Assessment of cardiovascular abnormalities and the effect of rosuvastatin treatment in patients with systemic sclerosis

by Orsolya Timár, MD

Supervisor: Gabriella Szűcs, MD, PhD, DSc

UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF CLINICAL MEDICINE

DEBRECEN, 2014
Assessment of cardiovascular abnormalities and the effect of rosuvastatin treatment in patients with systemic sclerosis

by Orsolya Timár, MD

Doctoral School of Clinical Medicine, University of Debrecen

Supervisor: Gabriella Szűcs, MD, PhD, DSc

Head of the Examination Committee: Prof. László Maródi MD, PhD, DSc
Members of the Examination Committee: László Kovács, MD, PhD
Zoltán Szabó MD, PhD

The Examination takes place at the Department of Infectology and Pediatric Immunology, Faculty of Medicine, University of Debrecen at 11:00 a.m. on the 1 December, 2014.

Head of the Defense Committee: Prof. László Maródi MD, PhD, DSc
Reviewers: András Komócsi MD, PhD, DSc
Tünde Tarr, MD, PhD

Members of the Defense Committee: László Kovács, MD, PhD
Zoltán Szabó MD, PhD

The PhD Defense takes place at the Auguszta Lecture Hall, Auguszta Center, Faculty of Medicine, University of Debrecen at 2:00 p.m. on the 1 December, 2014.
1. INTRODUCTION

1.1. Systemic sclerosis

Systemic sclerosis (SSc) is a chronic connective tissue disease (CTD) characterized by autoimmune features, functional microvascular impairment and fibrosis of the skin and several internal organs, such as lungs, kidneys, gastrointestinal (GI) tract and the heart. Present diagnostic criteria to identify SSc patients were established in 1980 by the American College of Rheumatology (ACR). Current prevalence of SSc in Hungary is around 9 ‰, significantly higher than previously presumed. Despite earlier diagnosis and emerging novel treatment options, today, SSc remains one of the most devastating among CTD-s, with a 10-year survival of about 65%. In addition to SSc-related cardiac (heart failure and arrhythmias), pulmonary (interstitial lung disease, pulmonary arterial hypertension) and renal disease, lately, non-SSc related causes of death such as cardiovascular disease, malignancies and infections became more frequent, accounting for almost a half of all cases. Cardiac and extrapulmonary vascular involvement is now responsible for one-third of total mortality in SSc, conveying the greatest risk in the following years for SSc patients among all causes.

1.2. Pathophysiology and clinical presentation of vascular disease in SSc

Despite extensive research in the field of rheumatology and well-coordinated clinical management of SSc, the exact pathomechanism of this disease is still unclear. A weak genetic predisposition and certain environmental factors (i.e. exposure to organic solvents or silica, epoxy resins, aromatic carbonhydrogenes, bleomycine, etc.) have been identified which can trigger the disorder.

Functional abnormalities, such as endothelial dysfunction, disequilibrium of vascular tone, defective angiogenesis, overexpression of cellular adhesion molecules (CAMs) and structural vascular pathology, pathological electronmicroscopic and histological structure, in particular endothelial cell, pericyte and basement membrane disorders, are the hallmark of the disease and appear in the initial phase of disease pathogenesis. The vascular endothelium is an important regulator of vessel wall homeostasis, maintaining relaxation of smooth muscle tone and limiting oxidative stress via nitric oxide (NO), prostacyclin (PGI2), endothelin-1 (ET-1) release and influencing vascular angiotensin II (AT-II) activity. One source of NO is from conversion of L-arginine by the endothelial NO synthase (eNOS), another source is the inducible NO synthase of macrophages, which can also produce NO in case of various immunological impulses. In addition, the endothelium plays an important role in the maintenance and control of vascular permeability to plasma components and platelets, as well as white blood cell adhesion, aggregation and thrombosis. “Endothelial activation” and
“endothelial dysfunction” describe changes in normal homeostatic control of the endothelium in response to harmful stimuli, resulting in phenotypic changes, such as expression of various cellular adhesion molecules, inflammatory cytokine production, vasoconstriction, increased oxidative stress and produce of pro-thrombotic substances. These pathophysiologic changes are detectable via functional imaging techniques such as impaired flow-mediated vasodilation (FMD) of the brachial artery in the clinical setting.

An imbalance between physiologic vasoconstrictors and vasodilators setting the vascular tone evolves resulting in a shift in favour of vasoconstriction in SSc. The potent vasoconstrictor ET-1 is increased while levels of the vasodilators, NO and PGI2 are decreased in the disease. Increased $\alpha_2$ adrenergic receptor ($\alpha$-AR) activation has also been demonstrated to contribute to impaired vasodilation in SSc. The $\alpha$-2c AR-subtype is translocated in response to cold exposure from the Golgi apparatus to the cell membrane and undergoes rapid selective activation. Cold stimuli lead to smooth muscle cell (SMC) mitochondrial reactive oxygen species (ROS) production and Rho/Rac kinase activation (ROCK pathway). The ROCK pathway, in turn, takes part in $\alpha$-2c AR translocation, but also fibroblast differentiation and extracellular matrix (ECM) production, which is why it has also been suggested as a therapeutic target in SSc.

Simultaneously with an increased presence of proangiogenic factors, most importantly vascular endothelial growth factor (VEGF), but also platelet-derived (PDGF), placental (PIGF) and fibroblast growth factors (FGF-2), or ET-1 in the sera of SSc patients, enhanced release of angiostatic molecules (angiostatin, CXC chemokine ligand 4 (CXCL4), thrombospondin and IL-4) have been described. The pathological imbalance between angiogenic and angiostatic mediators, abundant interferon $\alpha$ (IFN-$\alpha$) signaling of dermal epithelial cells and inhibition of normal vasculogenesis by SSc fibroblasts all favour impaired neovascularization in SSc leading to skin ulcers and other pathologies.

