

This Provisional PDF corresponds to the article as it appeared upon acceptance. Copyedited and fully formatted PDF and full text (HTML) versions will be made available soon.

Rosuvastatin improves impaired endothelial function, lowers high sensitivity C-reactive protein, complement and immunocomplex production in patients with systemic sclerosis: a prospective case-series study

Arthritis Research & Therapy 2013, **15**:R105 doi:10.1186/ar4285

Orsolya Timar (orsolyatimar@gmail.com)
Zoltan Szekanecz (szekanecz.zoltan@med.unideb.hu)
Gyorgy Kerekes (gkerekess@gmail.com)
Judit Vegh (veghju@freemail.hu)
Anna V. Olah (olaha@med.unideb.hu)
Gabor Nagy (nagy.gabor@unideb.hu)
Zoltan Csiki (csikiz@gmail.com)
Katalin Danko (katalin.danko@gmail.com)
Szilvia Szamosi (szamosi.szilvi@freemail.hu)
Agnes Nemeth (nemethagi1982@yahoo.com)
Pal Soltesz (soltesz@iibel.dote.hu)
Gabriella Szucs (szucs.gabriella@med.unideb.hu)

ISSN 1478-6354

Article type Research article

Submission date 14 August 2012

Acceptance date 15 May 2013

Publication date 4 September 2013

Article URL <http://arthritis-research.com/content/15/5/R105>

This peer-reviewed article can be downloaded, printed and distributed freely for any purposes (see copyright notice below).

Articles in *Arthritis Research & Therapy* are listed in PubMed and archived at PubMed Central.

For information about publishing your research in *Arthritis Research & Therapy* go to

<http://arthritis-research.com/authors/instructions/>

© 2013 Timar *et al.*

This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Rosuvastatin improves impaired endothelial function, lowers high sensitivity CRP, complement and immunocomplex production in patients with systemic sclerosis – a prospective case-series study

Orsolya Timár¹, Zoltán Szekanecz^{2*}, György Kerekes¹, Judit Végh¹, Anna V. Oláh³, Gábor Nagy¹, Zoltán Csiki¹, Katalin Dankó¹, Szilvia Szamosi², Ágnes Németh², Pál Soltész¹, Gabriella Szücs²

¹ Departments of Clinical Immunology and Angiology, Institute of Medicine, University of Debrecen Medical and Health Science Center, Móricz str 22, Debrecen, 4032, Hungary.

Phone/fax: +36 52 414969;

² Department of Rheumatology, Institute of Medicine, University of Debrecen Medical and Health Science Center, Nagyerdei str 98, Debrecen, 4032, Hungary. Phone/fax: +36 52

255091, szekanecz.zoltan@med.unideb.hu;

³ Department of Laboratory Medicine, University of Debrecen Medical and Health Science Center, Nagyerdei str 98, Debrecen, 4032, Hungary. Phone/fax: +36 52 411600;

*Corresponding author

Abstract

Introduction. To study the effect of rosuvastatin on endothelial and macrovascular function, cardiovascular risk factors and the complement pathway in patients with systemic sclerosis (SSc).

Methods. Altogether 28 patients with SSc underwent laboratory and complex vascular assessments before and after 6 months of 20mg rosuvastatin treatment. Flow-mediated dilation (FMD) of the brachial artery, as well as carotid artery intima-media thickness (ccIMT), carotid-femoral and aorto-femoral pulse wave-velocity (PWV) were analyzed by ECG-synchronized ultrasound. Ankle-brachial index (ABI) was determined by Doppler, forearm skin microcirculation was assessed by Laser Doppler perfusion monitoring.

Results. Brachial artery FMD significantly improved upon rosuvastatin therapy ($2.2\pm 3.3\%$ before vs. $5.7\pm 3.9\%$ after treatment, $p=0.0002$). With regards to patient subsets, FMD significantly improved in the 21 lcSSc patients (from 2.1% to 5.6%; $p=0.001$). In the 7 dcSSc patients, we observed a tendency of improvement in FMD (from 3% to 6%; $p=0.25$). Changes in PWV, ccIMT and ABI were not significant. Mean triglyceride (1.7 ± 0.97 vs. 1.3 ± 0.46 mmol/l, $p=0.0004$), total cholesterol (5.3 ± 1.6 mmol/l vs. 4.2 ± 1.3 mmol/l, $p=0.0003$), LDL-C (3.0 ± 1.3 vs. 2.2 ± 1.0 mmol/l, $p=0.005$) and CRP levels (5.1 ± 5.2 vs. 3.4 ± 2.7 , $p=0.01$) levels significantly decreased after rosuvastatin treatment. Mean C3, C4 and IC levels also decreased significantly as compared to pretreatment values.

Conclusions. Six-month rosuvastatin therapy improves endothelial function and lowers CRP, C3, C4 and IC levels indicating possible favourable effects of this statin on the cardiovascular and immune system in SSc.

Keywords: rosuvastatin, systemic sclerosis, atherosclerosis, cardiovascular, endothelial function, flow-mediated vasodilation, arterial stiffness, pulse-wave velocity

Introduction

Systemic sclerosis (SSc) is a systemic autoimmune disease of uncertain etiology characterized by progressive fibrosis of the skin, the small blood vessels and various internal organs. Population-based and other cohort studies have emphasized that survival is decreased in patients with SSc [1-3]. Several reports including our previous studies, have found macrovascular abnormalities in SSc patients. These include endothelial dysfunction indicated by abnormally low flow-mediated dilation (FMD) of the brachial artery [4, 5], increased carotid intima-media thickness (IMT) and increased arterial stiffness [6-8]. Statins may improve endothelial dysfunction, arterial stiffness and reduce levels of inflammatory markers in various conditions including chronic kidney disease, rheumatoid arthritis and dyslipidemia. In SSc, the vascular effects of atorvastatin, simvastatin and pravastatin have been investigated so far [9]. Atorvastatin exerted beneficial effects on microvascular function, digital ulcers, soluble markers of endothelial function, as well as FMD [10, 11]. Simvastatin and pravastatin reduced the production of soluble endothelial activation markers [12, 13].

