High cardiovascular risk in mixed connective tissue disease: evaluation of macrovascular involvement and its predictors by aortic pulse wave velocity

K. Triantafyllias[1], M. de Blasi[1], F. Lütgendorf[1], L. Cavagna[2], M. Stortz[3], J. Weinmann-Menke[3], S. Konstantinides[4], P.R. Galle[5], A. Schwarting[1,3]

[1]ACURA Rheumatology Clinics, Bad Kreuznach, Germany; [2]Rheumatology Unit, University and IRCCS Policlinico S. Matteo Foundation, Pavia, Italy; [3]Division of Rheumatology and Clinical Immunology, [4]Center for Thrombosis and Hemostasis, [5]Division of Gastroenterology, Department of Internal Medicine I, University Medical Center of the Johannes Gutenberg University Mainz, Germany.

Abstract

Objective

Macrovascular involvement and cardiovascular (CV) risk has not been sufficiently studied in mixed connective tissue disease (MCTD). In particular, the gold standard assessment method of aortic stiffness carotid-femoral pulse wave velocity (cfPWV) has never been evaluated in patients with this disease. The aims of the present study were therefore to examine cfPWV in MCTD and to evaluate its associations with MCTD-associated markers and traditional CV risk factors.

Methods

Measurements of cfPWV were performed in 43 MCTD patients and 107 healthy controls. The difference between cfPWV in the two groups was statistically examined and subsequently controlled for the effect of possible confounding factors. The association of cfPWV with MCTD-associated organ involvement, routine laboratory parameters and immunoserological markers was also evaluated. Finally, the relationship of cfPWV with medications and traditional CV risk factors was examined.

Results

Adjusted statistical analyses for confounding factors showed significantly higher cfPWV values in MCTD patients in comparison to controls ($p_{adj}<0.001$). cfPWV correlated in both the patients and the control group significantly with age (rho=0.69, p<0.001 and rho=0.67, p<0.001 respectively) and diastolic arterial pressure ($p_{adj}=0.024$ and $p_{adj}=0.032$ respectively). Moreover, cfPWV correlated in the control group with systolic and mean arterial pressure ($p_{adj}<0.001$ and p=0.002 respectively). Finally, higher cfPWV values could be documented in the subset of MCTD patients without lung involvement ($p_{adj}=0.007$).

Conclusion

Patients with MCTD have significantly higher aortic stiffness and thus CV risk in comparison to controls. Except for the disease itself, age and blood pressure were the main predictors of cfPWV.

Key words

mixed connective tissue disease, cardiovascular risk, vascular stiffness, pulse wave velocity, comorbidity

Konstantinos Triantafyllias, MD, Dr. med.
Michele de Blasi, Dipl. Ing.
Freya Lütgendorf, MD, Dr. med.
Lorenzo Cavagna, MD
Marco Stortz, doctoral candidate
Julia Weinmann-Menke, MD, Dr. med.
Stavros Konstantinides, MD, Dr. med., Prof.
Peter R. Galle, MD, Dr. med. Prof.
Andreas Schwarting, MD, Dr. med., Prof.

Please address correspondence and reprint requests to:
Dr Konstantinos Triantafyllias,
ACURA Rheumatology Clinics,
Kaiser-Wilhelm-Str. 9-11,
55543 Bad Kreuznach, Germany.
E-mail: ktriantafyllias@gmail.com

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Introduction

Mixed connective tissue disease (MCTD) is a chronic autoimmune overlap disease characterised by ribonucleoprotein U1 (U1-RNP) antibodies and selected clinical characteristics of systemic lupus erythematosus (SLE), systemic sclerosis (SSc) and Polymyositis (PM) (1). Raynaud's phenomenon (RP), arthritis, myositis and swollen (''puffy'') hands are features with high incidence among patients with MCTD (2). Moreover, up to 50% of patients with MCTD can develop interstitial lung disease (ILD) (3), whereas prevalence of pulmonary hypertension (PH) seems to be low (*i.e.* 2% in an unselected MCTD European population) (4). The annual incidence of MCTD varies according to the examined cohort from 0.2 to 1.9 per 100,000 (5–7), by a prevalence of 3.8–6.4. per 100,000 adults (5, 8).

As similarly described in further overlap syndromes, diagnosis of MCTD can often be troublesome through the sequential appearance of clinical features and the lack of widely accepted diagnostic criteria (9, 10). Except for that, MCTD is in comparison to other autoimmune diseases not well examined in terms of treatment strategies and comorbidities such as cardiovascular (CV) disease. In general, descriptions of cardiac involvement in patients with MCTD vary highly (from 13% to 65%) depending on the methodology of the research done (11). The most common cardiac manifestations are pericarditis (29%) and mitral valve prolapse (26%) followed by myocarditis, conduction disturbances and filling abnormalities of the left ventricle (12, 13). Furthermore, MCTD-associated vascular disease includes RP of the coronary vessels, acral ulcers and abnormalities of the nailfold capillaries (14-16). Interestingly enough, approximately 40% of MCTD patients have asymptomatic CV disease (CVD), whereas 20% of mortality in MCTD has been attributed to cardiac causes (11).

