

# Normal Range of Neutrophile to Lymphocyte Ratio in Healthy, Chronic Kidney Disease Stage 4 - 5 and Hemodialysis Participant in a Sub-Saharan African Context

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## Abstract

**Background:** Neutrophile to lymphocyte ratio (NLR) is a well-established marker of inflammation. It has been reported to be high in chronic kidney disease (CKD) and it is described as a prognosis factor in hemodialysis patients. However, limited data are available about the normal range of NLR in healthy adults as well as CKD patients including hemodialysis black Africans in Sub-Saharan countries. We sought to study NLR in healthy and advanced CKD in a single Cameroonian health facility. **Methods:** Blood samples were obtained from blood donors, CKD stage 4 and non-dialysis stage 5, and hemodialysis patients for more than 6 months. Patients with confounding factors such as positive CRP, infection, and recent use of steroids were excluded. RNL means standard deviations (SDs), and 95% confidence intervals (95% CI) were determined. RNL range was defined as percentiles P2.5 to P97.5. **Results:** A total of 102 participants were included. Mean age was  $40.45 \pm 9.97$  years and 58.8% were male. Age and sex distribution were similar in the three groups. Leucopenia and neutropenia were common in all the groups. Means NLR was  $1.29 \pm 0.57$  (95% CI 1.08 - 1.49) range between 0.55 to 2.67;  $2.31 \pm 1.3$  (95% CI 1.75 - 2.88) with range between 0.69 to 4.31 and  $2.14 \pm 0.98$  (95% CI 1.85 - 2.44) with range between 0.77 to 4.32 respectively in controls, CKD, and hemodialysis participants. NLR was comparable in CKD and hemodialysis patients ( $p = 0.99$ ). Compared to controls, NLR was significantly elevated in CKD ( $p = 0.043$ ) and hemodialysis patients ( $p < 0.001$ ). **Conclusion:** Our data suggest that the normal range for NLR in adult nongeriatric healthy and

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advanced CKD patients including those on chronic hemodialysis may vary between 0.55 to 2.67 and 0.69 to 4.32 respectively.

## Keywords

NLR, CKD, Hemodialysis, Cameroon

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## 1. Introduction

Chronic kidney disease (CKD) is one of the major public health concerns of the 21st century. It is estimated to affect more than 850 million individuals worldwide and it is one of a small number of non-communicable diseases that have shown an increase in associated death and morbidity over the past 2 decades [1] [2] [3]. Indeed, disability-adjusted life years linked to CKD increased from 21.5 million in 1990 to 41.54 million in 2019 [3] and the global all-age mortality attributed to CKD increased by 41.5% between 1990 and 2017. Moreover, CKD became the 12<sup>th</sup> leading cause of death in the world in 2019 compared to the 36th rank in 1990 and it will be the 5th leading cause of death worldwide in 2040 [1].

Inflammation is common in patient with CKD and is more frequent in advanced stage. It is a major contributor to mortality, especially through cardiovascular complications which are the leading cause of death in CKD [4] [5]. Inflammation is also associated with progression of CKD, development of insulin resistance, protein energy wasting, anemia as well as bone and mineral abnormality and malignancy [5] [6] [7]. Many biomarkers of inflammation have been investigated to evaluate the low-grade chronic inflammation associated with CKD or event for early detection of CKD including C-reactive protein (CRP), interleukin 8 and 18 or Tumor necrosis factors receptor 1 and 2 [8] [9] [10]. However, most of them are not available in clinical practice and are expensive.

