

Association between homocysteine levels and arterial stiffness in women with systemic sclerosis

A. Álvarez-Cienfuegos¹, L. Cantero-Nieto², J.A. García-Gómez³,
J.M. Sabio⁴, M.A. González-Gay⁵⁻⁷, N. Ortego-Centeno⁸⁻¹⁰

¹Department of Rheumatology, Hospital Vega-Baja, Alicante, Spain;

²Department of Emergency, Hospital Santa Ana, Granada, Spain;

³Infectious Diseases Unit, Hospital General de Elche, Alicante, Spain;

⁴Systemic Autoimmune Diseases Unit, Hospital Virgen de las Nieves, Granada, Spain;

⁵Epidemiology, Genetics and Atherosclerosis Research Group on Systemic Inflammatory Diseases, Rheumatology Division, IDIVAL, Santander, Spain;

⁶School of Medicine, University of Cantabria, Santander, Spain;

⁷Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa;

⁸Systemic Autoimmune Diseases Unit, Hospital Universitario San Cecilio, Granada, Spain;

⁹School of Medicine, University of Granada, Spain;

¹⁰Instituto de Investigación Biosanitaria, IBS, Granada, Spain.

Antonio Alvarez-Cienfuegos, MD, PhD
Lucia Cantero-Nieto, MD

Jose Alberto Garcia-Gomez, PhD

Jose Mario Sabio, MD, PhD

Miguel A. González-Gay, MD, PhD

Norberto Ortego-Centeno, MD, PhD

Please address correspondence to:

Antonio Alvarez de Cienfuegos,

Department of Rheumatology,

Hospital Vega-Baja,

Crta. Orihuela-Almoradi, s/n,

03314 Orihuela (Alicante), Spain.

E-mail: antonioalvarezdc@gmail.com

Received on May 22, 2020; accepted in revised form on July 21, 2020.

Clin Exp Rheumatol 2020; 38 (Suppl. 125): S92-S97.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2020.

Key words: homocysteine, systemic sclerosis, pulse wave velocity, brain natriuretic peptide, arterial stiffness

Competing interests: see page S-96.

ABSTRACT

Objective. The purpose of this study was to evaluate homocysteine (Hcy) serum levels in women with systemic sclerosis (SSc) compared with healthy controls and to examine possible associations between Hcy and markers of arterial stiffness.

Methods. A cross-sectional study was performed at a single hospital between November 2017 and May 2019: 62 women with SSc and 62 age- and sex-matched healthy controls were enrolled. Pulse wave velocity (PWV) was measured non-invasively along the carotid-femoral arterial segment. Serum Hcy was analysed using immunonephelometric method.

Results. There was a significant difference in Hcy serum levels between SSc female patients and healthy controls (11.9 ± 3.3 vs. 10.3 ± 2.3 $\mu\text{mol/ml}$, $p=0.002$). Serum levels of Hcy were positively correlated with PWV ($r=0.28$, $p<0.05$), brain natriuretic peptide (BNP) ($r=0.36$, $p<0.05$) and disease duration ($r=0.38$, $p<0.05$), within the SSc group. In addition, in the linear regression model, higher Hcy concentrations were associated with higher PWV [$\beta=0.74$ 95% CI (0.085, 1.394); $p=0.027$], BNP [$\beta=0.04$ 95% CI (0.014, 0.072); $p=0.004$] and disease duration [$\beta=0.18$ 95% CI (0.070, 0.300); $p=0.002$]. In multiple linear regression model adjusting for covariants, Hcy remained positively related to the PWV [$\beta=0.033$ 95% CI (0.003, 0.062); $p=0.031$].

Conclusion. Our findings revealed a positive correlation between Hcy serum levels and PWV, which indicates that high levels of Hcy may predispose to the development of vascular stiffness in patients with SSc.

Introduction

Systemic sclerosis (SSc) is a multisystemic disease featured by microvascu-

lar and immunological disorders along with an excessive accumulation of the components of the connective tissue (collagen, fibronectin, glycosaminoglycans, proteoglycans) that cause cutaneous sclerosis and fibrosis of different organs (1). Vascular involvement in SSc is thought to be the result of immune inflammatory processes that activate and injure the vascular endothelium (2). Such immune vascular injury could increase the risk of arteriopathy. Homocysteine (Hcy), a nonessential sulfur-containing amino acid, is derived from methionine (3). It is formed during the conversion of methionine to cysteine. Several studies have reported that serum Hcy levels are elevated in SSc patients compared to healthy controls (4-7). Elevated Hcy has been shown to be a moderately strong and independent cardiovascular risk factor in healthy populations (8). This association appears to be particularly strong in older people, in which the level of Hcy has even been shown to be a better predictor of cardiovascular mortality than models based on classic Framingham risk factors (age, gender, total cholesterol, high density lipoprotein cholesterol, smoking habits, and systolic blood pressure) (9). Hcy also has been associated with arterial stiffness (10). Arterial stiffness can reflect arterial elasticity and the burden of arteriosclerosis (11). Pulse wave velocity (PWV) is regarded as the gold standard measurement of large artery stiffness and is one of the markers of hypertension-mediated organ damage, and should be assessed among patients with hypertension according to the guidelines of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) (12). Previous meta-analyses have revealed that PWV is an independent predictor of the development of cardiovascular

