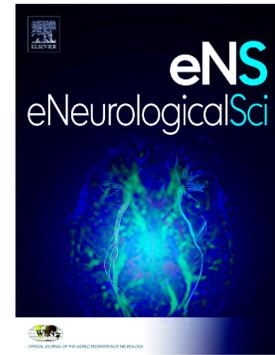


## Journal Pre-proof

Endothelial dysfunction and carotid atherosclerosis in Malawian adults: A cross-sectional study

Joseph KAMTCHUM-TATUENE, Gloria  
M.W.A.N.G.A.L.I.K.A. KACHINGWE, Henry C.  
MWANDUMBA, Tom SOLOMON, Laura A. BENJAMIN



PII: S2405-6502(20)30031-9

DOI: <https://doi.org/10.1016/j.ensci.2020.100252>

Reference: ENSCI 100252

To appear in: *eNeurologicalSci*

Received date: 14 May 2020

Revised date: 4 June 2020

Accepted date: 23 June 2020

Please cite this article as: J. KAMTCHUM-TATUENE, G.M.W.A.N.G.A.L.I.K.A. KACHINGWE, H.C. MWANDUMBA, et al., Endothelial dysfunction and carotid atherosclerosis in Malawian adults: A cross-sectional study, *eNeurologicalSci* (2020), <https://doi.org/10.1016/j.ensci.2020.100252>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier.

**Endothelial dysfunction and carotid atherosclerosis in Malawian adults: a cross-sectional study<sup>1</sup>**

Joseph KAMTCHUM-TATUENE<sup>1,2</sup>, Gloria MWANGALIKA KACHINGWE<sup>1</sup>, Henry C. MWANDUMBA<sup>1,3</sup>, Tom SOLOMON<sup>4</sup>, and Laura A. BENJAMIN<sup>4,5</sup>

<sup>1</sup> *Malawi-Liverpool-Wellcome Trust Clinical Research Programme, University of Malawi College of Medicine, Blantyre, Malawi*

<sup>2</sup> *Neuroscience and Mental Health Institute, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada*

<sup>3</sup> *Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

<sup>4</sup> *Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom*

<sup>5</sup> *Institute of Neurology, University College of London, London, United Kingdom*

**Corresponding author:** Dr. Joseph KAMTCHUM TATUENE, Neuroscience and Mental Health Institute, Faculty of Medicine and Dentistry, University of Alberta, 4-065 Katz Group Centre, 114 street and 87 Avenue Northwest, Edmonton, Alberta T6G 2E1, Canada; Tel: +1 780 248 1457; E-mail: kamtchum@ualberta.ca

---

<sup>1</sup> Abbreviations:

**cIMT** Carotid intima-media thickness,

**CV** Coefficient of variability

**ELISA** Enzyme-linked immunosorbent assay,

**HIV** Human immunodeficiency virus,

**ICAM-1** Intercellular adhesion molecule 1,

**PAI-1** Plasminogen activator inhibitor 1,

**sTM** soluble thrombomodulin,

**VEGF** Vascular endothelial growth factor,

**Word count in abstract:** 190

**Word count in main text:** 1000

**Running Head:** Endothelial dysfunction and carotid atherosclerosis in Malawian adults

**Number of tables and figures:** 2/2

**Declaration of interest:** None

## ABSTRACT

**Background and objective:** In sub-Saharan Africa, data on prevalence, risk factors and pathobiology of carotid atherosclerosis are scarce. We aimed to investigate the relationship between biomarkers of endothelial dysfunction and carotid atherosclerosis.

**Methods:** Carotid ultrasound was performed in 66 patients. Plasma concentration of ICAM-1, PAI-1, VEGF, and soluble thrombomodulin were measured by ELISA. A univariable logistic regression analysis was performed to study the relationship between carotid atherosclerosis, biomarkers of endothelial dysfunction, and various demographic and clinical parameters of the participants.

**Results:** The mean age of the participants was 58.7 years (95% CI: 54.4 – 63.1). Carotid atherosclerosis was diagnosed in 39.4% (95% CI: 27.6 – 52.2). In the univariable logistic regression, the following factors were associated with carotid atherosclerosis: age > 45 years (OR = 12.0, 95% CI: 1.4 – 98.8,  $p = 0.02$ ), hypertension (OR = 3.8, 95% CI: 1.2 – 12.1,  $p = 0.02$ ), and high-level of soluble thrombomodulin (OR = 3.4, 95% CI: 1.2 – 10.0,  $p = 0.02$ ).

