Telomere length, endothelial activation and carotid atherosclerosis in black and white African patients with rheumatoid arthritis

A.R. Raymond[1], R.L. Brooksbank[1], A.M.E. Millen[1], G.R. Norton[1], A. Solomon[2], A.J. Woodiwiss[1], L. Tsang[1], P.H. Dessein[1,3], M.A. Gonzalez-Gay[1,4]

[1]Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; [2]Department of Rheumatology, Charlotte Maxeke Johannesburg Academic Hospital, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; [3]Rheumatology Division, Universitair Ziekenhuis and Vrije Universiteit, Brussels, Belgium; [4]Epidemiology, Genetics and Atherosclerosis Research Group on Systemic Inflammatory Diseases, Rheumatology Division, Hospital Universitario Marques de Valdecilla, IDIVAL, Santander, Spain.

Abstract

Objective

Our objective was to examine associations of traditional and non-traditional cardiovascular risk factors with relative leukocyte telomere length and confounder adjusted relationships of relative telomere length with endothelial activation and carotid atherosclerosis in black and white African patients with rheumatoid arthritis (RA).

Methods

Relative telomere length of leukocyte DNA in whole blood was determined using quantitative RT-PCR in 205 (101 black) African patients with RA.

Results

In demographic characteristic adjusted analysis, relative telomere length tended to be larger in black compared to white patients (median (IQR)=0.54 (0.42-0.54) and 0.48 (0.37-0.60) (p=0.07), respectively). In black patients, waist circumference, systolic, diastolic and mean blood pressure were associated with relative telomere length (β (SE)=-0.00270 (0.00114) (p=0.02), -0.00185 (0.00060) (p=0.003), -0.00243 (0.00112) (p=0.03) and -0.00225 (0.00075) (p=0.003), respectively); in white patients, age, anti-cyclic citrullinated antibody positivity, biologic agent use, a cholesterol-HDL cholesterol ratio of >4 and the number of major traditional risk factors were related to relative telomere length (β (SE) =-0.00242 (0.00113) (p=0.03), 0.06629 (0.03374) (p=0.05), -0.09321 (0.04310) (p=0.03), 0.08225 (0.03420) (p=0.02) and 0.04046 (0.01719) (p=0.02), respectively). One SD increase in relative telomere length was associated with carotid plaque (OR (95% CI)=1.65 (0.99-2.75) (p=0.05)) and vascular cell adhesion molecule-1 concentrations (β (SE)=-0.05031 (0.02480) (p=0.04)) in black and white patients, respectively.

Conclusion

This study disclosed paradoxically direct relationships between relative telomere length and cardiovascular risk factors in white and atherosclerosis in black African RA patients. The role of relative telomere length in cardiovascular risk and its stratification in RA requires longitudinal investigation.

Key words

telomere length, rheumatoid arthritis, endothelial activation, atherosclerosis

*Andrew R. Raymond, PhD**
*Richard L. Brooksbank, PhD**
Aletta M.E. Millen, PhD
Gavin R. Norton, MBBCH, PhD
Ahmed Solomon, MBBCH, FCP (SA)
Angela J. Woodiwiss, PhD
Linda Tsang
*Patrick H. Dessein, MD, FCP (SA), FRCP (UK), PhD**
*Miguel A. Gonzalez-Gay, MD, PhD**

**These authors contributed equally to the study.*

Please address correspondence to:
Patrick H. Dessein,
Cardiovascular Pathophysiology and Genomics Research Unit,
School of Physiology,
Faculty of Health Sciences,
University of the Witwatersrand,
P.O. Box 1012, Melville 2109,
Johannesburg, South Africa.
E-mail: patrick.dessein22@gmail.com

Received on September 13, 2015; accepted in revised form on February 15, 2016.

Funding: this study was supported by the South African Medical Research Council (grant no. MRC2008_DES), the National Research Foundation and the Iris Ellen Hodges Cardiovascular Trust. Research carried out by M.A. Gonzalez-Gay was supported by grants from "Fondo de Investigaciones Sanitarias" PI06/0024, PS09/00748 and PI12/00060 (Spain) and the RETICS Program, RD08/0075 and RD12/0009/0013 (RIER) from "Instituto de Salud Carlos III" (ISCIII) (Spain).

Competing interests: none declared.

Introduction

Telomeres are complex DNA-protein structures that cap the ends of linear chromosomes and protect chromosomes against nuclease degradation, end-to-end fusion and cellular senescence (1). In somatic cells, telomeres typically shorten with each cellular division and this process is counteracted by telomerase (2, 3). Therefore, telomere length is a marker of biologic ageing. Inflammation associates with increased cellular division and thereby enhances telomere shortening (4, 5). Additionally, oxidative stress induces telomere attrition (4).

Telomere length is reduced in persons with traditional cardiovascular disease (CVD) risk factors and shortened telomere length is associated with prevalent and incident ischaemic heart disease, independent of traditional risk factors (6-11). Inhibition of telomere function in endothelial cells results in increased production of molecules that are involved in atherogenesis (12). This indicates that reduced telomere length can be causally linked to CVD (12).

Rheumatoid arthritis (RA) is a chronic inflammatory disease that markedly increases CVD risk (13-15). Patients with RA further experience increased oxidative stress (16). Traditional and non-traditional cardiovascular risk factors including high grade inflammation and genetic factors contribute to the increased cardiovascular risk associated with RA (17-19). Naïve CD4 T cells undergo accelerated telomere loss in RA (20). These findings are implicated in the pathophysiology of RA (20). Steer and colleagues (16) recently reported that peripheral leukocyte telomere length was also reduced in RA. Shortened leukocyte telomere length was influenced by HLA-DR1 genotype but not related to disease duration and markers of inflammation (16). However, the role of reduced telomere length in the enhanced CVD risk amongst patients with RA awaits investigation.