Among cellular and structural abnormalities, platelet activation is linked strongly to fibrosis and immune activation in SSc. Platelet activation following vascular dysfunction leads to production of thromboxane A2 (TXA2) and other vasoactive mediators contributing to vasoconstriction and thrombosis. Fibrotic tissue further enhances platelet activation via type I collagen, whereas platelets promote fibrosis via serotonin (5-HT), PDGF, transforming growth factor $\beta$ (TGF-$\beta$) and lisophospholipid production. 5-HT is a molecule of outstanding importance in SSc, since it has recently been reported to activate fibroblast collagen production via the receptor 5HT$_{2B}$. In mouse models, inhibition of this receptor blocked fibrosis, indicating clinical relevance of the above pathway in disease pathogenesis and therapy.
Vasculopathy characteristic of SSc appears to be distinct both in histopathological presentation, localisation and in cellular-molecular background from the accelerated atherosclerosis observable in SLE or RA. Subintimal proliferation and fibrosis, along with preserved media and leukocyte infiltration of the vessel wall characterizes pathologic SSc vessels. Limited cutaneous SSc (lcSSc) is thought to be more prone to peripheral vascular involvement, while the diffuse form (dcSSc) of the disease is characterized by increased prevalence of internal organ involvement such as PAH and scleroderma renal crisis. The first location of functional peripheral vascular impairment and very often the first clinical sign of the disease is Raynaud phenomena (RP) of the finger capillaries. RP exerts triphasic pattern in SSc including pallor, numbness and pain of the fingers followed by cyanosis and hyperaemia of the acral skin caused by a disproportionate response of thermoregulatory, small and middle sized peripheral vessels to various stimuli such as cold or emotional stress. RP can be present for years before SSc develops. RP in SSc affects 95% of patients, and in 25-39% of patients is accompanied by digital ulcers and scarring. In addition to microvascular dysfunction, small to medium size arteriopathy resulting in an obliterative vasculopathy have been described in various types of muscular arteries in SSc such as the pulmonary arterioles, the arcuate and interlobular arteries of the kidney as well as the retinal vessels or arteries of the genitals resulting in sexual dysfunction in both sexes. Arteriopathy or arteriosclerosis of the great elastic arteries, the aorta and its main branches (brachiocephalic artery, left common carotid and subclavian artery) as well as the main pulmonary artery is unusual in SSc unless the disease is combined with e.g. hypertension or traditional risk factors such as advanced age.

Cardiac involvement is also in part a result of vascular abnormalities in SSc. Primarily, SSc can affect the conduction system, the myocardium (fibrosis, less frequently carditis), the pericardium, as well as the coronary and small to medium sized arteries of the heart (obstructive arteriopathy, reduced coronary reserve flow). Valvular involvement is atypical in SSc, thus it is of minor significance. SSc-related cardiac diseases secondary to PAH, interstitial lung disease or renal involvement as well as complications of non-SSc related diseases such as systemic hypertension, general atherosclerosis might also develop in SSc, but ventricular systolic/diastolic dysfunction in these cases is not a primary manifestation of the disease.

1.3. Therapeutic options for the SSc-affected vasculature: the role of statins

Based on evidence and clinical experience of the European League Against Rheumatism (EULAR), currently recommended therapy of SSc stands on three bases: immunosuppressive therapies, anti-fibrotic therapy and vasculoprotection. This latter group
includes dihydropyridine-type calcium-channel blockers, iv. prostacyclin for digital vascular symptoms and ulcers, ET-1 receptor antagonists, PDE5 inhibitors and the prostanooid epoprostenol for therapy of PAH. ACE-inhibitors have been recommended in SSc, mainly due to their renoprotective effects. For patient with rheumatic diseases with great atherosclerotic burden, such as RA or spondyloarthriti, EULAR recommends administration of statin therapy, when necessary, to decrease CV risk. However, in SSc, no such recommendation exists today.

Since the 1990s, increasing body of evidence has accumulated to support that statins, originally used as lipid-lowering drugs via HMG-CoA-reductase inhibition, may exert multiple anti-atherogenic effects. Statins may reduce arterial stiffness, improve endothelial function by increasing NO bioavailability (via eNOS upregulation) and may convey antioxidant (NADPH oxidase inhibition), anti-inflammatory or potentially immunomodulating effects. In addition, stabilization of the atherosclerotic plaque, decreased vascular SMC migration and proliferation, and inhibition of platelet aggregation have been highlighted as favourable non-lipid effects of statins. Additionally, statin effects include reduction in CRP, IL-6, TNF-α and NF-κβ levels, decreasing coagulation activity, as well as normalisation of sympathetic outflow. Anti-inflammatory effects of statins are partly explained by a recently described non-mevalonate pathway effect of statins, for example, by binding to a novel regulatory integrin site, statins are able to block adhesion and costimulation of leukocytes.

Several clinical studies assessing the potential effect of statins on arterial stiffness, subclinical signs of atherosclerosis or arterial calcification reported a favourable CV outcome in the general population or in patients with a scope of diseases involving vascular pathology. Consequently, in the past decades an increasing number of studies addressed the effects of various statins in SSc. Although the pleiotropic effects of rosuvastatin in reduction of CV risk in the general population have been analysed, previously there was no data available about the clinical effects of rosuvastatin on SSc patients. Given a highly efficient statin with prior controlled studies indicating its protective effect in increased risk as well as intermediate-risk, symptom-free individuals, we decided to assess CV effects of rosuvastatin in a selected group of patients with SSc.
2. OBJECTIVES

1. To assess the markers of endothelial function, namely flow-mediated vasodilation (FMD) and nitrate-mediated vasodilation (NMD) as well as to look for clinical signs of subclinical atherosclerosis via measurement of ccIMT in a cohort of patients with SSc compared with individually matched healthy controls.

2. To search for a relationship between the above parameters and age, SSc duration, organ involvement or autoantibody positivity.

3. To examine arterial stiffness, a risk factor for CV disease and a sign of large vessel involvement, in patients with SSc and compare these values to healthy controls determining PWV and AIx by an automated oscillometric method.

4. To compare PWV and AIx results in limited and diffuse disease subsets and to investigate age-dependency of the above parameters as well as their correlation with disease duration in SSc.

5. To investigate the effect of 6-month rosuvastatin treatment on different macrovascular parameters: the endothelial functional marker FMD, arterial stiffness examined by PWV, peripheral arterial disease screened by ankle-brachial index (ABI) and ccIMT or presence of a carotid plaque as a sign of general atherosclerosis. In addition, we assessed the possible effects of 6 month rosuvastatin therapy on microvascular function of the skin by measuring forearm cutaneous blood flow by Laser Doppler perfusion imaging (LDPI) before and after treatment.

6. To determine effects of rosuvastatin on serum inflammatory markers (CRP, ESR), the endothelial marker vWF antigen, complement (C3, C4) and immune complex (IC) levels, as well as basic laboratory parameters (serum lipid levels, renal function, liver enzymes, complete blood count) preceding and following 6-month rosuvastatin treatment in patients with SSc.

7. To determine and characterize cardiac involvement in SSc by resting conventional and pulsed wave tissue Doppler echocardiography, resting ECG and 24-hour Holter ECG monitoring. To follow basic echocardiographic parameters such as baseline and post-treatment left ventricular ejection fraction (EF), indices of diastolic function, right ventricular
function, and right ventricular pressure as well as signs of valvulopathy preceding and following 6-month rosuvastatin therapy in SSc patients. Our goal was to search for a relation between presence of arrhythmias and echocardiographic abnormalities in SSc. In addition, we sought to follow-up physical fitness by repeated 6-minute walk tests before and after rosuvastatin therapy.
3. PATIENTS AND METHODS

3.1. Patients and study protocols

3.1.1. Endothelial function and ccIMT in SSc

In the first study, 29 randomly selected SSc patients (female: male ratio=25:4; mean age ±S.D.: 51.8±10 years, lc:dcSSc=19:10) and 29 healthy controls aged 49.3±6 years were included after screening. The diagnosis of SSc was established in all three studies according to the SSc criteria described in 1980 by the American College of Rheumatology (ACR). The average disease duration of SSc patients was 9.43±3.78 years. In both patients and controls, traditional CV risk factors including age, body mass index (BMI), plasma lipid profile, as well as systolic and diastolic blood pressure were determined. Each patient and a corresponding control subject were matched according to age and risk factor status. Considering that we wished to study subclinical surrogate markers of atherosclerosis as well as endothelial dysfunction, exclusion criteria included existing CV disease, diabetes mellitus, cigarette smoking, obesity (BMI ≥30), vasculitis, acute or chronic infection as well as renal failure (defined as serum creatinine levels >117 µmol/l).

