SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Evaluation of vascular markers in systemic lupus erythematosus and catastrophic antiphospholipid syndrome

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Head of the **Examination Committee**:	Prof. Andrea Szegedi, MD, PhD, DSc
Members of the Examination Committee:	Prof. Zoltán Járai, MD, PhD
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The Examination takes place at the Library of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, at 11:00 am, 12th December, 2023.

Head of the **Defense Committee**:	Prof. Andrea Szegedi, MD, PhD, DSc
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Members of the Defense Committee:	Prof. Zoltán Járai, MD, PhD
	Szilvia Szamosi, MD, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, at 13:00, 12th December, 2023.

INTRODUCTION

Epidemiology of systemic lupus erythematosus

SLE is a heterogeneous, multi-organ, chronic autoimmune disease characterised by alternating relapses and remissions in the classic form. SLE is predominantly a disease of young women in childbearing potential. SLE can affect almost any organ system. The most common is polyarthritis, skin symptoms, including acute and subacute cutaneous lupus. In addition, cardiac, pulmonary, haematological and, less frequently, ophthalmological and gastrointestinal manifestations may occur. The prognosis of the disease is mainly determined by renal involvement, lupus nephritis and neurophychiatric manifestations. A number of autoantibodies to intrinsic cellular structures or cells may be detected in the blood of patients. The marker antibody of the disease is the antibody against double-stranded DNA, which not only helps in the diagnosis but also usually follows the activity of the disease. SLE is diagnosed based on the 2019 EULAR/ACR classification criteria. the enrty ciróteria is the Antinuclear antibody (ANA) positivity which is required for diagnosis. It classifies 7 possible clinical manifestations (constitutional, haematological, musculoskeletal, mucocutaneous, acid membranes, renal, and neuropsychiatric) and 3 immunological markers (SLE-specific antibodies, antiphospholipid antibodies, complement system abnormalities) into separate groups. If each manifestation is positive, it scores differently and if the patient scores more than 10 points with ANA positivity, a diagnosis of SLE can be set up. The therapy of SLE is a complex task, aiming to manage flares to prevent organ damage, improve the patient's quality of life, induce and maintain remission while minimising the side effects of treatment.

Accelerated atherosclerosis in SLE

One of the main causes of mortality in SLE is the development of cardiovascular disease. In SLE, the prevalence of ischaemic heart disease ranges from 3.8% to 16%, depending on the study. The risk of stroke is 2-8 times higher in SLE compared to the general population. Depending on the diagnostic method, subclinical atherosclerosis can be detected in 30-40% of patients with SLE. Carotid intima-media thickness (cIMT) is significantly higher in the SLE patient group under 55 years of age. Traditional risk factors such as smoking, hypertension, obesity or diabetes mellitus are also present in patients with SLE, but the high prevalence of cardiovascular disease cannot be explained by Framingham risk factors alone. In addition to traditional risk factors, a complex immuno-inflammatory process may be responsible for the

development of accelerated atherosclerosis in SLE. The presence of SLE is an independent risk factor for the development of endothelial dysfunction.

Research in recent years has shown that a complex process of innate and adaptive immunity and endothelial cell damage is involved in the development of accelerated vascular damage in SLE. The accelerated atherosclerosis seen in SLE is described by the appearance and progression of plaques, stimulation and activation of endothelial cells, and the appearance of neutrophil granulocytes. This complex process is mediated by innate and adaptive immunity, antibody production and complement activation. Proinflammatory cytokines are involved in the development of early atherosclerosis. In SLE, IFN-I, including IFN -α and IFN-β, is mainly responsible for cardiovascular morbidity. Serum levels of vascular endothelial growth factor (VEGF) correlate with disease activity and higher cIMT. It is suggested that VEGF levels may be a novel marker of early atherosclerosis in SLE.

Assessment of atherosclerosis in SLE

To assess atherosclerosis and vasculopathy in SLE, different non-invasive, ultrasound- based imaging techniques are used. While common carotid intima-media thickness (cIMT) is an early indicator of generalized atherosclerosis, brachial artery flow-mediated dilation (FMD) based on B-mode ultrasound assesses endothelium-dependent vasodilation. Furthermore, vascular stiffness is reflected by pulse-wave velocity (PWV) and augmentation index (Aix) In women with SLE, Cypiene et al. found that PWV and Aix were significantly higher, while FMD was not different from controls. In adolescents with SLE, Boros et al. examined arterial stiffness and found that central PWV and characteristic impedance were elevated, while IMT, FMD, and myocardial perfusion were in the normal range. However, others have detected early endothelial dysfunction indicated by low FMD in SLE. In a previous study, SLE has been associated with increased arterial stiffness and higher cIMT, while another indicated that plasma TG is an independent predictor of carotid atherosclerosis in women with SLE.