Given these considerations, we examined the associations of traditional and non-traditional cardiovascular risk factors with relative leukocyte telomere length, and potential confounder adjusted relations of relative telomere length with surrogate markers of atherogenesis and carotid atherosclerosis in a large cohort of black and white African patients with RA.

Materials and methods

Patients

The present study was performed according to the principles outlined in the Helsinki declaration. The study protocol (approval number: M06-07-33) was approved by the Human Research Ethics Committee (Medical) from the University of the Witwatersrand in Johannesburg, South Africa, and participants gave informed, written consent. Two hundred and five consecutive African patients (101 black and 104 white) that met the 1988 American College of Rheumatology and 2010 American College of Rheumatology/EULAR criteria for RA (21, 22) were enrolled. All invited participants agreed to participate. Anti-cyclic citrullinated peptide antibody (anti-CCP) titers were recorded in 127 (77.4%) of participants; data were missing in fewer than 5% of any of the other recorded characteristics.

Assessments

Patient characteristics were recorded employing previously reported methods (23, 24). Briefly, we recorded demographic features and height, weight and waist and hip circumference were measured using standard approaches. The body mass index (BMI) was calculated and abdominal obesity and fat distribution were estimated by waist circumference and waist-hip ratio respectively. We recorded disease duration, rheumatoid factor and anti-CCP status. Disease activity was assessed by the Clinical Disease Activity Index (CDAI) and the Disease Activity Score in 28 joints (DAS28). Extra-articular manifestations included the current or previously recorded (hospital record review) presence of pericarditis, pleuritis, Felty's syndrome, cutaneous vasculitis, neuropathy, scleritis or episcleritis, retinal vasculitis, glomerulonephritis, vasculitis affecting other organs, amyloidosis, keratoconjunctivitis sicca, xerostomia, Sjogren's syndrome, pulmonary fibrosis, bronchiolitis obliterans organising pneumonia, cervical

myelopathy, subcutaneous nodules and rheumatoid nodules in other locations. C-reactive protein concentrations were determined using immunoturbidimetric methods and those of interleukin-6 by ELISA. Standard laboratory blood tests of erythrocyte sedimentation rate, renal and liver function, haematological parameters, lipids and glucose were performed. The glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (25).

Recorded conventional CVD risk factors included smoking status (current and ever), systolic, diastolic and mean blood pressure, lipid levels and ratios, and glucose concentrations. Hypertension was defined as an average systolic blood pressure ≥140 or/and diastolic blood pressure ≥90 mmHg or/and current use of antihypertensive medications. Dyslipidaemia was diagnosed when the atherogenic index, *i.e.* the cholesterol-HDL cholesterol ratio was >4 (15). Diabetes was identified as the use of glucose lowering agents or a fasting plasma glucose ≥7 mmol/l. Cardiovascular drugs included lipid lowering drugs, antihypertensives, oral glucose lowering agents and insulin.

We measured early endothelial activation molecule concentrations including those of soluble E-selectin, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1), as well as angiopoietin 2 using solid-phase sandwich ELISA (Quantikine®HS, R & D Systems, Inc., Minneapolis, MN, USA). Their lower detection limits were 0.009 ng/l, 0.6 ng/l, 0.096 ng/l, 5.0 pg/ml and 1.2 pg/ml respectively; their inter- and intra-assay coefficients of variation were 7.9 and 5.8, 7.0 and 3.1, 5.5 and 4.6, 5.7 and 5.8, and 8.9 and 5.9%, respectively.

BAS (see acknowledgement) and AS performed the carotid artery ultrasound measurements in private and public healthcare patients, respectively. Both operators obtained images of at least 1cm length of the distal common carotid arteries for measurement of the intima-media thickness of the far wall from an optimal angle of incidence defined as the longitudinal angle of approach where both branches of the internal and external carotid artery are visualised simultaneously (26) and with high resolution B-mode ultrasound (Image Point, Hewlett Packard, Andover, MA, USA and SonoCalc IMT, Sonosite Inc, Bothell, Wash, USA used by BAS and AS, respectively) employing linear array 7.5 MHz probes. The details of the methodology used by BAS were reported previously (27). The equipment used by AS involves the application of a unique semi-automated border detection program that was previously found to provide highly reproducible results (26). The intima-media thicknesses in the left and right common carotid artery were measured and the cIMT was defined as the mean of these. Carotid artery plaque was defined as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding intima-media thickness value, or demonstrates a thickness of >1.5 mm as measured from the media-adventitia interface to the intima-lumen interface (28). Both operators were blinded to the cardiovascular risk profiles of the patients. Repeat ultrasound examinations by both operators on 23 patients revealed Spearman correlations between repeat cIMT measurements of 0.983 and 0.956 for BAS and AS, respectively, and the correlation between measurements made by BAS and AS was 0.926. Amongst all measurements made by both operators, the mean (SD) cIMT was 0.775 (0.250) and 0.793 (0.266) (p=0.24 by paired Student t test) for BAS and AS, respectively. Both operators identified carotid artery bulb or/and internal carotid artery plaque in 11 of these 23 patients with full agreement.