Despite the evidence of high CV-associated mortality in MCTD, data concerning validated markers of CV risk are scarce and originate mainly from very few studies focusing on endothelial function and carotid arteriosclerosis. Further well established markers of CV risk such as arterial stiffness have not been examined in MCTD. Particularly, stiffness of the aorta, a modifiable, independent predictor of CV risk which can be measured via carotid femoral pulse wave velocity, has been assessed by our group and other researchers in various rheumatologic diseases but not in MCTD (17-19). The predictive value of this marker concerning CV events has been repeatedly shown in the general population and cfPWV continues to be described as the gold standard for the measurement of aortic stiffness (20).

Thus, the aim of this study was to test the hypothesis of an increased aortic stiffness in patients with MCTD in comparison to healthy controls and to examine the possible correlations of cfPWV with clinical and laboratory MCTD-associated parameters and selected traditional CV risk factors.

Patients and methods

Study populations

43 consecutive patients with MCTD underwent a cfPWV examination during their stay in our inpatient Rheumatology Clinic in Bad Kreuznach, Germany as a part of the diagnostic process (start of the recruitment on June 2016). For the control group, the same examination was conducted in 107 individuals without underlying rheumatologic diseases (co-workers of our hospital and of the University Medical Center of Mainz, Germany who freely responded to an open call for participation in the study). Exclusion criteria in both groups were: Malignancy, pregnancy, age <18 years, kidney failure, class III obesity (BMI >40 kg/m^2) and diabetes mellitus. Moreover, subjects who received glucocorticoids and/or had RP were excluded from the control group.

All included patients met the Alarcón-Segovia criteria for MCTD (21). Patients gave their informed consent and the assessment was reviewed and approved by the Local Standing Committee for Clinical Studies in adherence to the Declaration of Helsinki.

Data collection

In addition to epidemiological data (gender, age, weight, height), in both groups we documented cigarette smok-

ing, use of anti-hypertensive drugs and use of statins. We also calculated body mass index (BMI: weight in kilograms divided by the square of the height in meters) and placed an obesity cut off to a BMI greater than 30 kg/m^2.

Patients were asked to rate the current activity of MCTD on a scale ranging from not ''active at all'' (0 mm) to ''extremely active'' (100 mm). This visual analogue scale represented patient global assessment similarly to the scale used on the Disease Activity Score 28 (DAS28). Pulmonary function tests and chest x-rays in two planes were performed in order to control for interstitial lung disease (ILD). In case of pathologic results in these two examinations and/or presentation of symptoms and signs of interstitial lung disease (ILD) (*i.e.* cough, dyspnea) a high resolution CT scan was additionally performed. Presence of RP was assessed by a question about finger/toe colour changes by low temperatures or emotional stress and/or by a cold test. Cutaneous manifestations and joint erosions of the hands and feet (x-ray in two planes) were also reported. Furthermore, patients were asked about the duration of their disease, the use of immunosuppressive drugs and glucocorticoids. Laboratory parameters such as differential blood counts, inflammation markers [C-reactive protein (CRP), erythrocyte sedimentation rate (ESR)] and renal parameters [glomerular filtration rate (GFR), creatinine] were routinely tested and documented. Finally, immunoserological markers were assessed by well established methods: Antinuclear antibodies were detected on the HEp2 cell by indirect immunofluoresence. ELISA testing was used for the assessment of serum concentrations of antibodies such as rheumatoid factor (RF), double-stranded DNA (ds-DNA), SSA-Ro, SSB-La, Sm, Scl-70, U1-RNP and Jo-1. An immunonephelometric assay was used for the assessment of complement counts.

Carotid-femoral pulse wave velocity and median blood pressure measurements

cfPWV examination was performed by trained and experienced medical stuff using a validated non-invasive oscillometric device (Vicorder®, SMT medical GmbH&Co).

During the procedure, a pad containing a small bladder was placed around the neck of the patient and particularly over the carotid artery. A cuff (similar to blood pressure cuffs) was then strapped at the thigh of the patient. The bladder of the neck pad and the cuff inflated as the test started. After deflation pressure waves from the carotid and the femoral artery which appeared on the screen of a connected laptop were recorded simultaneously and the time delay between carotid and femoral wave was determined.

The examination protocol of cfPWV was in accordance with the instructions of the manufacturer of the device (SMT medical GmbH&Co) and the expert consensus document on arterial stiffness (20): cfPWV was measured as the velocity value calculated through 0.8 x of the distance between the common carotid artery and right femoral artery in meters (m) divided by the time that one pulse wave needed to cover this distance in seconds ($\Delta s/\Delta t$) (m/s) (''foot-to-foot'' velocity method). cfPWV assessments were performed in supine position after a minimum of 10 minutes of rest in a room with controlled conditions (temperature, noise). The average value of 3 measurements was documented.

