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RESEARCH ARTICLE

THE RELATIONSHIP BETWEEN HEMATOLOGICAL INDICES AND SUBCLINICAL ATHEROSCLEROSIS IN HYPERTENSIVE ADULTS IN SOUTHERN NIGERIA

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ABSTRACT

Background: Atherosclerosis is a primary cause of cardiovascular morbidity and mortality. Atherosclerosis is characterized by infiltration of inflammatory cells from circulating blood. Blood cell activation could play an important role in subclinical atherosclerosis. Carotid intima-media thickness (CIMT) is an established tool for the detection and assessment of subclinical atherosclerosis. We analyzed the relationship between blood cellular markers and quantitative measures of carotid wall atherosclerosis as determined by CIMT in hypertensive patients attending the cardiology clinic of the UPTH.

Methods: 144 Hypertensive subjects and 72 age- and sex- matched controls were recruited. Their waist circumference, body mass indices and fasting lipid profile were determined. The complete blood count of the entire study population was also determined. Diabetics were excluded. CIMT was measured in all study subjects using standard protocol. Results were subjected to linear, multiple, and logistic regression analyses.

Results: The mean white cell count among the hypertensive subjects was significantly higher than that of the control group ($p=0.029$). The mean erythrocyte sedimentation rate (ESR) of the hypertensive subjects was also significantly higher than that of the controls ($p<0.001$). The subjects with elevated CIMT had significantly higher white cell counts than their counterparts with normal CIMT ($p=0.005$). The individuals with increased CIMT had significantly lower monocyte count than those with normal CIMT ($p=0.004$). Univariate linear regression showed that total white cell count and monocyte count were associated with increased CIMT. Binary logistic regression showed that the monocyte count was predictive of carotid atherosclerosis. An ROC curve analysis showed that a cutoff value for WCC of 3.55×10^9/L yielded a 93% sensitivity and 94% specificity for predicting the presence of carotid atherosclerosis in these hypertensive patients.

Conclusion: A significantly positive association between white cell count and atherosclerotic changes in the vascular walls of a hypertensive Nigerian adult population was observed.

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INTRODUCTION

Atherosclerotic cardiovascular disease is a major cause of morbidity and mortality world-wide, accounting for more than 19 million deaths per year [1]. Atherosclerotic patients present with a significant overlapping of vascular disorders including peripheral arterial disease (PAD), coronary heart disease and carotid arterial disease and cerebral disease. Atherosclerosis is a systemic disease affecting large and medium-sized arteries with lipid and fibrous accumulation within the intimal layer.

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The genesis and progression of atherosclerotic plaques are accompanied by the release of a series of mediators of inflammation with significant chemotactic activity. These mediators carry the potential to be utilized as biomarkers, defined as measurable proteins, peptides, genes or metabolic products that represent ongoing biological processes in an organism at a given time [2]. Biomarkers are indicators of disease states and encompass a spectrum of molecules and cellular components with certain characteristics [3]. The development of an atherosclerotic plaque begins with the recruitment of blood-borne inflammatory cells at sites of lipid deposition [4] or arterial injury [5]. Local rheological factors, such as low and oscillatory (with vortices) blood-to-wall shear stress dictate the location of atherosclerotic plaques to

characteristic points along the vasculature [6, 7]. Atherosclerosis shares features with diseases caused by chronic inflammation [8]. Inflammation is intimately linked with disease activity, as the numbers of monocyte-macrophages infiltrating the plaque and their location at plaque rupture-sensitive sites is related to plaque vulnerability [9-11]. Macrophage differentiation is acknowledged as critical for the development of atherosclerosis [12]. Several serum inflammatory markers have been proposed as tools for risk assessment in patients bearing atherosclerotic lesions of the carotid artery. Among them, notable examples include fibrinogen, serum amyloid A (SAA), interleukin- 6 (IL-6), and lipoprotein-associated phospholipase A2 (Lp-PLA2). The most widely used in current clinical practice however remains high sensitivity C-reactive protein (hs-CRP), which has been shown to reproducibly predict the risk of stroke in several large epidemiological studies, including the Physicians' Health Study [13], women's health Study [14], and the Framingham Heart Study [15]. These biomarkers are however beyond the reach of the average health-care consumer in the resource-poor environment of sub-Saharan Africa. The white blood cell count in peripheral blood is usually increased in inflammatory and infectious conditions and could also be affected in plaque inflammation. Higher leukocyte count is associated with a greater cardiovascular risk. Furthermore, total white blood cell count and sub-populations including granulocytes, monocytes, and bone marrow derived precursors have been implicated in atherosclerotic cardiovascular disease (ACVD) [16, 17].