It is not fully clear which of the multiple mechanisms of statins may be involved in SSc-related vascular changes. Although a pronounced reduction of LDL-cholesterol is present in patients undergoing statin therapy, additional mechanisms have been suggested to improve endothelium-dependent vasodilation in case of HMG-coA reductase inhibitor therapy. A key point is promoting activity of the endothelial NO synthase. This can either be caused by a weakened interaction between the endothelial NO synthase (eNOS) and caveolin-1, or the association of e-NOS and hsp90, as well as by an upregulation of eNOS mRNA by inhibition of the Rho kinase pathway or a reduction in ICAM-1 and P-selectin levels, which also result in increased endothelial NO production [14].

In this study, we wished to determine the effects of rosuvastatin, a potent reducer of total (TC) and LDL cholesterol (LDL-C) [15, 16], on serum inflammatory markers and complement levels, on endothelial and macrovascular function, as well as on arterial stiffness in patients with SSc. In addition, we determined the possible effects of rosuvastatin on microvascular function, by assessing cutaneous blood flow. The primary endpoint of the study was rosuvastatin effects on the vasculature (FMD, ccIMT, PWV, ABI), while the secondary outcome included laboratory marker changes (lipids, CRP, immune complexes and complement). The assessment of acute phase reactants and complement has been included in scleroderma activity index [17].

Materials and methods

Patients

SSc patients undergoing follow-ups at our institution were randomly screened for inclusion and exclusion criteria described below. Altogether 28 patients including 25 females and 3 males were eligible for the study. The diagnosis was established according to the 1980 ACR criteria for SSc [18]. The mean age of patients was 60.4 ± 11.0 years (range: 34-83 years), the mean disease duration was 13.6 ± 7.7 years (range 2-30 years). Among these patients, 21 (75%) had the limited (lcSSc) and 7 (25%) had the diffuse cutaneous form of SSc (dcSSc). Clinical manifestations of SSc included Raynaud's phenomenon (96%), distal skin manifestations including sclerosis and ulcers (68%), proximal skin involvement (25%), cardiac manifestations including conduction defects, atrial fibrillation, arrhythmias (25%), gastrointestinal manifestations, such as esophageal dysmotility, gastroesophageal reflux (54%), renal involvement (4%) and sicca syndrome (10%). Altogether 6 patients (22%) had an overlap syndrome with another autoimmune disease: two patients had polydermatomyositis, one had systemic lupus erythematosus and 3 had rheumatoid arthritis in addition to SSc. The patients' medications are listed in Table 1. All recruited patients were non-smokers and their mean body mass index (BMI) was 23.1 ± 4.1 kg/m².

Patients were included in the study if lcSSc or dcSSc was present, and the patients exhibited microvascular symptoms including new digital ulcers, active Raynaud's symptoms despite ongoing therapy. Patients had not been on any lipid lowering drug therapy for the past 6 months prior to this study.

Exclusion criteria included hyperglycemia, acute systemic infection, uncontrolled hypertension, carotid sinus hyperesthesia, permanent atrial fibrillation, an ejection fraction (EF) less than 50% as determined by echocardiography, severe pulmonary arterial hypertension (PAH), active ulcers at any of the measurement sites or lack of patients' informed consent.

Approximately 60 patients were screened for the study. Thus, 28 patients meeting the inclusion criteria were included. Any patients meeting any exclusion criteria, those on vasoactive drugs, such as prostanoids or patients undergoing frequent treatment modifications were excluded.

Written informed consent was obtained from all subjects. The study was performed according to the Declaration of Helsinki under the auspices of the University of Debrecen

Research Ethics Committee and the ETT-TUKEB central Ethics Committee (No. 14804-1/2011-1018EKU)..

Study protocol

Both laboratory analyses and the clinical examinations described below were performed on two occasions, directly before and after the rosuvastatin treatment period. Each patient received 20 mg rosuvastatin daily for 6 months. All patients tolerated the drugs well and all of them could complete the study. On the day of vascular assessments, blood samples were drawn between 7 and 8 a.m. after an 8-hour fasting period. Samples were stored at room temperature and analyses were performed within 2 hours. Von Willebrand factor antigen (vWF) samples were stored on ice until analysis. Before vascular assessments, the use of vasoactive and antioxidant drugs, as well as alcohol or caffeine consumption were suspended for 24 hours. Examinations were performed under standardized conditions after a 10-minute resting period in a recumbent position. Vascular assessments were carried out in a quiet, darkened study room with a temperature of 22 ± 1 °C according to the recommendations of Laurent et al [19]. All assessments were performed by a single observer (O.T.)

Vascular assessments

Brachial artery FMD assessment was performed by the same skilled operator according to the methodology described by Corretti et al [20] under standardized conditions [21] using a 10 MHz linear array transducer of a HP Sonos 5500 (Hewlett Packard) ultrasound equipment. Briefly, longitudinal section of the brachial artery of supine patients 4-7 cms proximal from the antecubital fossa was obtained at end-diastole (upon R wave of ECG) and the arterial diameter (BAD_{basal}), namely the distance between the proximal and distal media-adventitia borders, was measured. Results of five repeated measurements were averaged. After 4.5 minutes of distal (forearm) blood pressure cuff occlusion with 50 Hgmm suprasystolic pressure, reactive hyperaemia-induced maximal diameters (BAD_{max}) were measured within 3 minutes after release. Images were digitized for further documentation. FMD was calculated as $[(BAD_{\text{max}} - BAD_{\text{basal}}) / BAD_{\text{basal}}] \times 100$ and expressed in % compared to the basal diameter. The intraobserver variability of FMD was excellent: the calculated coefficient of variation (CV) and intraclass correlation coefficient (ICC) were 5% and 0.935, respectively, indicating very good reproducibility.