Subsequently, traditional measurements of systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) were performed over the brachial artery and mean arterial pressure (MAP) was calculated by the formula

$$MAP=DAP+\tfrac{1}{3}(SAP-DAP)$$

Statistical analysis

The assumption of normality of distribution was evaluated through the Shapiro-Wilk numerical test and a graphical method (quantile-quantile plots). Continuous variables were presented as mean ± standard deviation (SD) when they were normally distributed and as median (25th and 75th percentiles) when they were skewed. Categorical variables were summarised as absolute (n) and relative (%) frequencies. Comparison of categorical variables was performed through chi-squared test.

The difference of cfPWV values between MCTD patients and controls was evaluated through *t*-test as cfPWV was normally distributed. This test was also used to evaluate the association between cfPWV and categorical variables with two categories. In order to assess the correlation between cfPWV and continuous characteristics, the Spearman's or the Pearson's correlation coefficient (*rho* and *r* respectively) were used.

Furthermore, the difference of cfPWV values between MCTD and control group after controlling for possible confounding factors was examined through multiple linear regression analysis. To search for confounding factors we calculated the B coefficient of cfPWV before and after the inclusion of different variables in the multivariate regression analysis model. We considered a variable to be a confounder of cfPWV if its inclusion caused a change of the B coefficient of ≥10% (assumed as the maximum superior limit). A probability value below 0.05 was considered statistically significant. All statistical calculations were performed using the SPSS v. 23.0 software (SPSS Inc, Chicago, Il, USA).

Results

We performed cfPWV measurements in 43 patients with MCTD and 107 control subjects (females: 83.7% *vs*. 90.5% respectively; p=0.242) (Table I). Average age of MCTD patients did not differ from control subjects (p=0.221) (Table I). Furthermore, groups of patients and controls were not significantly different regarding the percentage of smokers, MAP and use of statins (all; p>0.05) (Table I). However, patients had higher BMI values than controls (p=0.025) and thus a higher percentage of obesity (p=0.002) (Table I). Furthermore, patients group included statistically significantly more subjects receiving antihypertensive drugs in comparison to the control group (p=0.001) (Table I) and patients had in average higher heart rate (p<0.001) (Table I).

Association between group status (MCTD vs. control) and cfPWV

cfPWV average was statistically significantly higher in the patients group

Table I. Descriptive characteristics by group.

	Controls (n=105)	Patients (n=43)	Significance (*p*-value)
cfPWV (m/s)¶	6.78 ± 1.07	7.68 ± 1.29	<0.001*
Age (years)†	50 (39.50 – 56.50)	51.00 (44.00 – 58.00)	0.221
Gender (female)	95 (90.5%)	36 (83.7%)	0.242
Nicotine (smokers)	21 (20%)	4 (9.3%)	0.115
Anti-hypertensives	18 (17.1%)	18 (41.9%)	0.001*
BMI†	23.73 (21.06 – 27.05)	25.00 (22.15 – 31.38)	0.025*
Obesity (yes)	13 (12.4%)	15 (34.9%)	0.002
SAP¶ (mmHg)	123.47 ± 16.6	121.33 ± 17.01	0.480
DAP† (mmHg)	80 (70 – 85)	80 (70 – 90)	0.283
MAP¶ (mmHg)	93.14 ± 10.30	89.85 ± 12.10	0.097
Heart rate¶ (/min)	66.50 ± 9.24	74.57 ± 9.29	<0.001*
Cholesterol¶ (mg/dl)	-	186.20 ± 48.08	-
HDL† (mg/dl)	-	59.40 ± 18.98	-
LDL† (mg/dl)	-	116.94 ± 35.08	-
Disease duration† (years)	-	10 (5.25-18)	-
CRP† (mg/l)	-	2.96 (1.01-4.47)	-
ESR† (mm/h)	-	21.50 (10.50-35.50)	-
ANA†	-	945 (280-2560)	-
U1-RNP † (U/ml)	-	100 (37.15-100)	-
ds-DNA† (IU/ml)	-	9.8 (7.4-15.4)	-
RF (positive)	-	24 (61,5%)	-
C3† g/l	-	1.3 (1.11-1.55)	-
C4¶ g/l	-	0.22 ± 0.064	-
GFR¶ (ml/min/1.73)	-	94.65 ± 18.83	-
Creatinine¶ (mg/dl)	-	0.78 ± 0.13	-
Glucocorticoids (yes)	-	21 (48.8%)	-
Immunosuppressants (yes)	-	34 (79.1%)	-
Cutaneous (yes)	-	16 (38.1%)	
scleroderma		7/16 (43.75%)	
cutaneous lupus		5/16 (31.25%)	
finger-fissures		3/16 (18.75%)	
panniculitis		1/16 (6.25%)	-
ILD (yes)	-	13 (31.7%)	-
Raynaud's (yes)	-	34 (87.2%)	-
Erosions (yes)	-	7 (20%)	-
VAS¶ (/mm)	-	40.16 ± 25.22	-