In a meta-analysis of seven prospective studies comparing the top with the bottom third of the value distribution, leukocytes were observed to be a valuable marker of coronary disease [18]. Despite the wealth of knowledge on the involvement of infiltrating blood cells in atherosclerosis, little is known about the relationship between circulating white blood cells and carotid artery wall characteristics among hypertensives in the African sub-region. Of note, early atherosclerosis, measured as carotid intima-media thickness (CIMT), correlates with the risk of cardiovascular events such as cerebrovascular disease and coronary artery disease in the general population [19]. It has been reported that patients with the highest "inflammatory load" had the greater CIMT progression [20], further underlining the relevance of serum inflammatory biomarkers as surrogates that could reflect processes associated with atherosclerotic disease progression such as strokes. To our knowledge, there are no studies that had explored the relationships between circulating blood cells and carotid intima-media thickness (CIMT) as measured by carotid ultrasonography in Southern Nigeria. Therefore, we analyzed the association of multiple cellular pro-inflammatory indices with CIMT to see if these biomarkers of inflammation could serve as surrogate markers of atherosclerosis and so enhance cardiovascular risk stratification.

METHODS

Study population

Study subjects were randomly recruited from newly-diagnosed hypertensive patients attending the general out-patients, and medical out-patients clinics of the University of Port-Harcourt Teaching Hospital from January 2016 to August 2016. Cases were also excluded if they had previously been previously diagnosed hypertensive and on anti-hypertensive medication. Diabetics were also excluded from the study.

All participants underwent a routine clinical examination, hematological, biochemical examination and carotid ultrasonography. Finally, 144 newly-diagnosed hypertensive subjects were recruited as cases. Seventy-two apparently healthy age- and sex-matched individuals were randomly selected from hospital staff and patients' relatives and were classified as controls. Written informed consent was obtained from participants and the ethical committee of the hospital.

Demographic and clinical characteristics

Demographic and clinical characteristics such as age and gender were obtained by a structured questionnaire. Blood pressure was measured with a standard mercury sphygmomanometer. Height, weight, waist circumference, hip circumference were measured manually. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Waist-to-hip ratio was also calculated.

Laboratory examination

Fasting venous blood were collected and analyzed in the chemical pathology and hematology laboratories of the University of Port Harcourt Teaching Hospital for serum uric acid, lipid profile, blood glucose and a complete blood count. Fasting cholesterol and triglyceride levels were measured using the enzymatic method. Fasting HDL-C was measured with the precipitation method. LDL-C values were calculated using the Friedewald equation when triglyceride level was less than 4.0mmol/L: LDL-C= TCH- (HDL-C+TG/2.2) [21].

Carotid ultrasonography

The study was performed by the same operator who was blinded to the clinical information of the subjects, using Aloka Prosound SSD 4000 echocardiography machine equipped with a 7.5 MHz imaging transducer. Both the left and right carotid arteries were evaluated. The common carotid artery was carefully scanned utilizing standard protocol to identify the thickest CIMT. Intima-media thickness was defined as the distance between the leading edge of the lumen-intima and the leading edge of the media-adventitia. Mean value of the three determinations was calculated and the final values of IMT were averaged by the left and right mean IMT values. A normal CIMT was defined as values between 0.5-1.0mm [22].

Statistical analysis

Data was expressed as mean± standard deviations and frequencies as a percentage. Continuous variables were compared with the Students t-test. Proportions or categorical variables were compared with the Chi-square test. Relations among continuous variables were assessed using Pearson correlation coefficient and linear regression analysis. Multiple logistic models were constructed to elucidate the independent determinants of CIMT. The odds ratio and 95% confidence intervals were calculated. Finally, the screening ability of various hematological indices to identify individuals with carotid atherosclerosis was explored using receiver operating characteristic curve (ROC) analysis. Plots of sensitivity (true positives) versus 1 minus specificity (false positives) were constructed for each of the parameters. The area under the curve (AUC) of the ROC and 95% confidence intervals (CIs) were used to determine which index showed the highest accuracy in screening carotid atherosclerosis. The AUC is a measure of discrimination, and the AUC of 0.5, 0.6≤AUC < 7, 7≤AUC < 0.8, 0.8≤AUC < 0.9, and≥0.9 corresponded to no

discrimination, poor, acceptable, excellent, and outstanding discrimination, respectively [23]. All analyses were performed by SPSS statistical software (version 19.0, SPSS Inc). *P* values of <0.05 were considered statistically significant.