disease (CVD), adverse cardiovascular events and all-cause mortality (13–15). At present, PWV is widely used in both clinical practice and epidemiological studies due to its feasibility and clinical significance.

The purpose of this study was to analyse Hcy in a cohort of women with SSc and to investigate possible associations between Hcy serum levels with markers of arterial stiffness.

Materials and methods

Study subjects

This cross-sectional study was performed at San Cecilio Hospital, Granada, Spain, from November 2017 to May 2019. We prospectively enrolled 62 consecutive female patients affected by SSc ≥ 18 years old attending our Systemic Autoimmune Diseases Unit. In addition, a control group of 62 healthy women age-matched, recruited mainly among non-medical staff of our hospital that attended their annual medical health examination and who were invited to participate were included. All patients included in this study had normal serum creatinine (Cr) levels, and met the 2013 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria for SSc (16). Individuals with prevalent cardiovascular disease (acute myocardial infarction, angina pectoris, stroke, or peripheral arterial disease) were excluded.

At the clinic visit, participants completed questionnaires about their lifestyle characteristics, medical history, and current medication used. Informed consent was obtained for all subjects, and the study was approved by the Research Ethics Committee of Hospital Clínico Universitario San Cecilio in Granada, Spain, and conducted in accordance with the guidelines in the Declaration of Helsinki.

Cardiovascular assessment

Current smokers were defined as those who reported having smoked ≥ 1 cigarette per day regularly during the year preceding the examination. Waist circumference, weight, and height were measured; and body mass index (BMI) was calculated as weight (kg)/height

(m^2). Two recordings of blood pressure were obtained from the right arm of the seated subjects; measurements were taken in 5-min intervals, then mean values were calculated. Hypertension was defined as a mean of 3 independent measures of blood pressure $\geq 140/90$ mmHg or current use of antihypertensive drugs. Type 2 diabetes mellitus (T2DM) was defined by self-reported use of insulin, or oral hypoglycaemic medications, or a fasting glucose level ≥ 126 mg/dl. Kidney function was assessed using the estimated glomerular filtration rate (eGFR) calculated by the CKD-Epi study equation (17).

In our study, we assessed arterial stiffness by determining carotid–femoral pulse wave velocity (CFPWV), as previously described (18). Briefly, after 15–20 min in a supine posture, we measured brachial blood pressure and arterial tonometry with an electrocardiogram obtained from the carotid and femoral arteries using a custom transducer (Cardiovascular Engineering, Inc.) (18). Transit distance from the carotid to femoral arteries was assessed with body surface measurements from the suprasternal notch to the carotid and femoral pulse recording sites. We computed CFPWV as the pulse wave transit distance divided by the transit time of the pulse wave from the carotid to femoral arteries, with adjustment for parallel transmission of the arterial pulse wave in the brachiocephalic artery and aortic arch (18). Higher CFPWV indicates greater arterial stiffness.

Laboratory measurements

In all the cases, a fasting blood sample was taken in the morning, and was stored at -70°C until the assays were performed.

The sera were tested for creatinine, CRP, BNP and Hcy. Creatinine was determined by Jaffe method (Siemens Healthcare Diagnostic Inc. NY, USA). CRP was measured by turbidimetric immunoassay (Siemens Healthcare Diagnostic Inc. NY, USA). BNP was quantified in heparinised plasma using a solid-phase two-site chemiluminescent immunometric assay (Biomérieux, France). Serum Hcy (Siemens Healthcare Diagnostic Inc. NY, USA)

were measured by immunonephelometric method according to the manufacturer's recommendations. Antinuclear antibodies were assessed using ELISA kits produced by Generic Assay Dahlwitz Germany. Fasting plasma glucose was measured in fresh specimens with a hexokinase reagent kit (Siemens Healthcare Diagnostic Inc. NY, USA). Total cholesterol and triglyceride levels were determined by fully enzymatic techniques. High-density lipoprotein (HDL) was determined after precipitation of apolipoprotein B (apoB)–containing lipoproteins with magnesium sulphate and dextran sulphate. Low-density lipoprotein (LDL) was calculated using the Friedewald formula. All other routine serum biochemical parameters were measured at the Department of Clinical Chemistry, San Cecilio Hospital.

