

University of Groningen

Premature atherosclerosis in systemic autoimmune diseases

Leeuw, Karina de

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2008

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Leeuw, K. D. (2008). *Premature atherosclerosis in systemic autoimmune diseases*. s.n.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

CHAPTER 5

ACCUMULATION OF ADVANCED GLYCATION ENDPRODUCTS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Karina de Leeuw¹, Reindert Graaff³, Rindert de Vries⁴, Robin P Dullaart⁴,
Andries J Smit², Cees G Kallenberg¹, and Marc Bijl¹

Departments of Internal Medicine, ¹Rheumatology & Clinical Immunology,
²Vascular diseases, ³Biomedical Engineering, and ⁴Endocrinology,
University Medical Center Groningen, University of Groningen,
The Netherlands

Rheumatology 2007, 46: 1151 - 1156

ABSTRACT

Objectives. To investigate whether advanced glycation endproducts (AGEs) are increased in patients with systemic lupus erythematosus (SLE), and are related to atherosclerosis, which is accelerated in SLE, and its traditional and non-traditional disease-related risk factors.

Methods. Fifty-five SLE patients with quiescent disease and 55 age- and sex-matched controls were included. The amount of skin autofluorescence (AF), as a measure for the accumulation of AGEs, was assessed by the excitation-emission matrix scanner (AF-EEMS). Traditional risk factors and disease-related factors were recorded. Plasma levels of C-reactive protein (CRP), as a marker for systemic inflammation, were assessed. Intima-media thickness (IMT) of the common carotid artery was determined by ultrasound.

Results. Skin AF-EEMS was increased in SLE patients as compared to controls (1.50 ± 0.5 a.u. vs. 1.28 ± 0.4 a.u., $p = 0.006$). Regarding all included risk factors, univariate analyses in patients revealed that AF-EEMS was associated with age ($r = 0.48$, $p < 0.001$), IMT ($r = 0.35$, $p = 0.01$), creatinine ($r = 0.29$, $p = 0.03$), damage index ($r = 0.29$, $p = 0.03$), and disease duration ($r = 0.32$, $p = 0.02$). In multivariate analysis, age and disease duration were independent predictors of accumulation of AGEs in SLE ($p < 0.001$, $p = 0.03$, respectively).

Conclusions. AGEs are increased in SLE compared to controls. Our findings indicate that AGE accumulation is associated with disease duration and might contribute to the development of accelerated atherosclerosis in SLE and, therefore, could be used for assessment of risk for long-term vascular complications.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with a chronic relapsing course. Increasing evidence shows that SLE patients have an increased risk for cardiovascular disease (CVD) due to accelerated atherosclerosis. The accumulation of advanced glycation endproducts (AGEs) is recognized as one of the factors contributing to the progression of atherosclerosis.¹ AGEs are a class of compounds resulting from glycation and oxidation of proteins, lipids or nucleic acids. Glycation is the non-enzymatic addition or insertion of saccharide derivatives to these molecules, leading to the formation of intermediary Schiff bases and Amadori products, and, finally, to irreversible AGEs.² The formation of AGEs occurs ubiquitously and irreversibly, and accelerated formation and accumulation of these compounds in diabetes contributes to their accelerated atherosclerosis.³⁻⁵ AGEs can also be formed in other conditions, such as chronic inflammation and renal failure.^{6,7} Inflammation leads to oxidative stress and consequent formation of reactive carbonyl compounds, which are partly transformed in AGEs. Apart from AGEs formation in the extracellular matrix, resulting in a decreased elasticity and increased thickness and rigidity of the vascular wall, the interaction of AGEs with a range of receptors, including the receptor for AGE (RAGE), has been implicated in endothelial dysfunction.⁸ The interaction induces oxidative stress and activation of intracellular signaling, causing secretion of cytokines and expression of cell adhesion molecules, vasoconstriction by reducing the production of nitric oxide, and coagulation.⁹⁻¹³ In a model of accelerated and advanced atherosclerosis in diabetic mice, treatment with soluble RAGE completely suppressed diabetic atherosclerosis.¹⁴ Furthermore, several clinical studies suggest that AGEs can predict long-term vascular complications.¹⁵⁻¹⁸ For example, serum AGEs have been shown to positively predict cardiovascular mortality in women without diabetes.¹⁶ Meerwaldt *et al* demonstrated that AGEs accumulation, as determined by measuring skin autofluorescence (AF), was an independent and strong predictor of cardiovascular mortality in diabetic and hemodialysis patients.^{17,18}

In systemic autoimmune diseases, such as SLE, increased AGEs formation can be expected, as inflammation is common in these conditions. Furthermore, comparable to diabetes mellitus, accelerated atherosclerosis occurs in SLE as well and cannot be fully explained by the prevalence of traditional risk factors for CVD.¹⁹⁻²⁸

Based on these data, we hypothesized that tissue accumulation of AGEs is associated with accelerated atherosclerosis in SLE. Thus, we evaluated the presence of AGEs by

measuring skin AF in relation to the presence of early atherosclerosis, as measured by intima-media thickness (IMT). Assuming accumulation of AGEs in patients, we investigated whether the extent of AGEs accumulation correlates with traditional and non-traditional risk factors for CVD, including disease-related factors, such as disease duration and damage.