Neutrophil to lymphocyte ratio (NLR) is a simple, cost-effective, and easily available inflammatory biomarker. It is calculated as a simple ratio between neutrophil and lymphocyte counts measured in peripheral blood. It has been shown as an emerging marker of the relationship between immune system and disease [11]. An elevated NLR can be observed in conditions that activated systemic inflammatory response (SIR) including bacterial and fungal infections [12] [13], acute stroke [14], myocardial infarction [15], and cancer [16]. NLR is also an established prognostic factor of mortality in several diseases such as sepsis, pneumonia, heart disease, chronic lower respiratory disease, and kidney disease [17]. The increase in NLR after acute physiological stress is earlier (<6 hours) than other laboratory parameters such as CRP or white blood cell count. Indeed, NLR could be a better marker of acute stress and permit early diagnosis of severe pathologies such as sepsis or cardiovascular event [11]. However, the normal cut-off value of NLR is still under debate. In normal adult population, it may vary between 0.78 to 3.92 and may be higher in elderly, males, HIV, active hematological disorder, or exogenous steroid intake [18] [19] [20]. NLR also seems to

be elevated in CKD compared to a healthy population [21]. In CKD patients, it can help for early diagnosis of SIR, especially sepsis. It may also be a useful predictor of progression to end-stage kidney disease [22] [23]. We sought to study NLR in adult healthy and advanced CKD in a single Cameroonian health facility.

## 2. Material and Methods

### 2.1. Study Design

This was a cross-sectional study conducted at the Douala General Hospital (DGH) for 4 months (1 January 2023 to 30 April 2023). The DGH is a tertiary health facility located in the economic capital of Cameroon. It has 320 beds with a well-equipped laboratory, a blood bank, and radiology services. It is a nephrology reference center in the region and the hemodialysis facility is the oldest and the biggest in the country.

### 2.2. Participants

Adult (>18 years) and consent patients with stage 4 and nondialysis 5 CKD and chronic hemodialysis patients followed in the nephrology service of the DGH were included. Consent-eligible blood donors were recruited at the blood bank for healthy participants. Eligible blood donors were adults between 18 to 60 years, with no comorbidity including hypertension, obesity, diabetes, HIV, hepatitis B or C, without anemia, in good health at the time of blood donation and who were accepted for blood donation after completing the national blood donor questionnaire. Participants were matched according to sex and age. As people of more than 60 years are usually not eligible as blood donors in our setting, and NLR is influenced by elderly, participants  $\geq 60$  years were excluded. We also excluded participants with acute disease, recent hospitalization, positive CRP, infection including HIV, HBV and HCV, diabetes, cancer (active or history), connective tissue disease, heart failure, hematologic disorder, use of steroids during the previous 6 months; since all those conditions can modify leucocyte or lymphocyte count and are knowing to modify NLR. For hemodialysis patients, patients on dialysis for less than 6 months, and those using catheters as vascular access were also excluded.

### 2.3. Procedure

All Participants were interviewed, and medical records of CKD and hemodialysis patients were retrieved to rule out any of the exclusion criteria. A venous blood sample was then collected via a peripheral vein for non-dialysis participants. For hemodialysis patients, blood samples were collected via the arteriovenous fistula at the beginning of the dialysis before connection to the generator and the injection of heparin. A qualitative latex CRP test was done and patients with positive CRP were all excluded. Full blood count (FBC) was performed immediately after blood sampling using the URIT 3000 plus automate.

NLR was calculated by divide the absolute neutrophile count by the absolute lymphocyte count. NLR means, standard deviations (SDs), 95% confidence intervals (95% CI) were determined and NLR range was defined as 2.5<sup>th</sup> to 97.5<sup>th</sup> percentiles for each group.

Other FBC data were also collected: hemoglobin level, leucocyte count, mean corpuscular volume, mean corpuscular concentration in hemoglobin and platelet. Platelet to lymphocyte ratio (PLR) was also calculated in the same manner as NLR.

Anemia was defined as hemoglobin level < 12 g/dl in woman and 13 g/dl in male. It was classified as moderate for hemoglobin level between 10 to 8 g/dl and severe for hemoglobin level lower or equal to 7.9 g/dl.