Statistical analysis

Data were analysed by statistical software SPSS 21 (Chicago, IL, USA), using independent samples *t*-test, Mann-Whitney U-test, and Chi-square test when appropriate. Spearman's coefficient and Pearson's correlation were calculated as suitable to determine the correlation between the biochemical parameters, these correlation coefficients are statistical measures of the strength of a monotonic relationship between paired data and that correlation does not imply causation. The correlation between the two variables is denoted by the letter *r* and quantified with a number, which varies between -1 and $+1$. Zero means there is no correlation, where 1 means a complete or perfect correlation. The sign of the *r* shows the direction of the correlation. A negative *r* means that the variables are inversely related. The strength of the correlation increases both from 0 to $+1$, and 0 to -1 , using the following interpretation: 0.00–0.19 is “very weak”, 0.20–0.39 is “weak”, 0.40–0.59 is “moderate”, 0.60–0.79 is “strong” and 0.80–1 is “very strong”. *P*-values of less than 0.05 were considered statistically significant. Sample size was estimated for a 0.05% alpha risk. The quantitative data were shown as mean $\pm$ standard deviation (SD) and median

(Q1–Q3) as suitable. To test if we can admit that the distribution is normal, we use the Shapiro-Wilk test. Linear regression was used to examine the cross-sectional associations of serum Hcy concentrations with PWV, BNP and disease duration. Multiple regression analysis was performed to determine the relationship between PWV, age, smoking, BMI, blood pressure, as well as serum levels of Hcy, HDL-c and glucose.

Results

Characteristics of the study subjects

The main features of the 62 women with SSc and 62 controls included in this study are shown in Table I. The mean age (SD) of the patients was 53.2±10.1 years. The majority were Caucasian (90.5%). The mean disease duration was 8.8±6.9 years. Forty-four (70.9%) patients had a limited form of the disease, 11 (17.7%) had a diffuse form, and 7 (11.3%) had SSc without skin involvement. Twelve (19%) patients had calcinosis and all patients had Raynaud Phenomenon. Three patients were treated with methotrexate. In addition, a total of 62 healthy women were included in our study as controls; mean age (SD) 52.7±9.7 years. Most of them were also Caucasian (98.3%).

Laboratory results

Laboratory tests of the patients and healthy controls included in the present study are shown in Table II. The mean CRP, ESR and BNP in SSc patients were 0.4±0.4 mg/dl, 21.1±16.0 mm/1st hour, and 41.2±27.5 pg/ml, respectively.

Antinuclear antibodies (ANAs) were detected in 54 patients (87.1%), anti-centromere antibodies in 34 patients (54.8%), anti-Scl-70 antibodies in 6 patients (9.6%), and anti-RNA-polymerase III antibodies in 4 patients (6.4%).

Serum Hcy concentrations were significantly higher in the SSc patients than those in the control group: [11.9±3.3 vs. 10.3±2.3, µmol/l; $p=0.002$] (Fig. 1).

Cardiovascular disease risk factors

As shown in Table I, patients had a mean BMI of 26.3±4.9 kg/m², waist circumference of 83.5±11.0 cm and

Table I. Characteristics of women with systemic sclerosis and healthy controls.

	SSc mean ± SD	HC mean ± SD	p-value
Age, years	53.2 ± 10.1	52.7 ± 9.7	0.71
Height, cm	159.5 ± 5.6	160.9 ± 7.1	0.23
Body weight, kg	66.8 ± 12.0	67.2 ± 12.0	0.90
Body mass index, kg/m ²	26.3 ± 4.9	25.9 ± 4.3	0.90
Waist circumference, cm	83.5 ± 11.0	83.1 ± 13.4	0.64
PWV, m/s	7.5 ± 1.2	7.3 ± 1.4	0.53
Current smokers, n (%)	11 (17.7)	15 (24.2)	0.04
Hypertension, n (%)	8 (12.9)	11 (17.7)	0.87
Angiotensin II receptor blockers, n (%)	5 (8.1)	5 (8.1)	1
ACE inhibitors, n (%)	2 (3.2)	3 (4.8)	1
Diuretics, n (%)	1 (1.6)	2 (3.2)	1
Calcium-channel blockers, n (%)	20 (32.2)	1 (1.6)	<0.001
Diabetes mellitus, n (%)	2 (3.2)	2 (3.2)	0.62
Dyslipidemia, n (%)	21 (33.8)	14 (22.5)	0.17
Prednisone, mg/day	6.5 ± 3.5	-	-
Methotrexate, n (%)	3 (4.8)	-	-
Disease duration, year	8.8 ± 6.9	-	-
lcSSc, n (%)	44 (70.9)	-	-
dcSSc, n (%)	11 (17.7)	-	-
SSc without skin involvement	7 (11.3)	-	-

ACE: angiotensin-converting enzyme; dcSSc: diffuse cutaneous SSc; HC: healthy control; lcSSc: limited cutaneous SSc; PWV: pulse wave velocity; SD: standard deviation; SSc: systemic sclerosis.