Aorto-femoral pulse wave velocity (c-fPWV) was measured as previously described elsewhere [22] on a HP Sonos 5500 ultrasound equipment with simultaneous ECG recording. Carotid-femoral pulse wave velocity (c-fPWV) was measured during the same examination with minor changes compared to the a-f PWV assessment. C-f PWV was determined between the left common carotid artery, 1 cm proximal to the bifurcation and the right common femoral artery at the level of the inguinal ligament using the linear array transducer. The distance between the two sites was defined as the difference between the sternal jugulum - carotid measurement point distance and the sternal jugulum-femoral measurement point distance. C-f PWV was again the quotient of the distance and the transit time, the time difference between the foot-to-foot intervals gained at the two measurement sites [23-25].

Common carotid artery intima-media thickness (ccIMT) was measured according to current guidelines [26] by the same examiner with the HP Sonos 5500 ultrasound equipment described above. Briefly, longitudinal B-mode views of the common carotid arteries were taken by medio-lateral probe position, then high magnification images were frozen at end-diastole. ccIMT, defined as the distance between the first and second echogenic lines corresponding the intima-lumen and media-adventitia interfaces, respectively, was measured offline 1 cm from the carotid bulb or the nearest plaque-free segment on the far wall according to the leading edge principle. Ten measurements were performed and averaged on either side and the value of ccIMT was expressed in millimeters. The intraobserver variability of ccIMT was excellent: the calculated CV and ICC were 4.2% and 0.98, respectively, indicating very good reproducibility.

Assessment of ankle-brachial index (ABI) was carried out according to TASC II guidelines [27]. A 10-12 cm sphygmomanometer cuff was placed just above the ankle and a handheld CW Doppler instrument (Vasodop 8 MHz, MediCAD Ltd, Miskolc, Hungary) was used to measure the systolic pressure of the posterior tibial and dorsal pedal artery of each leg. The higher of these pressures was divided by the higher brachial systolic blood pressure value to form the ABI.

Microvascular skin perfusion was assessed by Laser Doppler (LD) perfusion monitoring [28]. During this examination, a 780 nm wavelength laser beam penetrates the skin to a depth of 1-1.5 mm and a fraction of the light is scattered back by moving blood cells resulting in a frequency shift according to the Doppler principle. Therefore a signal proportional to tissue perfusion is generated. Provocation tests such as heat, postocclusive reactive hyperemia (PORH) or biochemical agents applied by iontophoresis allow for testing

skin reactivity. We applied a standard laser probe (PF 408) fixed in a straight probe holder (PH 08) of a Periflux PF 4001 LD flowmeter (Perimed AB, Järfälla, Sweden). The LD apparatus was connected to a laptop, which displayed recordings and saved the information for further offline analysis by the Perisoft for Windows (Ver. 2.5.5) software (Perimed AB). Measurements were carried out with the patients in a supine position. The probes were placed on the volar side of mid-forearm skin, avoiding superficial subcutaneous blood vessels and basal blood flow was recorded for 8 minutes. Afterwards, during continuous recording of skin blood flow, upper arm was obstructed for a three minute period by a 10-12 cm sphygmomanometer cuff inflated to 50 Hgmm suprasystolic pressure, then cuffs were suddenly deflated and forearm skin blood flow was further recorded for 5 minutes. During this period, blood flow returned to baseline. Blood flow (basal, peak, biological zero) was expressed in arbitrary perfusion units (PU) and relative changes compared to basal values were assessed, time to half before hyperaemia (TH1, sec), time to maximum flow (TM, sec) and time to half recovery (TH2, sec) were analysed, and the occlusion areas (AO), hyperaemic areas under curve (AH, PU*sec), as well as hyperemia repayment (AH/AO) were determined. In addition, we assessed the slope of the curve as it reached maximum perfusion after cuff release (acceleration slope) and upon return to basal skin flow (deceleration slope).

Laboratory analyses

Serum biochemical markers and high sensitivity CRP (hsCRP) were analysed on a Modular P-800 analyser (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 measured using enzyme kinetic UV assay, serum creatinine was determined by the compensated Jaffe kinetic method. Estimated GFR was calculated from the serum creatinine by the MDRD 175 (Modification of Diet in Renal Disease study group) formula. HsCRP was assessed by wide range immunoturbidimetric assay, hsCRP levels >5 mg/l were considered elevated.

Plasma levels of circulating vWF, a marker of endothelial cell activation was determined 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, the degree of agglutination 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 a Sysmex XE-2100D automated haematology analyzer (Sysmex Corp., Kobe, Japan). Erythrocyte sedimentation rate (ESR) was determined by the Westergren method.

Circulating immune complexes (IC) were detected by polyethylene glycol precipitation method. Serum complement C3 and C4 levels were measured by nephelometry on a Siemens-Dade-Behring BN-II nephelometer. Laboratory reference ranges were 0.9-1.8 g/l for C3, 0.1-0.4 g/l for C4 and an extinction of 0-170 for IC.

Statistical analysis

Due to the nature of the study (before/after comparison), paired one-tailed/two-tailed *t*-tests were used for statistical evaluation. P values < 0.05 were considered significant. Data represent a normal distribution, as shown by the Kolmogorov-Smirnov test. Correlations were assessed using SPSS software version 11.0. The Pearson correlation coefficients were determined and *r* values at the *p*<0.05 level were considered significant.

Results

Effects of rosuvastatin on micro-and macrovascular function

FMD significantly improved after 6 months of rosuvastatin therapy ($2.3\pm 3.3\%$ before vs $5.7\pm 3.9\%$ after treatment, *p*=0.0002) (Table 2). Altogether 23 patients responded with an increase in occlusion-provoked vasodilation of the brachial artery following rosuvastatin treatment FMD and serum cholesterol, LDL or HDL level changes.

With regards to patient subsets, FMD significantly improved in the 21 lcSSc patients, from 2.1% to 5.6% (*p*=0.001). In the 7 dcSSc patients, we observed a tendency of improvement in FMD, from 3% to 6% (*p*=0.25). The non-significant change in dcSSc may be the result of low patient number (data not shown).