Dyslipidaemia in SLE

In patients with SLE, Atta et al. discovered the prevalence of dyslipidemia to be more than 70%. This dyslipidemia is characterized by elevated plasma triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and apolipoprotein B100 (ApoB100), as well as decreased plasma concentrations of high-density lipoprotein cholesterol (HDL-C) resulting in a proatherogenic

lipid profile. Previous studies reported this characteristic “lupus pattern” of lipoproteins in SLE usually occurring in the active phases of the disease. Furthermore, the LDL particle size in SLE is significantly smaller than in controls. Most lipid abnormalities observed in SLE might be explained by an accumulation of TG-rich lipoproteins, namely chylomicrons and very low-density lipoprotein (VLDL) particles. In their catabolism, both lipoproteins undergo degradation by lipoprotein lipase (LPL). In patients with SLE, a low LPL-activity results in the accumulation of chylomicrons and VLDLs, leading to high plasma TG and low HDL-C. Beside LPL, the apolipoprotein C3–angiopoietin-like protein 4 axis is also disrupted in SLE, resulting in significantly lower ApoC3, and significantly higher ANGPLT4. Both of these are particularly important as regulators of triglyceride transport and are novel therapeutic targets.

SLE associated with APS

Antiphospholipid antibodies are detected in 20-40% of patients with SLE, and clinical manifestations of APS are seen in 50-70% of these patients. Antiphospholipid antibodies include a number of antibodies, of which lupus anticoagulant (LA), anti-cardiolipin, anti-β2glikoprotein are among the classification criteria for APS.

Clinical presentation of catastrophic antiphospholipid syndrome

Around 1% of patients with APS develop a severe, high mortality syndrome characterised by a rapid onset of a multi-organ thrombotic process. In the first descriptions, the mortality rate was 50%, hence the name catastrophic antiphospholipid syndrome (CAPS). Because of the rarity of the syndrome, the CAPS Registry was created in 2000 to compile the clinical and laboratory parameters and therapy.

CAPS management

The management of CAPS remains challenging and is currently associated with high mortality... Analysis of treatment data uploaded to the CAPS registry has played a major role in the development of the current treatment recommendation. The three pillars of treatment are anticoagulation, treatment of the precipitating factor and supportive therapy. For CAPS associated with SLE, initiation of cyclophosphamide is recommended. The best survival rate is achieved with a combination of anticoagulation, glucocorticoid, plasmapheresis and/or IVIG. This triple combination treatment is also recommended by the 2014 Task Force. Among biological therapies, good results have been reported with rituximab and eculizumab.

AIMS

In our study, the aim was in 50 active young SLE patients and 50 age and sex-matched control group

- to analyse lipid profile and lipoprotein subfractions
- to investigate biochemical and inflammatory markers of disease activity
- comparison of laboratory measurments with non-invasive imaging techniques (cIMT, FMD, PWV, Aix) used in assessment of early atherosclerosis.

Secondary, our aim was to aim to analyze the case presentation of SLE associated CAPS
- to present the diagnostic pathway of catastrophic antiphospholipid syndrome
- to evaluate the impact of histopathologically confirmed renal B-cell infiltration on clinical outcome
- the use and therapeutic role of anti-CD20 monoclonal antibody in CAPS.

METHODS

Clinically active SLE patients and control population

51 clinically active SLE patients (44 females and 7 males), who are treated at the Division of Clinical Immunology, Department of Internal Medicine, University of Debrecen, and 41 age- and gender-matched control subjects (36 females and 5 males) were enrolled in the study. Patients fulfilled the 2019 EULAR/ACR Classification Criteria for Systemic Lupus Erythematosus. SLE Disease Activity Index (SLEDAI) was also calculated to stratify the activity of SLE (0 = no activity, 1–5 = mild, 6–10 = moderate, 11–19 = high, and 20 ≥ very high activity, respectively). Exclusion criteria were active lupus nephritis, pregnancy, and malignant disease. Neither the study population, nor the control group had any previous major cardiovascular event (acute myocardial infarction, ischemic stroke, or significant carotid artery stenosis).