**Conclusions:** There is an association between high levels of soluble thrombomodulin and carotid atherosclerosis in Malawian adults. Further studies with a larger sample size are needed to confirm our findings in other African populations.

**Keywords:** Endothelial dysfunction, HIV infection, Africa, stroke, atherosclerosis, carotid intima-media thickness.

## 1. INTRODUCTION

Several studies carried out in Western countries have established that endothelial dysfunction triggered by various cardiovascular risk factors is the *primum movens* of the arterial remodelling process leading to atherosclerosis and then atherothrombotic events [1, 2]. In sub-Saharan Africa, data on prevalence, risk factors and pathobiology of carotid atherosclerosis are scarce. To address this knowledge gap, we have conducted several prospective studies in Malawi, a country with > 10% prevalence of HIV [3-6]. In one study, we showed that endothelial dysfunction persists despite antiretroviral therapy and could, therefore, contribute to the development of atherosclerosis [5]. In a subsequent study, we reported a 39.4% prevalence of carotid atherosclerosis in a sample of 66 patients presenting with an acute stroke-like syndrome [7]. Plasma samples were also collected from the patients to investigate the relationship between biomarkers of endothelial dysfunction and carotid atherosclerosis. Here, we present and discuss the findings of these complimentary analyses.

## 2. METHODS

### 2.1 Selection of patients and ultrasound assessment

Participants were recruited consecutively from April to August 2017 at the Queen Elizabeth Central Hospital which is the largest referral hospital in Malawi. The presence of any cardiovascular risk factor was recorded according to previously reported definitions [7].

Carotid ultrasound examination was performed on the day of enrolment (JKT) using the CX50 portable ultrasound device (Philips, Amsterdam, NL). Details of the examination protocol and criteria for the interpretation of findings were reported previously [7]. Briefly, the examination comprised a semi-automated assessment of the carotid intima-media thickness (cIMT) and a grading of any existing stenosis. Carotid atherosclerosis was defined as abnormal intima-media thickness or presence of carotid plaque with or without stenosis.

In order to define abnormal cIMT, the following age-specific cut-offs were applied:  $< 0.50$  mm (30-39 years old), 0.65 mm (40-49 years old), 0.70 mm (50-59 years old), 0.75 mm (60-69 years old), and 0.80 mm ( $\geq 70$  years old). In the absence of reference values specific to African populations, the reference cut-off in each age category was chosen close to the upper limit of the 95% confidence interval of the mean cIMT for participants without cardiovascular risk factors enrolled in the PARC study (Paroi Artérielle et Risque Cardiovasculaire) [8]. Results of the PARC study suggest that the normal aging of the arterial vessel wall is associated with intima-media thickening by approximately 0.5 mm per decade. In patients with cardiovascular risk factors, the cIMT is typically higher than the indicated cut-off in each age category and is dependent on the number of cardiovascular risk factors as illustrated in the Mannheim consensus guidelines [9].

## **2.2 Measurement of biomarkers of endothelial dysfunction**

We used ELISA kits from Abcam<sup>®</sup> (Abcam plc, Cambridge, United Kingdom) to measure the plasma concentrations of biomarkers of endothelial activation (intercellular adhesion molecule -ICAM-1), endothelial damage (plasminogen activator inhibitor 1 - PAI-1), angiogenesis

(VEGF), and coagulopathy (soluble thrombomodulin – sTM). SimpleStep<sup>®</sup> ELISA kits were used for ICAM-1, PAI-1, and sTM while the standard kit was used for VEGF. All samples were run in duplicates and samples with an individual coefficient of variability (CV) greater than 25% were retested in duplicates.

All the ELISA tests were performed according to the manufacturer's instructions with exception of the washing steps that were performed with an automated washer (ASYS Atlantis<sup>®</sup>, Biochrom Ltd, Cambridge, UK) using a mixture of phosphate buffer saline and Tween 20 (0.05% v/v). The plates were read at 450 nm using an automated microplate reader (EZ Read<sup>®</sup>, Biochrom Ltd, Cambridge, UK). The standard curves were built using 4-parameter logistic regression. The mean intra-assay CV for ICAM-1, PAI-1, VEGF, and sTM was 3.3%, 3.2%, 8.0%, and 3.1% respectively (accepted value < 10%). The mean inter-assay CV for ICAM-1, PAI-1, VEGF, and sTM was 4.1%, 4.1%, 9.0%, and 2.8% respectively (accepted value < 15%).