In 11 of the 28 patients (39.3%), baseline carotid-femoral PWV (c-fPWV) values were above the average reference values of age-, lipid- and blood-pressure-status-matched European patients [29] (Table 2). Neither aorto-femoral, nor carotid-femoral PWV showed significant improvement upon rosuvastatin treatment (a-f PWV: 8.8 ± 2.2 m/s before, vs.

8.3±2.1 m/s after therapy, p=0.15; c-f PWV: 8.7±2.6 m/s before vs. 8.1±1.9 m/s after treatment, p=0.1) (Table 2). However, by the end of rosuvastatin treatment, only 5/28 patients (17.9%) had c-fPWV above the mentioned reference values.

The mean ABI, indicator of peripheral arterial disease, was 1.1±0.2 on both sides and remained unchanged after rosuvastatin therapy (Table 2).

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. After rosuvastatin therapy, these values were 0.68±0.14 mm (p=0.38) and 0.70±0.17 mm (p=0.3), respectively (Table 2). Thus, statin treatment did not result in any improvement in carotid atherosclerosis.

Laser Doppler 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 vs. 10.0±10.3 U/sec; p=0.081; deceleration slope: -1.13±0.92 U/s vs -0.64±1.09 U/s; p=0.021) (Table 2). Neither basal, peak or biological zero skin perfusion, nor AH or any of the time characteristics (TM, TH1, TH2) showed significant changes compared to pretreatment values (data not shown).

Laboratory parameters

The 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).

Baseline serum lipid levels indicated that 10% of patients had hypertriglyceridaemia (TG >2.3 mmol/l), 50% had hypercholesterolemia (total cholesterol>5.2 mmol/l) 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 females/males, respectively). Reference values were determined as recommended for the medium cardiovascular risk group based on the European SCORE chart [30].

Among blood chemistry values, lipid parameters showed significant improvement after 6 months of rosuvastatin therapy. Mean TG levels decreased from 1.70± 0.97 mmol/l to 1.30±0.46 mmol/l following therapy (p=0.0004). Total cholesterol decreased from 5.3±1.6 mmol/l to 4.2±1.3 mmol/l (P=0.0003), LDL-C levels decreased from 3.0±1.3 mmol/l to

2.2±1.0 mmol/ (P=0.0046), while mean HDL-C levels remained unchanged (1.5±0.8 mmol/l before vs. 1.5±0.6 mmol/l after therapy, p=0.33) (Table 3). Non-HDL cholesterol levels also displayed a significant decrease after statin therapy (3.8±1.5 vs. 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). Erythrocyte sedimentation rate (ESR), renal function tests and full blood counts exerted no biologically relevant changes upon statin therapy as compared to baseline values (Table 3).

Baseline circulating vWF antigen levels were abnormally high in 63% of patients and although mean vWF antigen levels showed a slight decrease after rosuvastatin treatment (209±90% vs. 193±76%), this change remained statistically insignificant (p=0.09) (Table 3).

Serum immune complex levels (IC) were initially elevated and levels returned to normal after rosuvastatin therapy (extinction: 183.6 vs. 135.5, respectively, p=0.007), while C3 (1.81 vs. 1.62 g/l) and C4 levels (0.33 vs. 0.27 g/l) displayed a significant decrease after rosuvastatin treatment (p=0.001) within the reference range.

There were no significant differences in any laboratory parameters with regards to rosuvastatin treatment in lcSSc vs dcSSc (data not shown).

Discussions

We have previously described impaired FMD in SSc patients compared to age- and sex-matched controls [5]. Our current pretreatment FMD results in scleroderma patients are in concordance with our previous measurements. The detected significant improvement in endothelial function following 6 months of rosuvastatin treatment, however, is a novel finding which had not been demonstrated in SSc patients before. Comparing this effect of rosuvastatin to vascular effects of atorvastatin treatment reported by other investigators [31-34], it is likely that the two statins are similar with respect to their effect on endothelial function (FMD). Whether favourable effects of rosuvastatin on endothelial function is also accompanied by improvement in clinical vascular symptoms such as Raynaud's phenomenon, digital ulceration or Rodnan skin score in SSc has yet to be investigated.

The duration of therapy is a decisive feature of successive statin treatment in SSc. While in one study [33] an 8-week atorvastatin (20 mg) treatment in SSc exhibited no effect on endothelial function as assessed by Laser Doppler imaging, another [34] described beneficial effects of 24 month atorvastatin (10 mg) therapy on Raynaud's phenomenon of SSc

patients. Thus, the beneficial effects of statins on the vasculature, as well as on the underlying inflammatory disease may be expected only after 6 months or more.

As recently reviewed [35], there is an ongoing debate about the extent and presence of atherosclerosis in patients with SSc. By assessing ccIMT, PWV, FMD and ABI we wished to confer further data to this debate.

In our present study, the mean pretreatment values of right and left ccIMT were within the 25th and 75th percentile range of the given age group as described in large-scale European cohort studies [36, 37]. Our current findings support the results of studies reporting normal ccIMT in scleroderma patients and it seems these studies outnumber those detecting abnormally high ccIMT values [35]. Baseline ABI values were also normal in this study, in concordance with other studies describing either normal or mildly reduced ABI in SSc patients [35]. Regarding changes after 6 months of rosuvastatin therapy, our assumptions of favourable outcome were based on the results of previously conducted clinical trials with rosuvastatin on nonrheumatic patients and on a few pilot studies with rosuvastatin in patients with RA or hyperlipidaemia. Two-year treatment with high-dose rosuvastatin has been shown to result in regression of coronary atherosclerosis, as documented by intravascular ultrasound and quantitative coronary angiography [38, 39]. In the METEOR study, 40 mg rosuvastatin resulted in significant reduction in the rate progression of maximum ccIMT values over 2 years in middle-aged patients with subclinical atherosclerosis [40]. Yet, our present findings suggest that 6 months of rosuvastatin (20 mg) therapy yields no change in mean ccIMT in scleroderma patients. The reasons why we could not detect similar changes as the investigators in the METEOR study could be the lower dose and shorter duration of rosuvastatin therapy.