Sample Collection and Biochemical Measurements

All venous blood samples were drawn after a 12 h fasting. Routine laboratory parameters, including high-sensitivity C-reactive protein (hsCRP), total cholesterol, TG, HDL-C, LDL-C, apolipoprotein A1 (ApoA1), and ApoB100 levels were determined from fresh sera from the same vendor. Anti-double-stranded deoxyribonucleic acid (dsDNA), anti- beta-2-glycoprotein I (B2GPI), anticardiolipin (aCL), anti-Sm/RNP (Hycor Biomedical, Garden Grove, CA, USA), anti-Sjögren's- syndrome-related antigen A (SSA), and anti-Sjögren's-syndrome-related antigen B (SSB) autoantibodies were determined by enzyme-linked immunosorbent assays (ELISA). Complement 3 and 4 (C3 and C4) were measured by nephelometric methods. An in-house hemolytic immunoassay was used for measuring CH50, which is a functional test for classical pathway complement activity. All laboratory measurements have taken place at the Department of Laboratory Medicine, University of Debrecen.

Interleukine-6 (IL-6) Measurement

Serum IL-6 was determined with a commercially available quantitative sandwich enzyme immunoassay technique (R&D Systems, Abington, United Kingdom). Values are expressed as pg/mL with 1.7–4.4% and 2.0–3.7% intra- and inter-assay precision, respectively.

Lipoprotein Subfraction Analyses

Lipid electrophoreses were performed with a Lipoprint system for the analyses of LDL and HDL lipoprotein subfractions. During LDL subfraction analysis, up to seven LDL subfractions were distributed based on their size between the VLDL and HDL peaks. Mid-C, Mid-B, and Mid-A mainly corresponded with intermediate density lipoprotein (IDL) particles (IDL-C, IDL-B, and IDL-A). Proportion of large LDL (large LDL %) was defined as the sum of the percentage of LDL1 and LDL2, whereas proportion of small LDL (small-dense LDL %) was defined as the sum of LDL3–LDL7.

During HDL subfraction analysis, 10 HDL subfractions were distributed based on their size between the LDL and albumin peaks. The three major classes were calculated as the sum of HDL1–HDL3 (large HDL), HDL4–HDL7 (intermediate HDL), and HDL8–HDL10 (small HDL).

Computer tomography (CT)

CT examinations were taken at the University of Debrecen, Medical Imaging Clinic – Radiology.

Histological examination

Renal biopsy revealed thrombotic microangiopathy. Histopathological examination included trichrome staining, fibrin immunofluorescence staining and double immunofluorescence staining for T and B cells, CD34 and anti-C9 antibody and electron microscopy.

Histopathology examinations were taken at the University of Debrecen, Department of Pathology.

Flow-Mediated Vasodilation of the Brachial Artery (FMD)

Examinations were performed under standardized conditions, after 8 h of fasting and after an 18 h cessation of smoking, coffee, and tea. Tests were performed on the right brachial artery with a high-resolution duplex ultrasound using a 5–10 MHz linear transducer (Phillips HD11XE; Tampa, FL, USA). FMD was expressed in percentages indicating the change of diameter triggered by reactive hyperemia compared to resting diameter.

Determination of Carotid Intima/Media Ratio (cIMT)

Measurement of IMT was also performed with duplex ultrasound using a 5–10 MHz linear transducer. We examined both carotid arteries. If no plaque formation was detectable measurements were performed 1 cm below the carotid bulb. IMT was defined by determining the distance between the first (lumen–intima border) and second (media–adventitia border) echogenic line visible in the carotid artery. We performed 10 measurements on both sides, then the average value on each side, and then the mean IMT was calculated. Results were presented in centimeters.

Analysis of Stiffness Parameters

Determination of Aix and PWV was performed with an Arteriograph system (TensioMed Kft., Budapest, Hungary). The method of measurement is based on the principle that myocardial contraction produces a pulse wave in the aorta which is reflected from the aortic bifurcation. As a result, a second (reflected) wave can be observed during the systole (late systolic peak). Aix can be calculated as the difference between the early and late systolic peak pressure, divided by the late systolic peak pressure. To estimate the distance between the aortic arch and bifurcation, we measured the distance between the jugular fossa and the symphysis, then used the distance to calculate PWV as the ratio of the jugulo–symphyseal distance and RT S35. Dimension of the calculated ratio is m/s.