The study protocol was the following: all patients and controls were fasting and had been asked to suspend alcohol, tobacco, antioxidant and vasoactive drug intake for at least 24 hours prior to the assessments. On the morning of the vascular examinations, a fasting blood sample was drawn for renal functional parameters and serum lipid profile and participants underwent the common carotid intima-media thickness (ccIMT), brachial artery flow-mediated dilation (FMD) and nitrate mediated dilation (NMD) examinations as detailed below.

3.1.2. AIx and PWV in SSc

In the second cross-sectional study, a total of 46 consecutive patients of female preponderance, appearing for regular checkup at our clinic were screened, and 40 patients (with a female to male ratio of 36:4) were found eligible for the study. Inclusion and exclusion criteria were identical to those applied in Study 1. In addition, patients with constant arrhythmias were excluded in the present study due to methodological reasons. A written consent form from each participant as well as an Institutional Review Board approval was obtained. The mean age of eligible patients was 58.0 ± 12.3 years (range: 33–81 years), who dominantly suffered from the lcSSc form of the disease (31/40 patients). For comparison, we studied 35, age- and sex- matched healthy controls (female: male ratio=32:3, mean age 53.0 ± 10.5 years [range: 30–77 years]). The average disease duration in the patient group was
12.5 ± 6.7 years (range: 1–27 years). Traditional risk factors for CV disease, such as age, BMI, lipid levels, as well as systolic and diastolic blood pressure were assessed and differences between the patient and control group were found non-significant (data not shown).

On the morning of the vascular examinations, both patients and controls underwent a physical examination to exclude acute infection or arrhythmias and to determine baseline blood pressure and BMI values. Subsequently, fasting blood samples were drawn for serum LDL-, HDL-, total cholesterol and triglyceride levels as well as serum creatinine. Following a resting period in a quiet study room, arterial stiffness parameters AIx and PWV were determined.

3.1.3. Effects of rosuvastatin on cardiovascular and biomarkers in SSc

SSc patients arriving for regular checkup to our institution were randomly screened for inclusion and exclusion criteria as detailed below. Following screening, 28 patients were found eligible for the study (female: male ratio=25:4, median age: 60.4±11 years [range: 34–83 years]). The majority of patients (25/29) had intermediate disease duration or late disease, thus mean disease duration was 13.6±7.7 years (range: 2–30 years). Altogether 75% of patients suffered from lcSSc, while 25% had the diffuse form of the disease.

Clinical manifestations in order of decreasing prevalence among patients were RP (96%), pulmonary involvement including ILD (63%) or mild PAH (7%), distal skin manifestations including sclerosis and ulcers (68%), GI manifestations (54%), proximal skin involvement (32%), known cardiac manifestations (25%), sicca syndrome (10%), renal involvement (4%). In 6 patients (22%), SSc overlapped with another CTD, namely dermato-polymyositis (2 patients), SLE (1 patient) or RA (3 patients). All recruited patients were non-smokers with a mean BMI of 23.1 ± 4.1 kg/m2.

Inclusion criteria consisted of presence of microvascular symptoms such as new digital ulcers, active RP despite ongoing therapy, and informed consent of patients. Exclusion criteria included hyperglycemia, acute systemic infection, uncontrolled hypertension, carotid sinus hyperesthesia, permanent atrial fibrillation, an EF<50% as determined by previous echocardiography, severe PAH, smoking, active ulcers at any of the measurement site as well as vasoactive drug treatment such as prostanoids, or lipid-lowering drug treatment in the previous 6 months prior to screening, or patients requiring frequent therapeutic adjustments.

All examinations, including laboratory analyses as well as the functional and structural vascular assessments described below, were performed on two occasions, directly before and after the rosuvastatin treatment period. Each patient received 20 mg rosuvastatin per day for 6
months. All patients tolerated the drug well and we experienced no drop-outs. On the day of vascular assessments, a blood samples were drawn between 7 and 8 a.m. following an 8-hour fasting period. Samples were stored at room temperature and analyses were performed within 2 hours. vWF antigen samples were stored on ice until analysis. Alcohol, caffeine consumption, as well as administration of vasoactive and antioxidant drugs were suspended for 24 hours prior to examination. Assessments were performed under standardized conditions after a 10-minute resting period in supine position. Vascular assessments were carried out in a quiet, darkened study room with a steady temperature (22±1°C) and each vascular assessment was performed by the same examiner (OT). Patients underwent detailed echocardiography, ccIMT measurements, ultrasound-based aorto-femoral and carotid-femoral PWV measurements, brachial artery FMD assessment, ankle-brachial index and post-occlusive reactive hyperemia testing during laser Doppler forearm skin perfusion monitoring. In addition, a resting 12-lead ECG was obtained and 24-hour Holter ECG monitoring as well as a 6-minute walk test was performed. Following baseline measurements, each patient received 20 mg rosuvastatin per day for 6 months. All patients tolerated the drug well and we experienced no drop-outs. Measurements were repeated following 6 months under conditions identical to those of baseline assessments.

3.2. Vascular examinations

3.2.1. Brachial FMD and NMD

Brachial FMD and NMD were determined according to the 2002 guidelines by the American College of Cardiology under standardized conditions. Following a 30-minute resting period, at 21°C, ultrasound examinations were performed by a single, trained assessor with a HP Sonos 5500 ultrasound equipment and a 10 Mhz linear array transducer. Baseline brachial artery diameters (BAD\textsubscript{basal}) were obtained about 4-7 cm proximally to the cubital fossa, taking into account individual anatomical variations. BAD measurements were repeated 5 times and averaged. Subsequently, a pneumatic cuff was inflated over the forearm to a suprasystolic pressure for a total interval of 4.5 minutes. Upon deflation, BAD, as well as maximum flow velocity, were again assessed for 90 seconds, and maximal arterial diameter (BAD\textsubscript{max}) was determined. FMD was expressed as the percent change from the baseline value \((\frac{\text{BAD}_{\text{max}}-\text{BAD}_{\text{basal}}}{\text{BAD}_{\text{basal}}}\times100)\).