Leukocyte deoxyribonucleic acid (DNA) was extracted from whole blood samples using NucleoSpin Blood kits (Macherey-Nagel, Dueren, Germany) as per manufacturer's instructions. A quantitative real-time polymerase chain reaction (PCR) method was then used to determine relative telomere length of leukocyte DNA (27). Briefly, this method determines the factor by which telomere repeat copy number (T) differs from the copy number of a single copy gene (S). Samples were amplified in duplicate in two different PCR amplifications: one to determine telomere copy number and the other to determine 36B4 copy (the single copy gene) number. All PCR amplifications were performed using an ABI PRISM 7900HT Real-Time PCR system (Applied Biosystems, Foster, United States) with SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, United States). Relative leukocyte telomere length was then expressed as the difference in the number of PCR cycles for the single copy gene amplification to produce the equivalent amount of fluorescence as compared to the corresponding telomere amplification (T/S ratio). A standard sample was included in each plate and was used to standardise the entire sample T/S ratios. This real-time quantitative PCR method has been validated and shown to give a measure of relative telomere length, which is proportional to mean telomere length as determined by Southern blotting (9, 29).

Data management and analysis

Dichotomous variables are expressed as proportions or percentages and continuous variables as mean (SD), or median (interquartile range) when non-normally distributed. Non-normally distributed characteristics were also logarithmically transformed prior to their inclusion in multivariable statistical analysis.

In line with current disparate epidemiological transition stages amongst black and white Africans, cardiovascular risk factor profiles and their associations with atherosclerosis consistently differ amongst both groups (30-32). Therefore, we analysed the data separately in black and white patients with RA. Univariate relationships of patient characteristics with relative telomere length were assessed in simple linear regression models or by using the Mann-Whitney U test as appropriate. Associations of age and sex with telomere length were then re-assessed by entering the respective characteristics together in single mixed regression models; associations of other characteristics with telomere length were evaluated in models with adjustment for demographic characteristics.

The independent relationships between telomere length and endothelial activation molecule concentrations as well as cIMT and plaque were identified in mixed regression models; the inclusion of potential confounders in these models was based on findings in the previous analysis (age and sex adjusted associations of patient characteristics with telomere length), collinearity and biological plausibility.

Statistical computations were made using the GB Stat programme (Dynamic Microsystems, Inc, Silverspring, Maryland, USA) and SAS software, version 9.1 (The SAS Institute, Cary, NC). Significance was set at p-value ≤0.05.

Results

Recorded characteristics

The recorded patient characteristics are given in Table I. Compared to their white counterparts, black patients with RA were more frequently women and experienced more active and severe (joint deformities) RA, but less often had extraarticular disease. All patients used conventional disease modifying agents for rheumatic disease (DMARD). Methotrexate was more frequently used by black RA patients. Apart from methotrexate, chloroquine, leflunomide, sulphasalazine, azathioprine, tetracycline, cyclophosphamide and penicillamine were employed by 80.2% and 55.8%, 18.8% and 39.4%, 24.8% and 14.4%, 19.8% and 11.5%, 10.9% and 12.5%, 5.9% and 1.0% and 4.0% and 2.9% of black and white Africans with RA. Biologic agents were used in white RA patients only and included tumour necrosis factor-α inhibitors (n=8) and rituximab (n=1). Black Africans with RA had a larger BMI and waist circumference, more frequent hypertension and diabetes, and smoked less often. The overall conventional CVD risk burden as represented by the number of conventional risk factors was larger in black compared to white patients with RA. Endothelial activation molecule concentrations were either similar (VCAM-1), larger (E-selectin and angiopoietin 2) or smaller (ICAM-1 and MCP-1) amongst black and white patients with RA. The atherosclerosis extent was similar amongst both groups.

Table I. Recorded characteristics in black and white patients with RA.