Table II. Serum Hcy and biochemistry of the patients and healthy controls.

	SSc mean ± SD	HC mean ± SD	p-value
Hcy, µmol/l	11.9 ± 3.3	10.3 ± 2.3	0.002
Serum phosphate, mg/dl	3.6 ± 0.5	3.4 ± 0.5	0.04
Serum calcium, mg/dl	9.5 ± 0.3	9.4 ± 0.4	0.05
Glucose, mg/dl	88.4 ± 9.4	92.1 ± 13.0	0.07
Cholesterol, mg/dl	199.8 ± 34.9	211.8 ± 37.3	0.06
LDL-C, mg/dl	122.1 ± 32.0	129.7 ± 30.7	0.17
HDL-C, mg/dl	57.8 ± 15.5	63.3 ± 13.1	0.03
Triglycerides, mg/dl	105.2 ± 54.1	107.7 ± 53.9	0.79
Uric acid, mg/dl	4.6 ± 1.1	4.6 ± 1.3	0.91
BNP, pg/ml	41.2 ± 27.5	28.3 ± 19.9	0.003
Serum creatinine, mg/dl	0.7 ± 0.8	0.7 ± 0.2	0.12
eGFR, ml/min	93.0 ± 17.2	97.0 ± 13.2	0.19
CRP, mg/dl	0.4 ± 0.4	0.2 ± 0.1	0.007
ESR, mm/h	21.1 ± 16.0	11.3 ± 10.2	0.001
ANAs, n (%)	54 (87.1)	-	-
Anti-centromere, n (%)	34 (54.8)	-	-
Anti-Scl70, n (%)	6 (9.6)	-	-
Anti-RNA-polymerase III, n (%)	4 (6.4)	-	-

ANA: antinuclear antibodies; BNP: brain natriuretic peptide; CRP: C-reactive protein; eGFR: estimated glomerular filtration rate; ESR: erythrocyte sedimentation rate; HC: healthy control; HDL: high-density lipoprotein; Hcy: homocysteine; LDL: low-density lipoprotein; SD: standard deviation; SSc: systemic sclerosis.

PWV of 7.5±1.2 m/s. Eleven (17.7%) of them are current smokers. Two patients (3.2%) had diabetes mellitus. Healthy controls had a mean BMI of 25.9±4.3 kg/m², waist circumference of 83.1±13.4 cm and PWV of 7.3±1.4 m/s. Fifteen (24.2%) of them are current smokers. Two controls (3.2%) had diabetes mellitus.

Association between Hcy levels and cardiovascular risk factors or disease features in patients with SSc

Table III shows the correlation coefficients between Hcy and other markers in patients with SSc. Hcy serum levels showed a statistically significant positive weak correlation with PWV ($r=0.28$, $p=0.02$), BNP ($r=0.36$, $p=0.004$) and

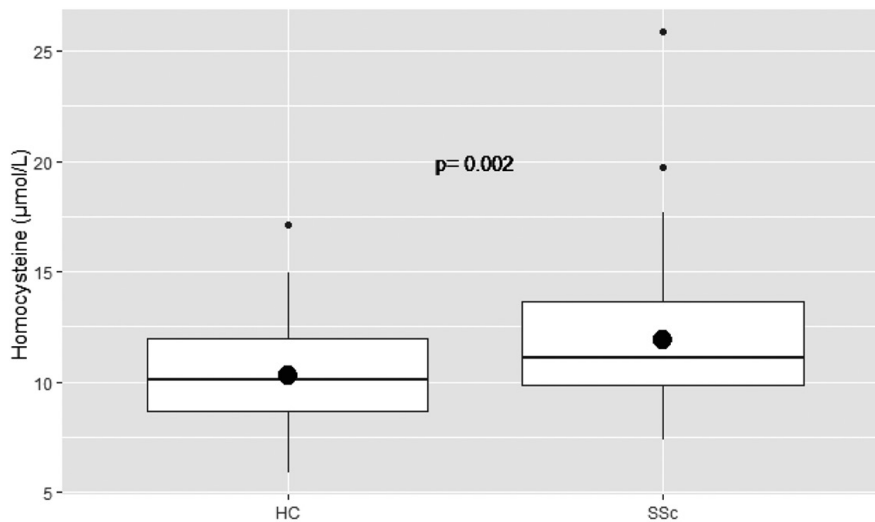


Fig. 1. Median serum concentration of homocysteine in systemic sclerosis (SSc) patients and healthy controls (HC). 2-tailed Mann Whitney U-test for unpaired sample.