NMD assessments were carried out after a 15 minute resting period as follows: baseline BAD were again obtained as described above. Thereafter, 400µg (1 spray exposition) of sublingual nitroglycerine was administered to the patient and changes in BAD were recorded constantly for 4 minutes. Maximal BAD values were used for estimation of NMD,
again, as percent change from the baseline. Reproducibility of the method was assessed in our laboratory resulting in a variation coefficient of 5%, and an intraclass coefficient of 0.935, which is considered excellent.

3.2.2. ccIMT

To assess ccIMT, we visualized and screened both right and left common carotid arteries including the carotid bulb by the same duplex ultrasound system we used for our FMD and NMD measurements (HP Sonos 5500, 10 MHz). Finally, ECG gated, end-diastolic (R synchronized) longitudinal sectional images of the common carotid artery were saved. Offline measurements were performed 1 cm proximal to the carotid bulb in the far wall of the artery using the leading edge method. ccIMT was defined as the distance between the first (intima-lumen) and second (media-adventitia border) echogenic lines starting from the lumen, an average of 10 measurements were used for ccIMT calculation. ccIMT values were expressed in mm. The intraobserver variability of ccIMT in our laboratory was excellent: the calculated CV and ICC were 4.2% and 0.98, respectively, indicating very good reproducibility.

3.2.3. Assessment of Alx and PWV

The shape of the pressure waveform originating from the left ventricle is influenced by the flexibility and distensibility of the aorta and its proximal branches as well as by reflection from anatomic branching points and changes in peripheral vascular resistance. Thus, we assessed two parameters, PWV, the velocity of the propagating pulse wave in the central elastic arteries, and Alx, a value which expresses the ratio of augmented pressure compared to the pulse pressure characterizing arterial stiffness.

In the arterial stiffness study, measurements were performed by the arteriograph system (Tensiomed™ Ltd., Budapest, Hungary), which had previously been validated by comparison to the standard SphygmoCor and Complior systems, as well as to invasive measurements of arterial stiffness. During the examination, first a systemic blood pressure was automatically measured with a right arm cuff, subsequently a pressure waveform was obtained by the same cuff with an oscillometric method at 35 mmHg suprasystolic pressure. Thereafter, the curve was analysed automatically to calculate brachial and central Alx as well as PWV. PWV was calculated as the quotient of the distance between the jugular fossa and symphysis and RT S35 (reflection time at 35 mmHg suprasystolic pressure). Arteriograph uses the jugular fossa–symphysis distance as a surrogate for the length of the descending aorta between the aortic trunk and the bifurcation. Reproducibility of arterial stiffness measurement results was ensured by maintaining constant, neutral (21°C) room temperature, performance
of measurements in a supine position following a 10-minute resting period, suspension of tobacco and caffeine consumption for at least 10 hours, adhering to expert consensus recommendations.

In the rosuvastatin study, aorto-femoral and carotid-femoral PWV (a-f PWV, c-f PWV) was measured on a HP Sonos 5500 ultrasound equipment. Briefly, suprasternal (aorta pulsed wave Doppler signal, 2-4 MHz phased array transducer) and femoral images (common femoral artery pulse Doppler signal at the level of the inguinal ligament, assessed by a 5-10 MHz linear array transducer) were obtained during simultaneous ECG recording. Pulsed Doppler analysis with 5 mm sample volume at 150 mm/s sweep speed was performed over the beginning of the aorta descendens and common femoral artery over two breathing cycles (10-12 cardiac cycles). The distance between the suprasternal notch and the aortic measuring site (d1) as well as distance between the suprasternal notch and the femoral measuring site (d2) was measured, and time delay between the R wave and feet of the ECG gated aortic and femoral signals was used as pulse transit time. a-fPWV was calculated as a ratio of (d2-d1)/(t2-t1) and expressed in m/s. c-fPWV assessments were performed similarly, with minor differences. The two measuring points of c-fPWV were the left common carotid artery (1 cm proximal to the the carotid bulb) and the right common femoral artery (see a-f PWV measurement). Pulse transit times were again calculated in relation to the ECG signal using the foot-to-foot method and the distance between the two sites was defined as the difference between the jugular notch jugulum-carotid measurement point and the jugular notch-femoral measurement point distance. c-fPWV was again the quotient of the distance and the transit time.

3.2.4. Assessment of ankle-brachial index

Ankle-brachial index (ABI) was assessed corresponding to inter-society consensus guidelines. with a handheld CW Doppler instrument (Vasodop 8 MHz, MediCAD Ltd, Miskolc, Hungary). The higher of the lower limb (posterior tibial and dorsal pedel) artery pressures was divided by the higher brachial systolic blood pressure value to form the ABI.

3.2.5. Laser Doppler Perfusion Monitoring

Microvascular skin perfusion was assessed by post-occlusive reactive hyperemia testing during Laser Doppler Perfusion Monitoring (LDPM) applying a standard laser probe (PF 408, wavelength:780nm, skin penetration: 1-1.5mm) fixed in a straight probe holder (PH 08) of a Periflux PF 4001 LD flowmeter (Perimed AB, Järfällä, Sweden). Measurements were carried out with the patients resting in supine position on the volar side of the mid-forearm
skin. Following 8 minutes of basal blood flow recording, upper arm obstruction was applied for 3 minutes (by 50 mmHg suprasystolic pressure, biological zero flow). Subsequently, the cuff was suddenly deflated (peak) and forearm skin blood flow was further recorded for five minutes during which flow returned to baseline. We analysed blood flow (expressed in arbitrary perfusion units, PU) relatively to baseline values and also assessed the kinetics of the PORH response.

3.3. Cardiac examinations

3.3.1. Standard echocardiography and tissue Doppler myocardial imaging

Echocardiography was performed by a skilled echocardiographer under the supervision of a cardiologist before and after therapy, as described by D’Andrea et al. Two-dimensional (parasternal long- and short axis views, as well as apical 3 chamber and four chamber views and assessment of the portal vein), M-mode and Doppler imaging were performed and basic echocardiographic parameters were determined with patients resting in left decubitus position. Images obtained by a 2-4 MHz phased array transducer of a HP Sonos 5500 ultrasound equipment were analysed over 3 cardiac cycles, and the average of three measurements was used. Septal and lateral wall thickness was analyzed at end-diastole in the parasternal short-axis view. Left ventricular mass, (LVM), indexed for height, was calculated according to Devereux by the following formula:

\[
LVM = 0.8 \times [1.04 \times ((LVID + PWT + IVST)^3 - LVID^3)] + 0.6
\]

(LVID: left ventricular internal diameter, IVST: thickness of the intraventricular septum, PWT: posterior wall thickness, 1.04 is the specific gravity of the myocardium, 0.8 is a correction factor.) All measurements were performed R synchron at end-diastole and expressed in cms. Left ventricular EF, in absence of regional wall motion disturbances or asynchrony, was approximated by the Teicholz formula. Right ventricular (RV) end-diastolic diameter was measured on the apical 4-chamber view at the middle level. Global systolic function was approximated by measurement of tricuspid annular plane systolic excursion (TAPSE), calculated by the difference between the end-diastolic and end-systolic excursion of the tricuspidal annulus on an M-mode picture (measured in mm).