Characteristics	Black (n=101)	White (n=104)	p-value
Demographic characteristics			
Age (years)	56.6 (9.4)	58.5 (11.5)	0.3
Female sex	**90.0**	**76.0**	**0.01**
Anthropometry			
Body mass index (kg/m^2)	**29.7 (6.6)**	**25.8 (4.6)**	**<0.0001**
Waist circumference (cm)	**93.1 (13.4)**	**89.7 (13.1)**	**0.03**
Waist-hip ratio	0.84 (0.78-0.89)	0.88 (0.81-0.94)	0.2
Cardiovascular agents			
Antihypertensives	**54.5**	**40.3**	**0.02**
Statins	**18.8**	**36.5**	**0.009**
Ezetimibe	0	2.0	…
Oral glucose lowering agents	**14.9**	**4.8**	**0.03**
Insulin	2.0	1.0	0.5
RA characteristics			
Disease duration (years)	13.0 (8.9)	14.2 (9.0)	0.2
RF positive	75.2	73.8	0.7
Anti CCP positive	77.4	77.9	0.9
HAQ-DI	**0.625 (0.125-1.000)**	**0.250 (0.000-0.750)**	**0.0008**
Clinical Disease Activity Index	**11.4 (4.8-16.1)**	**5.3 (0.9-11.8)**	**<0.0001**
DAS28	**3.0 (1.3)**	**2.0 (1.4)**	**0.0005**
Erythrocyte sedimentation rate (mm/hr)	**22 (9-39)**	**6 (2-14)**	**<0.0001**
C-reactive protein (mg/l)	**7.0 (4.0-14.5)**	**3.7 (1.5-9.2)**	**0.002**
Interleukin-6 (pg/ml)	3.7 (2.5-6.2)	3.3 (2.0-5.8)	0.6
Leukocytes (n/nl)	5.7 (4.6-7.4)	6.0 (4.9-7.7)	0.3
Deformed joints (number)	**8 (3-15)**	**4 (0-16)**	**0.0007**
Extraarticular manifestation	**3.0**	**11.5**	**0.03**
Synthetic disease-modifying agents			
Number	**2.5 (1.0)**	**2.3 (0.9)**	**0.02**
Methotrexate	**90.1**	**77.9**	**0.02**
Biologic agent use	0	11.9	…
Prednisone use			
Current	2.0	2.0	1.0
Ever	41.6	42.3	0.7
Glomerular filtration rate (ml/min/1.73 m^2)	**89 (17)**	**95 (17)**	**0.002**
Conventional CV risk factors			
Smoking			
Current	**3.0**	**10.7**	**0.05**
Ever	**12.9**	**49.5**	**<0.0001**
Hypertension	**74.3**	**46.2**	**<0.0001**
Systolic blood pressure (mmHg)	**140 (25)**	**129 (17)**	**0.0008**
Diastolic blood pressure (mmHg)	**86 (14)**	**79 (9)**	**<0.0001**
Mean blood pressure (mmHg)	**122 (20)**	**112 (13)**	**0.0002**
Total cholesterol (mmol/l)	**4.6 (0.9)**	**4.9 (1.1)**	**0.003**
HDL cholesterol (mmol/l)	**1.50 (1.30-1.80)**	**1.58 (1.30-1.94)**	**0.01**
LDL cholesterol (mmol/l)	**2.5 (0.7)**	**2.8 (0.9)**	**0.01**
Triglycerides (mmol/l)	1.0 (0.7-1.4)	1.1 (0.9-1.4)	0.8
C-HDL C ratio	3.2 (1.0)	3.2 (1.0)	0.7
C-HDL C ratio>4	21.4	16.7	0.4
Non-HDL cholesterol (mmol/l)	3.1 (0.8)	3.3 (1.0)	0.06
Diabetes	**19.8**	**7.7**	**0.006**
Glucose (mmol/l)	**4.9 (4.5-5.4)**	**4.7 (4.4-5.1)**	**0.002**
Major conventional risk factors (number)	**1.2 (0.9)**	**0.8 (0.7)**	**0.0001**
Endothelial activation molecules			
Early endothelial activation			
E-selectin (ng/ml)	**41.8 (19.4)**	**36.1 (16.6)**	**0.05**
VCAM-1 (ng/ml)	880 (714-1 074)	858 (634-1 069)	0.1
ICAM-1 (ng/ml)	**237 (177-317)**	**312 (255-389)**	**<0.0001**
MCP-1 (pg/ml)	**347 (235-629)**	**479 (325-706)**	**0.004**
Angiopoietin 2 (pg/ml)	**2 705 (2 232-3 524)**	**2 387 (1 958-3 150)**	**0.02**
Carotid atherosclerosis			
Intima-media thickness (mm)	0.706 (0.085)	0.720 (0.132)	0.8
Plaque	35.6	43.3	0.8
Relative leukocyte telomere length (T/S)	0.54 (0.42-0.64)	0.48 (0.37-0.60)	0.07

Categorical variables are expressed as proportions and continuous characteristics as mean (SD) or median (interquartile range) as appropriate. Comparisons between black and white Africans were assessed in age and sex adjusted mixed regression models, as well as lipid lowering and antihypertensive agents in models that included lipid and blood pressure variables, respectively; in the model on disease duration, age at disease onset rather than age at the time of the study was entered. Significant associations are shown in bold. RA: rheumatoid arthritis; RF: rheumatoid factor; CCP: cyclic citrullinated peptide antibody; n: number; HAQ-DI: Health Assessment Questionnaire-disability index; DAS: Disease Activity Score; CV: cardiovascular; HDL: high density lipoprotein; LDl: low density lipoprotein; C: cholesterol; VCAM: vascular cell adhesion molecule; ICAM: intercellular adhesion molecules; MCP: monocyte chemoattractant protein; Log: logarithmically transformed.

In univariate analysis, the relative leukocyte telomere length was longer in black compared to white patients (0.54 (0.42-0.64) *vs*. 0.48 (0.37–0.60), *p*=0.02); this difference still approached significance after adjustment for demographic characteristics (*p*=0.07).

Associations of clinical characteristics with relative telomere length

In black patients, univariate analysis revealed that waist circumference and systolic, diastolic and mean blood pressure were associated with relative telomere length (β (SE)=-0.00244 (0.00115) (*p*=0.04), -0.00190 (0.00061) (*p*=0.002), -0.00264 (0.00110) (*p*=0.02) and -0.00234 (0.00075) (*p*=0.002), respectively); relative telomere length was also longer in black women compared to men (median (IQR)=0.55 (0.45–0.66) versus 0.40 (0.33–0.50) (*p*=0.03)) and in those without compared to with a cholesterol-HDL cholesterol ratio >4 (median (IQR)=0.55 (0.45–0.67) *vs*. 0.45 (0.38–0.56) (*p*=0.04)).

In univariate analysis amongst white patients, age and the number of major conventional cardiovascular risk factors were associated with relative telomere length (β (SE)=-0.00242 (0.00111) (*p*=0.03) and 0.03918 (0.01747) (*p*=0.03), respectively); relative telomere length was also longer in white patients that did not compared to those that did use biologic agents (median (SE)=0.50 (0.40–0.60) versus 0.40 (0.31–0.47) (*p*=0.05)), smokers compared to non-smokers (median (SE)=0.60 (0.46–0.69) *vs*. 0.47 (0.37–0.58) (*p*=0.03)) and those with compared to those without a cholesterol-HDL cholesterol ratio >4 (median (IQR)=0.58 (0.46–0.64) *vs*. 0.46 (0.37–0.58) (*p*=0.01)).

Table II shows the age and sex adjusted associations of recorded clinical characteristics with relative leukocyte telomere length. In black African patients with RA, waist circumference, systolic, diastolic and mean blood pressure was inversely related to relative leukocyte telomere length.

