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Heterogeneity of peripheral blood monocytes, endothelial dysfunction and subclinical atherosclerosis in patients with Systemic Lupus Erythematosus

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Abstract

Background—Systemic Lupus Erythematosus (SLE) is characterized by increased cardiovascular morbidity and mortality. SLE patients have increased prevalence of subclinical atherosclerosis, although the mechanisms of this observation remain unclear. Considering the emerging role of monocytes in atherosclerosis, we aimed to investigate the relationship between subclinical atherosclerosis, endothelial dysfunction and the phenotype of peripheral blood monocytes in SLE patients.

Methods—We characterized the phenotype of monocyte subsets defined by the expression of CD14 and CD16 in 42 patients with SLE and 42 non-SLE controls. Using ultrasonography, intima-media thickness (IMT) of carotid arteries and brachial artery flow mediated dilatation (FMD) as well as nitroglycerin induced dilatation (NMD) were assessed.

Results—Patients with SLE had significantly, but only modestly, increased intima-media thickness when compared with non-SLE controls (median (25th/75th percentile) 0.65 (0.60/0.71) mm vs 0.60 (0.56/0.68) mm; $p<0.05$). Importantly, in spite of early atherosclerotic complications in the studied SLE group, marked endothelial dysfunction was observed. CD14dimCD16+ proinflammatory cell subpopulation was positively correlated with intima-media thickness in SLE patients. This phenomenon was not observed in control subjects. Interestingly, endothelial dysfunction assessed by FMD was not correlated with any of the studied monocyte subsets.

Conclusions—Our observations suggest that CD14dimCD16+ monocytes are associated with subclinical atherosclerosis in SLE, although the mechanism appears to be independent of endothelial dysfunction.

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Keywords

intima-media thickness; flow mediated dilatation; monocyte subsets; SLE

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Introduction

Systemic Lupus Erythematosus (SLE) is an autoimmune disease, characterized by increased cardiovascular morbidity and mortality1. Already in the late 1970's it was noted that myocardial infarction and cardiovascular diseases were the most important causes of death in patients with SLE, even more than in other rheumatoid disease2. Indeed, the incidence of myocardial infraction is over 50 times higher in women with SLE than in healthy subjects3. This is a result of increased prevalence of atherosclerosis in SLE4–7. While increase in subclinical atherosclerosis translates to increased cardiovascular risk, although the mechanism of this observation in SLE remains unclear. Nevertheless, some studies demonstrated that atherosclerosis in SLE subjects was only modestly increased or even absent8, 9. Recently, it has been postulated that systemic inflammation and the presence of auto-antibodies against endothelial cell membrane antigens induces a proinflammatory phenotype of endothelium, resulting in endothelial dysfunction 10, 11. While this is an interesting possibility, vascular disease appears to be a universal feature of SLE. This is already observed at early stages of the disease, in patients with low disease activity and is not primarily associated with auto-antibody mediated damage. Therefore, it is very likely that a general inflammatory activation leads to initiation of endothelial dysfunction and atherosclerosis in SLE patients.

One of the prominent inflammatory features associated with SLE is the dysfunction of peripheral blood monocytes12. In particular, imbalance within the monocyte subsets defined by the expression of CD14 and CD16 antigens may be important13. Classical, quiescent, monocytes are strongly positive for CD14 (LPS receptor) but do not express Fc gamma receptor III CD16. In contrast, monocytes co-expressing CD16 and CD14 are proinflammatory and pro-atherogenic, although their nature varies depending on the level of CD14 expression. Cells with low expression of CD14 i.e. CD14dimCD16+, represent a mature macrophage-like monocyte, being an important source of TNF-alpha while the role of CD14highCD16+ monocytes is less clear. CD14highCD16+ and CD14dimCD16+ may be associated with increased cardiovascular disease14–16 as well as with myocardial dysfunction and recovery following myocardial infarction17–19, although their relationship to subclinical atherosclerosis is less clearly defined. Interestingly, we have recently observed that CD14high and CD14dim monocytes are increased in patients with early rheumatoid arthritis20. It has been demonstrated that CD14highCD16-, CD14highCD16+ and CD14dimCD16+ are differentially regulated in SLE, but the importance of this observation to cardiovascular risk and atherosclerosis remains unknown 21.

Therefore, it is very interesting to postulate that monocyte subpopulations in SLE are associated with the development of vascular disease. The aim of the present study was to evaluate the relationship between subclinical atherosclerosis assessed as intima-media

thickness, endothelial dysfunction and the phenotype of peripheral blood monocytes in SLE patients.