Statistical Methods

Statistical analyses were performed using the Statistica 13.5.0.17 software (TIBCO Software Inc. Palo Alto, CA, USA) and GraphPad Prism 6.01 (GraphPad Prism Software Inc., San Diego, CA, USA). The Kolmogorov–Smirnov test was used to determine the normality of data. Student's unpaired t-test and the Mann–Whitney u-test were performed to describe the difference between continuous variables. The Chi-squared test was used to analyze the difference between binominal variables. Data were expressed as mean ± SD or median (upper quartile–lower quartile) in case of normal and non-normal distribution, respectively. Pearson's correlation was used to analyze the relationship between continuous variables. A multiple regression analysis (backward stepwise method) was performed to determine the best independent predictor of accelerated atherosclerosis. $p \leq 0.05$ probability values were considered statistically significant.

RESULTS

Anthropometric and SLE-related clinical data alongside inflammatory markers and the results of imaging techniques

The average age and sex ratio of patients with SLE did not differ from the control group. There was no significant difference between the two groups in traditional cardiovascular risk factors such as BMI, smoking, hypertension, diabetes mellitus. All patients with SLE received corticosteroid treatment, 49% received chloroquine and 62.7% received some form of immunosuppressive treatment. None of the patients received biological therapy. Three patients had lupus nephritis but were in remission at the time of the study. Patients with SLE had significantly higher levels of CRP and IL-6 compared with the control group. In terms of imaging studies characterising early atherosclerosis, Aix was significantly lower in the control group than in patients with SLE.

Examination of lipid levels and subfractions

SLE patients had significantly higher plasma TG and ApoB100 concentrations, with lower HDL-C and ApoA1 compared to the control group. Higher total IDL, IDL-B, and IDL- C subfractions were found in SLE. There were no significant differences in LDL subfractions. Significantly lower concentrations of large, intermediate, and small HDL subfractions were found in patients with SLE compared to controls.

Correlation of lipid subfractions and vascular imaging techniques

Aix positively correlated with VLDL (r = 0,31, p = 0,04), IDL-C (r = 0,41, p = 0,006), and IDL-B subfractions (r = 0,29; p = 0,05) in subjects with SLE. Marginally significant associations were found between Aix and LDL1 (r = 0,29, p = 0,059), TG (r = 0,27, p = 0,078), total cholesterol (r = 0,30, p = 0,058), and ApoB100 (r = 0,29, p = 0,057) in the patient group. Similar to Aix, PWV showed positive correlations with the concentrations of VLDL (r = 0,41, p = 0,007), IDL-C (r = 0,4, p = 0,004), IDL-B (r = 0,35, p = 00,2) and LDL-1 (r = 0,31, p = 0,04) subfractions as well as with TG (r = 0,31, p = 0,04) in SLE.

There were significant negative correlations between hsCRP (r = −0.4; p = 0,006), TG (r = −0,36; p = 0,02), LDL-C (r = −0,31; p = 0,03), ApoB100 (r = −0,34; p = 0,02), VLDL (r = −0,36; p = 0,01), LDL-2 subfraction (r = −0,32; p = 0,03) and FMD in patients.

cIMT showed significant negative correlation with C4 (r = −0,4; p = 0,005) in patients. Patients with SLE were divided into two groups based on SLEDAI; subjects with mild and moderate disease activity comprised the low (SLEDAI = 0–10), and subjects with high and very high activity comprised the high disease activity group (SLEDAI < 11). C4 was significantly higher in the low SLEDAI group (0,165 vs. 0,103 g/L; p = 0,03); while ApoB100 and cIMT was significantly lower in the low SLEDAI group (0,82 vs. 1,03 g/L; p = 0,05 and 0,0478 vs. 0,0554 cm; p = 0,05, respectively).

Multiple regression analysis showed that IDL-C was an independent predictor of Aix (β = 0.,99; p = 0,0009). The model included age, TG, total cholesterol, VLDL, IDL-C, IDL-B, and LDL1.

We could not find significant correlations between the results of vascular diagnostic test (PWV, Aix, IMT, and FMD) and lipid or inflammatory parameters in the control population.

SLE associated CAPS

In September 2010 he the Caucasian male patient was diagnosed with multiple thromboses involving the right femoral vein, the common and external iliac veins, respectively. LA, IgG type aCL and IgG type ab2GPI antibo- dies were identified again. Oral anticoagulant therapy with vitamin K antagonist was started. In 2011 he was diagnosed with SLE based on photosensi- tivity, lupus erythema and the presence of anti- nuclear, anti-SSA, anti-SSB, anti-CL antibodies and hypocomplementemia as well, all reflecting positive LA. Hence, the diagnosis of APS associated with SLE was also established based on the clinical picture and laboratory findings. The maintenance therapy comprised corticosteroids, hydroxycloroquine and anticoagulation. During the following 2 years he had been in remission with no significant kidney abnormalities. However, he had mild symptoms of arthralgia, skin involvement and mild upper airway tract infections. During the autumn of 2013 he was hospitalized several times because of high blood sedimentation rate, massive proteinuria (10 g/day), striking pitting oedema on the legs, increasing levels of carbamide, creatinine, worsening anaemia and thrombocytopenia, respectively. After pulse corticosteroid treatments (3 x1000 mg), the platelet counts increased. However, due to deteriorating renal function and progressive proteinuria, indicating lupus activation, mycophenolate mofetil treatment was initiated. In January 2014 he was hospitalized again because of recurrent deep vein thrombosis in his right superficial femoral vein. Low molecular weight heparin (LMWH) therapy was administered. The therapy was modified due to recurrent thromboses and acti- vation of SLE: cyclophosphamide was