The following definitions were used:

- Leucopenia: total leucocyte count < 3000 cells/mm<sup>3</sup>,
- Leucocytosis: total leucocyte count >10,000 cells/mm<sup>3</sup>,
- Neutropenia: absolute neutrophile count < 1500 cells/mm<sup>3</sup>,
- Neutrophilia: absolute neutrophile count > 7000 cells/mm<sup>3</sup>,
- Lymphopenia: absolute lymphocyte count < 1000 cells/mm<sup>3</sup>,
- Lymphocytosis: absolute lymphocyte count > 4000 cells/mm<sup>3</sup>,
- Thrombopenia: platelets count < 150,000 cells/mm<sup>3</sup>,
- Thrombocytosis: platelets count > 500,000 cells/mm<sup>3</sup>.

## 2.4. Ethical Considerations

We obtained the agreement of the ethics and institutional committee of the University of Douala as well as administrative authorization from the DGH. Written informed consent was obtained for all participants. The study was carried out in strict compliance with ethical rules, in particular data confidentiality and respect for the privacy of participants.

## 2.5. Statistical Analyses

Continuous quantitative variables were presented as mean with standard deviation or median with interquartile range (IIQ25e-75e) depending on the distribution and the qualitative ones in the form of proportion. Chi-test was used to compare qualitative data and student test or non-parametric equivalent test for quantitative data. Pearson correlation was also used to identify the association between NLR and other variables. p-value was <0.05. Data were analyzed using SPSS 23.

## 3. Results

A total of 102 patients were included: healthy participants n = 33; CKD stage 4 - 5 patients n = 23 and hemodialysis patients n = 46, (**Table 1**). Mean age was 40.45 ± 9.97 years and 58.8% (n = 60) were male. Age and sex distribution were similar in the 3 groups. Hypertension was the sole comorbidity in patients with CKD (n = 15, 65.2%) and hemodialysis patients (n = 41, 89.13%). Concerning

**Table 1.** Distribution of sociodemographic and clinical data according to type of participants.

Variables	Healthy (n = 33)	CKD (n = 23)	Hemodialysis (n = 46)	P
Sex (Male)	19	11	30	0.44
Age* (Years)	41.2 ± 9.7	40.65 ± 9.8	39.82 ± 10.35	0.83
Marital status (Married)	15 (45.45)	11 (47.8)	21 (45.65)	0.9
Hypertension	-	15 (65.2)	41 (89.13)	-
Stage of CKD				-
4	-	10 (43.48)		-
5	-	13 (56.52)	46 (100)	-
Length of follow** (Months)	-	1 [0.5 - 36]	-	-
Hemodialysis vintage	-	-	48 [24 - 84]	-
Etiology of CKD				-
CGN	-	4 (17.4)	10 (21.8)	-
Hypertension	-	7 (30.43)	6 (13)	-
CTIN	-	4 (17.4)	3 (6.5)	-
Unknown	-	8 (34.77)	27 (58.7)	-

\*Mean ± SD, \*\*Median [Interquartile 25<sup>th</sup> - 75<sup>th</sup>], CKD = chronic kidney disease, CGN = chronic glomerulonephritis, CTIN = chronic tubule-interstitial nephritis.

patients with CKD, most were in stage 5 (n = 13, 56.52%) and median length of follow-up was 1 month [0.5 - 36] with extreme of 0.25 to 77 months. For hemodialysis patients, median dialysis vintage was 48 months [24 - 84] with extremes of 7 to 216 months.

Mean leucocyte count (**Table 2**) was similar in the 3 groups with  $4527 \pm 1823$  cells/mm<sup>3</sup>;  $5235 \pm 1800$  cells/mm<sup>3</sup> and  $3937 \pm 1235$  cells/mm<sup>3</sup> respectively in healthy, CKD and hemodialysis participants (p = 0.06). Hemodialysis participants had the lowest lymphocyte and platelet counts ( $1174 \pm 549$  cells/mm<sup>3</sup> and  $171,543 \pm 51,920$  cells/mm<sup>3</sup>).