†Data are presented as median (inter quartile range) as they are not normally distributed. ¶Data are presented as mean ± standard deviation as they are normally distributed. The rest of data are presented as absolute (n) and relative frequency (%).
cfPWV: carotid femoral pulse wave velocity; BMI: body mass index; SAP: systolic arterial pressure; DAP: diastolic arterial pressure; MAP: mean arterial pressure; HDL: high density lipoprotein; LDL: low density lipoprotein; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; ANA: antinuclear antibodies; U1-RNP: ribonucleoprotein antibodies; ds-DNA: double stranded DNA antibodies; RF: rheumatoid factor; GFR: glomerular filtration rate; ILD: interstitial lung disease; VAS: visual analogue scale. *p<0.05.

compared to the control group [7.68 ± 1.29 *vs*. 6.78±1.07, p<0.001] (Fig. 1).
In the statistical analyses, age and MAP showed a possible confounding effect on the results. On the other hand, treatment with antihypertensive drugs or statins, heart rate and traditional cardiovascular risk factors such as nicotine use or obesity (or BMI) was not statistically identified as confounding factors. The age- and MAP- adjusted statistical model revealed that cfPWV remained statistically significantly higher in the patients group in comparison to the control group [0.788, 95%CI (0.474–1.103), p_{adj}<0.001]. Thus, MCTD patients have higher cfPWV values than the control group in the present study.

Associated factors of cfPWV within both groups

Among patients with MCTD, unadjusted statistical analyses showed significant associations of cfPWV with age (*rho*=0.69, p<0.001) (Fig. 2), SAP (r=0.45, p=0.003), DAP (r=0.36, p=0.019), MAP (r=0.44, p=0.004) (Fig. 2), antihypertensive therapy (8.26±1.26 *vs*. 7.26±1.18, p=0.011), disease duration (*rho*=0.36, p=0.023) and creatinine values (r=0.48, p=0.001). Some inverse correlations of cfPWV [ANA (*rho*= -0.35, p=0.022), U1-RNP (*rho*= -0.403, p=0.012) and GFR 0.012 (r= -0.38, p=0.012)] could also be seen. Furthermore, MCTD patients without ILD showed higher cfPWV values in comparison to their counter partners with ILD (7.02±1.27 *vs*. 7.76±0.98, p=0.049).
On the other hand, there were no statistically significant relationships between cfPWV and gender, nicotine, obesity (or BMI), statin take, heart rate, inflammation markers (CRP and ESR), ds-DNA, RF, C3, C4, blood count changes, take of prednisolone or immunosuppressants, RP, erosions (all; p>0.05) (Table II).
Among controls, it was found that cfPWV significantly correlated with age (*rho*=0.66, p<0.001) (Fig. 2), SAP (*rho*=0.46, p<0.001), DAP (*rho*=0.45, p<0.001), MAP (r=0.49, p<0.001) (Fig. 2) and BMI (*rho*=0.24, p=0.015). On the other hand, nicotine use, statin or antihypertensive therapy, gender, obesity and heart rate did not significantly correlate with cfPWV (all; p>0.05) (Table III).
To control the statistically significant correlations, we performed statistical adjustments for age (a known crucial influence factor of cfPWV) in both groups using a multiple linear regression analysis model. After this adjustment, correlation between cfPWV and DAP remained statistically significant in both the patients and the control group [0.033, 95%CI (0.005–0.061), p_{adj}=0.024 and (0.020, 95%CI (0.002–0.038), p_{adj}=0.032, respectively].
Moreover, inverse association between cfPWV and ILD remained statistically significant (-0.736, 95%CI (-1.257–-0.215), p_{adj}=0.007), whereas relationships of cfPWV with other examined variables in the same group became statistically non-significant (SAP, MAP, antihypertensive therapy, disease duration, GFR, ANA, U1-RNP) (all p_{adj}>0.05) (Table II). Interestingly, cfPWV kept on correlating with creatinine in a statistically significant manner in the patients group [2.619, 95%CI (0.050–5.189), p_{adj}=0.046]. However, this correlation was controlled through an additional statistical adjustment for

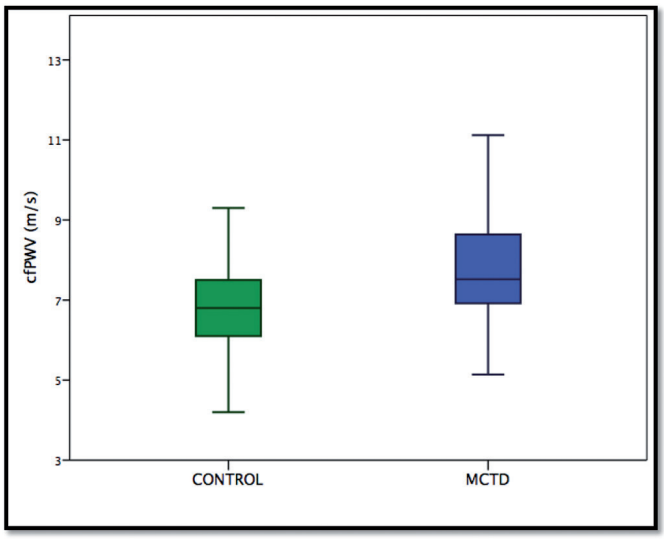

Fig. 1. cfPWV (m/s) in the control and in the patient group.
cfPWV: carotid femoral pulse wave velocity; MCTD: mixed connective tissue disease