Conventional Doppler assessment of left ventricular inflow was performed with the sample volume placed at the tip of the mitral valve leaflets from the apical 4-chamber view. Global diastolic left ventricular function was determined by peak velocities of E and A wave (m/s) and the E/A ratio, DT (deceleration time) of the E wave (msec) as described by Ho et al. In addition, LV TEI index was calculated by the \((x-e)/e\) formula where \(x\) corresponds to
the time interval between the cessation of the mitral inflow and the beginning of the next E wave, and e is the left ventricular ejection time measured from the apical 5-chamber view with the PW sample volume just below the aortic valve.

Conventional Doppler RV diastolic indices were determined in apical 4-chamber view, placing the sample volume at the tips of tricuspid valve leaflets: E and A peak velocities (m/s) and their ratios were calculated. Global right ventricular filling was characterized by assessment of E and A peak velocities (m/s), E/A ratio and E wave deceleration time. RV TEI Index was calculated after measurement of the time interval (x) between the end of the tricuspid A wave and the beginning of tricuspid inflow (E) and the duration of the right ventricular ejection wave (e) from the parasternal short-axis view at the level of the aortic valve with the formula (x-e)/e.

Non-invasive measurement of the pulmonary artery systolic pressure was calculated in all the patients of the study using continuous wave Doppler recordings of tricuspid regurgitation, according to the modified Bernoulli equation. In particular, pulmonary artery systolic pressure was considered as equal to 4 times the square of the peak velocity of the tricuspid jet, plus the right atrial pressure. Inferior vena cava diameters and inspiratory collapse were measured from the subcostal view.

Tissue Doppler myocardial imaging was performed similarly to the technique described by d’Andrea et al, by spectral pulsed Doppler signal filters. Systolic mitral annular velocity (Sa), determined at the 4 ventricular sites, and two diastolic velocities early (Ea) and atrial (Aa) as well as tricuspid annular peak systolic velocities were measured and expressed in cm/s. After obtaining a good apical 4-chamber view, a pulsed Doppler sample volume of 5 mm was placed on both the LV and the RV lateral walls, at the base, middle and apex and the level of mitral and tricuspid annulus, respectively. Myocardial peak velocity of the systolic wave Sm (m/s), as well as myocardial early diastolic (Em) and atrial (Am) peak velocities (m/s) and Em/Am ratio, were measured. Right ventricular peak systolic velocity (the highest of the pulsed tissue Doppler velocities recorded at either the base, middle or apical level) was registered and evaluated for each patient.

3.3.2. ECG monitoring

Resting 12-channel ECG as well as 24-hour 3-channel ECG monitoring (Cardiospy, Software Ver 4.1, Labtech Kft., Debrecen, Hungary) was performed according to institutional standard practice. Arrhythmias were categorized as extrasystole, supraventricular tachycardia (>4 beats, without atrial fibrillation or flutter), atrial fibrillation/flutter (>4 beats), pause (>3 seconds was regarded as significant), atrioventricular block (I. degree, Mobitz type I. of II, or
third-degree atrioventricular block), ventricular tachycardia (>4 beats), or polymorphic ventricular tachycardia/ventricular fibrillation.

3.3.3. Evaluation of physical condition

The 6-minute walk test was performed prior to rosuvastatin therapy and at end of the treatment period according to American Thoracic Society Statement Guidelines.

3.4. Laboratory analyses

Serum biochemical markers and high sensitivity CRP (hsCRP) were measured using a Modular P-800 analyzer (Roche Ltd, Mannheim, Germany). Serum total cholesterol, triglyceride and uric acid levels were determined by enzymatic colorimetric assay, HDL and LDL-cholesterol were analysed by homogenous enzymatic assay. Serum glucose and urea levels were assessed using enzyme kinetic UV assay, serum creatinine was measured by the compensated Jaffe kinetic method. Estimated GFR was calculated from serum creatinine by the MDRD 175 (Modification of Diet in Renal Disease study group) formula. hsCRP was determined by wide range immunoturbidimetric assay, hsCRP levels> 5 mg/l were considered elevated. Plasma levels of circulating vWF antigen, a marker of endothelial cell activation were measured by STA Liatest vWF immunoturbidimetric assay using microlatex particles coated with polyclonal rabbit anti-human vWF antibodies (Diagnostica Stago, Asnieres, France). After mixing the reagent with plasma, degree of agglutination was evaluated which was proportional to the amount of vWF present in the plasma sample. The reference range for the test is 50-160%. Hematological parameters including hemoglobin (Hgb), white blood cell and platelet counts were determined using an automated hematology analyzer (Sysmex XE-2100D, Sysmex Corp., Kobe, Japan). Erythrocyte sedimentation rate (ESR) was determined by the Westergren method. All the above laboratory measurements were performed by the Department of Laboratory Medicine at the University of Debrecen.

Circulating immune complexes (IC) were detected by the polyethylene glycol precipitation method. Serum complement C3 and C4 levels were measured by nephelometry on a Siemens-Dade-Behring BN-II nephelometer. Anti-ENA and anti-Scl-70 autoantibodies were detected by indirect immunofluorescence staining and ELISA technique. Immunolaboratory measurements were all performed by the Regional Immunological Laboratory of the Institute of Medicine at the University of Debrecen.
3.5. Statistical analysis

Results of our cross-sectional as well as longitudinal studies were expressed as the mean±S.D. in case of a normal distribution. Statistical analysis was performed with the help of the SPSS version 11.0 Software, normal distribution was determined by the Kolmogorov-Smirnov test. In case of a normal distribution, statistical analysis was carried out by Student’s paired, one- or two-tailed t-test, according to the individual analysed parameter. Nonparametric distribution was analysed using the Mann-Whitney test. A p value less than 0.05 was considered significant. Normally distributed parameters were correlated using Pearson’s correlation coefficient, an r value at the p <0.05 level were considered significant. If the distribution of the parameters was not normal, Spearman-test was used to search for correlations. In case of a correlation, the independent variables were plotted in a frame of reference, and the type of correlation was described. In case of a linear correlation, the equation and slope of the function, as well as the regression coefficient (R) value and level of significance (p) were determined.
4. RESULTS

4.1. Endothelial function and ccIMT in SSc

Although there was no statistically significant difference between patients and controls regarding age, blood pressure or cholesterol levels, FMD in SSc patients was significantly lower (4.82±3.76% vs. 8.86±3.56%, p<0.01), however, NMD was comparable to results of controls. ccIMT did not differ significantly in patients (0.67±0.26 mm) vs. controls (0.57±0.09mm, p=0.067). Comparison of the lcSSc and dcSSc form of the disease revealed no differences in FMD, NMD, or ccIMT between the two groups.