RESULTS

Clinical characteristics

Table 1 summarizes the clinical characteristics and biochemical parameters of the individuals. The age of the study participants with hypertension ranged between 20 and 86 years with a mean age of 51.4±12.9 years. 59.7% of the participants were in the 40-59 years' age-group.

Table 1. Baseline Clinical Characteristics of Study Population

Variables	Cases (n=144) Mean±SD	Controls (n=72) Mean±SD	P value
Age (years)	51.40±12.9	48.47±12.8	0.118
BMI (Kg/m^2)	29.47±4.87	27.17±4.98	0.001
WC (cm)	97.51±11.9	86.11±18.5	<0.001
SBP (mmHg)	149.0±22.5	115.0±11.3	<0.001
DBP (mmHg)	92.95±13.6	70.61±9.12	<0.001
TCH (mmol/L)	5.09±1.19	4.61±0.68	0.002
TG (mmol/L)	1.18±0.48	0.92±0.41	<0.001
HDL-C (mmol/L)	0.89±0.12	1..07±0.51	0.004
LDL-C (mmol/l)	3.50±1.06	3.30±0.66	0.151
CIMT (mm)	0.79±0.19	0.62±0.77	<0.001

BMI= body mass index; WC= waist circumference; SBP= systolic blood pressure; DBP= diastolic blood pressure; TC= total cholesterol, TG= triglycerides, HDL-c= high density lipoprotein cholesterol, LDL-c= low density lipoprotein cholesterol; CIMT=Carotid intima-media thickness.

Table 2. Baseline Hematological Characteristics of Study Population

Variables	Cases (n=144) Mean±SD	Controls (n=72) Mean±SD	P value
PCV (%)	38.96±5.35	40.81±5.10	0.021
ESR (mm/hr)	28.06±20.48	7.73±8.40	<0.001
WCC x 10^9/L	5.99±1.64	5.47±1.39	0.029
PLT x10^9/L	240.74±75.31	276.00±0.00	0.644
Neutrophils (%)	51.0±11.89	50.41±11.45	0.715
Monocytes (%)	2.25±2.75	2.70±2.16	0.256
Lymphocytes (%)	44.32±12.00	45.9±11.91	0.388
Eosinophils (%)	1.32±1.82	1.35±1.86	0.934

Table 3. Baseline Clinical Characteristics with respect to Carotid Intima-Media Thickness

Variables	Normal CIMT Mean±SD	Elevated CIMT Mean±SD	P value
Age (years)	49.05±12.46	63.30±10.55	<0.001
BMI (Kg/m^2)	28.66±5.14	29.42±3.50	0.521
WC (cm)	93.39±15.85	98.15±8.06	0.187
SBP (mmHg)	135.58±24.69	160.00±18.35	<0.001
DBP (mmHg)	84.61±16.04	95.50±13.95	0.004
TCH (mmol/L)	4.94±1.06	4.97±1.09	0.900
TG (mmol/L)	1.08±0.47	1.24±0.53	0.180
HDL-C (mmol/L)	1.07±0.33	1.00±0.45	0.550
LDL-C (mmol/l)	3.33±1.06	3.45±0.96	0.604

BMI= body mass index; WC= waist circumference; SBP= systolic blood pressure; DBP= diastolic blood pressure; TC= total cholesterol, TG= triglycerides, HDL-c= high density lipoprotein cholesterol, LDL-c= low density lipoprotein cholesterol.

The mean age of the control population was 47.9±14.7 years with a range of 24 – 82 years. The case and controls were matched for age (p=0.083). The mean age of the subjects with elevated CIMT was statistically higher than that of the subjects with normal CIMT (p<0.001) (See Table 3). The mean body mass index (BMI) was significantly higher in the cases compared to the controls (p=0.001).