Fig. 2. (below) Correlations of cfPWV (m/s) with age (years) and MAP (mm Hg) in the control and the MCTD group (all; *p<0.05).
cfPWV: carotid femoral pulse wave velocity; MAP: mean arterial pressure; MCTD: mixed connective tissue disease.

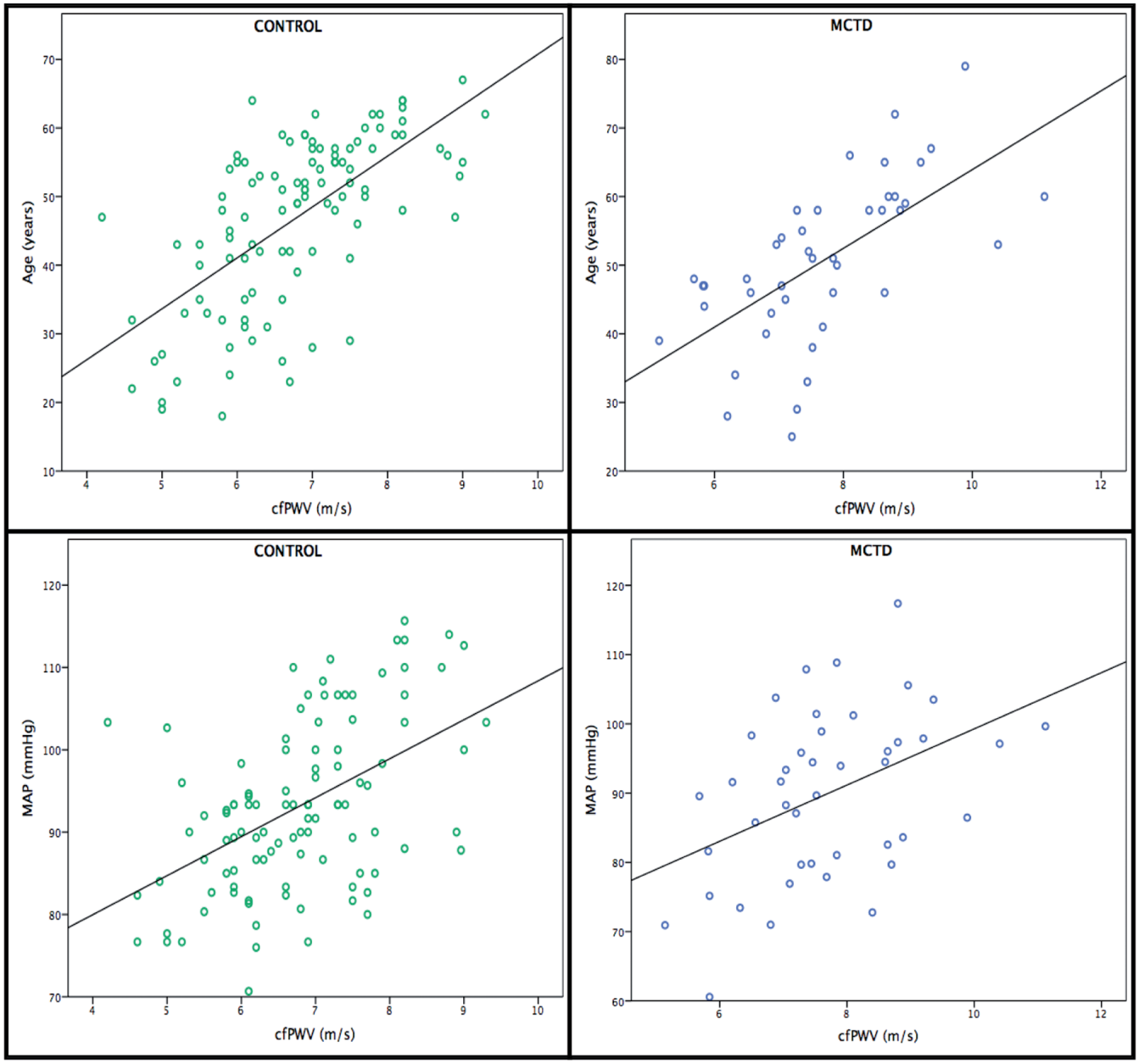

Table II. Association between MCTD patients characteristics and cfPWV.