4.2. Relation between FMD, NMD, ccIMT and age or clinical data

In SSc patients, ccIMT exerted a significant correlation with age (r=0.470; p=0.013), and disease duration (r=0.472, p=0.011). An inverse correlation was observed between NMD and age (r=0.492; p=0.012), but no correlation was found between NMD and disease duration. Neither age, nor disease duration correlated with FMD results in SSc. Interestingly, FMD, NMD and ccIMT did not correlate with each other, nor with the presence or absence of any of the assessed organ manifestations (RP: 100%, GI: 72%, pulmonary: 66%, cardiac: 66%, digital ulcers: 45%, renal: 10%) or autoantibody (Ab) positivity (anti-Scl-70: 44.8%, anti-centromere Ab: 10%).

In the control group, ccIMT also showed a significant linear correlation with age (r=0.61; P=0.003), but neither FMD (r=0.264, P=0.082) nor NMD (r=0.032,P=0.870) correlated with age.

4.3. PWV and AIx in SSc

Both PWV (9.67± 2.08 m/s vs. 8.00 ± 1.46 m/s, p = 0.00017) and AIx (9.02± 30.32 in SSc vs. –41.15 ± 22.5, p < 0.0001) were substantially higher in SSc patients compared to controls.

4.4. Arterial stiffness in relation to clinical data: disease subtype, age and disease duration

Upon comparison of the lcSSc group (n=9) with the dcSSc patients (n=31), PWV of the limited group was significantly elevated (10.04 ± 2.01 m/s vs 8.39 ± 1.87 m/s, respectively; p = 0.034), while no statistically significant difference regarding AIx results (11.75±24.5 vs. -4±36%, p=0.3) was observed. Differences between lcSSc and dcSSc patients with respect to serum lipid profile as well as disease duration were nonsignificant, however,
patients with the limited form of the disease included in our study were significantly older (mean age of 61.7±10.6 vs 45.2±8.73 years, respectively, p<0.005.) On the other hand, no statistically significant difference regarding Alx results were observed between patients belonging to different disease subsets.

A positive correlation was found between Alx and PWV in patients with SSc (r = 0.32, p = 0.045). In addition, Alx, as well as PWV showed significant positive correlations with advancing age in patients with SSc (r = 0.31, p = 0.048 and r = 0.36, p = 0.021, respectively).

PWV also showed a significant positive correlation with disease duration in SSc patients (r = 0.40, p=0.011) In contrast, Alx showed no correlation with disease duration nor could we detect a relationship between serum lipid levels and either of the assessed arterial stiffness parameters (data not shown).

4.5. Effects of rosuvastatin on micro-and macrovascular parameters

Brachial artery FMD significantly improved after six months of rosuvastatin therapy (2.3% ± 3.3% before versus 5.7% ± 3.9% after treatment, P= 0.0002). Although mean PWV values decreased, neither a-fPWV nor c-fPWV showed a statistically significant improvement upon rosuvastatin treatment (a-f PWV: 8.8 ± 2.2 m/s before versus 8.3 ± 2.1 m/s after therapy, p=0.15; c-f PWV: 8.7 ± 2.6 m/s before versus 8.1 ± 1.9 m/s after treatment, p= 0.1). Compared to 11/28 patients pretreatment, by the end of rosuvastatin treatment, only 5/28 patients (17.9%) had a c-fPWV result above the reference values of age-, lipid- and blood-pressure-status-matched European patients.

Mean ABI, as indicator of PAD, was 1.1 ± 0.2 on both sides and remained unchanged after rosuvastatin therapy. Ultrasound analysis of the common carotid arteries revealed a mean ccIMT of 0.68 ± 0.14 mm on the right and 0.72 ± 0.17 mm on the left side at baseline. Additionally, in 6/28 patients (21.4%), a carotid plaque causing no or nonsignificant stenosis was observed, which remained unchanged after therapy. After rosuvastatin therapy, cc IMT values were 0.68 ± 0.14 mm (P= 0.38) and 0.70 ± 0.17 mm (p=0.3), respectively. Statin treatment did not result in any improvement in carotid atherosclerosis. Total number of patients with abnormal carotid or ABI findings was 8/28 (28.6%).

Laser Doppler perfusion analysis of the forearm skin flow during PORH testing revealed decreases in the acceleration and deceleration slope of the curves following rosuvastatin therapy compared to pretreatment values (acceleration slope: 14.6 ± 14.8 versus 10.0 ± 10.3 U/second,P= 0.081; deceleration slope: -1.13 ± 0.92 U/second versus -0.64 ± 1.09
U/second, \( P = 0.021 \) (Table 10). Neither basal, nor peak, or biological zero skin perfusion, nor AH nor any of the time characteristics (TM, TH1, TH2) showed significant changes compared to pretreatment values (data not shown).

4.6. Rosuvastatin’s effects on laboratory parameters

Presence of antinuclear autoantibodies (ANA) among patients was the following: 26/28 patients (93%) were ANA positive, 12/28 patients (43%) had antibodies against extractable nuclear antigen (ENA), 1/28 (4%) against nuclear ribonucleoproteins (RNP), none against Smith antigen (Sm), 3/28 patients (11%) against SS-A (Ro) antigen, none against SS-B (La). 12/28 patients (43%) tested positive for antibodies against topoisomerase I (Scl-70), and 2/28 (7%) were positive for antibodies against histidyl-tRNA synthetase (Jo-1).

Serum IC levels were initially elevated and levels returned to normal after rosuvastatin therapy (extinction: 183.6 versus 135.5, respectively, \( P = 0.007 \)), while C3 (1.81 versus 1.62 g/L) and C4 levels (0.33 versus 0.27 g/L) displayed a significant decrease after rosuvastatin treatment (\( P = 0.001 \)) within the reference range. Baseline circulating levels of the endothelial marker vWF antigen were abnormally high in 63% of patients and were unaffected by rosuvastatin treatment (209 ± 90% versus 193 ± 76%, \( p = 0.09 \)).

Baseline serum lipid levels indicated that 10% of patients had hypertriglyceridaemia (TG >2.3 mmol/L), 50% had hypercholesterolemia (total cholesterol >5.2 mmol/IL) and 32% had elevated LDL-C levels (>3.4 mmol/L). At baseline, 11 out of 28 patients (39%) had low HDL-C levels (<1.2/<1.0 mmol/L for women/men, respectively). Reference values were determined as recommended for the medium cardiovascular risk group based on the European SCORE chart. Serum total cholesterol, LDL-cholesterol and triglyceride levels, as well as atherogenic index decreased significantly upon rosuvastatin treatment. Non-HDL cholesterol levels also displayed a significant decrease after statin therapy (3.8 ± 1.5 versus 2.5 ± 1.3 mmol/L, \( p = 0.0003 \)). Among acute phase reactants, hsCRP levels showed a significant decrease, from 5.1 ± 5.2 mg/L to 3.4 ± 2.7 mg/L (\( P = 0.01 \)). ESR, renal function tests and full blood counts showed no biologically relevant changes upon statin therapy as compared to baseline values.