The frequency of PWV values above mean European reference values [29] decreased from 11/28 to 5/28 following rosuvastatin therapy. Somewhat surprisingly, a-f and c-fPWV were elevated only in 39% of patients in this study and mean values exhibited no statistically significant changes following rosuvastatin therapy. Multiple explanations including concomitant use of calcium channel blockers and/or ACE-inhibitors (Table 1), the duration of statin therapy may arise. In order to interpret our results appropriately, repetition of these examinations with longer treatment periods and comparison with PWV progression of nontreated patients will be required.

Adding Laser Doppler measurements to our study protocol was decided as some studies had previously indicated impaired cutaneous vasodilatory response to ischemia in SSc patients [41]. Our Laser Doppler flowmetry measurements revealed significantly slower

deceleration slope during PORH testing after rosuvastatin of the forearm skin compared to pretreatment values. Yet, the relevance of these slopes in determining microcirculation has not yet been fully determined.

Among laboratory parameters, in our current study marked reductions in total TG, total and non-HDL cholesterol as well as LDL-C cholesterol were observed after 6 months of rosuvastatin treatment. HDL-C levels remained unchanged after therapy, however, the frequency of HDL-C levels below normal decreased. The changes in total cholesterol and LDL-levels correspond to the expectations based on results of clinical trials conducted previously to assess efficacy of rosuvastatin in comparison with other statins [15, 16, 42]. A possible explanation for not observing significant changes in HDL-C levels may be the fact that the majority of SSc patients exerted HDL-C levels within normal range at baseline.

Among inflammatory markers, hsCRP levels significantly decreased following rosuvastatin treatment. Rosuvastatin reduces CRP levels in low cardiovascular risk individuals in the general population [43]. There is substantial evidence that hsCRP is an independent cardiovascular risk factor in the general population, but its predictive value in early stages of atherosclerosis is doubtful [44, 45]. To our best knowledge, this is the first study to show that CRP levels improve after 6 months of 20 mg rosuvastatin therapy in patients with SSc.

Levels of circulating vWF antigen have been found elevated in patients with Raynaud's phenomenon and SSc [46] as a sign of endothelial injury. In our patient cohort, rosuvastatin treatment resulted in slight, non-significant decrease in elevated circulating vWF levels.

Although the exact pathomechanism of SSc is yet unknown, lately, activation of the complement system has been suggested and immune complex deposition, particularly in the perivascular and subendothelial region has been described [47]. In our current study we observed elevated serum immune complex levels returning to normal following rosuvastatin treatment. In addition, C3 and C4 levels showed a significant decrease after rosuvastatin treatment.

Conclusions

In conclusion, rosuvastatin improves brachial artery FMD, corrects dyslipidemia and decreases CRP, complement 3 and 4 and immune complex levels, while it may not reduce arterial stiffness or carotid atherosclerosis following a 6-month treatment period in SSc

patients with intermediate cardiovascular risk. More long-term studies carried out in larger patient cohorts are needed to determine the place of rosuvastatin and other statins in the therapy of SSc and to find the right agent capable of improving cardiovascular outcome in these patients.

Key messages

- Accelerated atherosclerosis and vasculopathy have been associated with systemic sclerosis
- Statins may have beneficial effects on vascular function in SSc
- In our study, rosuvastatin improved endothelial function (FMD) and decreased CRP levels suggesting a possible link between inflammation and vasculopathy in SSc
- complement 3, 4 and immune complex levels also decreased upon rosuvastatin treatment

Abbreviations

ABI: ankle-brachial index, ACE: angiotensin converting enzyme, ACR: American College of Rheumatology, ANA: antinuclear antibody, BAD: brachial artery diameter, BMI: body mass index, ccIMT: common carotid intima-media thickness, C3, C4: complement 3 and 4 factors, CRP: C-reactive protein, CV: coefficient of variation, ECG: electrocardiogram, EF: ejection fraction, ENA: extractable nuclear antigen, eNOS: endothelial NO synthase, ESR: erythrocyte sedimentation rate, FMD: flow-mediated vasodilation, HDL: high density lipoprotein, IC: immune complex, ICAM-1: intercellular adhesion molecule 1, ICC: intraclass coefficient of correlation, IMT: intima-media thickness, LD: laser doppler, LDL: low density lipoprotein, NO: nitric oxide, PAH: pulmonary arterial hypertension, PORH: post-occlusive reactive hyperemia, PU: perfusion unit, PWV: pulse-wave velocity, RNP: ribonucleoprotein, Sm: Smith antigen, SSc: systemic sclerosis, TG: triglyceride, vWF: von Willebrand factor

Competing interests

All authors declare they have no competing interests.

Author contributions

All authors read and approved the final manuscript.

O.T.: First author, involved in patient recruitment, vascular imaging, data analysis and paper writing, Z.S.: Senior researcher involved in data analysis, paper writing and supervision (head of department), G.K.: cardiologist, involved in vascular imaging assessments, as well as data analysis and paper writing, J.V.: cardiologist, involved in vascular imaging assessments and data analysis, A.V.O.: laboratory medicine

personnel, laboratory assessments, G.N.: immunolaboratory assessments and data analysis, Z.C.: immunologist, some vascular assessments and paper writing, K.D.: involved in patient recruitment and examination of patients including overlap patients, S.S.: scleroderma patient recruitment and examination, Á.N.: clinical resident involved in patient examination and data analysis, P.S.: head of cardiology study group, vascular imaging assessment supervision, G.S.: head of rheumatology study group, patient recruitment, supervision of the whole project, paper writing

Acknowledgements

This work was supported by research grant ETT 315/2009 (Z.S.) from the Medical Research Council of Hungary (Z.S.); and by the TÁMOP 4.2.1/B-09/1/KONV-2010-0007 project co-financed by the European Union and the European Social Fund (Z.S.). The authors would like to thank the staff of the Third Department of Internal Medicine and those of the Department of Rheumatology for their help in blood sample acquisitions and patient management. Many thanks to the staff of the Laboratory of Immunology for help in laboratory examinations and sample storage. We wish to deliver our special thanks to Ms Andrea Sipos, Ms Ildiko Pappne Farkas and Dr. Renata Laczik for their assistance in the vascular examinations and to Ms Katalin Hodosi for her help in the statistical analysis.