initiated; in turn, the proteinuria diminished and the renal func tion improved slightly. In March 2014 he was hospitalized for the next cycle of cyclophosphamide therapy. However, ultimately he could not be given it because of the progressive decreasing platelet count. Regarding his physical status, swollen lilac lips, epigastric pain, oedema of the lower right extremity and diarrhoea were observed. The clinical pic- ture and the laboratory tests confirmed the diagnosis of gastrointestinal infection. Metronidazole was initiated. In the next few days the gastrointestinal symptoms diminished; however, the renal failure, anaemia and thrombocytopenia progressed and in the quantitative blood smear fragmentocytes were detected. Subsequently, paresis of the left upper extremity appeared. Skull computed tomography (CT) showed ischaemic lesion at regions of the right medial cerebral artery (Figure 1(a)). Due to exceeding gastrointestinal pain, abdominal CT was performed, which detected thrombus in the lumen of the coeliac trunk, infarction in the spleen, and in the right kidney. Laboratory tests revealed anti- dsDNA and ab2GPI positivities. As a differential diagnosis thrombotic thrombocytopenic purpura/ haemolytic uraemic syndrome was considered; however, it was ruled out by the negative results on ADAMTS 13 and anti-factor H antibody assessment, respectively.

Histopathology of renal biopsy

Renal biopsy revealed thrombotic nephropathy. Special (trichrome) staining confirmed the presence of microthrombi which occluded the glomerular capillaries and arterioles. Overall, the biopsy specimen was identified as class IIb lupus nephritis of the International Society of Nephrology/Renal Pathology Society classifications. chronic mesangioproliferative glomerular damage exhibit- ing thrombotic occlusions of the capillary tufts reflecting SLE-associated CAPS. Immunofluorescent staining for fibrin confirmed that the glomerular capillary tuft occlusions and the interstitial vascular obstructions correspond to multiple fibrin-rich thrombi, suggestive of a renal manifestation of CAPS. Electron microscopy also exhibited glomeruli with basement membrane (BM) thickening and endothelial-subendothelial damage associated with CAPS. Moreover, higher magnification on electron microscopy identified splitting and dissection of the endothelial cells from the BM signified by electronlucent subendothelial amorphous material deposition admixed with tissue debris. Double immunofluorescent labelling of kidney biopsy using anti-complement C9 antibody in combination with the endothelial marker CD34 identified the presence of the membrane-attack complex of the complement cascade that showed unam- biguous evidence of endothelial and subendothelial C9 depositions,

predominantly along the glomerular capillary tufts and in part within the vasculature of the interstitial spaces. Finally, double immunofluorescent staining for the T and B-cells demonstrated the immune-inflammatory cel- lular composition of the SLE-associated CAPS in kidney tissue of this case: both the T-cells and the B-lymphocytes could be identified to have roles in the pathomechanism of the vascular damage resulting in thrombotic occlusions.

CAPS management in our case history

Based on the clinical features together with the applied histology and laboratory findings we could establish that CAPS on the grounds of SLE, which was induced by gastrointestinal infection, most likely developed by perpetuating the complement cascade activation with T and B-lymphocytes mobilization, resulting in multiple thromboses and consecutive ischaemic tissue damage in an amplified fashion. The primary goal of the therapeutic plan was to decelerate the thrombotic cascade and to suppress inflammatory cytokines. LMWH treatment was continued but in lower dosage (2 □ 0.2 ml) in order to avoid bleeding in the ischaemic lesion of the territory at the right medial cerebral artery. The dose of corticosteroid was increased to 80mg per day. Plasma exchange treatment (40ml/kg) was performed six times. Haemodiafiltration treatment was performed three times because of the acute renal failure and increasing levels of carbamide and creatinine. After six plasma exchange cycles, along with the corticosteroid and LMWH treat- ment, the paresis resolved slightly and the haemato- logical parameters began to normalize. However, no significant improvement could be achieved in renal function. The therapeutic regime was there- fore supplemented with rituximab; 1000 mg of rituximab was given two times and the treatment led to a significant improvement of the acute renal failure, showing the efficacy of therapy on renal failure. After 5 weeks the patient was discharged from hospital with normal laboratory parameters and good general health.