4.7. Cardiac effects of rosuvastatin

4.7.1. Echocardiographic parameters before and after rosuvastatin therapy

Standard echocardiography detected normal left ventricular dimensions along with normal left ventricular systolic function as indicated by the EF (60.5 vs. 61.7%) both before and
following 6-month rosuvastatin treatment. 25% of patients presented with mild elevations in systolic PA pressure (>37 mm of Hg), possibly indicating PAH. Right ventricular diastolic diameters were also normal (26.7 mm± 4.5) at baseline and increased post-treatment (28.4±5.4 Hgmm, p=0.023).

Severe valvulopathy was infrequent among the assessed SSc patients. Altogether 5/28 patients had mitral valve prolapse, 18/28 patients had mitral insufficiency, the majority of cases being mild to moderate (<Grade II). Only 2 out of 18 patients had grade II mitral regurgitation. Three patients had mild aortic regurgitation, one patient had a nonsignificant aortic stenosis with a mean gradient of 13.5 mmHg and a peak gradient of 25.2 mmHg.

Left ventricular diastolic function was abnormal in 81% of patients at baseline. 17/28 patients had impaired relaxation upon interrogation of mitral valve inflow patterns, 3/28 had moderate and 2/28 had severe diastolic dysfunction (restrictive pattern). Frequency and severity of diastolic dysfunction remained unchanged after rosuvastatin therapy.

4.7.2. Arrhythmias, conduction disturbances in SSc and the effect of rosuvastatin on ECG alterations

Single abnormalities of conduction or rhythm were infrequent among SSc patients, adding these up resulted in a frequency of conduction disturbances of 23% (6/26 patients), abnormal basic rhythm or major arrhythmias in 42% (11/26) and abnormal HRV (heart-rate variability, indicating possible autonomic dysfunction) in 23% (6/26) of patients. Major arrhythmias were defined subjectively as presence of any of the following: supraventricular run or tachycardia, coupled ventricular extrasystole or ventricular run/tachycardia, a pause longer than 1500 msec or a frequency lower than 60 beats per minute. Upon comparison of ECG-s prior to and following rosuvastatin treatment, we observed no significant changes in ECG abnormalities.

4.7.3. Correlations between ECG abnormalities and vascular, laboratory or echocardiographic parameters

Our results indicate a strong relationship between physical fitness and normal ECG: patients with a normal ECG performed on average 388±82 m on the baseline 6-minute walk test while among patients with ECG abnormalities this number was 284±103 meters (p<0.05), and this difference remained significant after rosuvastatin therapy as well. Not surprisingly, vasoreactivity during the FMD test was smaller in the abnormal ECG-group representing impaired endothelial function, however, this difference did not reach statistical significance (3.9 ± 3.1 in the normal group vs. 1.7±3.2% in the abnormal ECG group, p=0.06). Patients
with abnormal ECG-s had higher pulse-wave velocities (both aorto-femoral and carotid-femoral), corresponding to increased arterial stiffness compared to patients with normal ECG-s, however, only after rosuvastatin treatment did this difference reach statistical significance (c-fPWV was 8.6±1.7 m/s, vs. 6.7±1.6 in the two groups, respectively, p=0.012). Presence of a manifest carotid plaque (6/28 patients) stood in no correlation with supraventricular or ventricular arrhythmias, or ST-T segment abnormalities. However, decreased heart rate variability was significantly more frequent in the carotid atherosclerosis patient group (p=0.044, OR 8.5, CI: 1.13-63.87.)

Among the assessed laboratory parameters, ESR, CRP and vWF levels or atherogenic indices were in no significant relation with ECG abnormalities of SSc patients. CRP levels of patients with abnormal ECG-s demonstrated a tendency of being higher than CRP-s of normal ECG patients (3.9±2.8 vs. 1.87± 1.28 mg/l, p=0.059.)

We analysed the relationship between right ventricular functional echocardiographic parameters and ECG abnormalities and got the following results. Presence of supraventricular arrhythmias correlated inversely with TAPSE (24.68±4.4 vs. 21.02 ±3.6mm, p=0.031), tricuspid E wave, and right ventricular E/A ratio (1.23±0.27 vs. 0.89±0.29, p=0.004) and a positive correlation was found between SVARY and left atrial enlargement (longitudinal LA diameter 41.5±4.5 vs. 52±10.5 mm).

4.3.3.4. Results of exercise testing

Based on our results, rosuvastatin did not influence physical fitness, as assessed by 6-minute walk test in SSc patients. 6-minute walk-test results prior to and following rosuvastatin treatment were 311±108 vs. 316±118 meters, respectively (p=0.11).
5. DISCUSSION

In a significant proportion of autoimmune rheumatic diseases including RA, SLE or polymyositis accelerated atherosclerosis and early cardiovascular disease (presenting as early onset endothelial dysfunction, subclinical atherosclerosis, increased arterial stiffness etc.) are major influencing factors of morbidity and mortality.

At the time of conducting our cross-sectional study of FMD and ccIMT in SSc in 2007, there was little information about endothelial dysfunction in SSc despite increasing number of reports on SSc-related vascular involvement. Our results of decreased brachial FMD in a Framingham–risk matched study group indicate early functional impairment of the endothelium typical of SSc preceding overt carotid atherosclerosis. The inverse correlation of NMD and positive correlation of ccIMT with age indicates a deteriorating endothelium-independent vasodilation and advancing atherosclerosis with higher age in SSc. The correlation of ccIMT with disease duration could in theory be due to more advanced age, since age and disease duration may also be related to each other. In this patient cohort, however, age and disease duration were independent, suggesting that ccIMT is related to systemic sclerosis itself.

The clinical significance of impaired endothelium-dependent vasodilation parallel to maintained nitroglycerine-dependent vasoreactivity is on one hand a possible therapeutic option for introduction of exogenous NO-donors and nitroglycerine in SSc therapy to reduce SSc patients’ vascular symptoms and perhaps cardiovascular risk. On the other hand, preserved endothelium independent vasodilation is a major argument supporting the dominant role of regulating vasoactive molecules as opposed to structural vascular alterations (vessel wall abnormalities, obstructive vasculopathy) in the pathomechanism of SSc, otherwise vasodilation would be impaired even in presence of exogenous vasoactive agents.

