of Yaounde and Yaounde University Teaching Hospital, Cameroon
REVIEW RETURNED	08-Jun-2018

GENERAL COMMENTS	This study is of great importance in Africa setting presenting the haematological profile of CKD. The results are not innovative but give a picture of the situation in Africa which is something to be encouraged. Concerning abstract, the author's mentioned only red blood cell profile which is not suitable for the paper title. I suggest to add data of white blood cell and platelets which are present in the text. In the results section, as the authors presented the profile according to eGFR stage, I suggest them to add also for WBC parameters (L/N ratio, lymphocytes and neutrophils count) mainly, and if necessary for platelets too. As the authors used the data of VMH study, if they can add C-reactive protein and lipid profile data, it will give an added value to the paper in order to find out any association of inflammation and haematological profile as well as the stage of CKD.

VERSION 1 – AUTHOR RESPONSE

RE: Response to reviewers' comments

REVIEWER #1:

Overall comments: The study sought to determine the haematological profile of screen-detected CKD participants and the association of various haematological parameters with eGFR. The authors make assumptions about CKD based on a single serum-creatinine level and provide no indication that the diagnosis is based on previous repeated measurements. This failure to fulfil the critical criteria for defining CKD using duration of disease has not been acknowledged hence conclusions on CKD in this study may be misleading. I have listed several concerns below that I believe need to be addressed to put more context in this contribution.

Thank you for the comment. Yes, we agree and refer you to lines 388-390 in our limitations section, "Our study also only included a single serum creatinine measure to determine the grade of kidney function and did not include estimates of albuminuria". According to KDIGO's clinical practice guideline for the evaluation and management of chronic kidney disease (2012), CKD is defined as a

GFR <60 ml/min/1.73 m^2 for ≥3months and/or increased urinary albumin excretion (≥30 mg/24h). However, worldwide (and not only in Africa), population-based prevalence and association studies in the field of CKD epidemiology and chronic NCDs in general, are based on a single time-point assessment; and not on repeated measurements; our team has extensively published on this topic in Africa. Furthermore, in the development and validation of both the MDRD and CKD-EPI equations, Levey et al [1 2] did not make use of repeated measures to predict CKD. No confusion should be made between clinical diagnosis of CKD for the purpose on initiating treatment to prevent the progression of the disease, and diagnosis of CKD in community-based epidemiological studies. As far as we know, only one community-based study has estimated the prevalence of CKD in Africa using repeated measurements [3]. In this study conducted in Cameroon and involving our team, repeated assessment was conducted only in participants with an initial abnormal test, and final prevalence estimates were within the range of those previously reported across Africa. Furthermore, about 5% of the participants were lost (including deaths) between the two-assessment time-points.

Specific comments:

1. **Abstract**

   The statement that 'little is known about the association between CKD and white blood cells' seems incorrect and must be qualified. Rather existing data should be recognized with gaps in knowledge/ understanding appropriately explained in the literature review section. Lines 27-28, page 2/25

   We have decided to remove the sentence which reads "…with little know about the association between CKD and white blood cells (WBC)", as it is not essential as part of the abstract. As mentioned by the reviewer, we do explain the gap in knowledge regarding the association between WBCs and CKD in the introduction section. Please refer to lines 104-114.

   Background:

   It is also unclear to what extent observed haematological abnormalities in this study population differ from the general population or other published studies. The effect of aging on both eGFR and haematological parameters is not discussed.

   To clarify, this is a study conducted in the general mixed-ancestry population in South Africa. Furthermore, the focus of the study was not to asses to what extent the haematological profile of our sample compared with that of another general population; but rather on how the profile varies according to kidney function within the same general population. In addition, though it is known that increasing age is associated with reduction in kidney function and deterioration in haematological profile, the focus on the effect of age may not be justified in this study as our analyses adjusted for age. However, please refer to line 85 where we have added age as a risk factor for CKD. "Chronic kidney disease (CKD) is a major global public health problem, estimated to affect more than 10% of the general adult population and up to 50% of some high-risk subpopulations, such as the elderly, those with non-communicable diseases (NCD),

including type 2 diabetes mellitus (T2D) and hypertension, and communicable diseases (CD), including *human immunodeficiency virus (HIV)/* acquired immunodeficiency syndrome (AIDS)".

2. Methods

a. Biochemical analysis and calculations.