Table III. Correlations between Hcy and study parameters in SSc patients.

	Correlation coefficient	p-value
Age	0.24	0.054
Height	-0.07	0.614
Body weight	0.04	0.745
Body mass index	0.08	0.559
Waist circumference	0.04	0.764
Hypertension	0.08	0.543
Smoking	0.17	0.189
Disease duration	0.38	0.002
PWV	0.28	0.027
Prednisone dose	-0.03	0.690
Methotrexate	-0.08	0.374
C-reactive protein	0.18	0.155
Erythrocyte sedimentation rate	0.05	0.721
Serum creatinine	0.38	0.002
Serum urea	0.35	0.005
eGFR	-0.3	0.007
Serum phosphate	0.18	0.165
Serum calcium	0.09	0.507
Glucose	-0.20	0.127
Cholesterol	0.14	0.283
LDL-C	0.11	0.414
HDL-C	-0.06	0.622
Triglycerides	0.20	0.116
Uric acid	0.19	0.146
BNP	0.36	0.004

BNP: brain natriuretic peptide; eGFR: estimated glomerular filtration rate; HDL: high density lipoprotein; Hcy: homocysteine; LDL: low density lipoprotein; PWV: pulse wave velocity; SSc: systemic sclerosis.

disease duration ($r=0.38$, $p=0.002$). However, there was no correlation of Hcy levels with age, BMI, waist circumference, or smoking status. In the linear regression model, higher Hcy concentrations were associated with higher PWV [$\beta=0.74$ 95% CI (0.085, 1.394); $p=0.027$], BNP [$\beta=0.04$ 95% CI (0.014, 0.072); $p=0.004$] and disease duration [$\beta=0.18$ 95% CI (0.070, 0.300); $p=0.002$] (Fig. 2).

Multiple linear regression analysis (Table IV) shows the possible influence of age, BMI, current smoking, blood pressure, serum Hcy, HDL-c and glucose levels in predicting PWV.

Discussion

Systemic sclerosis (SSc) is a multisystem disease characterised by vasculopathy and organ fibrosis (19). Cardiovascular complications in SSc include

peripheral vascular disease, cerebrovascular disease, coronary disease and primary myocardial disease (20).

Hyperhomocysteinaemia (HHcy) was recognised as a modifiable independent risk factor for coronary, cerebral and peripheral vascular diseases (21-23), and experimental studies have also suggested that Hcy induces vascular endothelium injury and leads to vascular damage (24). In our study, we found significantly elevated Hcy serum levels in patients with SSc compared with healthy controls, in accordance with several reports (4-7). We also have found higher BNP serum levels in SSc female patients compared to healthy controls. Brain natriuretic peptide (BNP) level assessment has become a strong and well-recognised indicator of the cardiovascular risk in SSc (25). Elshamy *et al.* (26), reported a significant increase in the mean values of serum levels of N-terminal pro-brain natriuretic peptide in SSc patients compared to controls. Interestingly, we disclosed a significant positive correlation between Hcy serum levels and BNP in our patients with SSc, independent of age. Washio *et al.* (27) found that BNP levels were increased in the high Hcy group in patients with acute myocardial infarction.

Pulse wave velocity (PWV) is a well-validated index of arterial distensibility, large artery stiffness is a powerful and independent predictor of cardiovascular risk (28, 29). In our study, there was no significant difference in PWV between SSc patients and healthy controls that were in agreement with the study of Bartoloni *et al.* (30), this result may be biased by the higher proportion of smokers in the control group or by the higher proportion of patients under calcium-channel blockers treatment (31, 32).

Smooth muscle tone influences the stiffness of the elastic and muscular arteries, and removal of the vascular endothelium modifies large artery mechanics *in vivo* (33, 34), suggesting a degree of functional regulation of large artery stiffness by endothelium-derived vasoactive mediators. In the present study, we report for first time that high Hcy serum levels were positively as-