Severe anemia was more common among hemodialysis patients compare to CKD (54.34% n = 25 vs 26% n = 6, p < 0.01). Microcytosis was common in all patients (n = 82, 80.4%) including healthy participants (**Table 3**). Microcytic anemia was most common in CKD and hemodialysis patients (n = 14, 60.86% and n = 36, 78.26% respectively).

Leucocytosis, neutrophilia, lymphocytosis, and thrombocytosis were not observed in any group. Leucopenia and neutropenia were comparable among groups while lymphopenia and thrombopenia were mainly found in hemodialysis patients (**Table 3**).

Mean NLR (**Figure 1**) was  $1.29 \pm 0.57$  (95% CI 1.08 - 1.49) range between 0.55 to 2.67;  $2.31 \pm 1.3$  (95% CI 1.75 - 2.88) with range between 0.69 to 4.31 and 2.14

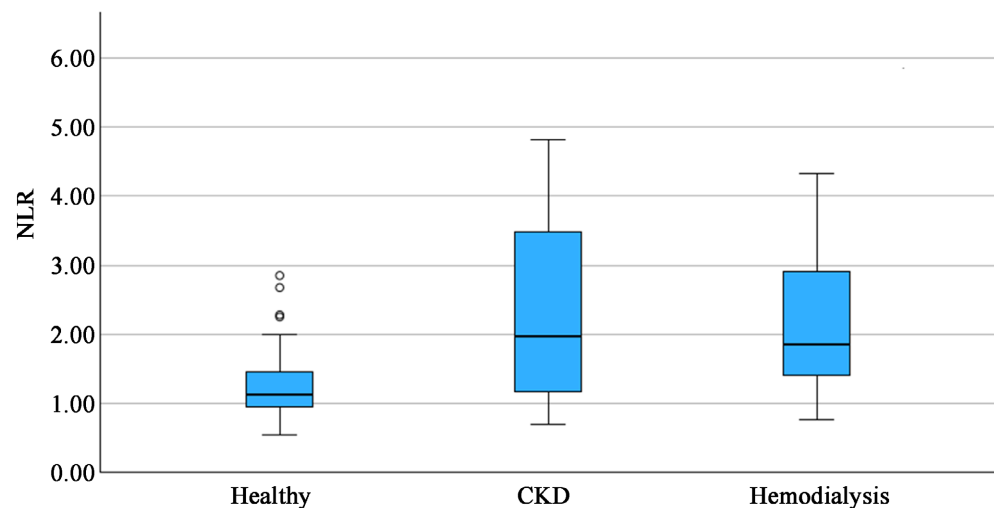
**Table 2.** Comparison of mean ± SD hematologic data according to type of participants.

Variables	Healthy (n = 33)	CKD (n = 23)	Hemodialysis (n = 46)	P
Hemoglobin (g/dl)	14.24 ± 1.46	10.30 ± 3.05	8.09 ± 1.34	<0.001
VGM (fl)	76.6 ± 7.46	71.85 ± 5,51	76.2 ± 6,64	0.022
CCMH	33.41 ± 1.2	34.73 ± 3.15	34.1 ± 1.42	0.04
Leucocytes (cell/mm <sup>3</sup> )	4527 ± 1823	5235 ± 1800	3937 ± 1235	0.06
Neutrophiles* (cell/mm <sup>3</sup> )	1915 [1502 - 3450]	3256 [2173 - 4879]	2090 [1710 - 2540]	0.78
Lymphocytes (cell/mm <sup>3</sup> )	1835 ± 663	1591 ± 576	1174 ± 549	<0.001
Platelets (cell/mm <sup>3</sup> )	197454 ± 52871	244652 ± 85913	171543 ± 51920	<0.001
NLR*	1.1 [0.93-1.5]	1.97[1.1-3.84]	1.81[1.4-2.9]	<0.001
PLR	116.12 ± 40.82	172.59 ± 74.47	166.78 ± 81.28	0.002

\*Median [interquartile 25<sup>th</sup>-75<sup>th</sup>].