### **2.3 Statistical analysis**

Participants' demographic and clinical parameters were summarized as proportions and means with 95% confidence interval for categorical and numerical variables, respectively. The relationship between plasma levels of biomarkers of endothelial dysfunction and cIMT was explored using the Spearman rank correlation test.

A univariable logistic regression analysis was performed to study the relationship between carotid atherosclerosis (dependent variable) and various demographic and clinical parameters of the participants. Following the approach in our previous publications [5, 6], age was dichotomized at 45 years while biomarkers were dichotomized at the third tertile of the distribution of plasma concentration values (low versus

high level). A multivariable analysis was not performed due to the small sample size. All statistical tests were two-tailed and statistical significance was defined as  $p \leq 0.05$ . Analyses were performed using the software STATA (version 13, StataCorp, College Station, TX, USA).

### 3. RESULTS

The mean age of the participants was 58.7 years (95% CI: 54.4 – 63.1). There were 22 HIV-positive patients and 77.3% were on antiretroviral therapy. HIV+ cases were younger (mean age 43.6 versus 66.3,  $p < 0.001$ ) and their mean CD4+ T cell count was  $223/\text{mm}^3$ . There was no difference in the levels of biomarkers between HIV-positive and HIV-negative patients. The baseline characteristics of the participants are summarized in Table 1.

There was no correlation between the plasma concentration of the biomarkers of endothelial dysfunction and the value of cIMT (Spearman rank correlation coefficient of -0.24, -0.03, 0.11, 0.11 for ICAM-1, PAI-1, sTM, and VEGF, respectively).

In the univariable logistic regression (Table 2), the following factors were associated with carotid atherosclerosis: age  $> 45$  years (OR = 12.0, 95% CI: 1.4 – 98.8,  $p = 0.02$ ), hypertension (OR = 3.8, 95% CI: 1.2 – 12.1,  $p = 0.02$ ), and high-level of soluble thrombomodulin (OR = 3.4, 95% CI: 1.2 – 10.0,  $p = 0.02$ ).

### 4. DISCUSSION

This study is the first to investigate the relationship between biomarkers of endothelial dysfunction and carotid atherosclerosis in sub-Saharan Africa. The association between high levels of soluble thrombomodulin and carotid atherosclerosis is in line with findings in Western

populations [10, 11]. Soluble thrombomodulin is an endothelial membrane glycoprotein also known as CD141. It is a marker of endothelial injury and higher plasma levels have been associated with other risk factors of atherosclerosis, notably age and hypertension [12]. In this study, patients with carotid atherosclerosis had a higher mean age and a higher prevalence of hypertension, which could contribute to the association between high levels of soluble thrombomodulin and carotid atherosclerosis. Due to the small sample size of this exploratory study, we could not reliably explore the independence of the association in a multivariable analysis. Further studies with a larger sample size are, therefore, needed to confirm our findings in African populations and investigate the performance of biomarkers of endothelial dysfunction as predictors of cardiovascular events. The small sample size likely also explains why our analysis did not show an association between carotid atherosclerosis and other established drivers of endothelial dysfunction and atherosclerosis, notably smoking, diabetes mellitus, adiposity, and HIV infection [13-16]. Specifically, for HIV-positive patients who are younger, longitudinal studies are needed to investigate if the persistence of HIV-induced endothelial dysfunction has a significant and independent impact on the incidence of carotid atherosclerosis and whether antiretroviral or anti-inflammatory drugs are useful to prevent its progression and cerebrovascular consequences. Such studies should ideally be performed on large sample size populations and follow international consensus recommendations for the assessment of subclinical and clinical atherosclerosis in order to avoid generating more heterogeneous or contradictory results [17].

**Acknowledgement:** The authors are grateful to: 1) All patients and their families for consenting to take part in this study, 2) Prof. Robert Heyderman for his contribution to the development of the research protocol and for sponsoring the application for funding, 3) Prof. Stephen

Gordon for providing local sponsorship for the study and working space for our research team, 4) The staff and students of the Queen Elizabeth Central Hospital, the Malawi-Liverpool-Wellcome Trust Clinical Research Programme and the Wellcome Trust Centre for Global Health Research for their various contributions to the success of this study.