Materials and Methods

Patients and controls

Forty-two patients diagnosed with SLE (36F/6M; aged 44±14, range 19-75 years) according to the American College of Rheumatology Criteria and 42 non-SLE controls (37F/5M; aged 41±11, range 22-68 years) were included in the study. Average Systemic Lupus Erythematosus disease activity index (SLEDAI)22 was 8.02±8.11. SLE patients were treated using typical immunosuppression schedules with prednisone, hydroxychloroquine/chloroquine, azathioprine, mycophenolate mofetil or cyclophosphamide. Subjects were carefully screened for risk factors for atherosclerosis, defined as follows: hypercholesterolemia (total plasma cholesterol level >5 mmol/L or treatment with statins); diabetes (fasting glucose level ≥7 mmol/L or HbA1c >6.5% or current treatment with insulin or oral hypoglycemic agents); hypertension (≥140/90 mmHg or treatment with antihypertensive agents), and smoking (current/within last 6 months). Coronary artery disease (CAD) was defined by clinical history of CAD, particularly myocardial infarction, angina, angioplasty, or coronary artery bypass grafting, previous angiography because of CAD or abnormal results of coronary angiography. Characteristics of patients with SLE and controls are shown in the Table 1. Control group was recruited from our general medical clinic, from subjects attending regular health check-ups and was matched for age, sex and major risk factors including hypertension, diabetes, hypercholesterolemia, CAD, PAD, TIA, MI. As expected nephritis and proteinuria were significantly more common in SLE subjects. All subjects gave written informed consent and the study was approved by the Local Ethics Committee of the Jagiellonian University(KBET/28/B/2011).

Flow cytometry analysis of antigen expression on peripheral blood monocytes

Surface antigens were studied on peripheral blood mononuclear cells (PBMC) as previously described20, 21. Briefly, PBMCs were isolated by gradient centrifugation using Lymphocyte Separation Medium LSM 1077 (PAA Laboratories GmbH, Austria) from EDTA-treated blood. PBMC were further suspended in phosphate buffer saline (PBS) containing 1% heat inactivated fetal bovine serum (FBS) (Gibco, Life Technologies, USA) and were used immediately after isolation. 250000 PBMCs were stained for 20 minutes with fluorochrome-conjugated monoclonal antibodies: anti-CD14-PerCP (MΦP9), anti-CD16-APC-H7 (3G8), anti-HLA-DR-PE-Cy7 (L243) (BD, Pharmingen, CA, USA). After staining, cells were washed twice with PBS containing 1% FBS.

Cells were processed in the FACS Verse flow cytometer and analyzed using FlowJo software (TreeStar, USA). Monocytes were gated according to FSC (forward scatter) and SSC (side scatter) signals as described previously21. Subsequently, cells were gated in a HLA-DR/CD14 plot to exclude HLA-DR negative natural killer cells. Finally, we analyzed cells for CD14 and CD16 expression, which allowed for discrimination of major monocyte subpopulations as described23. Absolute monocyte number/mm^3 was calculated20.

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Assessment of endothelial function

Endothelial function was assessed by flow mediated dilatation (FMD) measurement of the brachial artery diameter, before and after 5 minute long brachial artery occlusion (using sphygmomanometer cuff), as described previously5. Measurements were performed in a dimmed, temperature-controlled room using a Toshiba Xario Ultrasound System and an 8MHz linear transducer. Nitroglycerine mediated dilatation (NMD) was measured to study non-endothelium dependent vasodilatation by assessing vessel diameter before use of nitroglycerine (Nitromint, aerosol, 400μg s.l.) and 1, 2 and 5 minutes after sublingual nitroglycerin application5. Images were digitally recorded and analyzed using ImagePro Plus software.

Measurement of carotid artery intima-media thickness (IMT)

Intima-media thickness measurements were taken in accordance with commonly used standard methods5 at 12 different points (2 cm below common carotid artery sinuses, ca. every 1cm, omitting visible carotid plaques), on the right and left common carotid arteries. The distance from the border between the artery lumen and carotid artery intima and second bright m-line (border between media and adventitia) was measured5. Images were digitally recorded and analyzed using ImagePro Plus. Mean and maximal IMT were calculated (IMTmean and IMTmax, respectively).

Statistical analysis

Analysis was performed using Statsoft Statistica software or IBM SPSS Statistics. Compliance in the distribution of variables with normal distribution was verified using the Shapiro-Wilk test. Data are presented as means±SD. Differences of means were compared among groups by two-way ANOVA and Bonferroni's post-hoc test. When appropriate, Student's t-test was used and considered significant when $p<0.05$. Non-normally distributed variables were analyzed using non-parametric Mann–Whitney U test and are presented as median [10th–90th percentile].

Analysis of correlations between FMD, NMD, IMT, was performed using Spearman test.