	rho/r B coeff. (95% CI)	Significance (*p*-value)		Mean (± SD) B coeff. (95% CI)	Significance (*p*-value)
Age† *(years)*	0.698	<0.001*	Nicotine *(no)*	7.67 ± 1.34	0.984
			-//- *(yes)*	7.67 ± 0.81	
	--	--		-	-
SAP¶ *(mmHg)*	0.446	0.003*	Anti-hypertensives *(no)*	7.26 ± 1.18	0.011
			-//- *(yes)*	8.26 ± 1.20	
	B coeff. 0.018, 95% CI (-0.01 – 0.038)	0.067‡		B coeff. 0.259, 95% CI(-0.47 – 0.98)	0.477‡
DAP¶ *(mmHg)*	0.356	0.019*	Gender *(Female)*	7.62 ± 1.38	0.515
			-//- *(Male)*	7.97 ± 0.80	
	B coeff. 0.033, 95%CI (0.005 – 0.061)	0.024*‡		-	-
MAP¶ *(mmHg)*	0.435	0.004*	BMI *(<30)*	7.55 ± 1.06	0.431
			-//- *(30-34.99)*	7.92 ± 1.66	
	B coeff. 0.024, 95%CI (-0.004 – 0.052)	0.088‡		-	-
BMI† *(kg/m²)*	0.104	0.509	Statins *(no)*	7.63 ± 1.32	0.441
			-//- *(yes)*	8.16 ± 1.07	
	-	-		-	-
Cholesterol¶ *(mg/dl)*	0.085	0.596	RF *(negative)*	7.74 ± 1.71	0.783
			-//- *(positive)*	7.60 ± 1.09	
	-	-		-	-
HDL¶ *(mg/dl)*	0.024	0.893	Prednisolone *(no)*	7.84 ± 1.33	0.412
			-//- *(yes)*	7.51 ± 1.27	
	-	-		-	-
LDL¶ *(mg/dl)*	-0.01	0.994	Immunosuppresants *(no)*	8.12 ± 1.60	0.259
			-//- *(yes)*	*7.56* ±1.20	
	-	-		-	-
Heart rate¶ *(/min)*	0.064	0.685	ILD *(no)*	7.76 ± 0.98	0.049
			-//- *(yes)*	7.02 ± 1.27	
	-	-		B coeff. -0.736, 95%CI(-1.257 – -0.215)	0.007
CRP† *(mg/l)*	0.236	0.133	Raynaud's *(no)*	8.23 ± 0.77	0.321
			-//- *(yes)*	7.60 ± 1.36	
	-	-		-	-
ESR† *(mm/h)*	-0.225	0.162	Erosions *(no)*	7.42 ±1.10	0.082
			-//- *(yes)*	8.29± 1.40	
	-	-		-	-
Disease duration¶ *(years)*	0.355	0.023*	Cutaneous *(no)*	7.52 ± 1.12	0.597
			-//- *(yes)*	7.72 ± 1.35	
	B coeff. 0.020, 95%CI (-0.023 – 0.063)	0.350‡		-	-
ANA†	-0.354 B coeff. 0.0, 95%CI (0.0 – 0.0)	0.22 0.080‡			
U1-RNP† (U/ml)	-0.403 B coeff. -0.007, 95%CI(-0.016 – 0.001)	0.012* 0.092‡			
C3† *(g/l)*	-0.173 -	0.293 -			
C4¶ *(g/l)*	0.198 -	0.227 -			
Anti-ds-DNA† *(IU/ml)*	0.008 -	0.961 -			
GFR¶ (ml/min/1.73)	-0.383 B coeff. -0.008, 95%CI (-0.028 – 0.011)	0.012* 0.385‡			
Creatinine¶ (mg/dl)	0.479 B coeff. 2.619, 95%CI (0.050 – 5.189)	0.001 0.046*‡			

Spearmann's († not normal distribution) und Pearson's (¶ normal distribution) tests were performed to investigate the relationships between cfPWV and quantitative patients characteristics.

t-test was used to investigate the relationships between cfPWV and qualitative patients characteristics.

‡ *p*-values adjusted for age by multiple linear regression (B coefficient 95% CI).

cfPWV: carotid femoral pulse wave velocity; BMI: body mass index; SAP: systolic arterial pressure; DAP: diastolic arterial pressure; MAP: mean arterial pressure; HDL: high density lipoprotein; LDL: low density lipoprotein; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; ANA: antinuclear antibodies; U1-RNP: ribonucleoprotein antibodies; anti-ds-DNA: double stranded DNA antibodies; GFR: glomerular filtration rate; RF: rheumatoid factor. *$p<0.05$.