In white Africans with RA, age and biologic agent use were inversely associated with relative leukocyte telomere length, whereas anti-CCP positivity, cholesterol-HDL cholesterol ratio >4 and number of major conventional CVD risk factors were directly related to relative leukocyte telomere length.

The slopes of the relations of systolic, diastolic and mean blood pressure,

Table II. Disease characteristics and cardiovascular risk factors associated with relative leukocyte telomere length*.

	Age and sex adjusted models			
	Black RA patients		White RA patients	
Variables	β (SE)	*p*-value	β (SE)	*p*-value
Demographic characteristics				
Age	-0.00100 (0.00166)	0.5	**-0.00242 (0.00113)**	**0.03**
Gender	0.09606 (0.05163)	0.07	0.00120 (0.03008)	1.0
Anthropometry				
Body mass index	-0.00341 (0.00244)	0.2	-0.00011 (0.00285)	1.0
Waist circumference	**-0.00270 (0.00114)**	**0.02**	-0.00025 (0.00105)	0.8
Waist-hip ratio*	0.00796 (0.31231)	1.0	-0.01067 (0.03570)	1.0
Cardiovascular drugs				
Antihypertensives	-0.05281 (0.03089)	0.09	0.05348 (0.02778)	0.06
Statins	0.00747 (0.03939)	0.9	-0.00422 (0.02717)	0.9
Ezetimibe	…	…	-0.16172 (0.09290)	0.08
Oral glucose lowering agents	-0.04030 (0.04318)	0.4	-0.03379 (0.06014)	0.6
Insulin	0.01445 (0.11055)	0.9	-0.12753 (0.13311)	0.3
RA characteristics				
Disease duration	0.00171 (0.00210)	0.4	-0.00341 (0.00176)	0.06
RF positive	0.00850 (0.03574)	0.8	0.04431 (0.02937)	0.1
Anti CCP positive	-0.03582 (0.05238)	0.5	**0.06629 (0.03374)**	**0.05**
HAQ-DI*	0.02972 (0.10651)	0.8	0.02207 (0.08915)	0.8
Clinical Disease Activity Index*	0.04381 (0.04287)	0.3	-0.01215 (0.02678)	0.7
DAS28	0.00879 (0.01180)	0.5	0.00017 (0.00937)	1.0
Erythrocyte sedimentation rate*	0.05837 (0.03753)	0.1	0.01247 (0.02992)	0.7
C-reactive protein*	-0.02404 (0.03377)	0.5	0.00096 (0.02385)	1.0
Interleukin-6*	-0.01238 (0.05611)	0.8	-0.00789 (0.04004)	1.0
Leukocytes*	-0.03452 (0.10005)	0.7	-0.02807 (0.09022)	0.8
Deformed joints*	0.04629 (0.03743)	0.2	-0.02024 (0.02203)	0.4
Extraarticular manifestation	-0.05743 (0.09126)	0.5	-0.04223 (0.04002)	0.3
Synthetic disease-modifying agents				
Number	-0.00127 (0.01607)	0.9	-0.01677 (0.01501)	0.3
Methotrexate	-0.05690 (0.05344)	0.3	-0.01990 (0.03085)	0.5
Biologic agent use	…	…	**-0.09321 (0.04310)**	**0.03**
Prednisone use				
Current	0.04269 (0.11057)	0.7	0.04824 (0.09349)	0.6
Ever	0.02501 (0.03156)	0.4	-0.00107 (0.02605)	1.0
Glomerular filtration rate	-0.00003 (0.00106)	1.0	0.00004 (0.00087)	1.0
Conventional CV risk factors				
Smoking				
Current	-0.04489 (0.09138)	0.6	0.06652 (0.04168)	0.1
Ever	0.00320 (0.04941)	1.0	0.00740 (0.02676)	0.8
Hypertension	-0.04140 (0.03550)	0.2	0.02647 (0.02628)	0.3
Systolic blood pressure	**-0.00185 (0.00060)**	**0.003**	0.00103 (0.00076)	0.2
Diastolic blood pressure	**-0.00243 (0.00112)**	**0.03**	0.00204 (0.00142)	0.2
Mean blood pressure	**-0.00225 (0.00075)**	**0.003**	0.00140 (0.00096)	0.2
Total cholesterol	0.01510 (0.01846)	0.4	-0.00085 (0.01306)	0.9
HDL cholesterol*	0.11758 (0.12576)	0.4	-0.14971 (0.11158)	0.2
LDL cholesterol	0.01204 (0.02179)	0.6	0.00857 (0.01438)	0.6
Triglycerides*	-0.02904 (0.06713)	0.7	-0.06390 (0.07321)	0.4
C-HDL C ratio	-0.00580 (0.01622)	0.7	0.01645 (0.01293)	0.2
C-HDL C ratio>4	-0.04850 (0.03819)	0.2	**0.08225 (0.03420)**	**0.02**
Non-HDL cholesterol	0.00958 (0.01878)	0.6	0.00631 (0.01281)	0.6
Diabetes	-0.00550 (0.03865)	0.9	-0.02673 (0.04974)	0.6
Glucose*	0.01090 (0.10338)	0.9	-0.08506 (0.16255)	0.6
Major conventional risk factors	-0.02327 (0.01881)	0.2	**0.04046 (0.01719)**	**0.02**

Associations were determined in age and sex adjusted mixed regression models. Significant associations are shown in bold. RA: rheumatoid arthritis; β: beta; SE: standard error; RF: rheumatoid factor; CCP: cyclic citrullinated peptide; HAQ-DI: Health Assessment Questionnaire-disability index; DAS: Disease Activity Score; CV: cardiovascular; HDL: high density lipoprotein; LDL: low density lipoprotein; C: cholesterol. *Logarithmically transformed.