PATIENTS AND METHODS

Patients

Fifty-five consecutive patients fulfilling the American College of Rheumatology (ACR) criteria for SLE²⁹, who attended the out-patient clinic of the University Medical Center Groningen, were included (table 1). Exclusion criteria were pregnancy, diabetes mellitus and active disease within 4 months before participation, defined as SLE disease activity index (SLEDAI) >4.³⁰ Fifty-five age and sex-matched healthy subjects were recruited as controls. All patients and controls were Caucasians, except for two Asian patients. Characteristics are given in table 2. The local research ethics committee gave approval for the study and informed consent was obtained from each participant.

Information was obtained regarding the presence of CVD, defined as a history of ischemic heart disease (ICD-9 classification 410-414), cerebrovascular accidents or peripheral vascular disease based on medical records. Hypertension was defined as systolic arterial pressure above 140 mmHg and/or diastolic arterial pressure above 90 mmHg, or use of antihypertensive drugs, prescribed with the aim to reduce blood pressure. Dyslipidemia was diagnosed if plasma cholesterol exceeded 6.21 mmol/l, plasma LDL cholesterol exceeded 3.36 mmol/l, plasma triglycerides exceeded 2.26 mmol/l, or use of lipid-lowering drugs.³¹ Furthermore, body mass index (BMI) and smoking status were recorded.

Current recommendations on the prevention of CVD emphasize that intervention should be based on the individual's total burden of risk for CVD rather than on the level of any particular risk factor. To obtain an estimation of the overall cardiovascular risk the SCORE (systematic coronary risk evaluation) risk analysis was used.³² This estimation of ten year risk of fatal CVD is based on the traditional risk factors gender, age, total cholesterol level, systolic blood pressure and smoking status. As younger persons are essentially risk free within the next ten years, using the SCORE adjusted for that age would give a wrong impression of the long-term risk for young people with a high number of risk factors. The cardiovascular risk estimation was, therefore,

Table 1. Disease-related factors

	Patients (n = 55)
Cumulative ACR criteria, n (%)	
Malar rash	28 (51%)
Discoid rash	16 (29%)
Photosensitivity	24 (44%)
Oral ulcers	11 (20%)
Arthritis	32 (58%)
Serositis	23 (42%)
Renal disorder	26 (47%)
Neurologic disorder	3 (5%)
Hematologic disorder	39 (71%)
Immunologic disorder	47 (85%)
Antinuclear antibody	55 (100%)
Duration of disease, months	145 (72-217)
SLEDAI	2 (0-2)
SLICC/ACR DI	1 (0-2)
Without damage caused by CVD	0 (0-1)
Presence of manifest CVD, n (%)	13 (24%)
Anti ds-DNA, Farr	7 (0-23)
Cumulative prednisolone dose, g	20 (13-39)
Cumulative hydroxychloroquine dose, g	730 (364-1188)
Cumulative azathioprine dose, g	237 (122-347)

Data are expressed as median (interquartile range).

extrapolated to the age of 60 years to circumvent such an underestimation.

Furthermore, we assessed disease-related factors that might influence the development of atherosclerosis. Next to disease duration, disease-related damage was determined at the time of enrolment in the study using the systemic lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SLICC/ACR DI).³³ The use of prednisolone, hydroxychloroquine and azathioprine were categorized as ever or never used, and quantified as cumulative dose and time of treatment.

Measurements of advanced glycation endproducts

Tissue AGEs accumulation can be assessed as skin AF, following the principles of the AGE-reader, which is a validated and non-invasive technique.^{34;35} Repeated measurements on one day in controls and diabetic patients showed an overall Altman error percentage of 5%. In this study, an adapted set-up of the AGE-reader was used,

namely the excitation-emission matrix scanner (EEMS), which is a technique to determine skin AF (AF-EEMS), which has the additional potential to discriminate between AF spectra of different fluorophores. This technique and set-up has been described elsewhere.³⁶ Briefly, approximately 4 cm² of the skin of the ventral site of the lower arm is illuminated using a 75 W Xenon lamp and a 0.2 m f/4 monochromator (PTI, New Jersey, USA). As in the AGE-reader, a fiber placed under an angle of 45° collects the light coming back from the skin, and leads it to a spectrometer. The measurement was performed at non-lesional skin, to prevent influences by the presence of skin disease in SLE patients. Skin color can also influence the measurement, as the skin reflection will decrease, when there is more pigment in the skin. The skin reflection ideally should be >6% to perform an adequate measurement. In this study, skin reflection of all patients and controls was >6%, except for 2 patients and 1 control. However, when we excluded these 3 measurements, and performed all analyses again, the same results were found. Therefore, we did not exclude these data.