The mean BMI of the cases was 29.47± 4.87kg/m^2 and it was 22.17± 4.98kg/m^2 among the controls. The mean waist circumference among the cases was 97.51± 11.9cm compared to 86.11± 18.5cm for the control cohorts. The mean waist circumference was significantly higher among the cases compared to the control (p<0.001). The systolic blood pressure (SBP) among the cases ranged from 100mmHg to 200mmHg with a mean of 149.0± 22.5mmHg.The diastolic blood pressure (DBP) among the cases ranged from 60-130mmHg with a mean of 92.95± 13.6mmHg.The mean SBP and DBP of the controls were 115.0± 11.3mmHg and 70.61±9.12mmHg respectively. There was a statistically significant difference in the mean SBP and DBP of the cases and controls (p< 0.001 and p< 0.001 respectively). We also found that the mean systolic and diastolic blood pressures of the subjects with elevated CIMT were significantly higher than those with normal CIMT (See Table 3). Table 2 shows the baseline hematological indices of the study population. The mean packed cell volume (PCV) of the cases was significantly lower than that of the control group (p=0.021). The mean white cell count among the cases was 5.99±1.64x 10^9/L compared to 5.47± 1.39x 10$^{9/L}$ for the control cohorts. The mean white cell count was significantly higher among the cases compared to the control (p=0.029).

Table 4. Baseline Hematological Characteristics with respect to Carotid Intima-Media Thickness

Variables	Normal CIMT Mean±SD	Elevated CIMT Mean±SD	P-value
PCV (%)	39.95±5.39	36.65±5.94	0.015
ESR (mm/hr)	18.71±19.27	18.50±11.00	0.983
WCC x 10^9/L	5.70±1.54	6.81±1.52	0.005
PLT x10^9/L	237.34±69.08	273.00±113.18	0.238
Neutrophils (%)	50.40±11.50	54.13±13.39	0.225
Monocytes (%)	2.58±2.57	0.69±1.35	0.004
Lymphocytes (%)	45.18±11.64	43.31±15.03	0.551
Eosinophils (%)	1.35±1.89	1.13±1.20	0.642

PCV=Packed cell volume; ESR=Erythrocyte sedimentation rate; WCC=White cell count; PLT=Platelets count.

Table 5. Linear regression analysis for predictors of Carotid Intima-Media Thickness

Univariate analysis			
PREDICTOR	B	OR	p-value
Age	0.006	0.414	<0.001
SBP	0.003	0.459	<0.001
LDL-C	-0.029	-0.152	0.029
WCC	0.031	0.257	<0.001
Monocytes count	-0.018	-0.241	0.001

B=Coefficient of regression; OR=Odds ratio; SBP=Systolic blood pressure; LDL-C=Low density lipoprotein cholesterol; WCC=White cell count.

Table 6. Binary logistic regression analysis for predictors of Carotid Intima-Media Thickness

Multivariate analysis			
PREDICTOR	B	OR	p-value
Age groups	-19.886		
20-39 years	-1.500		
40-59 years	0.315	0.000	0.997
60-79 years	0.047	0.223	0.231
SBP	-0.114	1.370	0.811
LDL-C	0.255	1.048	0.008
WCC	-0.463	0.892	0.705
Monocytes count		1.291	0.186
		0.629	0.04

B=Coefficient of regression; OR=Odds ratio; SBP=Systolic blood pressure; LDL-C=Low density lipoprotein cholesterol; WCC=White cell count.