### **Statement of Ethics**

This work was conducted as part of the study of Biomarkers of Stroke and Arterial diseases in Malawian Adults (BIOSTATA). The study protocol was approved by the Human Research Ethics Committees of the Liverpool School of Tropical Medicine (16-035) and the University Of Malawi College Of Medicine (P.06/16/1974). A signed informed consent was obtained from all the patients enrolled or their next of kin. The research was conducted in accordance with the World Medical Association Declaration of Helsinki.

**Declarations of interest:** None

### **Funding Sources**

This research was supported by the Wellcome Trust [grant number 106829/Z/15/Z to JK-T].

The funder had no role in study design; data collection, analysis and interpretation; writing of the report; and decision to submit the article for publication.

### **Author contributions**

**Conceptualization:** Joseph Kamtchum-Tatuene, Laura A. Benjamin

**Data curation:** Joseph Kamtchum-Tatuene

**Formal analysis:** Joseph Kamtchum-Tatuene

**Funding acquisition:** Joseph Kamtchum-Tatuene, Henry C. Mwandumba, Tom Solomon, Laura A. Benjamin

**Investigation:** Joseph Kamtchum-Tatuene, Gloria Mwangalika-Kachingwe

**Methodology:** Joseph Kamtchum-Tatuene

**Project administration:** Joseph Kamtchum-Tatuene

**Supervision:** Henry C. Mwandumba, Laura A. Benjamin

**Validation:** Henry C. Mwandumba, Tom Solomon, Laura A. Benjamin

**Visualization:** Joseph Kamtchum-Tatuene

**Writing – original draft:** Joseph Kamtchum-Tatuene

**Writing – review and editing:** Gloria Mwangalika-Kachingwe, Henry C. Mwandumba, Tom Solomon, Laura A. Benjamin

## REFERENCES

1. **Ruparelia N, Chai JT, Fisher EA, Choudhary RP.** Inflammatory processes in cardiovascular disease: a route to targeted therapies. *Nat Rev Cardiol* 2017;**14**:133-144.
2. **Munzel T, Sinning C, Post F, Warnholtz A, Schulz E.** Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. *Ann Med* 2008;**40**:180-196.

3. **Benjamin LA, Corbett EL, Connor MD, Mzinganjira H, Kampondeni S, Choko A, et al.** HIV, antiretroviral treatment, hypertension, and stroke in Malawian adults: A case-control study. *Neurology* 2016;**86**:324-333.
4. **Benjamin LA, Allain TJ, Mzinganjira H, Connor MD, Smith C, Lucas S, et al.** The Role of Human Immunodeficiency Virus-Associated Vasculopathy in the Etiology of Stroke. *J Infect Dis* 2017;**216**:545-553.
5. **Kamtchum-Tatuene J, Mwandumba H, Al-Bayati Z, Flatley J, Griffiths M, Solomon T, et al.** HIV is associated with endothelial activation despite ART, in a sub-Saharan African setting. *Neurol Neuroimmunol Neuroinflamm* 2019;**6**:e531.
6. **Kamtchum-Tatuene J, Al-Bayati Z, Mwandumba HC, Solomon T, Christman RE, Benjamin LA.** Serum concentration of anti-Cytomegalovirus IgG and ischaemic stroke in patients with advanced HIV infection in Malawi. *PLoS One* 2018;**13**:e0208040.
7. **Kamtchum-Tatuene J, Mwandumba HC, Mwangalika Kachingwe G, Bonnett LJ, Kayange N, Solomon T, et al.** A cross-sectional feasibility study of neurovascular ultrasound in Malawian adults with acute stroke-like syndrome. *PLoS One* 2020;**15**:e0229033.
8. **Touboul PJ, Labreuche J, Vicaut E, Belliard JN, Cohen S, Kownator S, et al.** Country-based reference values and impact of cardiovascular risk factors on carotid intima-media thickness in a French population: the 'Paroi Arterielle et Risque Cardio-Vasculaire' (PARC) Study. *Cerebrovasc Dis* 2009;**27**:361-367.
9. **Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al.** Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and

20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. *Cerebrovasc Dis* 2012;**34**:290-296.

10. **Salomaa V, Matei C, Aleksic N, Sansores-Garcia L, Folsom AR, Juneja H, et al.** Cross-sectional association of soluble thrombomodulin with mild peripheral artery disease; the ARIC study. *Atherosclerosis Risk in Communities. Atherosclerosis* 2001;**157**:309-314.