Table III. Measures of endothelial activation and carotid atherosclerosis independently associated with relative leukocyte telomere length* (1 SD increase).

	Age and sex adjusted models					
	Black RA patients			White RA patients		
Variables	β (SE)		*p*-value	β (SE)		*p*-value
Endothelial activation						
Early						
E-selectin	-1.81753	(2.05874)	0.4	1.76749	(2.58705)	0.5
VCAM-1*	0.02002	(0.01509)	0.2	**-0.05031**	**(0.02480)**	**0.04**
ICAM-1*	-0.00787	(0.01933)	0.7	-0.00583	(0.02306)	0.8
MCP-1*	-0.00184	(0.03015)	0.9	0.00021	(0.03758)	1.0
Angiopoietin 2	-0.00870	(0.01598)	0.6	-0.04330	(0.03050)	0.2
Carotid atherosclerosis						
Intima-media thickness	0.00490	(0.00824)	0.6	0.06289	(0.10908)	0.6
	OR	(95%CI)†	*p*	OR	(96%CI)‡	*p*
Plaque	**1.65**	**(0.99-2.75)**	**0.05**	1.31	(0.79-2.15)	0.3

Associations were determined in mixed regression models. SD: standard deviation; RA: rheumatoid arthritis; β: regression coefficient; SE: standard error; VCAM: vascular cell adhesion molecule; ICAM: intercellular adhesion molecule; MCP: monocyte chemoattractant protein; OR: odds ratio; CI: confidence interval. *Logarithmically transformed. †Adjusted for age, sex, waist circumference and systolic blood pressure. ‡Adjusted for age, sex, number of major cardiovascular risk factors, anti-cyclic citrullinated peptide positivity and biologic agent use.

cholesterol-HDL cholesterol >4 and major conventional CVD risk factors with relative leukocyte telomere length differed amongst black and white Africans with RA (p=0.03, 0.01, 0.003, 0.01 and 0.01, respectively, by unpaired Student t-test).

Relationships of relative leukocyte telomere length with endothelial activation and carotid atherosclerosis

In univariate analysis amongst black patients, relative telomere length tended to be larger in those with compared to those without carotid plaque (median (SE)=0.56 (0.46–0.68) *vs*. 0.52 (0.40–0.68) (p=0.06)). In white patients, relative telomere length (1 SD increase) was associated with logarithmically transformed VCAM-1 concentrations (β (SE)=-0.04966 (0.01834)) (p=0.008).

As shown in Table III, in confounder adjusted analysis, relative leukocyte telomere length was associated with plaque (OR (95% CI) = 1.65 (0.99–2.75) for 1 SD increase in relative telomere length, p=0.05) in black African patients with RA. Amongst white RA patients, relative leukocyte telomere length was inversely related to VCAM-1 concentrations. The slopes of the relationship of relative leukocyte telomere length with VCAM-1 concentrations differed amongst black and white Africans with RA (p=0.02). Replacement of the number of major cardiovascular risk factors by cholesterol-HDL cholesterol ratio in the models applied in white patients did not change the results (data not shown).

Additional adjustment for inflammatory markers in the models shown in Table III also did not materially alter the results (data not shown).

Discussion

In the present study, relative telomere length tended to be longer in black compared to white patients. Patient characteristics including RA features and traditional risk factors were associated with relative telomere length, which was further independently related to surrogate markers of atherogenesis and carotid plaque. These relations consistently differed amongst black and white Africans with RA.

In black RA patients, abdominal adiposity as estimated by waist circumference and blood pressure values were associated with shorter relative telomere length. An adverse impact of obesity and high blood pressure on telomere length has also been well documented in non-RA subjects (6, 7). However, we found that contrary to our expectations, a 1 SD increment in relative telomere length increased the odds ratio for plaque 1.65-fold independent of potential confounders. In the present context, it is of interest that Haque and colleagues (33) recently also reported that amongst 164 patients with lupus, carotid plaque prevalence was particularly increased in those with the higher tertile of telomere length. Congruent with results obtained in a previous investigation from our group (34), black African RA patients had markedly more severe disease than their white counterparts in the present study. Hence, in line with a suggested explanation offered by Haque and colleagues (33) for the paradoxical relation between telomere length and atherosclerosis in lupus, it is possible that patients with the shortest telomeres and atherosclerosis may have been too ill to attend our clinic or already have died and, therefore, their data was not included in our cross-sectionally designed study. Longitudinal studies are clearly needed to further dissect the relationship between relative telomere length and atherosclerosis in both lupus patients and black Africans with RA.

In white African RA patients, we found that, as reported in non-RA subjects (6), age was associated with shorter relative telomere length. Biologic agents were used in only 9 RA patients in whom the disease was refractory to at least 3 conventional DMARD. It is therefore likely that the inverse relation between biologic agent use and telomere length in our investigation represents an adverse influence of disease severity. As in the study by Steer and colleagues (16) on telomere length in RA, disease duration and activity including acute phase responses, and disease severity markers other than biologic agent use, were not associated with relative telomere length.