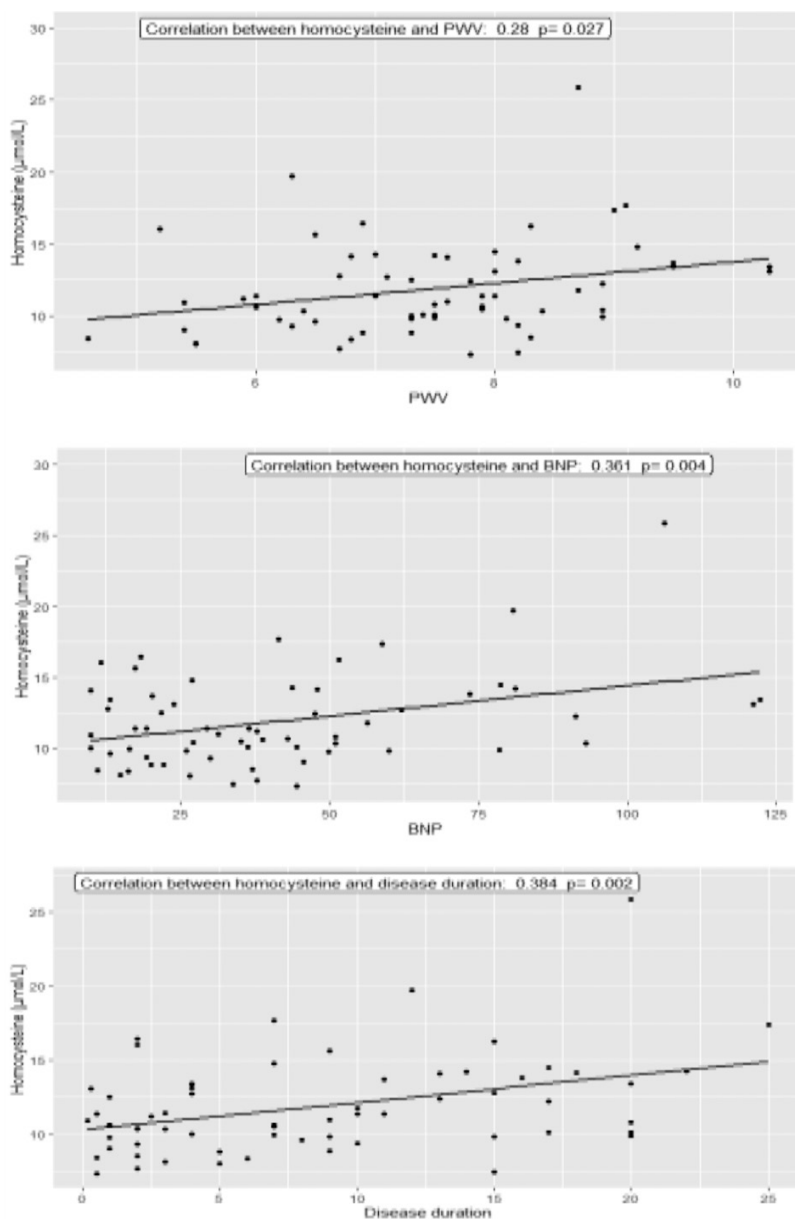


Fig. 2. Correlation between homocysteine and pulse wave velocity (PWV), brain natriuretic peptide (BNP) and disease duration.

Table IV. Multiple regression analysis of PWV with all other parameters in SSc patients.

	β	95%CI	p-value
Age	0.045	(0.02, 0.07)	0.001
Smoking	0.001	(-0.004, 0.007)	0.614
BMI	0.062	(0.03, 0.16)	0.214
Hypertension	0.040	(0, 0.08)	0.036
Hcy	0.033	(0.003, 0.062)	0.031
HDL-c	-0.051	(-0.105, 0.043)	0.023
Glucose	0.004	(0, 0.007)	0.035

BMI: body mass index; HDL: high density lipoprotein; Hcy: homocysteine; PWV: pulse wave velocity; SSc: systemic sclerosis.

sociated with PWV in SSc female patients. Arterial stiffness and Hcy are both powerful predictors of cardiovascular disease. Hcy may increase vascu-

lar stiffness by damaging elastin fibres, increasing collagen production through the activation of metalloproteinases, and stimulating smooth muscle cell

proliferation (35). Previous studies have reported a significant association of serum Hcy concentration with different indices of arterial stiffness, including pulse pressure and aortic stiffness as assessed by CFPWV in the general population (36-38). Zhang *et al.* (39) found that serum Hcy concentration was positively associated with artery stiffness, measured as CFPWV, in the elderly subjects. Xiao *et al.* (40) detected a significant positive association between serum Hcy and arterial stiffness, measured as CFPWV, in hypertensive subjects.

We also found a positive correlation between Hcy serum levels and disease duration in women with SSc. In this regard, Sabio *et al.* (41) described a positive correlation between Hcy serum levels and disease duration in women with Systemic Lupus Erythematosus.

There are several limitations in our study that should be considered. First, this study was a cross-sectional analysis that reflected the status of a population in a particular period. The cross-sectional design of this study does not allow drawing causal inferences. This study focused only on SSc women; therefore, the findings of this study cannot be generalised to men with SSc. The lack of data on folate and vitamin B12 status in these subjects made it impossible to analyse their potential influence on Hcy serum levels.