Table III. Association between control subjects characteristics and cfPWV.

	rho/r B coeff. (95% CI)	Significance (*p*-value)		Mean (± SD) B coeff. (95% CI)	Significance (*p*-value)
Age† *(years)*	0.66	<0.001*	Nicotine *(no)*	6.76 ± 1.13	0.699
			-//- *(yes)*	6.85 ± 0.78	
	-	-		-	-
SAP¶ *(mmHg)*	0.455	<0.001*	Anti-hypertensives *(no)*	6.70 ± 1.06	0.078
			-//- *(yes)*	7.19 ± 1.04	
	B coeff. 0.018, 95%CI (0.008 – 0.028)	<0.001*‡		-	-
DAP¶ *(mmHg)*	0.449	<0.001*	Gender *(female)*	6.74 ± 1.08	0.165
			-//- *(male)*	7.23 ± 0.90	
	B coeff. 0.020, 95%CI (0.002 – 0.038)	0.032*‡		-	-
MAP¶ *(mmHg)*	0.493	<0.001*	BMI *(<30)*	6.76 ± 1.11	0.606
			-//- *(30-34.99)*	6.93 ± 0.71	
	B coeff. 0.026, 95%CI (0.010 – 0.043)	0.002*‡		-	-
BMI† *(kg/m²)*	0.238	0.015*	Statins *(no)*	6.74 ± 1.06	0.048*
			-//- *(yes)*	7.70 ± 0.77	
	B coeff. 0.034, 95%CI (-0.008 – 0.075)	0.119‡		B coeff. 0.551, 95%CI (-0.200 – 1.302)	0.143‡
HDL¶ *(mg/dl)*	-0.247	0.095			
	-	-			
LDL¶ *(mg/dl)*	0.346	0.017*			
	B coeff. 0.0, 95%CI (-0.006 – 0.007)	0.899‡			
Heart rate¶ *(/min)*	0.487	0.069			
	-	-			
CRP† *(mg/l)*	0.288	0.050*			
	B coeff. 0.033, 95%CI (-0.013 – 0.080)	0.156‡			
Creatinine¶ (mg/dl)	0.132	0.377			
	-	-			

Spearmann's († not normal distribution) und Pearson's (¶ normal distribution) tests were performed to investigate the relationships between cfPWV and quantitative control subjects characteristics. *t*-test was used to investigate the relationships between cfPWV and qualitative control subjects characteristics. ‡*p*-values adjusted for age by multiple linear regression (B coefficient 95% CI). cfPWV: carotid femoral pulse wave velocity; BMI: body mass index; SAP: systolic arterial pressure; DAP: diastolic arterial pressure; MAP: mean arterial pressure. *$p<0.05$.

the effect of MAP (due to the known bi-directional relationship between blood pressure and kidney function) and did not reach the required statistical significance level [2.341, 95%CI (-0.217 – 4.90), p_{adj}=0.072].

Finally, in the control group, correlations between cfPWV and SAP and cfPWV and MAP remained significant [0.018, 95% CI, (0.008-0.028), p_{adj}<0.001 and 0.026, 95% CI (0.010-0.043), p_{adj}=0.002, respectively)], after adjustment for age (Table III).

Taken together, MCTD patients have increased cfPWV compared to the control group. cfPWV correlated in both groups significantly with age and DAP, in the control group with SAP and MAP and in MCTD (inversely) with ILD.

Discussion

Surrogate markers of CV risk and MCTD-associated angiopathy

Our data suggest that patients with MCTD have higher aortic stiffness than healthy controls, even after adjusting for confounding factors of cfPWV. To our knowledge, this is the first study to examine the gold standard assessment method of aortic stiffness in patients with MCTD. In general, literature lacks adequate data concerning surrogate markers of MCTD-associated CV risk. In particular, we are aware of one exploration in which Soltész *et al*. found impaired flow mediated dilation (FMD, a marker of endothelial function) and high carotid artery intima-media thickness (cIMT, a marker of carotid arteriosclerosis) in 50 patients with MCTD in comparison to 38 controls (22). Disease duration, apolipoprotein-A-levels and systolic blood pressure were associated with impaired endothelial function in this study. Moreover, it could be shown that serum levels of autoantibodies (anti-U1RNP, AECA and anti-CL) were significantly higher in MCTD patients who simultaneously had a cardiovascular disease (CVD) in comparison to the MCTD patients that had no CVD. These specific antibodies are believed to associate with endothelial dysfunction through an increase of proinflammatory and procoagulative effects (23) and have been found to correlate with cIMT (22). cIMT correlated in the same study with age, thrombomodulin, von Willebrand factor antigen and markers of inflammation (CRP, ESR).

Further CV risk related MCTD studies focused on the examination of different laboratory markers [*i.e*. vascular endothelial growth factor (VEGF), endostatin, paraoxonase activity (PON)] (15, 24, 25). In all of these explorations there was an indirect implication of increased risk of vascular disease in patients with MCTD. That is to say, in the study by Distler *et al*., a dysbalance of angiogenic and angiostatic factors (VEGF and endostatin respectively)

could be shown in patients with MCTD (24). Both VEGF (a mediator which correlates with PAH) and endostatin which has an antiangiogenic function and tends to restrain migration and proliferation of endothelial cells were increased in MCTD patients. The authors postulated that dysregulation of these two mediators could exacerbate MCTD-associated microangiopathy and thus contribute directly to the vascular disease of MCTD. Moreover, Reiseter *et al*. could show that increased endostatin levels in MCTD patients correlated with digital ulcers and higher all cause mortality (15).