DISCUSSION

Lipid levels, lipid subfractions and vascular parameters in SLE

Cardiovascular mortality is known to be significantly higher in patients with SLE and it has also been shown that although overall mortality from SLE has improved over the past decades, this reduction has not been observed in cardiovascular mortality. The significance of our study is that it is the first clinical study to simultaneously investigate variations in lipid profile and lipoprotein subfraction analysis and to compare these results with inflammatory markers and non-invasive imaging studies detecting early atherosclerosis.

While the central role of LDL-C in the development of atherosclerotic cardiovascular disease is well established, the role of triglyceride-rich lipoproteins and their remnants in this process remains poorly understood. Triglyceride-rich lipoproteins reduce autophosphorylation of focal adhesion kinases and thereby inhibit the phosphatidylinositol 3-kinase/protein kinase B (Akt) signaling pathway, leading to inactivation of nitric oxide synthase (NOS) and thus reduced endothelial nitric oxide (NO) synthesis. Furthermore, triglyceride-rich lipoproteins accumulated in plasma increase serum levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NOS. It should also be emphasised that these lipoproteins promote the release of endothelin-1 (ET-1), which induces vasoconstriction and smooth muscle cell proliferation by increasing vascular smooth muscle cell tone. Overall, a proinflammatory, protrombotic state is produced under increased oxidative stress. In addition, hyperviscosity and increased platelet aggregation, which may also be caused by triglyceride-rich lipoproteins, are involved in the development of the protrombotic state.

Although there have been previous human studies supporting the role of remnant lipoprotein particles, particularly IDL and small VLDL, as independent predictors of atherosclerosis severity or progression, their role in the accelerated atherosclerosis seen in SLE has not been clarified. In a previous study, it was described that a higher SLEDAI was associated with a higher proportion of atherogenic lipoproteins, especially small VLDL particles, and a significant correlation was found between the ApoB100:ApoA1 ratio and disease activity as indicated by SLEDAI. In our studies, we found significantly higher ApoB100 levels in the high/high SLEDAI patient group compared to the low/medium disease activity SLEDAI group. Our present results also support that inflammatory processes play a role in the development of

lipid abnormalities. Furthermore, we showed that the clinically active SLE patient group had significantly higher VLDL and significantly lower IDL subfraction levels.

Based on literature data, arterial stiffness was increased in patients with SLE compared to the control group. This has been confirmed by a recent meta-analysis of 49 studies by Mendosa et al [163]. Furthermore, studies have shown that arterial stiffness in SLE patients deteriorates in parallel with atherosclerotic plaque propagation. Another study described the correlation between stiffness parameters and cIMT. Elevated arterial stiffness thus highlights the increased cardiovascular risk and the importance of promptly initiating adequate cardiovascular risk-reducing therapy. Our study confirmed that the augmentation index describing arterial stiffness was significantly higher in the SLE patient population compared to the control group. The significant positive correlation between the augmentation index and VLDL, IDL-B and IDL-C also supports a role for triglyceride-rich lipoproteins in the process of early atherosclerosis. In multiple regression analysis, we showed that IDL-C is an independent predictor of Aix, a finding that further supports the role of triglyceride-rich lipoproteins in the pathomechanism of SLE-associated vascular complications.

In our study, cIMT was found to be significantly higher in the high/high disease activity group, supporting the role of inflammatory processes in atherosclerosis-induced arterial remodelling at a young age. Previous studies have shown significantly lower FMD in patients with SLE compared to the control group. No significant difference in FMD was found between the two groups, nor were there significant differences in the high/high and low/medium disease activity groups. However, we observed a significant negative correlation between FMD and CRP, Tg, LDL-C, ApoB100, VLDL and LDL2 in our SLE patient group. Our result, which differs from the literature, may be due to the environmental sensitive methodology detailed above.

Consistent with previous literature data, our present study did not show any significant association between non-invasive imaging modalities (cIMT, FMD, PWV, Aix) and lipid and inflammatory parameters in the control group. This may be explained by the fact that the control group was also mainly composed of young women [99,169]. The fact that we did not find a significant association between vascular imaging and lipid and inflammatory markers in the control group suggests that the abnormalities in vascular, lipid and inflammatory markers observed in the SLE patient population are disease specific and due to SLE-associated dyslipidemia and inflammation.