In conclusion, our findings revealed a positive correlation between Hcy serum levels and PWV, which indicates that high levels of Hcy may be involved in the development of vascular stiffness in patients with SSc.

Competing interests

M.A. González-Gay received grants/research support from Abbvie, MSD, Janssen and Roche, and had consultation fees/participation in company sponsored speakers bureau from Abbvie, Pfizer, Roche, Sanofi, Celgene and MSD. The other co-authors have declared no competing interests.

References

1. LEROY EC, BLACK C, FLEISCHMAJER R *et al.*: Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988; 15: 202-5.

2. HO M, VEALE D, EASTMOND C, NUKI G, BELCH J: Macrovascular disease and systemic sclerosis. *Ann Rheum Dis* 2000; 59: 39-43.
3. SELHUB J: Homocysteine metabolism. *Annu Rev Nutr* 1999; 19: 217-46.
4. CARAMASCHI P, MARTINELLI N, BIASI D *et al.*: Homocysteine plasma concentration is related to severity of lung impairment in scleroderma. *J Rheumatol* 2003; 30: 298-304.
5. CARAMASCHI P, CANESTRINI S, MARTINELLI N *et al.*: Scleroderma patients nailfold videocapillaroscopic patterns are associated with disease subset and disease severity. *Rheumatology* (Oxford) 2007; 46: 1566-9.
6. KHURMA V, MEYER C, PARK GS *et al.*: A pilot study of subclinical coronary atherosclerosis in systemic sclerosis: coronary artery calcification in cases and controls. *Arthritis Rheum* 2008; 59: 591-7.
7. MOTEGI S, TOKI S, YAMADA K, UCHIYAMA A, ISHIKAWA O: Elevated plasma homocysteine level is possibly associated with skin sclerosis in a series of Japanese patients with systemic sclerosis. *J Dermatol* 2014; 41: 986-91.
8. HOMOCYSTEINE STUDIES COLLABORATION: Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA* 2002; 288: 2015-22.
9. DE RUIJTER W, WESTENDORP RG, ASSENDELFT WJ *et al.*: Use of Framingham risk score and new biomarkers to predict cardiovascular mortality in older people: population based observational cohort study. *BMJ* 2009; 338: a3083.
10. VAN DIJK SC, SMULDERS YM, ENNEMAN AW *et al.*: Homocysteine level is associated with aortic stiffness in elderly: cross-sectional results from the B-PROOF study. *J Hypertens* 2013; 31: 952-9.
11. TOMIYAMA H: Arterial stiffness: is this a marker for the current status or future? *J Atheroscler Thromb* 2016; 23: 1026-7.
12. WILLIAMS B, MANCIA G, SPIERING W *et al.*: 2018 ESC/ESH guidelines for the management of arterial hypertension. *Eur Heart J* 2018; 39: 3021-104.
13. BEN-SHLOMO Y, SPEARS M, BOUSTRED C *et al.*: Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. *J Am Coll Cardiol* 2014; 63: 636-46.
14. OHKUMA T, NINOMIYA T, TOMIYAMA H *et al.*: Brachial-Ankle pulse wave velocity and the risk prediction of cardiovascular disease: an individual participant data meta-analysis. *Hypertension* 2017; 69: 1045-52.
15. VLACHOPOULOS C, AZNAOURIDIS K, TERTENTES-PRINTZIOS D *et al.*: Prediction of cardiovascular events and all-cause mortality with brachial-ankle elasticity index: a systematic review and meta-analysis. *Hypertension* 2012; 60: 556-62.
16. VAN DEN HOOGEN F, KHANNA D, FRANSEN J *et al.*: 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2013; 72: 1747-55.
17. NATIONAL KIDNEY FOUNDATION: K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; 39: S1-266.
18. MITCHELL GF, VAN BUCHEM MA, SIGURDSSON S *et al.*: Arterial stiffness, pressure and flow pulsatility and brain structure and function: the Age, Gene/Environment Susceptibility-Reykjavik study. *Brain* 2011; 134: 3398-407.
19. BARSOTTI S, ORLANDI M, CODULLO V *et al.*: One year in review 2019: systemic sclerosis. *Clin Exp Rheumatol* 2019; 37: (Suppl. 119): S3-14.
20. NGIAN GS, SAHHAR J, WICKS IP, VAN DORNUM S: Cardiovascular disease in systemic sclerosis – an emerging association? *Arthritis Res Ther* 2011; 13: 237.