Anti-CCP antibody positivity is associated with disease severity as well as enhanced CVD risk in RA (15). Also, traditional CVD risk factors including not only obesity, hypertension and diabetes but also dyslipidaemia are reportedly related to shortened telomere length in non-RA subjects (6-11). However, anti-CCP positivity as well as a cholesterol-HDL cholesterol ratio of >4 and the

number of traditional cardiovascular risk factors were associated with longer relative telomere length amongst white Africans with RA in the present study. In this regard, an inflammation related paradoxical relationship between lipid concentrations and CVD risk, labelled as the "lipid paradox", has been reported in RA (35, 36). In addition, mechanisms that are involved in determining telomere length are modifiable (37, 38). Therefore, whether RA patients can mount compensatory adaptive changes in metabolic pathways that result in increased telomere length in the presence of enhanced CVD risk and in an attempt to reduce this risk, comprises a hypothesis that merits to be tested in future studies. Indeed, this could provide a unifying mechanism underlying the various paradoxical relative telomere length-CVD risk relations as observed amongst both black and white African RA patients in the present study. Notably, Haque and colleagues (33) also found a positive association between triglyceride concentrations and telomere length in the lupus study that was alluded to previously. Taken together, our current findings and those reported by Haque and colleagues (33) further reinforce the need for future longitudinal studies in both lupus and RA.

In the present study, relative telomere length was inversely related to circulating VCAM-1 concentrations amongst white Africans with RA. In this regard, Minamino and colleagues (12) showed that inhibition of telomere function in human aortic endothelial cells results in cellular senescence with enhanced endothelial activation as was estimated by increased ICAM-1 expression and reduced endothelial nitric oxide synthase activity. This finding comprises evidence in support of a mechanistic role of reduced telomere length in enhanced CVD risk.

Relative telomere length tended to be longer in black compared to white African RA patients. Similarly, in the National Heart, Lung, and Blood Institute Family Heart Study and the Bogalusa Heart Study, leukocyte telomere length was longer in black compared to white Americans (39). The authors suggested that reduced replication rates of haematopoietic stem cells in black Americans may have explained their finding (39). In this regard, leukocyte counts were however similar amongst black and white Africans with RA and leukocyte counts were not related to relative telomere length in either group in the present investigation.

Apart from its cross-sectional design, a further limitation of the present study is that relative leukocyte telomere length may not necessarily reflect telomere length in cells located in the vasculature. Available evidence indicates that telomere length does correlate in different tissues amongst individuals (40). In conclusion, this study revealed inverse associations between relative telomere length and abdominal obesity and blood pressure values in black and biologic agent use and endothelial activation in white African patients with RA. However, the paradoxically direct or positive relationships between relative telomere length and cardiovascular risk factors in white and atherosclerosis in black African patients require further elucidation in future longitudinal and mechanistic studies in order to determine the role of relative telomere length in CVD risk and its stratification in RA.

References

1. BLACKHURN EH: Telomeres and telomerase: the means to the end. *Angew Chem Int Ed Engl* 2010; 49: 7405-21.
2. SHAMMAS MA: Telomeres, lifestyle, cancer and aging. *Curr Opin Clin Nutr Metab Care* 2011; 14: 28-34.
3. SHSCHERBAKOVA DM, ZVEREVA ME, SHPANCHENKO OV, DONTSOVA OA: Telomerase: structure and properties of the enzyme, characteristics of the yeast telomerase. *Mol Biol* (Moscow) 2006; 40: 580-94.
4. AVIV A: Telomeres and human aging: facts and fibs. *Sci Aging Knowledge Environ* 2004; 2004: e43.
5. RAYMOND AR, NORTON GR, HARDEN LM. WOODIWISS AJ, BROOKSBANK RL: Chronic inflammation reduces cardiac relative telomere length without altering left ventricular chamber function. *Int J Cardiol* 2014; 175: 367-9.
6. FITZPATRICK AL, KRONMAL RA, GARDNER JP *et al*.: Leukocyte telomere length and cardiovascular disease in the Cardiovascular Health Study. *Am J Epidemiol* 2006; 165: 14-21.
7. NORFDFJALL K, ELIASSON M, STEGMAYR B, MELANDER O, NILSSON P, ROOS G: Telomere length is associated with obesity parameters but with a gender difference. *Obesity* 2008; 16: 2682-9.
8. ADAIKALAKOTSWARI A, BALASUBRAMANYM M, RAVIKUMAR R, DEEPA R, MOHAN V: Association of telomere shortening with impaired glucose tolerance and diabetic macroangiopathy. *Atherosclerosis* 2007; 195: 83-9.
9. BROUILETTE SW, MOORE JS, MCMAHON AD *et al*.: Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. *Lancet* 2007; 369: 107-14.
10. HAYCOCK PC, HEYDON EE, KAPTOGE S, BUTTERWORTH AS, THOMPSON A, WILLEIT P: Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis. *Br Med J* 2014; 349: g4227.
11. CHEN W, GARDNER JP, KIMURA M *et al*.: Leukocyte telomere length is associated with HDL cholesterol levels: The Bogalusa heart study. *Atherosclerosis* 2009; 205: 620-5.
12. MINAMINA T, MIAUCHI H, YOSHIDA T, ISHIDA Y, YOSHIDA H, KOMURO I: Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. *Circulation* 2002; 105: 1541-4.
13. GONZALEZ-GAY MA, GONZALEZ-JUANATEY C, MARTIN J: Rheumatoid arthritis: a disease associated with accelerated atherogenesis. *Semin Arthritis Rheum* 2005; 35: 8-17.
14. AVINA-ZUBIETA JA, CHOI HK, SADATSAFAVI M *et al*.: Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies. *Arthritis Rheum* 2008; 59: 1690-7.
15. PETERS MJL, SYMMONS DPM, MCCAREY D *et al*.: EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. *Ann Rheum Dis* 2010; 69: 325-31.
16. STEER SE, WILLIAMS FMK, KATO B *et al*.: Reduced telomere length in rheumatoid arthritis is independent of disease activity and duration. *Ann Rheum Dis* 2007; 66: 476-80.
17. DESSEIN PH, NORTON GR, WOODIWISS AJ, JOFFE BI, WOLFE F: Influence of nonclassical cardiovascular risk factors on the accuracy of predicting subclinical atherosclerosis in rheumatoid arthritis. *J Rheumatol* 2007; 34: 943-51.
18. SOLOMON DH, KREMER J, CURTIS JR *et al*.: Explaining the cardiovascular risk associated with rheumatoid arthritis: traditional risk factors versus markers of rheumatoid arthritis severity. *Ann Rheum Dis* 2012; 69: 1920-5.
19. GONZALEZ-GAY MA, GONZALEZ-JUANATEY C, LOPEZ-DIA MJ *et al*.: HLA-DRB1 and persistent chronic inflammation contribute to cardiovascular events and mortality patients with rheumatoid arthritis. *Arthritis Rheum* 2007; 57: 125-32.
20. FUJII H, SHAO L, COLMEGNA I, GORONZY JJ, WEYAND CM: Telomerase insufficiency in rheumatoid arthritis. *Proc Natl Acad Sci USA* 2009; 106: 4360-5.
21. ARNETT FC, EDWORTHY SM, BLOCH DA *et al*.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.