Elevated aortic PWV has been found to be the best indicator of increased cardiovascular risk independently from traditional risk factors in a post hoc analysis of the Framingham heart study. Increased stiffness has also been found to correlate with disease duration in AI disorders SLE and RA. Our group has been among the first ones to assess indicators of systemic arterial stiffness in SSc and was the first to report about elevated PWV in SSc compared to healthy controls signifying large-vessel involvement in addition to microvascular disease in SSc. The correlation between PWV and SSc disease duration indicates the need for taking into consideration disease duration when planning vascular screening in SSc. In addition, higher PWV values characterize the limited form of the disease compared to dcSSc, suggesting more severe macrovascular involvement in the lcSSc subgroup.
In the past decades, an increasing number of studies addressed the effects of several statins in SSc as well as the pleiotropic effects of rosuvastatin in reduction of CV risk in the general population. The findings of endothelial dysfunction, increased PWV or the correlation of PWV with disease duration in SSc prompted us to test the clinical usefulness of rosuvastatin on cardiovascular and laboratory abnormalities in SSc. Significant improvement in endothelial function assessed following six months of rosuvastatin treatment is a novel finding which has not been demonstrated in SSc patients before. Significant decrease of serum CRP and complement levels within normal values after rosuvastatin treatment indicate diminished disease activity or perhaps also a decrease in cardiovascular risk. A return of elevated pretreatment immune complex levels to normal might be of significance in disease pathogenesis.

Lack of correlation between pre- and posttreatment atherogenic indices and FMD values suggests that FMD improvement is possibly explained by nonlipid statin effects, such as the mechanism of normalization of eNOS expression, decreasing serum levels of ADMA; enhancing adiponectin release or repression of MHC-II-mediated T-cell activation in endothelial cells or an inhibitory effect of rosuvastatin on the Rho kinase pathway, an important regulator of cutaneous vascular tone.

As opposed to impaired FMD, ABI, ccIMT, and PWV measurement results were not significantly elevated compared to European reference values. In addition, the latter three parameters did not change significantly after rosuvastatin treatment. It is somewhat surprising that PWV, a strong predictor of cardiovascular risk was unchanged by statin therapy, however, multiple explanations, such as the significant disease duration (cca 10 years) and the limited length of statin therapy may play a role.

Results regarding cardiac involvement in SSc suggest several conclusions. Whereas all patients had preserved left ventricular systolic function, LV diastolic function was abnormal as a sign of cardiac involvement in as many as 81.5% of patients. ECG analysis indicated that although individual arrhythmias are low in number, these add up to a significant frequency of electrocardiac abnormalities, which were not effected by rosuvastatin therapy. Rosuvastatin also left the most important left ventricular systolic, diastolic and right ventricular functional parameters (EF, TAPSE, mitral inflow patterns) unchanged. Clinical significance of the detected minor, although statistically significant decrease in left ventricular systolic diameter, the minor increase in posterior wall thickness or increased right ventricular end-diastolic diameter after rosuvastatin therapy is limited.

Pulmonary fibrosis and supraventricular arrhythmias correlated with markers of RV dysfunction (decreased TAPSE, lower E/A ratio - in case of SV arrhythmias) and left atrial
enlargement in SSc. The mechanism linking these abnormalities is probably the right ventricular overload caused by increased pulmonary resistance resulting in RV systolic and diastolic overload. The correlation between carotid atherosclerosis and decreased heart rate variability might be related to the atherosclerosis near the carotid body chemoreceptors influencing the sensory afferent function of the heart’s autonomic reflexes.
6. SUMMARY

During the assessment of cardiovascular abnormalities and the effects of rosuvastatin treatment in SSc we got the following novel results:

1. FMD is significantly lower in Hungarian SSc patients compared to healthy controls.

2. FMD, NMD, and carotis IMT do not correlate with internal organ involvement or autoantibody positivity.

3. Pulse wave velocity and augmentation index is significantly higher in SSc patients compared to controls indicating increased arterial stiffness in SSc.

4. Pulse wave velocity correlates with disease duration in SSc and PWV is higher in the limited form of the disease.

5. Rosuvastatin improves FMD, lowers serum high-sensitivity CRP and complement levels and decreases immune complex production in patients with SSc indicating a positive effect on disease activity and a possible decrease in cardiovascular risk.

6. The improvement of endothelial function, taking into account lack of correlation with the decrease in atherogenic index, is independent from the lipid-lowering effect of rosuvastatin.

7. 6-month rosuvastatin therapy has no effect on peripheral arterial disease frequency or severity and on the following angiologic parameters: ankle-brachial index, ccIMT, or forearm cutaneous blood flow as assessed by Laser Doppler perfusion monitoring.
7. ACKNOWLEDGEMENTS

I am grateful to my supervisor, Gabriella Szűcs, for her guidance and helpful remarks during my scientific work as well as for her steadfastness and devotion in the management of the hundreds of SSc patients throughout the years.

I would like to thank Prof. Pál Soltész for letting me join his team at the Angiology and Intensive Care Unit, for directing my attention to vascular abnormalities and for helping my work as a co-supervisor for a significant part of my PhD project.

I would like to thank Prof. Zoltán Szekanecz for his supportive supervision, synthesis of results, his help during the wording of the articles and prompt reactions whatever odd time I contacted him.

I wish to say thank you to Prof. Margit Zeher, Head of the IIIrd Department of Internal Medicine for enabling my scientific work at the department in addition to supervising my specialisation and activity in the field of internal medicine.

I am grateful to the former Head of the IIIrd Department of Internal Medicine, Prof. Gyula Szegedi for laying the foundations of current clinical research in the field of Immunology in our Department.

I wish to deliver special thanks to three colleagues, György Kerekes, Judit Végh and Franciska Tizedes for helping me by sharing their expertise in the field of cardiology and cardiovascular ultrasound imaging. I have learned a lot from them both scientifically and personally.

I appreciate the work and of the staff and nurses of the Third Department of Internal Medicine especially for the nurses at the Intensive Care Unit and of the Department of Rheumatology, for their help in blood sample acquisitions and patient management.

I wish to deliver special thanks to Andrea Sipos, Ildikó Pappné Farkas and Renáta Laczik as well as Henrietta Dér for their assistance in the vascular examinations and to Katalin Hodosi for her technical and personal support as well as her help in the statistical analysis. Thanks to Zsuzsa Oláh, Andrea Domján and Anita Márton for their swift help regarding administrative issues.

I am thankful to my co-authors, to my current and past colleagues at the Dpt. of Internal Medicine for helping out if research and clinical patient care posed tasks to be performed simultaneously.

Many thanks to the directors Prof. Sipka Sándor, Gábor Nagy and to the staff of the Laboratory of Immunology for their co-operation in laboratory examinations and sample storage. I thank the colleagues at KBMPI for their help with laboratory measurements.
And finally, I thank my family for their loving support and patience as well as for enduring the time I spent with science instead of them.
List of publications related to the dissertation


*These two authors contributed equally to this work.
List of other publications


* These two authors contributed equally to this work.

H- 4032 Debrecen, Egyetem tér 1. © E-mail publikaciod@lib.unideb.hu

30
Total IF of journals (all publications): 23.844
Total IF of journals (publications related to the dissertation): 11.629

The Candidate's publication data submitted to the IDEa Tudóstár have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

18 July, 2014