21. TAHERAGHDAM AA, DALIRAKBARI N, KHALILI M *et al.*: Hyperhomocysteinemia, low vitamin B12, and low folic acid: are risk factors of cerebral vascular thrombosis in northwest Iran? *J Res Med Sci* 2016; 21: 16.
22. YEH JK, CHEN CC, HSIEH MJ *et al.*: Impact of homocysteine level on long-term cardiovascular outcomes in patients after coronary artery stenting. *J Atheroscler Thromb* 2017; 24: 696-705.
23. KUNDI H, KIZILTUNC E, ATES I *et al.*: Association between plasma homocysteine levels and endorgan damage in newly diagnosed type 2 diabetes mellitus patients. *Endocr Res* 2017; 42: 36-41.
24. LAI WK, KAN MY: Homocysteine-induced endothelial dysfunction. *Ann Nutr Metab* 2015; 67: 1-12.
25. ALLANORE Y, MEUNE C: N-terminal pro brain natriuretic peptide: the new cornerstone of cardiovascular assessment in systemic sclerosis. *Clin Exp Rheumatol* 2009; 27 (Suppl. 54): S59-63.
26. ELSHAMY HA, IBRAHIM SE, FAROUK HM: N-terminal pro-brain natriuretic peptide in systemic sclerosis: new insights. *Eur J Dermatol* 2011; 21: 686-90.
27. WASHIO T, NOMOTO K, WATANABE I, TANI S, NAGAO K, HIRAYAMA A: Relationship between plasma Homocysteine levels and congestive heart failure in patients with acute myocardial infarction. *Int Heart J* 2011; 52: 224-8.
28. BLACHER J, ASMAR R, DJANE S, LONDON GM, SAFAR ME: Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension* 1999; 33: 1111-7.
29. BLACHER J, GUERIN AP, PANNIER B, MARCHAIS SJ, LONDON GM: Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. *Hypertension* 2001; 38: 938-42.
30. BARTOLONI E, PUCCI G, CANNARILE F *et al.*: Central hemodynamics and arterial stiffness in systemic sclerosis. *Hypertension* 2016; 68: 1504-11.
31. MAHMUD A, FEELY J: Effect of smoking on arterial stiffness and pulse pressure amplification. *Hypertension* 2003; 41: 183-7.
32. ONG K, DELERME S, PANNIER B *et al.*: Aortic stiffness is reduced beyond blood pressure lowering by short-term and long-term antihypertensive treatment: a meta-analysis of individual data in 294 patients. *J Hypertens* 2011; 29: 1034-42.
33. LEVY BI, BENESSIANO J, POITEVIN P, SAFAR ME: Endothelium-dependent mechanical properties of the carotid artery in WKY and SHR. Role of angiotensin-converting enzyme inhibition. *Circ Res* 1990; 66: 321-8.
34. BOUTOUYRIE P, BEZIE Y, LACOLLEY P *et al.*: *In vivo/in vitro* comparison of rat abdominal aorta wall viscosity. Influence of endothelial function. *Arterioscler Thromb Vasc Biol* 1997; 17: 1346-55.
35. SAMOUILLAN V, LAMY E, DANDURAND J *et al.*: Changes in the physical structure and chain dynamics of elastin network in homocysteinecultured arteries. *J Biomed Mater Res A* 2010; 93: 696-703.
36. FENG SQ, YE P, LUO LM *et al.*: Associations of plasma homocysteine and high-sensitivity C-reactive protein levels with arterial stiffness in Chinese population: a community-based study. *Chin Med J (Engl)* 2012; 125: 44-9.
37. MAYER O, FILIPOVSKÝ J, DOLEJSOVÁ M *et al.*: Mild hyperhomocysteinemia is associated with increased aortic stiffness in general population. *J Hum Hypertens* 2006; 20: 267-71.
38. VYSSOULIS G, KARPANOU E, KYVELOU SM *et al.*: Associations between plasma homocysteine levels, aortic stiffness and wave reflection in patients with arterial hypertension, isolated office hypertension and normotensive controls. *J Hum Hypertens* 2010; 24: 183-9.
39. ZHANG S, BAI YY, LUO LM, XIAO WK, WU HM, YE P: Association between serum homocysteine and arterial stiffness in elderly: a community-based study. *J Geriatr Cardiol* 2014; 11: 32-8.
40. XIAO W, BAI Y, YE P *et al.*: Plasma homocysteine is associated with aortic arterial stiffness but not wave reflection in chinese hypertensive subjects. *PLoS One* 2014; 9: e85938.
41. SABIO JM, VARGAS-HITOS JA, MARTINEZ-BORDONADO J *et al.*: Relationship between homocysteine levels and hypertension in systemic lupus erythematosus. *Arthritis Care Res* 2014; 66: 1528-35.