Results

SLE patients exhibit higher intima-media thickness and impaired endothelial function

Studied patients with Systemic Lupus Erythematosus have a higher intima-media thickness when compared to healthy subjects (median (25th/75th percentile) 0.65 (0.60/0.71) mm vs 0.60 (0.56/0.68) mm; $p<0.05$, Figure 1A). Similarly, maximal IMT was 0.76 (0.70/0.79) mm in SLE patients vs 0.68 (0.63/0.78) mm in healthy individuals, $p<0.01$ (Figure 1B). These values were only modestly increased and did not reach the range generally considered pathological (> 0.9mm), indicating that we assessed patients at an early stage of carotid atherosclerosis.

SLE patients exhibited impaired flow mediated dilatation in comparison to healthy subjects (median (25th/75th percentile) 9.71 (6.27/11.20) % vs 13.50 (11.85/15.88) %, $p<0.01$, Figure 1C), representing the presence of a significant degree of endothelial dysfunction in

SLE. At the same time, nitroglycerin mediated dilatation did not differ between healthy subjects and SLE patients (Figure 1D).

Monocytes from SLE patients exhibit a higher percentage of CD14highCD16+ cells

No significant differences were observed in the number of total peripheral blood monocytes between patients with SLE and healthy subjects (426±212 cells/mm^3 vs 396±137 cells/mm^3). Peripheral blood monocytes after labeling with monoclonal anti-CD14 and anti-CD16 antibodies can be separated into three functionally distinct subpopulations: CD14highCD16-, CD14highCD16+ and CD14dimCD16+. We compared the content of individual monocyte subpopulations between groups of patients and control subjects. SLE was associated with an increased percentage of CD14highCD16+ monocytes when compared to healthy subjects (8.81±4.24% vs 4.89±1.51%, $p<0.01$). Similarly, the absolute number of these cells was increased (37±24 cells/mm^3 vs 19±8 cells/mm^3, $p<0.01$, Table 2). At the same time, the percentage of classical, non-activated CD14highCD16- monocytes was slightly decreased in SLE (86±5.5% vs 89.1±2.55%, $p<0.01$), while this difference was not sufficient to reach statistical significance when calculated per mm^3. No significant difference was observed in either the percentage or absolute number of CD14dimCD16+ between SLE patients and control subjects (Table 2).

Correlations of monocyte subsets with subclinical atherosclerosis in SLE patients

We next assessed the relationships between monocyte subsets (CD14highCD16-, CD14highCD16+ and CD14dimCD16+) and Intima-Media Thickness in SLE patients and controls. The percentage and the absolute number/mm^3 of CD14dimCD16+ monocytes in SLE patients positively correlated with intima-media thickness ($r=0.33$, $p=0.03$; Figure 2), while other subpopulations did not display a significant relationship. This phenomenon was not observed in non-SLE controls, suggesting that CD14dimCD16+ monocytes may be specifically associated with the risk of atherosclerosis in patients with SLE. Studied population was too small for detecting sufficient effects in multivariate analysis. Type III sum of squares ANOVA has shown that after taking into account other possible predictors of IMT (smoking, hypertension, diabetes and hypercholesterolemia) only diabetes remained independently associated with IMT in this group and CD14dimCD16+ cells showed borderline association ($p=0.1$).

Relationships between monocyte subsets and endothelial dysfunction

As endothelial dysfunction is an important mechanism of atherosclerosis, and is observed in SLE, we next analyzed the relationships between individual monocyte subpopulations and flow mediated dilatation as well as nitroglycerin induced (non-endothelium dependent) dilatation. We did not observe significant correlations between monocyte subsets (CD14highCD16-, CD14highCD16+, CD14dimCD16+) and FMD or NMD in either the SLE subjects or the non-SLE controls (Figure 3).

Discussion

Patients with systemic lupus erythematosus (SLE) have a considerable risk for premature death due to coronary heart disease and accelerated atherosclerosis6, 24, 25, although some