SLE associated CAPS in our case history

CAPS is a rare manifestation ofAPS, mortality rate can reach up to 50%. Current guidelines suggest that the standard treatment of CAPS is a combination of anticoagulation with heparin, high-dose parenteral glucocorticoids, plasmapheresis and/or IVIG. Rituximab is a chimeric, anti-CD20 monoclonal antibody that causes immunosuppression by B cell depletion. It inhibits IgG production and promotes the emergence of a regulatory B cell population, which, in turn, provides negative feedback to autoimmune processes through IL-10 production. Studies have shown a significant decrease in anticardiolipin IgG titre following treatment with rituximab and cyclophosphamide. Some manifestations of CAPS are associated with the development of SIRS, in which proinflammatory cytokines (TNF-α, IL-1, IL-2, IL-6) play a major role. The B-cell depleting effect of rituximab may decrease the levels of these cytokines, which may be an additive factor for the beneficial effect of rituximab in CAPS . Case reports suggest that rituximab may be beneficial in the treatment of refractory or recurrent CAPS. Berman et al. analysed data from the CAPS registry , 20 patients received rituximab treatment. 12 patients received rituximab as second-line therapy, and 8 patients received first-line therapy in addition to standard medication. The indication for first-line treatment was lymphoma as the underlying disease in 2 patients, while 6 patients were already in critical condition at diagnosis. Rituximab was used as second-line therapy for refractory thrombocytopenia or newly manifesting thrombotic events. In terms of outcome, 4 patients died and 16 patients recovered from CAPS, showing a significant regression in terms of mortality, with mortality reduced to 20% in patients treated with rituximab. The mean follow-up of patients was 9.5 months, during which time no recurrent thrombotic event occurred. Regarding the antibody profile, half of the patients showed persistent antiphospholipid antibody positivity. Rymarz et al. presented the case of a 35-year-old woman with primary APS who developed CAPS. A renal biopsy confirmed thrombotic angiopathy. Anticoagulant treatment, glucocorticoid treatment was supplemented first with plasmapheresis and then with IVIG, good therapeutic response was achieved. Then, 3 months after the diagnosis of CAPS, an elevated titer of lups anticoagulant and elevated serum cretanin levels were again detected. Relapse of CAPS was successfully prevented with the administration of rituximab. In 2021, Stanescu et al. published the case of a 61-year-old man who developed SLE-associated CAPS. Among the clinical manifestations, I would highlight the acute kidney injury, which required temporary haemodialysis. Renal biopsy demonstrated thrombotic microangiopathy. In addition to standard treatment (anticoagulation, glucocorticoid

and plasmapheresis), rituximab was decided (2x1000 mg iv., 7 days apart), after which renal function normalized within 1 month.

We are among the first to publish the successful use of rituximab in CAPS with renal involvement. Our case was also added to the CAPS registry. Our case report and the literature review highlight the importance of biopsy. Histopathological and immunohistochemical examinations helped better understanding of the pathomechanism of CAPS. In our case, immunohistochemistry revealed intrarenal T- and B-lymphocyte accumulation and complement activation, which supports the efficacy of rituximab treatment and confirms its place in the treatment of CAPS.

SUMMARY, NEW SCIENTIFIC ACHIVMENTS

1. In terms of lipid parameters, significantly lower HDL and ApoA1 levels and significantly higher Tg and ApoB levels were found in patients with SLE.

2. Aix in SLE patients was significantly correlated with VLDL, IDL-B and IDL -C subfractions.

3. A slight but significant positive correlation was detected between Aix and LDL-1, Tg, total cholesterol and ApoB100 levels in SLE patients.

4. Significant positive correlations were also found between Tg, VLDL, IDL-C, IDL-B and LDL-1 subfractions for PWV, another parameter characterizing arterial stiffness in SLE patients

5. As for FMD, a significant negative correlation was found between CRP, Tg, LDL-C, ApoB100, VLDL and LDL-2 subfractions in the SLE patient group.

6. cIMT in the SLE population showed a significant negative correlation with C4 levels.

7. In subjects with mild and moderate disease activity comprised the low (SLEDAI = 0–10), we found that C4 levels were significantly higher, while ApoB100 and cIMT were significantly lower.

8. Stepwise multiple regression analysis showed that the IDL-C subfraction was an independent predictor of Aix.

SLE associated CAPS

12. We successfully used anti-CD20 monoclonal antibody therapy for first-line therapy of refractory acute kidney injury and histopathologically proven renal B-cell infiltration.