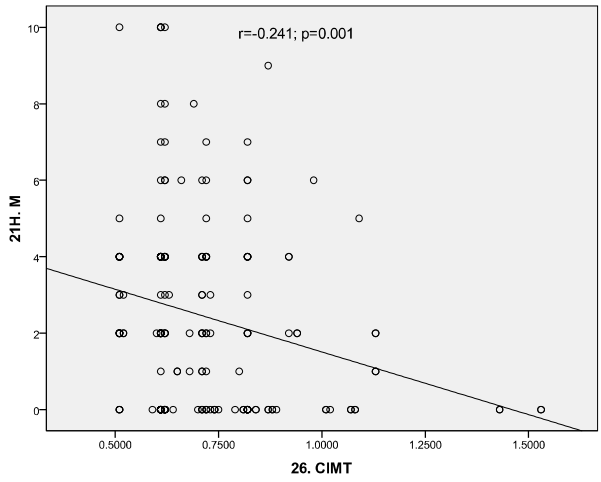

Figure 1. Correlations between monocytes count and CIMT

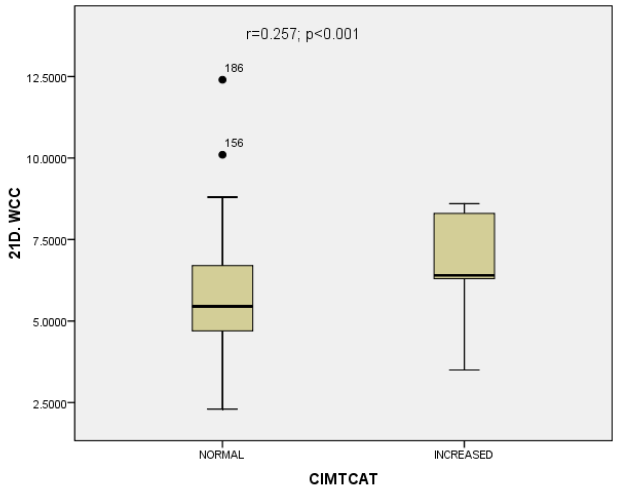

Figure 2. Correlations between white cell counts and CIMT

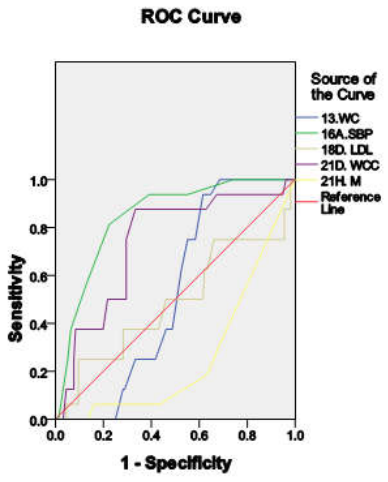

Figure 2. ROC curve for each variable predictive of increased CIMT

The mean erythrocyte sedimentation rate (ESR) of the cases was also significantly higher than that of the controls ($p<0.001$). This study revealed that the PCV of the subjects with normal CIMT was significantly higher than those with elevated CIMT (39.95±5.39% versus 36.65±5.99%; $p=0.015$). The subjects with elevated CIMT had significantly higher white cell counts than their counterparts with normal CIMT ($p=0.005$).

We also found that the individuals with increased CIMT had significantly lower monocyte count than those with normal CIMT ($p=0.004$) (See Table 4). The correlation between white cell count and CIMT was ($r=0.257$, $p<0.001$) and between monocyte count and CIMT was ($r=-0.241$, $p=0.001$) (See Figures 1 and 2). The other hematological indices failed to correlate with CIMT. Univariate linear regression analysis was done to evaluate the effect of age, SBP, LDL-C, white cell count and monocyte count on CIMT. It revealed that the CIMT was significantly and independently associated with all these risk factors for carotid atherosclerosis (Table 5). When binary logistic regression analysis of the whole population was performed and age groups, SBP, LDL-C, white cell count and monocyte count included in the statistical model, the monocyte count and SBP were the parameters predictive of carotid atherosclerosis (See Table 6). ROC curve analysis performed to detect the best cutoff value for white cell count in predicting subclinical carotid atherosclerosis in hypertensive patients revealed an AUC value of 0.736 (95% CI 0.613–0.860, $p = 0.0002$) (Figure 3). A cutoff value for WCC of 3.55x 10^9/L yielded a 93% sensitivity and 94% specificity for predicting the presence of carotid atherosclerosis in this hypertensive patient cohort. Monocyte count had an AUC of 0.262 (95% CI 0.152-372, $p=0.002$). A cut-off value of monocyte count of 0.50% yield a 32% sensitivity and 68% specificity for predicting the presence of carotid atherosclerosis. So monocyte count would be a poor screening tool, but was more specific for carotid atherosclerosis (Figure 3).

DISCUSSION

Hypertension is a major global health problem and public-health challenge, demanding a vast proportion of health care resources directly and indirectly because of its high and increasing prevalence and the concomitant risks of cardiovascular morbidity and mortality [24. 25]. Various risk factors have been implicated in the development of hypertension, some of which include genetic, environmental, psychosocial, and inflammatory factors [26, 27]. Links between inflammation and hypertension have been suggested in the past, with mounting evidence of more than a mere putative link between the two [28, 29]. Indeed, various inflammatory markers including high-sensitive C-reactive protein (hsCRP), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-a) and white blood cell (WBC) count have been studied and found to be associated with hypertension and its complications [30–32].