References

1. Czirjak L, Kumanovics G, Varju C, Nagy Z, Pakozdi A, Szekanecz Z, Szucs G: **Survival and causes of death in 366 Hungarian patients with systemic sclerosis.** *Ann Rheum Dis* 2008, **67**:59-63.
2. Czirjak L, Nagy Z, Szegedi G: **Survival analysis of 118 patients with systemic sclerosis.** *J Intern Med* 1993, **234**:335-337.
3. Arias-Nunez MC, Llorca J, Vazquez-Rodriguez TR, Gomez-Acebo I, Miranda-Filloo JA, Martin J, Gonzalez-Juanatey C, Gonzalez-Gay MA: **Systemic sclerosis in northwestern Spain: a 19-year epidemiologic study.** *Medicine (Baltimore)* 2008, **87**:272-280.
4. Cypiene A, Laucevicius A, Venalis A, Dadoniene J, Ryliskyte L, Petrulioniene Z, Kovaite M, Gintautas J: **The impact of systemic sclerosis on arterial wall stiffness parameters and endothelial function.** *Clin Rheumatol* 2008, **27**:1517-1522.
5. Szucs G, Timar O, Szekanecz Z, Der H, Kerekes G, Szamosi S, Shoenfeld Y, Szegedi G, Soltesz P: **Endothelial dysfunction precedes atherosclerosis in systemic sclerosis--relevance for prevention of vascular complications.** *Rheumatology (Oxford)* 2007, **46**:759-762.
6. Lekakis J, Mavrikakis M, Papamichael C, Papazoglou S, Economou O, Scotiniotis I, Stamatelopoulos K, Vemmos C, Stamatelopoulos S, Mouloupoulos S: **Short-term estrogen administration improves abnormal endothelial function in women with systemic sclerosis and Raynaud's phenomenon.** *Am Heart J* 1998, **136**:905-912.
7. Bartoli F, Blagojevic J, Bacci M, Fiori G, Tempestini A, Conforti ML, Guiducci S, Miniati I, Di Chicco M, Del Rosso A, Perfetto F, Castellani S, Pignone A, Cerinic MM: **Flow-mediated vasodilation and carotid intima-media thickness in systemic sclerosis.** *Ann N Y Acad Sci* 2007, **1108**:283-290.
8. Sherer Y, Cerinic MM, Bartoli F, Blagojevic J, Conforti ML, Gilburd B, Ehrenfeld M, Shoenfeld Y: **Early atherosclerosis and autoantibodies to heat-shock proteins and oxidized LDL in systemic sclerosis.** *Ann N Y Acad Sci* 2007, **1108**:259-267.

9. Davignon J: **Beneficial cardiovascular pleiotropic effects of statins.** *Circulation* 2004, **109** Suppl 1:III39-43.
10. Abou-Raya A, Abou-Raya S, Helmii M: **Statins as immunomodulators in systemic sclerosis.** *Ann N Y Acad Sci* 2007, **1110**:670-680.
11. Abou-Raya A, Abou-Raya S, Helmii M: **Statins: potentially useful in therapy of systemic sclerosis-related Raynaud's phenomenon and digital ulcers.** *J Rheumatol* 2008, **35**:1801-1808.
12. Furukawa S, Yasuda S, Amengual O, Horita T, Atsumi T, Koike T: **Protective effect of pravastatin on vascular endothelium in patients with systemic sclerosis: a pilot study.** *Ann Rheum Dis* 2006, **65**:1118-1120.
13. Del Papa N, Cortiana M, Vitali C, Silvestris I, Maglione W, Comina DP, Lucchi T, Cortelezzi A: **Simvastatin reduces endothelial activation and damage but is partially ineffective in inducing endothelial repair in systemic sclerosis.** *J Rheumatol* 2008, **35**:1323-1328.
14. Davignon J, Ganz P: **Role of endothelial dysfunction in atherosclerosis.** *Circulation* 2004, **109** Suppl 1:III27-32.
15. Jones PH, Davidson MH, Stein EA, Bays HE, McKenney JM, Miller E, Cain VA, Blasetto JW: **Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR* Trial).** *Am J Cardiol* 2003, **92**:152-160.
16. McKenney JM, Jones PH, Adamczyk MA, Cain VA, Bryzinski BS, Blasetto JW: **Comparison of the efficacy of rosuvastatin versus atorvastatin, simvastatin, and pravastatin in achieving lipid goals: results from the STELLAR trial.** *Curr Med Res Opin* 2003, **19**:689-698.
17. Valentini G, Bencivelli W, Bombardieri S, D'Angelo S, Della Rossa A, Silman AJ, Black CM, Czirkjak L, Nielsen H, Vlachoyiannopoulos PG: **European Scleroderma Study Group to define disease activity criteria for systemic sclerosis. III. Assessment of the construct validity of the preliminary activity criteria.** *Ann Rheum Dis* 2003, **62**:901-903.
18. **Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee.** *Arthritis Rheum* 1980, **23**:581-590.
19. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H: **Expert consensus document on arterial stiffness: methodological issues and clinical applications.** *Eur Heart J* 2006, **27**:2588-2605.
20. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R; International Brachial Artery Reactivity Task Force: **Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the International Brachial Artery Reactivity Task Force.** *Journal of the American College of Cardiology* 2002, **39**:257-265.
21. Donald AE, Halcox JP, Charakida M, Storry C, Wallace SM, Cole TJ, Friberg P, Deanfield JE: **Methodological approaches to optimize reproducibility and power in clinical studies of flow-mediated dilation.** *J Am Coll Cardiol* 2008, **51**:1959-1964.
22. Bodnar N, Kerekes G, Seres I, Paragh G, Kappelmayer J, Nemethne ZG, Szegedi G, Shoenfeld Y, Sipka S, Soltesz P, Szekanecz Z, Szanto S: **Assessment of subclinical vascular disease associated with ankylosing spondylitis.** *J Rheumatol* 2011, **38**:723-729.