13 Our case also demonstrates that rituximab treatment may be a treatment modality to consider for conventional therapy in refractory cases with catastrophic thrombotic syndrome

SUMMARY

SLE is associated with high cardiovascular morbidity and mortality. In addition to traditional and SLE-specific risk factors, a complex immuno-inflammatory process is responsible for the accelerated atherosclerosis in SLE. The role of LDL-C in the atherosclerosis pathway is well known, but less is known about the role of triglyceride-rich lipoproteins. Non-invasive imaging techniques are suitable for the characterisation of subclinical atherosclerosis. Previous studies have shown that cIMT is significantly higher in patients with SLE, and studies have also been performed with FMD characterizing endothelial dysfunction. The results of previous studies have confirmed that the augmentation index and pulse wave velocity are significantly higher in the SLE patients. This is the first study evaluating vascular diagnostic tests, lipid and lipoprotein subfraction profile and immuno-inflammatory markers of the SLE patients.

We have enrolled 51 active patients with SLE and 41 age- and sex-matched controls. To establish SLE diagnose, SLICC/ACR 2012 criteria was used, and reclassification has been taken according to the currently valid EULAR/ACR 2019 criteria. Exclusion criteria were active lupus nephritis, previous major cardiovascular disease or pregnancy. In addition to routine laboratory investigations (blood count, liver and kidney function, CRP), IL-6 and antibody profile were performed. Their lipid parameters were assessed by Lipoprint gelelectrophoresis. In addition, FMD, cIMT, Aix and PWV were determined.

Our results showed that there was no significant difference in traditional cardiovascular risk factors between the age- and sex-matched control group and the SLE group. Inflammatory markers (CRP, IL-6) were significantly higher in the SLE patient population compared to the control group. In terms of lipid parameters, significantly lower HDL-C level and significantly higher Tg, ApoA1 and ApoB levels were found in patients with SLE. Aix positively correlated with VLDL, IDL-C and IDL-B subfractions in subjects with SLE. Marginally but significant positive correlation was detected between Aix and LDL-1, Tg, total cholesterol and ApoB100 levels in SLE patients. For PWV, a significant positive correlation was also found between Tg, VLDL, IDL-C, IDL-B and LDL-1 subfractions. As for FMD, a significant negative correlation was found between CRP, Tg, LDL-C, ApoB100, VLDL and LDL-2 subfractions in the SLE patient group. cIMT showed a significant negative correlation with C4 levels, a marker of disease activity in SLE patients. In the low disease activity SLEDAI group, we found that C4 levels were significantly higher, whereas ApoB100 and cIMT were significantly lower.

Our results demonstrate the possible role of SLE-associated lipid abnormalities and imflammatory processes in early atherogenesis and underline the importance of vascular

parameter measurement, especially the determination of arterial stiffness parameters in SLE patients.

SLE is often associated with APS. CAPS is a rare, potentially life-threatening subset of APS. Usually a precipitating factor can be detected in the patomechanism of CAPS. One of the diagnostic criteria is the demonstration of histologically proven small vessel occlusion, the thrombotic microangiopathy. We were among the firsts to publish the efficacy of rituximab treatment in CAPS complicated by acute kidney injury. Immunohistochemical evidence of intrarenal T- and B-lymphocyte accumulation and complement activation demonstrates the efficacy of rituximab treatment and confirms its place in the treatment of CAPS.

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Doctoral School: Gyula Petrányi Doctoral School of Allergy and Clinical Immunology
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List of publications related to the dissertation

1. **Diószegi, Á.**, Lőrincz, H., Kaáli, E., Soltész, P., Perge, B., Varga, É., Harangi, M., Tarr, T.: Role of Altered Metabolism of Triglyceride-Rich Lipoprotein Particles in the Development of Vascular Dysfunction in Systemic Lupus Erythematosus.
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2. **Diószegi, Á.**, Tarr, T., Nagy-Vincze, M., Vass, M., Veisz, R., Bidiga, L., Dezső, B., Balla, J., Szodoray, P., Szekanecz, Z., Soltész, P.: Microthrombotic renal involvement in an SLE patient with concomitant catastrophic antiphospholipid syndrome: the beneficial effect of rituximab treatment.
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List of other publications

3. Kovács, B., Németh, Á., Daróczy, B., Karányi, Z., Maroda, L., **Diószegi, Á.**, Harangi, M., Páll, D.: Assessment of Hypertensive Patients' Complex Metabolic Status Using Data Mining Methods.
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