**Table 3.** Distribution of hematologic abnormality according to type of participants.

Variables	Healthy (n = 33)	CKD (n = 23)	Hemodialysis (n = 46)	p
Anemia	-	16 (69.6)	46 (100)	<0.001
Severe anemia	-	6 (26)	25 (54.34)	<0.001
Microcytosis	26 (78.8)	20 (87)	36(78.26)	0.418
leucopenia	3 (9)	1 (4.5)	9 (19.7)	0.15
Neutropenia	8 (24.24)	1 (4.5)	8 (17.4)	0.143
Lymphopenia	-	3 (13)	21 (45.65)	<0.001
Thrombopenia	3 (9)	1 (4.5)	13 (28.3)	0.016



**Figure 1.** Comparison of NLR between healthy, CKD and hemodialysis participants. CKD = Chronic kidney disease, NLR = Neutrophile to lymphocyte ratio.

$\pm 0.98$  (95% CI 1.85 - 2.44) with range between 0.77 to 4.32 respectively in healthy, CKD and hemodialysis participants. NLR was comparable in CKD and hemodialysis patients ( $p = 0.99$ ). Compared to healthy, NLR was significantly elevated in CKD ( $p = 0.043$ ) and hemodialysis patients ( $p < 0.001$ ).

NLR was positively correlated to PLR ( $p < 0.01$ ,  $r = 0.554$ ) and total leucocyte count ( $p = 0.03$ ,  $r = 0.354$ ) in all participants. In CKD patients, NLR was positively correlated with PLR ( $p = 0.01$ ,  $r = 0.526$ ), length of follow ( $p = 0.049$ ,  $r = 0.415$ ) and total leucocyte count ( $p = 0.034$ ,  $r = 0.444$ ). No correlation was found with platelet ( $p = 0.83$ ) or CKD etiologies ( $p = 0.56$ ). In hemodialysis patients, NLR was only positively correlated with PLR ( $p < 0.01$ ,  $r = 0.551$ ). No correlation with total leucocyte count ( $p = 0.068$ ), hemoglobin level ( $p = 0.77$ ), platelet count ( $p = 0.86$ ), hemodialysis vintage ( $p = 0.93$ ), or CKD etiologies ( $p = 0.78$ ) was found.

#### 4. Discussion

The aim of this study was to study NLR in healthy and advanced CKD participants. Leucopenia and neutropenia were similar in the three groups while lymphopenia and thrombopenia were mostly found in hemodialysis patients. NLR range respectively from 0.55 to 2.67; 0.69 to 4.31 and 0.77 to 4.32 in healthy, CKD stage 4 - 5 and hemodialysis participants. Compared to healthy participants, NLR was significantly elevated in CKD and hemodialysis patients. However, NLR was comparable among CKD and hemodialysis patients. A positive correlation was noted between NLR and PLR in CKD and hemodialysis patients and between NLR and total leucocytes in CKD.

NLR is a well-established inflammatory marker although there is still debate about its normal cut-off in normal population as well as specific population such as CKD. In Belgian adults healthy non geriatric patient, *Forget and al*, identified normal NLR between 0.78 to 3.53 [18]. In The Rotterdam study, reference intervals for NLR were 0.83 to 3.92 [19] which is higher than results of *Forget and al*, and may be explained by older age (*Forget* series 38 years vs Rotterdam 66 years). In a large American adult healthy cohort (mean age 47.56), *Azab and al*, found a mean NLR of 2.15 and African American participants had lower NLR compared to Caucasian [24]. Normal range of total leucocytes and neutrophils are known to be lower in African and may explain the lower NLR in black [25]. Indeed, in Sudan, *Mohamet and al*, identified reference range of NLR between 0.3 to 2.9 in a cohort of three hundred participants aged from 5 to 85 years [26]. In Nigeria, *Uduagbamen and al*, found a median NLR of 1.5 (range 0.9 - 2.1) in healthy adults with a mean age of 46 years [21]. In our series, we found a mean NLR was  $1.29 \pm 0.57$  with a range between 0.55 to 2.67 in healthy adult with a mean age of 40.65 years (range 19 - 56 years).