23. Calabria J, Torguet P, Garcia M, Garcia I, Martin N, Guasch B, Faur D, Valles M: **Doppler ultrasound in the measurement of pulse wave velocity: agreement with the Complior method.** *Cardiovasc Ultrasound* 2011, **9**:13.
24. Baguet JP, Kingwell BA, Dart AL, Shaw J, Ferrier KE, Jennings GL: **Analysis of the regional pulse wave velocity by Doppler: methodology and reproducibility.** *J Hum Hypertens* 2003, **17**:407-412.
25. Jiang B, Liu B, McNeill KL, Chowienczyk PJ: **Measurement of pulse wave velocity using pulse wave Doppler ultrasound: comparison with arterial tonometry.** *Ultrasound Med Biol* 2008, **34**:509-512.
26. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, Najjar SS, Rembold CM, Post WS: **Use of Carotid Ultrasound to Identify Subclinical Vascular Disease and Evaluate Cardiovascular Disease Risk: A Consensus Statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force Endorsed by the Society for Vascular Medicine.** *Journal of the American Society of Echocardiography* 2008, **21**:93-111.
27. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG, Bell K, Caporusso J, Durand-Zaleski I, Komori K, Lammer J, Liapis C, Novo S, Razavi M, Robbs J, Schaper N, Shigematsu H, Sapoval M, White C, White J, Clement D, Creager M, Jaff M, Mohler E 3rd, Rutherford RB, Sheehan P, Sillesen H, Rosenfield K: **Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II).** *Eur J Vasc Endovasc Surg* 2007, **33** Suppl 1:S1-75.
28. Cracowski JL, Minson CT, Salvat-Melis M, Halliwill JR: **Methodological issues in the assessment of skin microvascular endothelial function in humans.** *Trends Pharmacol Sci* 2006, **27**:503-508.
29. **Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. The Reference Values for Arterial Stiffness' Collaboration.** *Eur Heart J* 2010, **31**:2338-2350.
30. Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, De Bacquer D, Ducimetiere P, Jousilahti P, Keil U, Njølstad I, Oganov RG, Thomsen T, Tunstall-Pedoe H, Tverdal A, Wedel H, Whincup P, Wilhelmsen L, Graham IM; SCORE project group: **Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project.** *Eur Heart J* 2003, **24**:987-1003.
31. Tam LS, Li E, Shang Q, Tomlinson B, Lee V, Lee K, Li M, Kuan WP, Li T, Tseung L, Yip GW, Freedman B, Yu CM: **Effects of rosuvastatin on subclinical atherosclerosis and arterial stiffness in rheumatoid arthritis: a randomized controlled pilot trial.** *Scand J Rheumatol* 2011, **40**:411-421.
32. El-Barbary AM, Hussein MS, Rageh EM, Hamouda HE, Wagih AA, Ismail RG: **Effect of atorvastatin on inflammation and modification of vascular risk factors in rheumatoid arthritis.** *J Rheumatol* 2011, **38**:229-235.
33. Sadik HY, Moore TL, Vail A, Murray A, Anderson M, Blann A, Herrick AL: **Lack of effect of 8 weeks atorvastatin on microvascular endothelial function in patients with systemic sclerosis.** *Rheumatology (Oxford)* 2010, **49**:990-996.
34. Kuwana M, Okazaki Y, Kaburaki J: **Long-term beneficial effects of statins on vascular manifestations in patients with systemic sclerosis.** *Mod Rheumatol* 2009, **19**:530-535.
35. Nussinovitch U, Shoenfeld Y: **Atherosclerosis and macrovascular involvement in systemic sclerosis: myth or reality.** *Autoimmun Rev* 2011, **10**:259-266.

36. Rosvall M, Janzon L, Berglund G, Engstrom G, Hedblad B: **Incident coronary events and case fatality in relation to common carotid intima-media thickness.** *J Intern Med* 2005, **257**:430-437.
37. Lorenz MW, von Kegler S, Steinmetz H, Markus HS, Sitzer M: **Carotid intima-media thickening indicates a higher vascular risk across a wide age range: prospective data from the Carotid Atherosclerosis Progression Study (CAPS).** *Stroke* 2006, **37**:87-92.
38. Nissen SE, Nicholls SJ, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, Davignon J, Erbel R, Fruchart JC, Tardif JC, Schoenhagen P, Crowe T, Cain V, Wolski K, Goormastic M, Tuzcu EM; ASTEROID Investigators: **Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial.** *JAMA* 2006, **295**:1556-1565.
39. Ballantyne CM, Raichlen JS, Nicholls SJ, Erbel R, Tardif JC, Brener SJ, Cain VA, Nissen SE: **Effect of rosuvastatin therapy on coronary artery stenoses assessed by quantitative coronary angiography: a study to evaluate the effect of rosuvastatin on intravascular ultrasound-derived coronary atheroma burden.** *Circulation* 2008, **117**:2458-2466.
40. Crouse JR, 3rd, Raichlen JS, Riley WA, Evans GW, Palmer MK, O'Leary DH, Grobbee DE, Bots ML: **Effect of rosuvastatin on progression of carotid intima-media thickness in low-risk individuals with subclinical atherosclerosis: the METEOR Trial.** *JAMA* 2007, **297**:1344-1353.
41. La Civita L, Rossi M, Vaghegghini G, Storino FA, Credidio L, Pasero G, Giusti C, Ferri C: **Microvascular involvement in systemic sclerosis: laser Doppler evaluation of reactivity to acetylcholine and sodium nitroprusside by iontophoresis.** *Ann Rheum Dis* 1998, **57**:52-55.
42. Leiter LA, Rosenson RS, Stein E, Reckless JP, Schulte KL, Schleman M, Miller P, Palmer M, Sosef F: **Efficacy and safety of rosuvastatin 40 mg versus atorvastatin 80 mg in high-risk patients with hypercholesterolemia: results of the POLARIS study.** *Atherosclerosis* 2007, **194**:e154-164.
43. Peters SA, Palmer MK, Grobbee DE, Crouse JR, 3rd, O'Leary DH, Raichlen JS, Bots ML: **C-reactive protein lowering with rosuvastatin in the METEOR study.** *J Intern Med* 2010, **268**:155-161.
44. Ridker PM: **Clinical application of C-reactive protein for cardiovascular disease detection and prevention.** *Circulation* 2003, **107**:363-369.
45. Corrado E, Rizzo M, Coppola G, Fattouch K, Novo G, Marturana I, Ferrara F, Novo S: **An update on the role of markers of inflammation in atherosclerosis.** *J Atheroscler Thromb* 2010, **17**:1-11.
46. Herrick AL, Illingworth K, Blann A, Hay CR, Hollis S, Jayson MI: **Von Willebrand factor, thrombomodulin, thromboxane, beta-thromboglobulin and markers of fibrinolysis in primary Raynaud's phenomenon and systemic sclerosis.** *Ann Rheum Dis* 1996, **55**:122-127.
47. Chen M, Daha MR, Kallenberg CG: **The complement system in systemic autoimmune disease.** *J Autoimmun* 2010, **34**:J276-286.