A series of measurements is obtained for each subject, varying the peak excitation wavelength from 360 to 405 nm with 5 nm interval steps. Additionally, a white reference measurement is performed to obtain reflectance. Furthermore, dark measurements are performed before each measurement to correct for dark current noise of the detector-array. All actions are performed automatically using a LabView program (National Instruments, Austin, USA). For each interval step, AF-EEMS measurement is composed of the average of 50 (separate) scans. A whole series of measurements can be performed in about ten minutes. To derive the mean skin AF from the excitation-emission matrices, firstly the AF-EEMS values for each separated excitation wavelength are calculated by dividing the mean emitted intensity per nm (I_{em}) in the range between 420 and 600 nm by the mean reflected excitation intensity per nm (I_{ex}) between 300 and 420 nm for a given excitation wavelength and expressed in arbitrary units (a.u.), as was performed for the AGE-reader as well. Then, mean skin AF-EEMS is determined by calculating for each excitation step the contribution in excitation light intensity corresponding to that of a conventional AGE-reader lamp intensity spectrum with a maximum wavelength at 370 nm. The AF value is calculated off-line by automated analysis and is observer-independent.

Measurement of intima-media thickness

To measure the extent of early atherosclerosis, we measured IMT of the common carotid artery as this segment can be assessed with high reproducibility using B-mode ultrasound, as described before.³⁷

Blood analyses

Serum levels of lipids and creatinine were measured by routine techniques. Additional plasma samples for measuring levels of C-reactive protein (CRP) were stored at -20°C until analysis. CRP was measured using in-house enzyme-linked immunosorbent assays as described before.³⁷

In patients, antibodies to double stranded DNA (ds-DNA) were measured using Farr ammonium sulphate precipitation technique. Anticardiolipin antibodies and lupus anticoagulant were measured as described.³⁸ Antiphospholipid antibody status was considered positive if the levels of either IgG or IgM anticardiolipin antibodies were ≥ 40 GPL or MPL, respectively, or if lupus anticoagulant was present on more than one occasion.

Statistical methods

Except when stated otherwise, values were expressed as mean \pm standard deviation, when variables were normally distributed. In case of a non-normal distribution data were reported as median and interquartile range. Comparisons between patients and controls were made by two-sample t-tests or Mann-Whitney tests for continuous variables and by chi-square analysis for categorical variables. The univariate correlation between AF-EEMS values and other categorical variables was assessed by Pearson correlation coefficient, when variables were normally distributed. Otherwise, Spearman correlation coefficient was used. Significant correlations were defined as strong when the r value exceeded 0.5, and as moderate when the r value was between 0.26 and 0.5. To assess the influence of tested parameters on AF-EEMS, multiple linear regression analysis was performed with mean skin AF-EEMS as the dependent variable and all variables which were significantly correlated in univariate analyses as independent variables. Disease-related factors were transformed into dummy variables to perform this multivariate analysis. All analyses were performed using SPSS 12.0. A two-sided p value < 0.05 was considered to indicate statistical significance. We performed a power analysis based on the study of Meerwaldt *et al*, in which AF values

of 109 hemodialysis patients (2.4 ± 0.7 a.u.) were compared to AF values of 43 controls (1.0 ± 0.1 a.u.).¹⁷ We hypothesized that AF values of SLE patients might be in between the AF values of these patients and controls. Power analysis revealed that at least 49 patients and controls had to be included to detect a difference in AF of 0.4 a.u. with a standard deviation of 0.7 at a significance level of 0.05 with a power of 80%.

RESULTS

Clinical characteristics of patients and controls

Patients were comparable to controls regarding age, gender and BMI (table 2). The prevalence of hypertension was increased in SLE patients compared to controls. This difference was due to increased use of antihypertensive drugs among patients and not to increased level of actually measured blood pressure. No difference was found in the prevalence of dyslipidemia, however, more patients used lipid-lowering drugs than controls, probably an explanation for the increased lipid levels, including total, HDL and LDL cholesterol, found in controls. Furthermore, smoking was more frequently present among patients than controls. Although, as shown, traditional risk factors were