Specify whether creatinine levels were standardized or calibrated to be isotope dilution mass spectrometry (IDMS)-compatible, and relevant IDMS eGFR formulae were used. Lines 170-174.

As indicated in the relevant section, biochemical analyses were all performed at an ISO 15189 accredited Reference Pathology service. Such an accreditation, as certainly known to the reviewer, covers issues relating to compliance with calibration and good clinical laboratory practice. Standardized creatinine measurement is a global recommendation, implemented by our partner laboratory since 2009 (https://www.pathcare.co.za/webfiles/files/StandardisationOfCreatinineDetermination.pdf). We have now added this clarification to the paper (lines 175-178) where it reads: "Creatinine assays at our Partner pathology service are standardized to the internationally accepted reference method (isotope dilution mass spectrophotometry [IDMS]) since 2009 and eGFR estimators applicable to standardised creatinine values were used."

b. Classification

The NKF-KDOQI CKD definition requires that the kidney function abnormalities are present for at least 3 months. Clarify how this minimum duration was established in this study population. If only a single measurement was used (without any previous record), then that must be explicit in the manuscript and reference of reduced eGFR as equivalent to CKD re-considered. Lines 117-120, page 6/25 and 184-187, page 7/25. Line 192, page 7/25 should read: screen-detected hypertension, not hypertensive.

Thank you. This is dually noted. As mentioned in point #1 for reviewer #1, our study included a single serum creatinine measure at one single time point, which we list as a study limitation (lines 388-390). However, as mentioned previously, various population-based prevalence and association studies in the field of CKD epidemiology and chronic NCDs in general, are based on a single time-point assessment; and not on repeated measurement. Even in CKD prevalence studies in hospital settings, it is a very common practice worldwide to report estimates based on single assessment of the kidney function. As a case in point, in a recent global systematic review on the prevalence of CKD in HIV infected people (a highly medicalised population), we identified 61 studies from 60 countries around the world [4]. Only 21 (1/3rd) of these studies provided CKD prevalence estimates based on repeated measures of kidney function. Furthermore, we are not of the reviewer's opinion that we should change the terminology in our manuscript from 'CKD' to 'reduced eGFR'. Again, this is a population-

based, not a clinical study; the standards we have used to diagnosed CKD are well in-line with practices around the world to diagnose CKD in population-based surveys. The use of the unfamiliar 'reduced eGFR' can only confuse the readers who for a large majority will not be practicing nephrologists conversant with KDOQI recommendations for clinical practice, yet who also need to understand this paper in the context of many others available in the public domain on prevalent CKD in Africa [5] and elsewhere, based on single assessment of the kidney function. In addition, please refer to line 199 where we have changed "hypertensive" to "screen-detected hypertension".

3. **Results**

How have authors interpreted the prevalence of anaemia of 17% in those with normal kidney function? Table 2, Line 31, page 13/25. And could prevalence in the general population (whether high or low) influence the observations made in this study? Although aging has a significant effect on both eGFR as well as various haematological variables such as neutrophil: lymphocyte ratio, anaemia, etc. the associations with age have not been sufficiently explored. The eGFR models for each haematological parameter were adjusted for age but it is not clear whether interaction with age was evaluated in the observed relationships.

This study is from a general population sample. Indeed, we found that 17% of the sample population with normal kidney function had haemoglobin levels <13.5g/dL and <12g/dL for men and women, respectively. 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) [6] showed that 22% and 12.2% of adult females and males have anaemia. Please refer to lines 361-366, where we have added this interpretation. Therefore, with the haematological profile being generally similar to that of the general South African population from recent studies; there is no reason to support that differentials with the general population would explain our findings.

With relation to the age effect on both eGFR and haematological profile, which wasn't the focus of the present study; we are grateful the reviewer is acknowledging that we accounted for the effect of age in our linear regressions models. From a statistical perspective however, even this adjustment for age and gender narrows down to modelling complex terms of age and gender, and adding the interaction terms of age and eGFR will result in even more complex terms of age and gender, with regression coefficients that cannot be easily interpreted. Indeed, the GFR in this study is not measured, but rather estimated using equations that are complex functions of serum creatinine, age and gender [f(Scr/Age/gender)]. Therefore, entering age, gender and eGFR in the same model to predict red blood cells for instance can be operationalised as RBC = a*Age + b*Gender + c*f(Scr/Age/Gender), and by extension, modelling the interaction term of eGFR and Age in addition can be operationalised as RBC = a*Age + b*Gender + c*f(Scr/Age/Gender) + d*Age*f(Scr/Age/Gender).