Further laboratory parameters examined in MCTD are the arteriosclerosis promoter anti-Heat shock protein 60 (anti-Hsp 60) and the LDL-antioxidant paraoxonase (PON) (25). Anti-Hsp60 was found to be significantly higher in MCTD patients in comparison to healthy individuals and MCTD patients with CVD had significantly higher levels of this marker compared to their CVD-free counterpartners. Furthermore, patients with MCTD showed lower PON activity and lower high density lipoproteins in comparison to the control groups.

Cardiac disease in MCTD

Cardiac manifestations of MCTD were summarised in a systematic review of 11 case series performed by Ungprasert *et al*. (11). Even if prevalence of MCTD-related heart disease can be as high as 65%, symptomatic manifestations were observed only in approximately one third to one fourth of the patients pointing to high rates of asymptomatic disease. Myocarditis was described as a possible cause for MCTD typical conduction abnormalities and diastolic dysfunction, whereas mild pericarditis and mitral valve prolapse were mentioned as some of the most common cardiac MCTD manifestations. In this review 3 prospective studies were included and showed a mortality rate of 10.4% over a period of 13–15 years (13, 26, 27). Approximately one fifth of mortality was associated with cardiac disease. The authors concluded that arteriosclerosis and direct cardiac injury from MCTD were two of the most important causes of CV mortality.

In one of these prospective studies in particular, survival and prognostic indicators of MCTD were examined in a large cohort of patients for the time span between 1979 and 2011 (27). The overall 5-, 10- and 15-year survival rates were 98%, 96% and 88% respectively. From the 22 deaths occurred, 7 were attributed to acute CV events. Five patients had severe sclerosis of the coronary vessels followed by ischaemic cardiomyopathy and arrhythmia, whereas 2 patients died due to an acute myocardial infarction. In general, CV events in MCTD were characterised by poor prognosis and a strong association between CV events and the presence of anti-CL antibodies could be found. Furthermore anti-ß2-GPI were more frequently observed in patients with CV events and correlated with the magnitude of CVD. Finally, thrombotic events correlated with the presence of AECA and anti-CL IgG antibodies.

Established associations of cfPWV among patients and control subjects

In our group of patients, a direct correlation between age and cfPWV could be shown in both the control and the patients group. The strong association between age and cfPWV has been described in a large number of studies examining healthy populations (28-30) but also in cohorts of patients with various rheumatologic diseases (18, 31, 32). In fact, age is one of the most known influencing factors of cfPWV and thus its effect should be always taken into account in the search for confounding factors of cfPWV (33). Pathophysiologically seen, this can be explained through a change in the properties of the aortic wall in the course of the lifetime: aging is followed by a gradual replacement of the elastic component elastin through inelastic collagen fibres (34).

Moreover, blood pressure at the time of the cfPWV measurement is a crucial influence factor of aortic stiffness. Because of the elasticity of the aorta the vessel wall gets stretched through high blood pressure (distending pressure) (33, 35). This stretching causes an increase of stiffness which is represented by higher cfPWV values. For that reason MAP should be always taken into account when interpreting the results of cfPWV examinations (33).

Finally, in our study patients with ILD were found to have lower cfPWV values in comparison to patients without ILD. As a possible explanation for this finding we could postulate special treatment regimes used in MCTD patients with concomitant lung involvement. It is well known, that presence of ILD is often a reason to treat MCTD with immunosuppressive agents such as cyclophosphamide, mycophenolatemofetil or rituximab, even if data regarding their efficacy in MCTD lung involvement are scarce. As it has been shown in some well examined rheumatic diseases such as rheumatoid arthritis, immunosuppressive agents could have favourable effects on the CV system by improving endothelial function and reducing inflammation related arteriosclerosis leading to a decrease of arterial stiffness (36-38). Nevertheless, specific data regarding the effect of immunosuppressants on MCTD-associated vascular abnormalities are currently lacking and the size of our exploration did not allow an examination of the impact of every separate immunosuppressant agent on cfPWV. Thus, this could be seen as a limitation of our study. Moreover, presence of cardiovascular risk factors in both groups may have had a confounding effect on the results. Despite the fact that adjusted statistical analyses for several possible confounders were conducted the outcomes of the study should be interpreted with caution. A final possible limitation of our study originates from the fact that laboratory values of the healthy subjects were not assessed. Thus, CV-studies in MCTD that will take into account laboratory values of the control group are needed.

To conclude, this is the first report of higher aortic stiffness in patients with MCTD compared to healthy controls. Given the fact that cfPWV is a valid and reliable marker of CV risk, this finding could lead to an improvement of MCTD outcome through identification, close up monitoring and adequate treatment of patients at high risk. Control and confirmation of these results in future studies is important.

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