investigators have not shown accelerated atherosclerosis 9, while many studies show only modest increase8. Thus, the mechanisms of this increased risk remain unclear, although recent interest has focused on the importance of immune mechanisms of atherosclerosis and cardiovascular disease, which is particularly relevant to SLE in which immune dysfunction is of primary importance26. However, the exact nature of increased susceptibility to atherosclerosis in SLE is not currently known6, 25. In the studied population, with a relatively early stage of subclinical atherosclerosis (judged by IMT<0.9mm), we confirmed that SLE was associated with modestly increased carotid intima-media thickness when compared to control subjects. This is consistent with previous reports7, 25, although in the present study we observed only a modest increase in atherosclerosis, which may be related to the comparatively young age of studied patients. At the same time, we have observed that patients with SLE have strongly impaired flow-mediated vasodilatation (FMD) in response to reactive hyperemia in comparison to age- and sex- matched non-SLE individuals, and is particularly blunted in subjects with a high SLEDAI score (data not shown). This is consistent with previous data25, 27, 28. El-Magadami et al. have also shown that endothelial dysfunction in SLE patients correlated negatively with intima-media thickness and that endothelial dysfunction remained a significant predictor of IMT even after adjustment for other classic coronary heart disease risk factors29. We have shown that nitrate-mediated vasodilatation (NMD) was not reduced in SLE patients, which is in line with other studies27. Impaired FMD with preserved NMD suggests endothelial cell pathology, with vascular smooth muscle reactivity. Importantly, FMD is considered to be a good predictor of cardiovascular complications in lupus patients, but the reduction of FMD does not correlate with the extent of atherosclerotic development27. Previous studies have demonstrated that FMD decreases with increasing age and is impaired in subjects with hypertension, obesity, type 2 diabetes mellitus, hypercholesterolemia and in chronic smokers 30. The incidence of the hypertension and other risk factor in our study is comparable between SLE patients and control group to exclude the potential influence of those factors that can affect flow mediated dilatation. Moreover, it is important to note that our study was not designed or powered to compare incidence of hypertension or major risk factors/co-morbidities in SLE and general population. Karadag et al. examined novel cardiovascular risk factors and cardiac event predictors in inactive SLE patients, who did not have major cardiovascular risk factors. They found higher levels of hs-CRP and lower FMD in SLE patients when compared with the control group, suggesting that SLE patients without traditional major cardiovascular risk factors may have an increased risk of cardiovascular disease31. The mechanisms of increased susceptibility for atherosclerosis are complex in SLE and not fully recognized. These may include an autoimmune reaction with the generation of auto-antibodies, overproduction of pathogenic cytokines or formation of immune complexes. Recent data indicate that imbalances of monocyte subsets may be related to increased risk of cardiovascular complications in patients with acute coronary syndromes or CAD32. Indeed, monocyte subsets are affected by SLE, but little is known about how these changes in monocyte phenotype may be related to subclinical atherosclerosis or endothelial dysfunction observed in SLE patients. Chronic inflammation is closely linked to endothelial dysfunction, activation of immune cells and their subsequent migration into intima and sub-intimal layers of the vessel wall33. Peripheral blood monocytes constitute a heterogeneous group of cells with subpopulations characterized by high proinflammatory potential (CD14dimCD16+), a

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more quiescent phenotype (CD14highCD16-) and an intermediate phenotype (CD16highCD14+)21, 34. Imbalances between these subpopulations have been defined in various disease states including bacterial infections, rheumatoid arthritis, SLE and atherosclerosis35. Peripheral blood monocytes phenotype and functions may also change in relation to ageing36, 37, chronic inflammatory conditions or obesity 35, 38. Increased number of CD16+ monocytes was associated with cardiovascular disease and with the development of atherosclerosis16. The relationship between the frequency of CD16+ monocyte and intima-media thickness (IMT) as the marker of subclinical atherosclerosis was shown in patients with high cardiovascular risk suffering from chronic kidney disease14, 39. Recently, it has been described that CD16+ monocytes were significantly associated with obesity, IMT and subclinical atherosclerosis in low risk individuals38. CD16+ monocytes can be divided into CD14dim and CD14high cells, which display distinct phenotypic and functional properties34. The pathogenic role of these monocyte subsets in autoimmune disease and increased cardiovascular risk due to accelerated atherosclerosis is not fully understood. In the present study, we observed that patients with systemic lupus erythematosus have both a higher percentage and absolute number of CD14highCD16+ monocytes when compared to control subjects, which is interesting, as increased number of CD14highCD16+ monocytes is associated with cardiovascular events and death in a high-risk population of dialysis patients14 as well as in a population of non-dialysis patients with chronic kidney disease (CDK)39. Additional subgroups analysis between patients with nephritis and subjects without renal disturbances revealed no significant differences in the percentage of monocyte subsets in our study population. However, the limitation of this study is a relatively small group of patients with nephritis. CD14highCD16+ monocytes are correlated with the number of risk factors for atherosclerosis in patients with CAD32. On the other hand, increased levels of proinflammatory CD14dimCD16+ monocytes were observed in patients with coronary artery disease (CAD) when compared to controls16. Interestingly, increased subsets of CD14dimCD16+ cells were related to coronary plaque vulnerability in patients with stable angina pectoris40. Rogacev et al. demonstrated that CD14dimCD16+ monocytes were significantly associated with body mass index (BMI) and showed correlation with the traditional cardiovascular risk factors, whereas CD14highCD16- and CD14highCD16+ cells did not38. Pro-inflammatory CD14dimCD16+ monocytes correlate with IMT in renal transplant recipients and are independently associated with subclinical atherosclerosis in these patients41. In our study, we observed that both the percentage and absolute number of CD14dimCD16+ monocytes in SLE patients, but not in control subjects, positively correlated with subclinical atherosclerosis measured as intima-media thickness (IMT). At the same time, we have observed no relationship between flow-mediated dilatation and any of the three distinct subsets of monocytes including CD14highCD16-, CD14highCD16+ and CD14dimCD16+ in neither SLE subjects nor in controls. This suggests that pro-atherosclerotic properties of CD14dim cells may provide an additional mechanism for increased atherosclerosis susceptibility, which is independent of endothelial dysfunction.