4. **Discussion**

While there was no relationship with MCV/MCH/MCHC, is there any explanation for the positive trend observed with red cell distribution width (CKD-EPI-eGFR) as this has not been explained. Line 33, page 17/25 and lines 358-360, page 19/25.

We suspect that this observation is the consequence of the complex terms of age and gender we have alluded to in #3. When CKD-EPI eGFR is used to predict RDW in an unadjusted model, a negative association is found, as found in various other studies [7-9]. However, once age and sex are added to the model the association of eGFR with RDW becomes positive.

Was the association of obesity with N:L ratio evaluated as two-thirds of the population were classified overweight/obese?

We have adjusting for BMI in the N:L model with eGFR and found that this addition did not affect the N:L to eGFR relationship. For that reason, we have decided to not include it in the final version of the models presented in the main manuscript.

The role, in a resource-limited setting, of the increasing N:L ratio (while within the normal range) with falling eGFR has not been argued sufficiently. Lines 385-389, page 15/25.

Thanks for raising this point. With nearly a paragraph of the discussion dedicated to the relationship of N/L with eGFR, very sincerely, we are struggling to understand what additional point the reviewer is trying to make here; unless he is specific on what other context specific implication he/she feels should be included.

5. **Conclusion**

It is unclear how the authors arrive at the conclusion that the association of haematological measures with eGFR, suggests that the GFR estimates used measure true kidney function since that was not what they sought to determine in this study. Furthermore, other confounders in such a relationship were not assessed, other than age, especially haematinic/ nutritional deficiencies.

The aim of the study was to characterise the haematological profile of screen-detected CKD participants in a community-based sample, and to correlate the complete blood count measures with two commonly advocated kidney function estimators of CKD. From this study we could conclude that (please refer to lines 404-406 for added text) “the findings from our study are valuable as full blood count analysis are done routinely and are relatively affordable, taking into account the severely resource limited setting found in Africa and other low and middle-income countries.” However, we still agree with our conclusion that (please refer to lines 406-410) “though it still remains unclear whether the advocated kidney function estimators provide accurate estimates of CKD burden in African populations, the correlation of these estimates, with deteriorating profile of blood cell counts, suggests that these advocated GFR estimates, particularly the CKD-EPI equation, to some extent, measure

kidney function in African populations." However, we would disagree with the reviewer on the possible effect of unmeasured confounders on the observed association. Unmeasured confounders will have to occur in a differential way by CKD status to invalidate our conclusion. There is no reason to suggest that in a community-based sample, haematinic or nutritional deficiency will be more frequent in people with low eGFR than those without; or will affect haematological profile only in the context of low GFR. Furthermore, age is a driver of CKD and most chronic non-communicable diseases; that is everything else being equal, the prevalence of CKD and other NCDs will increase with age. Beyond adjustment for age (which is what we did), there is no other efficient way we can think of the account for the effect of age in analysis of cross-sectional survey data.

6. **Strengths and limitations.**

Single versus repeat measurement, if applicable. Haematinic deficiencies were not evaluated as the most common causes of anaemia, particularly that almost 10% of the population had macrocytic anaemia while almost 30% had microcytosis. This is a shortcoming in this study, especially in the older population evaluated, thus should be acknowledged, as it limits the conclusions that can be made about anaemia and severity of kidney function in this study.

Thank you for the recommendation. Please refer to lines 388-390 and lines 394-396, where we have added these limitations. However, in line with the development above we are still in disagreement with the reviewer on a number of points.

1) There is no plausible reason why in a population-based study haematinic deficiency (if present) would lead more to anaemia in people with low eGFR than in those with normal eGFR.
2) About 10% of our population with normal eGFR and anaemia (16.9% of the total without CKD) had macrocytic anaemia. That is 25 participants out of a total of 1470 participants without CKD, or 1.7% of the total 1470 people, AND NOT 10% as suggested by the reviewers, which would have translated into 147 people. The claims of the reviewer therefore is exaggerated as there is no reason why such a small proportion of a rather large sample would differentially affect our conclusions.
3) The same reasoning in #2 applies to the claim of 30% of the population having microcytic anaemia. It was 83 out of 1470 or 5.6% of the total population without CKD, representing 33% of 249 with anaemia in this subgroup. In a population-based study with a good proportion of women of reproductive age, for well-known reasons, such a proportion should be expected.
4) The median age of our study population was 50 years, that is half of this sample was 50 years of age or younger, and 75% were age 61 years or younger. Such a profile we are afraid doesn't qualify for the stigmatisation of the sample as 'older population' as done by the reviewer.