NLR had been shown to increase in CKD, especially in late stage. A study in Nigeria noted that NLR was significantly elevated in CKD stage 3 - 4 (2.6 to 7.2) compared to stage 1 - 2 (1.7 to 3.4) and healthy controls (0.9 to 2.1) [21]. *Yuan and al*, in Chinese CKD patients showed that high NLR (2.09) was more com-

mon in stage 3 and 4 [23] and *Yoshitomi et al*, in Japan found that high NLR using a cut-off of 1.86 was associated with poor renal outcome in CKD stage 1 - 4 [22]. In our cohort, CKD stage 4 - 5 had higher NLR compared to healthy participants. However, it was comparable with hemodialysis patients. Since most of them was in stage 5 and in needing of renal replacement therapy it could explain comparable NLR in both groups.

High NLR had also been associated with poor survival in hemodialysis patients. *Zhang and al*, found a NLR between 2.54 to 4.78 and patients with NLR > 3.42 had higher risk of all-cause mortality [27]. Another Chinese study found a NLR cut-off value of 4.56 (sensitivity 0.695 and specificity 0.60) for differentiated all-cause mortality rate [28]. In our series, mean NLR in hemodialysis participants was  $2.14 \pm 0.98$  (95% CI 1.85 - 2.44) with a range between 0.77 to 4.32. It was significantly elevated compared to healthy patients, but our values are lower than those found in other countries. Another study done in Cameroon among hemodialysis patients also found lowest NLR with a median NLR of 1.86 [1.37 - 2.42] which is similar to our result [29]. This may be explained by lower neutrophil count due to black origin as well as younger age (mean age 39.82) and less comorbidities of end stage kidney patients in our context. This data also suggests that they may have a better survival.

NLR is assumed to be an early marker of inflammation and a prognostic factor of conditions associated with SIR. It has been noted that NLR greater than 3.7 in general population in US was associated with overall mortality and mortality due to heart disease, pneumonia, and kidney disease [17]. *Zahorec* had proposed to consider NLR between 1 - 2 as normal; NLR between 2 - 3 as a grey zone and may reflect latent, subclinical or low-grade inflammation as in our series, elevated NLR in CKD and hemodialysis patient reflecting low grade chronic inflammation link to the disease. NLR between 3 - 7 may be link to mild to moderate inflammation as sepsis; NLR between 7 - 11 may express severe inflammation as severe sepsis or bacteremia while NLR between 11 - 17 may reflected critical immune-inflammatory reaction and stress with high intensity such as septic shock or multiple traumas. NLR between 17 to 23 or upper than 23 may be due to critical systemic inflammation and it is associated with worse outcome [30]. Other authors had proposed a cut-off of 5 for diagnosis of sepsis [31] and 10 to assess sepsis severity [11]. In our study, NLR ranged between 0.69 to 4.31 in CKD and 0.77 to 4.32 in hemodialysis patients. So, NLR cut-off > 5 or >10 could still be used in these patients for early detection of sepsis, especially among hemodialysis patients; knowing that sepsis is still the leading cause of mortality of these patients in our context [32].

As noted by other, NLR was correlated with total leucocytes count and PLR which are both also markers of inflammation [33].

The small size of our sample is the main limitation of this study. Since we sought to evaluate NLR in young adult with less confounding factors and CKD  $\pm$  hypertension as the sole comorbidity, many patients were excluded.



However, to our knowledge, this study is the first to provide data about normal range of NLR in healthy population and CKD including hemodialysis patient in our context.

## 5. Conclusion

NLR is higher in CKD including hemodialysis patients compared to healthy participants. Normal range of NLR may vary between 0.55 to 2.28 in healthy people, 0.69 to 4.31 in CKD stage 4-5 and 0.77 to 4.32 in hemodialysis patients in our context.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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