CD14dimCD16+ monocytes have been also previously shown to positively correlate with serum cholesterol and triglyceride levels in patients with hypercholesterolemia42. Interestingly, Mosig et al. demonstrated that CD14dimCD16+ monocytes from patients with

familial hypercholesterolemia preferentially exhibit increased uptake of oxidized LDL via CD36 and a higher adherence to activated endothelial cells in response to oxidized LDL and native LDL stimulation43. This observation supports the previous finding that CD14dimCD16+ monocytes show potent capacity to invade vascular lesions. CD16+ monocytes are also considered to have a more mature phenotype when compared to CD16- and exhibit features of tissue macrophages44. CD14dimCD16+ monocytes have a proinflammatory phenotype and they are the main producers of TNF-α in human blood, whereas the anti-inflammatory IL-10 is low or absent in these cells45. This is consistent with other observations that the serum concentrations of TNF-α were elevated in subjects with the highest quartiles of CD14dimCD16+ monocytes16. This provides several direct mechanisms, through which CD14dimCD16+ could be involved in the development of human atherosclerosis38, 39. It is likely elevated production of pro-inflammatory cytokines such as TNF-α in SLE46 is associated with increased IMT and possibly faster progression for atherosclerosis. This could explain why we observed association only in SLE but not in control population. It is also possible that CD14dimCD16+ monocytes work in concert with other pathophysiological mediators in SLE that are not present in controls to promote IMT.

In summary, our study shows that CD16+CD14dim monocytes may be an important subpopulation of pro-inflammatory monocytes related to increased development of atherosclerosis in SLE. While this is interesting, no clear relationship was found between these cells and endothelial dysfunction. This could suggest that pro-inflammatory effects of these monocytes on the vascular wall are independent of nitric oxide dependent endothelial dysfunction and could be an important treatment target47, 48. This could include direct interactions with other vascular cells 42. Future mechanistic studies in SLE subjects are necessary to further elucidate the mechanism of this observation.

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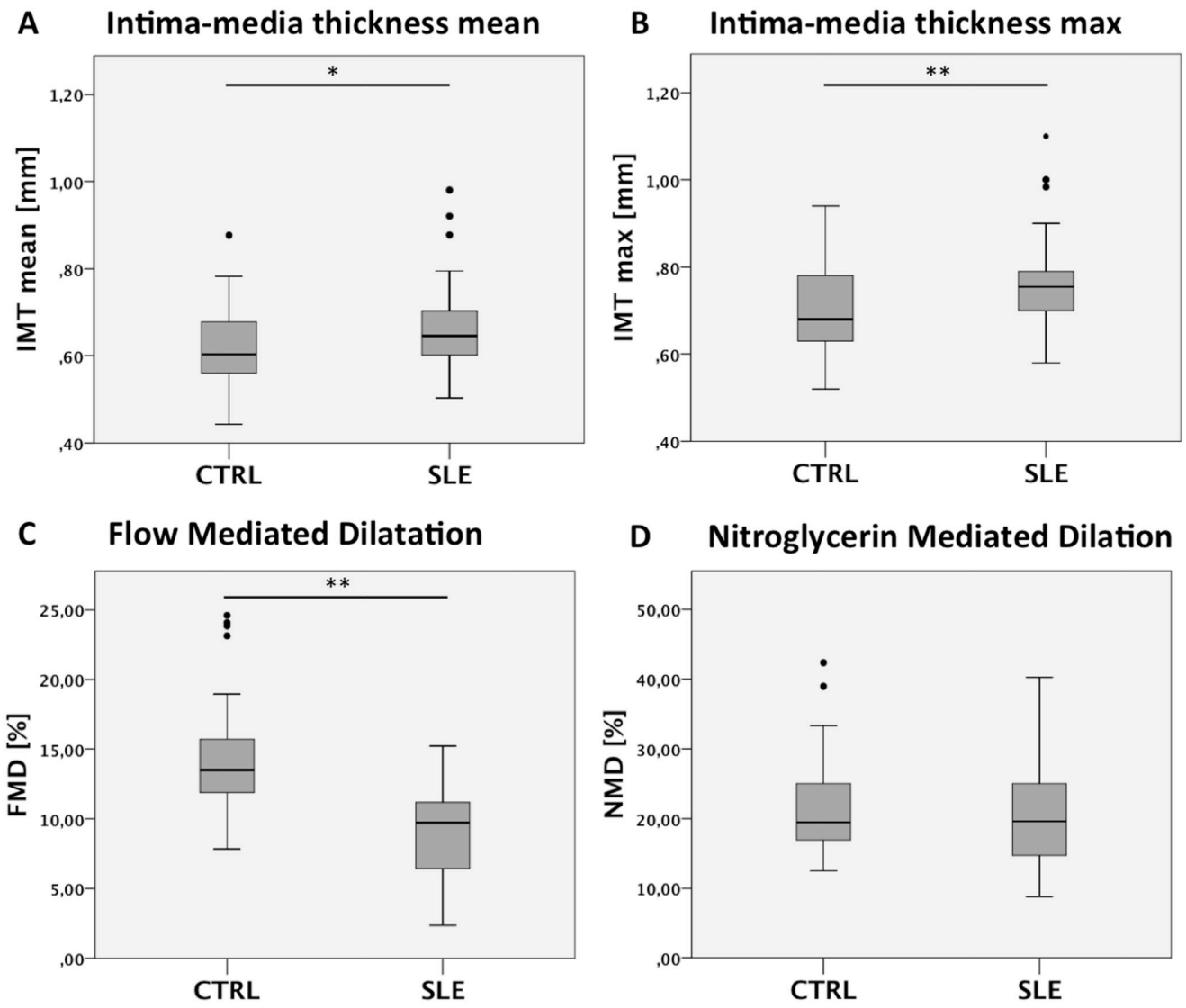