11. **Gerdes VE, Kremer Hovinga JA, Ten Cate H, Brandjes DP, Buller HR, Amsterdam Vascular Medicine G.** Soluble thrombomodulin in patients with established atherosclerosis. *J Thromb Haemost* 2004;**2**:200-201.

12. **Olivot JM, Labreuche J, Aiach M, Amarenco P, Investigators G.** Soluble thrombomodulin and brain infarction: case-control and prospective study. *Stroke* 2004;**35**:1946-1951.

13. **Arildsen H, Sorensen KE, Ingerslev JM, Ostergaard LJ, Naursen AL.** Endothelial dysfunction, increased inflammation, and activated coagulation in HIV-infected patients improve after initiation of highly active antiretroviral therapy. *HIV Med* 2013;**14**:1-9.

14. **Graham SM, Mwilu R, Liles WC.** Clinical utility of biomarkers of endothelial activation and coagulation for prognosis in HIV infection: a systematic review. *Virulence* 2013;**4**:564-571.

15. **Gresele P, Falcinelli E, Sebastiano M, Baldelli F.** Endothelial and platelet function alterations in HIV-infected patients. *Thromb Res* 2012;**129**:301-308.

16. **Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S.** Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. *Circ Res* 2016;**118**:535-546.

17. Vos AG, Idris NS, Barth RE, Klipstein-Grobusch K, Grobbee DE. Pro-Inflammatory Markers in Relation to Cardiovascular Disease in HIV Infection. A Systematic Review. PLoS One 2016;11:e0147484.

**Table 1:** Participants' baseline characteristics

Characteristics	Carotid atherosclerosis	
	Yes (n = 26)	No (n = 46)
Age (years)	63.0 (57.7 – 68.2)	56.1 (49.7 – 62.4)
Age > 45 years	25 (96.2)	27 (57.5)
Men	13 (50.0)	15 (37.5)
HIV positive	7 (27.0)	15 (37.5)
On ART	7 (26.9)	10 (25.0)
Smoking	3 (11.5)	5 (12.5)
Alcohol consumption	4 (15.4)	9 (22.5)
Hypercholesterolemia	3 (11.5)	0 (0.0)
Hypertension	21 (80.8)	21 (52.5)
Diabetes mellitus	5 (19.2)	6 (15.0)
Waist-hip ratio	0.9 (0.9 – 1.0)	0.9 (0.8 – 0.9)
CD4+ count	472.3 (377.7 – 566.9)	421.2 (320.2 – 522.2)
ICAM-1 (ng/mL)	377.4 (244.2 – 510.6)	404.7 (268.4 – 540.9)
PAI-1 (ng/mL)	43.0 (26.8 – 59.2)	49.3 (31.0 – 67.5)

sTM (ng/mL)	8.1 (5.6 – 10.6)	6.2 (5.1 – 7.4)
VEGF (pg/mL)	12.8 (0.0 – 26.7)	4.4 (0.9 – 8.0)

\* Data are presented as N (%) or mean (95%CI)

**Table 2:** Univariable analysis of factors associated with carotid atherosclerosis

Characteristic	Univariable analysis		
	OR	95% CI	p
HIV infection	0.6	0.2 – 1.8	0.38
Age > 45	12.0	1.4 – 98.8	0.02
Male	1.6	0.6 – 4.5	0.32
Hypertension	3.8	1.2 – 12.1	0.02
Diabetes mellitus	1.3	0.4 – 5.0	0.65
Smoking	0.9	0.2 – 4.2	0.91
Waist-hip ratio (3 <sup>rd</sup> tertile)	1.9	0.7 – 5.5	0.22
ART started	1.1	0.4 – 3.1	0.86
High level of ICAM-1	0.8	0.3 – 2.4	0.72
High level of PAI-1	1.1	0.4 – 3.1	0.86
High level of sTM	3.4	1.2 – 10.0	0.02
High level of VEGF	1.8	0.6 – 5.5	0.32

\* There were 3 patients with hypercholesterolemia and they all had carotid atherosclerosis

## HIGHLIGHTS

High levels of soluble thrombomodulin are associated with carotid atherosclerosis.

Age > 45 years and hypertension are also associated with carotid atherosclerosis.

Mannheim consensus criteria for assessing carotid intima-media thickness and plaque.