Table 1. Pharmacological therapy of SSc patients at inclusion

Medication	Number of patients	%
ARB/ACE inhibitors	21	75
Calcium channel blockers	16	57
Beta blockers	8	29
Corticosteroids	9	32
Pentoxifylline	23	82
Nitroglycerine	4	14
Vitamin E	8	29
Platelet Aggregation Inhibitors	16	57
H2-receptor blockers or proton-pump inhibitors	14	50
Immunosuppressive agents	3	11
NSAIDs	5	18
Bisphosphonates	3	11
Others (diuretics, tramadol, benzodiazepines, bronchodilators)	14	50

Table 2. Vascular assessments before and after rosuvastatin treatment in SSc patients

Parameter	Pre-treatment mean (S.D.)	Post-treatment mean (S.D.)	P value*
FMD (%)	2.3 (3.3)	5.7 (3.9)	0.0002
Right ccIMT (mm)	0.675 (0.144)	0.681 (0.142)	ns (0.38)
Left ccIMT (mm)	0.717 (0.172)	0.701 (0.165)	ns (0.3)
Carotid-femoral PWV (m/s)	8.7 (2.6)	8.1 (1.9)	ns (0.1)
Aorto-femoral PWV (m/s)	8.8 (2.2)	8.3 (2.1)	ns (0.15)
Right Ankle-Brachial Index	1.1 (0.16)	1.1 (0.27)	ns (0.4)
Left Ankle-Brachial Index	1.1 (0.14)	1.1 (0.19)	ns (0.4)
Laser Doppler acceleration slope (U/s)	14.6 (14.8)	10.0 (10.3)	ns (0.08)
Laser Doppler deceleration slope (U/s)	-1.13 (0.92)	-0.64 (1.09)	0.021

*Significant differences are indicated in bold. ns: non significant

Table 3. Laboratory parameters before and after rosuvastatin treatment in SSc patients

Parameter	Pre-treatment mean (S.D.)	Post-treatment mean (S.D.)	P value *
Erythrocyte sedimentation rate (mm/h)	21 (15.6)	24.7 (19)	ns (0.15)
CRP (mg/l)	5.1 (5.2)	3.4 (2.7)	0.01
Glucose (mmol/l)	5.3 (1.0)	5.5 (1.3)	ns (0.24)
Urea (mmol/l)	6.0 (2.4)	5.9 (2.5)	ns (0.5)
Creatinine (μ mol/l)	67.5 (19.7)	63 (16.9)	ns (0.062)
GFR (ml/min)	82.1 (14.0)	84.8 (10.8)	ns (0.078)
Triglyceride (mmol/l)	1.7 (0.97)	1.3 (0.46)	0.0004
Total cholesterol (mmol/l)	5.3 (1.57)	4.2 (1.28)	0.0003
LDL (mmol/l)	3.0 (1.3)	2.2 (1.0)	0.005
Non-HDL (mmol/l)	3.8 (1.5)	2.5 (1.3)	0.0003
HDL (mmol/l)	1.5 (0.84)	1.5 (0.6)	ns (0.33)
Uric acid (μ mol/l)	263 (56)	273 (77)	ns (0.22)
von Willebrand factor (%)	209 (90)	193 (75.6)	ns (0.092)
Hemoglobin (g/l)	125 (12.7)	126 (12.5)	ns (0.242)
White blood cell count ($10^9/l$)	6.7 (2.6)	7.1 (2.7)	ns (0.191)
Platelet count ($10^9/l$)	250 (62)	265 (64.5)	ns (0.064)
Complement 3 (g/l)	1.81 (0.4)	1.62 (0.32)	0.001
Complement 4 (g/l)	0.31 (0.13)	0.27 (0.1)	0.001
Immune complex (extinction)	183.6 (110)	135.5 (55)	0.005

*Significant differences are indicated in bold, ns: non-significant.

Author email addresses

Orsolya Timár orsolyatimar@gmail.com
Zoltán Szekanecz szekanecz.zoltan@med.unideb.hu
György Kerekes gkerekess@gmail.com
Judit Végh veghju@freemail.hu
Anna V. Oláh olaha@med.unideb.hu
Gábor Nagy nagy.gabor@unideb.hu
Zoltán Csiki csikiz@gmail.com
Katalin Dankó katalin.danko@gmail.com
Szilvia Szamosi szamosi.szilvi@freemail.hu
Ágnes Németh nemethagi1982@yahoo.com
Pál Soltész dr.soltesz.pal@gmail.com
Gabriella Szücs szucs.gabriella@med.unideb.hu