Figure 1. Subclinical atherosclerosis and endothelial function in SLE patients and non-SLE controls

Common carotid artery far wall intima-media thickness mean and max (Panel A and Panel B, respectively), flow- mediated brachial artery dilatation in response reactive hyperemia (FMD; Panel C) and nitroglycerin induced dilatation (NMD; Panel D) were measured by ultrasonography in SLE patients (SLE) and non-SLE controls matched for major risk factors for atherosclerosis (CTRL). Boxes represent the 25th and 75th percentiles and horizontal lines the median. Dots represent samples outside the 10/90 percentile bars. IMT-Intima-Media Thickness; FMD- Flow Mediated Dilatation; NMD- Nitroglycerin Mediated Dilatation; *, $p<0.05$ vs CTRL; **, $p<0.01$ vs CTRL

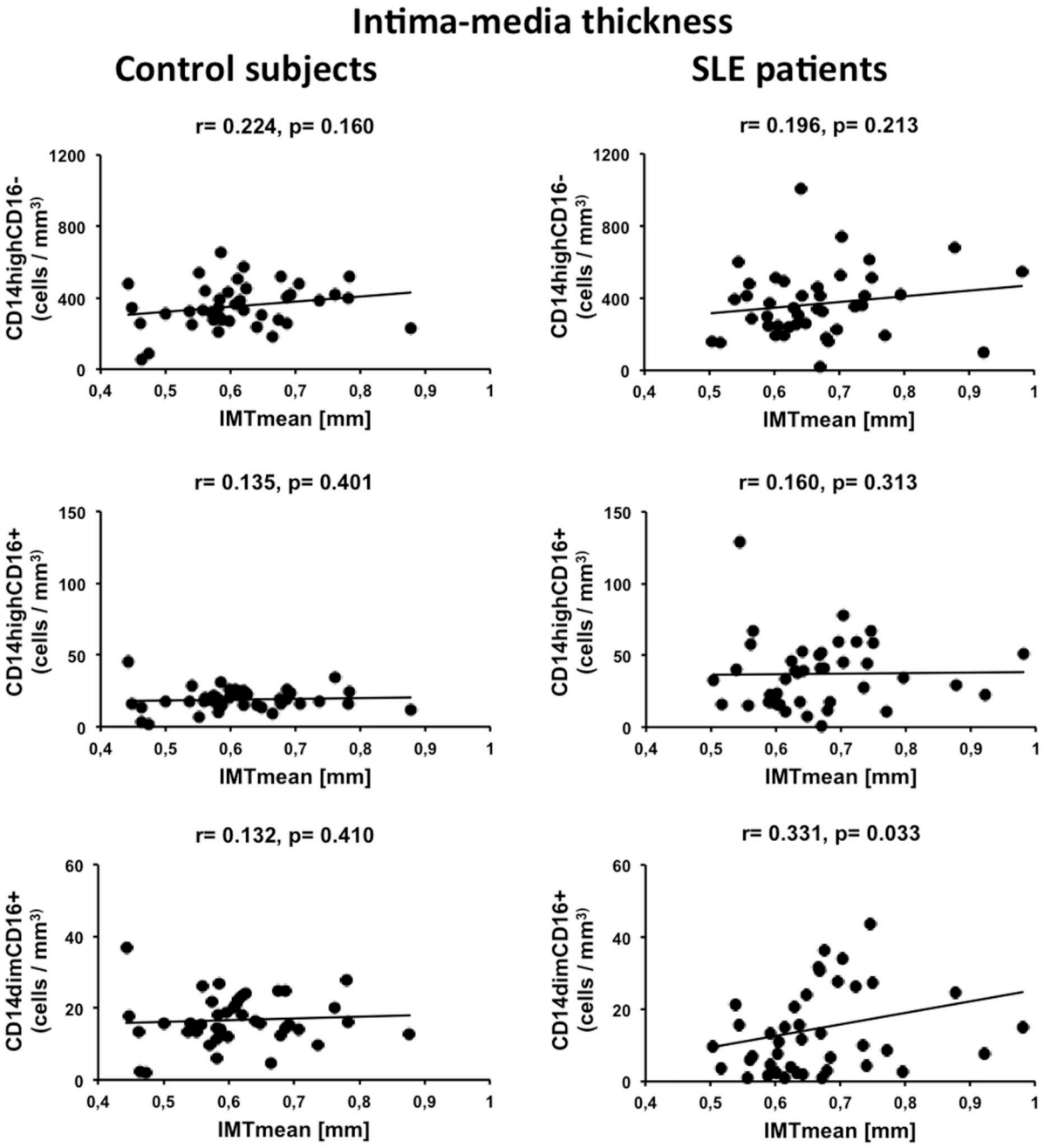

Figure 2. Relationship between monocyte subsets and subclinical atherosclerosis

The scatterplots show correlations between monocyte subsets: CD14highCD16-, CD14highCD16+, CD14dimCD16+ and Intima-media thickness (IMT) in non-SLE subjects (left panels) and SLE patients (right panels). Correlations were calculated using Spearman's rank order test.

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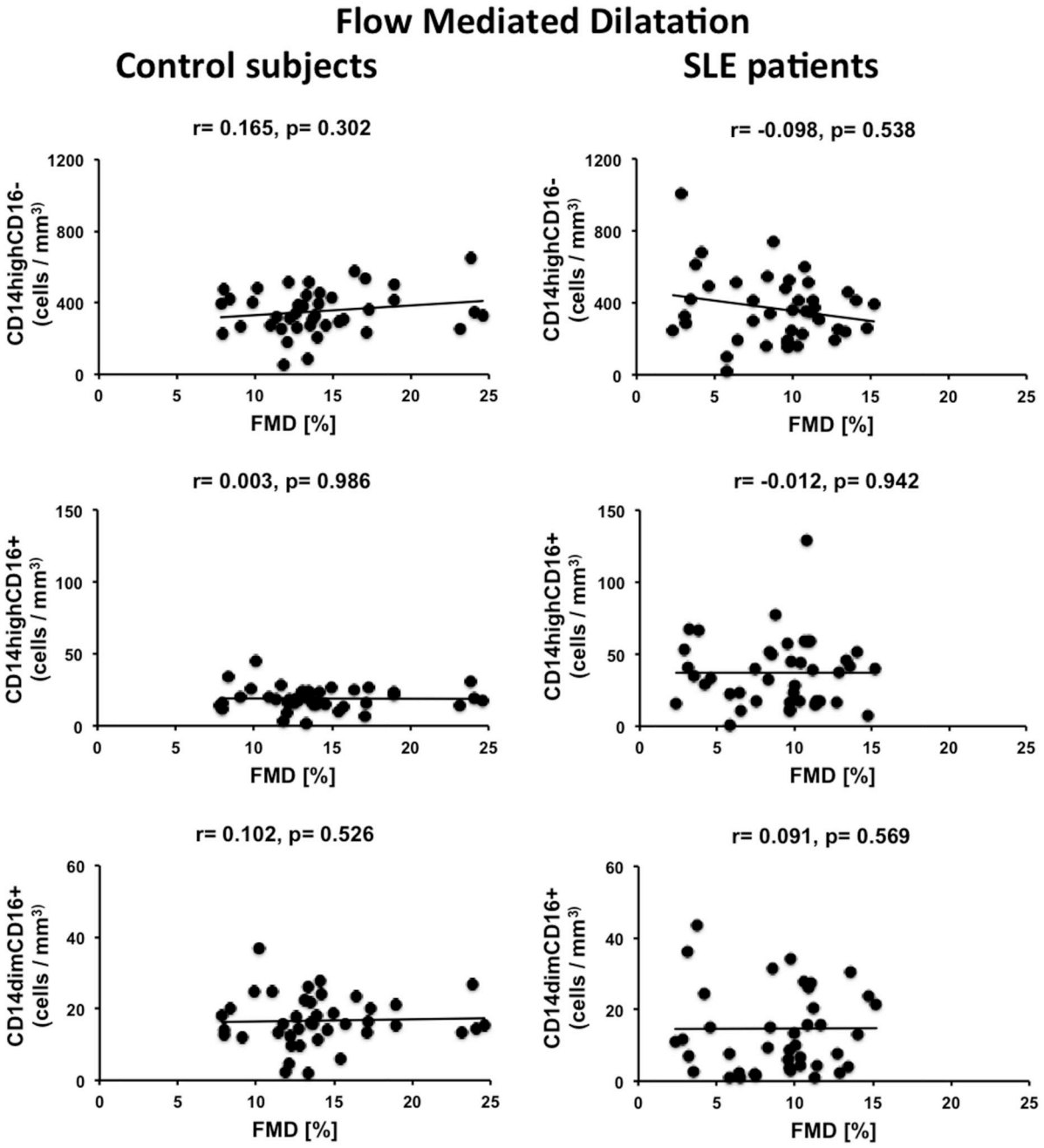