These reports are in concert with the finding in this present study which showed that the mean white cell count of the hypertensive subjects were significantly higher than those of the normotensive population. Two longitudinal studies [33, 34] investigating the association between WBC count and incidence of hypertension also noted an increased incidence of hypertension with ‘high normal’ WBC count compared to lower WBC counts. It has been hypothesized that elevated WBC counts cause a chronic low-grade inflammation that alters endothelial function, affecting nitric oxide and prostacyclin production and consequently, a loss of vasodilator, anti-thrombotic and anti-atherogenic properties of the vascular endothelium. Other postulated mechanisms include increased adherence of the stimulated leukocytes to the vascular endothelium, causing capillary leukocytosis and subsequent increased vascular resistance; a raised WBC count may therefore indicate increased catecholamine levels or

enhanced sympathetic nervous system activity, thus causing an increase in blood pressure and eventually resulting in sustained hypertension [35]. In addition, inflammation may play a key role in the initiation and development of hypertension via the proinflammatory actions of mediators such as adhesion molecules, chemokines, growth factors, heat shock proteins, endothelin-1 and angiotensin [36]. This study also showed that the mean ESR which is an inexpensive, well-validated marker of inflammation was significantly higher in the hypertensives than in the controls (See Table 2). This is similar to the observation by Mirsaeidi et al who reported that hypertensive patients had significantly higher ESR levels than normotensives [37]. The main and novel finding in this study was that white cell count is an independent predictor of future subclinical atherosclerosis. As far as we know, this is the first study aimed at defining the relationship between hematological components with subclinical carotid atherosclerosis among Nigerian black population. The fact that the white cell count was higher in persons with increased CIMT supports the concept that inflammation plays an etiological role in early atherogenesis. A similar finding was reported by Danesh et al who showed that there was a link between increased leukocyte counts with atherosclerosis [18]. This finding was further buttressed by the acceptable level of discrimination depicted by the AUC for white cell count. Inflammatory mechanisms may be important through all stages of atherogenesis, from the onset of fatty streaks to its progression to plaque rupture. White blood cells constitute the effector arm of the immune system, attending to both immune surveillance and prompt response to tissue damage. Several cell types are found among white blood cells, each with a different function and differentially activated by specific stimuli. A careful analysis of circulating leukocytes may thus provide a valuable tool to evaluate the inflammatory and immune status of the patient [38]: using this approach circulating white blood cells may well serve as biomarkers.

Among the different cells found to be altered in patients with atherosclerosis, mononuclear cells, both lymphocytes [38] and monocytes [39] subpopulations have been most frequently implicated. Oxidized low-density lipoprotein cholesterol acts chemotactically for monocytes and thus upregulate the expression of genes for macrophage colony-stimulating factor. This stimulates recruitment of monocytes from the peripheral blood to the intima of the artery wall and replication of monocyte-derived macrophages [40]. The trans-migration of the monocytes into the subendothelial spaces where they are converted to tissue macrophages might explain why the mean intravascular monocyte count for the subjects with increased CIMT were statistically less than those of the individuals with normal CIMT (See Table 4). Furthermore, this study showed that after binary logistic regression analysis monocyte count was a significant and independent predictor of subclinical atherosclerosis. In contrast, a study by Chapman et al. showed that plasma levels of IL-6, fibrinogen, and monocytes counts were associated with CIMT. However, the significance of association was lost upon correction for conventional risk factors [41]. This contradiction might be due to the background of the study population and the method of statistical analyses. Indeed, it has been shown that monocytes of recently symptomatic patients bear signs of activations. These patients were found to express high concentrations in the adhesion molecules CD11b and thrombospondin1 [42]. Interestingly, these same markers were shown to correlate with the presence of platelet- monocyte aggregates [42], which appear to be involved in the pathogenesis of atherosclerosis and atherothrombosis [38, 43].

Conclusion

The ESR is a well-validated and inexpensive tool for evaluating inflammation and is available at every outpatient clinic. Its elevation in the hypertensive cohorts probably supports the role of inflammation in hypertension.
This study also provides insight of the possible role of systemic inflammation in the development of carotid atherosclerosis as an increase in white cell count was predictive of an increase in CIMT. The inflammatory milieu surrounding the aetiopathogenesis of hypertension might also be its link with atherosclerosis. The identification of suitable and relatively inexpensive hematological biomarkers could therefore provide a useful adjunctive criterion in resource-poor settings to ensure better risk stratification of atherosclerotic vascular disease (ASVD) among hypertensive patients.

Limitations

- Only one WBC measurement was taken per patient for analysis, and whether an acute, brief inflammatory episode or chronic inflammation was responsible for the observed correlation could not be discriminated. Further prospective studies investigating the association between white cell count and subclinical carotid atherosclerosis in large population cohorts are probably required.
- We could not fully exclude individuals with relevant inflammation or infection.

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