REVIEWER #2:

This study tried to address an interesting research question but I think the methodology was not adequate and that gives serious limitations to its results. The relation should be eGFR explaining hematological profile but not the inverse. It is clinically not to try to predict CKD from only FBC analysis because there are many possible confounding factors that can modify hematologic profile ranging from common infections to hemoglobinopathies like sickle cell disease or beta-thalassemia. These diseases are known frequent in African patients but authors did not look at them in their study. So as long as you cannot have demonstrate that hematological abnormalities observed in some of the study participants are secondary to uremia-associated erythropoeisis disturbances, it will be very difficult to conclude that correlations found in this study confirmed in some extend validity of eGFR formulas in African populations. Moreover, like many other cross-studies looking at CKD, there is a high probability of "false positive cases" because one single Scr dosage can be erronously interpreted as it was nicely demonstrated in data from population-based cohort.

Thank you for your comments. We have since adapted our analysis such that eGFR explains the haematological indices. To clarify, the reviewers' main concern is that we did not account for confounding factors like infections and haemoglobinopathies (including sickle-cell disease and beta-thalassemia). There is no reason why those confounding factors (if present) would affect the haematological profile in a differential way between people with normal kidney function and those with low glomerular filtration rates, as the haemoglobinopathies referred to are common rather in endemic region for malaria, with a very low frequency in South Africa [10-13]. It would thus not be an issue in a mixed ancestry South African population. In the absence of other plausible reasons to explain why those with low eGFR in our sample displayed very high rate of anaemia, it is very appropriate to use the abundant existing knowledge to speculate that the observed high anaemia rate is likely specific to low eGFR. We can't understand why the reviewer would feel so uncomfortable with such an approach in a population-based study. We take all the precaution by saying that the observed correlation of eGFR with anaemia SUGGESTS that advocated GFR estimators to some extent approximate kidney function in African populations. We have nowhere used the word 'CONFIRMATION' as suggested by the reviewer. A confirmation can only come from a validation of eGFR against objectively measure GFR. Regarding the single measure of serum creatinine, we agree that according to KDIGO's clinical practice guidelines CKD can only be diagnosed after repeated measures. However, worldwide (and not only in Africa), population-based prevalence and association studies in the field of CKD epidemiology and chronic non-communicable diseases in general, are based on a single time-point assessment; and not on repeated measurements; our team has extensively published on this topic in Africa. Furthermore, the reviewer and colleagues have also recently published similar research including in clinical settings, using a single measure of serum creatinine to diagnose CKD [14-17]. Thus, no confusion should be made between clinical diagnosis of CKD for the purpose on initiating treatment to prevent the progression of the disease, and diagnosis of CKD in community-based epidemiological studies.

REVIEWER #3:

This study is of great importance in Africa setting presenting the haematological profile of CKD. The results are not innovative but give a picture of the situation in Africa which is something to be encouraged. Concerning abstract, the author's mentioned only red blood cell profile which is not suitable for the paper title. I suggest to add data of white blood cell and platelets which are present in the text. In the results section, as the authors presented the profile according to eGFR stage, I suggest them to add also for WBC parameters (L/N ratio, lymphocytes and neutrophils count) mainly, and if necessary for platelets too.

Thank you for the suggestion. Please refer to lines 36-41 where we have added WBC and platelet data to the results section.

As the authors used the data of VMH study, if they can add C- reactive protein and lipid profile data, it will give an added value to the paper in order to find out any association of inflammation and haematological profile as well as the stage of CKD.

We appreciate the suggestion of the reviewer, and have since added the CRP and lipid data. Please refer to Table 1.

VERSION 2 – REVIEW

REVIEWER	Sidy Seck Faculty of Health Sciences University Gaston Berger of Saint-Louis, Senegal
REVIEW RETURNED	15-Aug-2018

GENERAL COMMENTS	Hence the limitations about CKD classification issues are more discussed, i think the paper can be published.