Figure 3. Relationship between monocyte subsets and endothelial function

The scatterplots show correlations between monocyte subsets: CD14highCD16-, CD14highCD16+, CD14dimCD16+ and Flow mediated dilatation (FMD) in non-SLE controls (left panels) and SLE patients (right panels). Correlations were calculated using Spearman's rank order test.

Table 1
Clinical characteristics of patients with SLE and control subjects included in this study

	Control subjects	SLE patients	p value
N	42	42	
Sex (M:F)	5:37	6:36	NS
Age (mean±SD)	41±11	44±14	0.33
Current disease activity: Active (SLEDAI≥10) Non-active and modest activity (SLEDAI<10) SLEDAI (average)	0 n/a n/a	15 27 8.02±8.11	
Immunosuppression therapy: Hydroxychloroquine/chloroquine Azathioprine Mycophenolate mofetil Cyclophosphamide Prednisone/methylprednisone: <10mg >10mg	0 0 0 0 0 0	38 8 6 5 16 20	
Risk factors:			
Smoking (n; %)	4 (9.5%)	2 (4.76%)	0.40
Hypertension (n; %)	14 (33.33%)	13 (30.95%)	0.82
Diabetes (n; %)	2 (4.76%)	2 (4.76%)	1
BMI (mean±SD)	24.13±4.28	24.46±4.53	0.73
Hypercholesterolemia (n; %)	18 (42.86%)	17 (40.48%)	0.85
Cholesterol (mmol/L; mean±SD)	5.31±1.10	5.02±1.17	0.27
CAD (n; %)	4 (9.52%)	5 (11.90%)	0.73
PAD (n; %)	3 (7.14%)	3 (7.14%)	1
TIA (n: %)	1 (2.38%)	2 (4.76%)	0.56
IS (n: %)	0	1 (2.38%)	0.32
MI (n; %)	1 (2.38%)	1 (2.38%)	1
Nephritis	0	10 (23.81%)	0.0009
Proteinuria >0.5g/day	0	4 (9.52%)	0.04
Main mediators:			
Aspirin	6 (14.28%)	9 (21.43%)	0.40
ACE-I	8 (19.04%)	10 (23.81%)	0.60
β-Blocker	7 (16.67%)	11 (26.19%)	0.29
Statin	6 (14.28%)	9 (21.43%)	0.39

BMI — body mass index; CAD — coronary artery disease; PAD — peripheral arterial disease; TIA — transient ischemic attacks; IS- ischemic stroke; MI- myocardial infraction; ACE-I — Angiotensin Converting Enzyme Inhibitor.

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Table 2

Characteristics of monocyte subsets defined by expression of CD14 and CD16 in SLE patients and control subjects

	CD14highCD16- % (Absolute cell number/mm^3)	CD14highCD16+ % (Absolute cell number/mm^3)	CD14dimCD16+ % (Absolute cell number/mm^3)
Control subjects	**89.1±2.55** (353±125)	**4.89±1.51** (19±8)	**4.23±1.30** (17±7)
SLE patients	**86±5.5** ** (368±189)	**8.81±4.24** ** (37±24) **	**3.47±2.44** (15±13.5)

** -p<0.01 vs control subjects