22. ALETAHA D, NEOGI T, SILMAN AJ *et al*.: 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/ European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010; 69: 1580-8.
23. DESSEIN PH, WOODIWISS AJ, NORTON GR, TSANG L, SOLOMON A: Independent associations of total and high molecular weight adiponectin with cardiometabolic risk and surrogate markers of enhanced early atherogenesis in black and white patients with rheumatoid arthritis: a cross-sectional study. *Arthritis Res Ther* 2013; 15: R128.
24. DESSEIN PH, TSANG L, WOODIWISS AJ, NORTON GR, SOLOMON A: Circulating concentrations of the novel adipokine chemerin are associated with cardiovascular disease risk in rheumatoid arthritis. *J Rheumatol* 2014; 4: 1746-54.
25. LEVEY AS, STEVENS LA, SCHMID CH *et al*.: A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150: 604-12.
26. GEPNER AD, KORCARZ CE, AESCHLIMANN SE *et al*.: Validation of a carotid intima-media thickness border detection program for use in an office setting. *J Am Soc Echocardiogr* 2006; 19: 223-8.
27. DESSEIN PH, JOFFE BI, VELLER MG *et al*.: Traditional and nontraditional cardiovascular risk factors are associated with atherosclerosis in rheumatoid arthritis. *J Rheumatol* 2005; 32: 435-42.
28. TOUBOL PJ, HENNERICI MG, MEAIRS S *et al*.: Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. *Cerebrovasc Dis* 2007; 23: 75-80.
29. CAWTHON RM: Telomere measurement by quantitative PCR. *Nucleic Acids Res* 2002; 30: e47.
30. SOLOMON A, WOODIWISS AJ, ABDOOL-CARRIM AT, STEVENS BA, NORTON GR, DESSEIN PH: The carotid atherosclerosis burden and its relation to cardiovascular risk factors in black and white Africans with established rheumatoid arthritis: a cross-sectional study. *J Rheumatol* 2012; 39: 1789-896.
31. SOLOMON A, NORTON GR, WOODIWISS AJ, DESSEIN PH: Obesity and carotid atherosclerosis in African black and Caucasian women with rheumatoid arthritis: a cross-sectional study. *Arthritis Res Ther* 2012; 14: R67.
32. DESSEIN PH, NORTON GR, JOFFE BI, ABDOOL-CARRIM AT, WOODIWISS AJ, SOLOMON A: Metabolic cardiovascular risk burden and atherosclerosis in African black and Caucasian women with rheumatoid arthritis: a cross-sectional study. *Clin Exp Rheumatol* 2013; 31: 53-61.
33. HAQUE S, RAKIEH C, MARRIAGE F *et al*.: Shortened telomere length in patients with systemic lupus erythematosus. *Arthritis Rheum* 2013: 65: 1319-23.
34. SOLOMON A, CHRISTIAN BF, DESSEIN PH, STANWIX AE: The need for tighter rheumatoid arthritis control in a South African public health care center. *Semin Arthritis Rheum* 2005; 35: 122-31.
35. MYASOEDOVA E, CROWSON CS, KREMERS HM *et al*.: Lipid paradox in rheumatoid arthritis: the impact of inflammation on the risk of cardiovascular disease. *Ann Rheum Dis* 2011; 70: 482-7.
36. GONZALEZ-GAY MA, GONZALEZ-JUANATEY C: Inflammation and lipid profile in rheumatoid arthritis: bridging the apparent paradox. *Ann Rheum Dis* 2014: 73: 1281-3.
37. ORNISH D, LIN J, CHAN JM *et al*.: Effect of comprehensive lifestyle changes on telomerase activity and telomere length in men with biopsy-proven low-risk prostate cancer: a 5-year follow-up study of a descriptive pilot study. *Lancet* 2013; 14: 1112-20.
38. DENHAM J, NELSON CP, O'BRIEN BJ *et al*.: Longer leukocyte telomeres are associated with ultra-endurance exercise independent of cardiovascular risk factors. *PLoS One* 2013; 8: e6377.
39. HUNT SC, CHEN W, GARDNER JP *et al*.: Leukocyte telomeres are longer in African Americans than in whites: the National Heart, Lung, and Blood Institute Family Heart Study and Bogalusa Heart Study. *Aging Cell* 2008; 7: 451-8.
40. TABUKO K, IZUMIYAMA-SHIMOMURA N *et al*.: Telomere lengths are characteristic in each human individual. *Exp Gerontol* 2002; 37: 523-31.