Table 2. Clinical characteristics of patients and controls

	Patients (n = 55)	Controls (n = 55)	p value
Age, years	43 ± 12	43 ± 13	NS
Men, n (%)	8 (15%)	8 (15%)	NS
Body mass index, kg/m ²	24 ± 4	25 ± 4	NS
Smokers, n (%)	14 (25%)	2 (4%)	<0.01
Hypertension, n (%)*	24 (44%)	8 (15%)	0.001
Antihypertensive drugs, n (%)	21 (38%)	0	<0.001
Systolic blood pressure, mmHg	128 ± 17	125 ± 18	NS
Diastolic blood pressure, mmHg	80 ± 9	78 ± 10	NS
Dyslipidemia, n (%)	23 (42%)	14 (26%)	NS
Lipid-lowering drugs, n (%)	13 (24%)	0	<0.001
Cholesterol, mmol/l	4.7 ± 0.9	5.4 ± 1.0	<0.001
HDL, mmol/l	1.5 ± 0.3	2.0 ± 0.8	<0.001
LDL, mmol/l	2.6 ± 0.8	2.9 ± 1.2	0.05
SCORE extrapolated to 60 years, %	2.7 (1.9-4.3)	2.6 (1.8-4.2)	NS
Creatinine, μmol/l	82 (74-96)	75 (68-82)	0.01
CRP, mg/l	2.5 (1.1-6)	1.6 (0.4-2.8)	0.01

Unless otherwise indicated, data are expressed as mean ± standard deviation when normally distributed and as median (interquartile range) when non-normally distributed.

not equally distributed between patients and controls, the total cardiovascular risk, calculated using SCORE, did not differ between both groups. Patients had increased levels of creatinine and CRP compared to controls.

IMT did not differ between patients and controls (0.64 (0.57-0.72) mm vs. 0.67 (0.59-0.76) mm, $p = 0.52$). Disease-related factors of the patients are presented in table 1. Median duration of SLE was 145 months. At the time of measurement 27 (49%) patients were using prednisolone with a daily median dose of 7.5 mg (minimum-maximum: 3.75-15 mg). Prednisolone dose was not changed in the 3 months prior to measurements. Furthermore, 21 (38%) patients were using hydroxychloroquine with a daily median dose of 400 mg (200-800 mg), and 15 (27%) were using azathioprine (median: 100 mg a day, 50-150 mg). Two patients also received mycophenolate mofetil, one patient methotrexate and one patient cyclophosphamide.

Concerning antiphospholipid antibodies status, nine (16%) patients were positive for lupus anticoagulant and six (11%) for either IgG or IgM anticardiolipin antibodies.

Skin AF-EEMS in patients and controls

AF-EEMS values for each separated excitation wavelength were significantly increased in SLE compared to controls with ratio's SLE:CTL ranging from 1.15 to 1.19 ($p < 0.05$). Mean skin AF-EEMS was also significantly increased in patients compared to matched controls (1.50 ± 0.5 a.u. vs. 1.28 ± 0.4 a.u., $p = 0.006$).

Using data of both patients and controls, skin AF-EEMS did not differ between smokers ($n = 16$) and non-smokers ($n = 94$) (1.42 ± 0.4 a.u. vs. 1.38 ± 0.5 a.u., $p > 0.05$) or between male ($n = 16$) and female ($n = 94$) (1.55 ± 0.7 a.u. vs. 1.36 ± 0.4 a.u., $p > 0.05$). Also, no difference between smokers and non-smokers, or between male and female were found using data of patients and controls, separately (data not shown).

Comparing those patients with manifest CVD ($n = 13$) to those without manifest CVD ($n = 42$) skin AF-EEMS did not differ (1.54 ± 0.4 a.u. vs. 1.49 ± 0.6 a.u., $p > 0.05$).

Also, use of prednisolone did not influence skin AF-EEMS, as patients who used prednisolone ($n = 27$) did not differ from those who did not receive prednisolone ($n = 28$) (1.48 ± 0.5 a.u. vs. 1.52 ± 0.5 a.u., $p > 0.05$). Furthermore, patients with antiphospholipid antibodies ($n = 14$) did not have increased skin AF-EEMS compared to those without antiphospholipid antibodies ($n = 41$) (1.48 ± 0.4 a.u. vs. 1.51 ± 0.6 a.u., $p > 0.05$).

Factors potentially related to skin AF-EEMS

Results of univariate analyses between skin AF-EEMS and risk factors for CVD are given in table 3. In patients, moderate correlations were found between skin AF-EEMS and age, serum creatinine, mean IMT, SLICC/ACR DI, as well after correction for damage caused by CVD, and disease duration (figures 1 and 2A). After correction for age, by subtracting the values of age- and sex-matched controls, disease duration remained moderately correlated with mean skin AF-EEMS (figure 2B). In controls, a moderate correlation was found between skin AF-EEMS and age.

Multivariate analysis, including the independent variables age, gender, serum creatinine, mean IMT, SLICC/ACR DI and disease duration, revealed that age and disease duration longer than 10 years were independently associated with skin AF-EEMS (table 4).

Table 3. Univariate correlations between skin AF-EEMS and risk factors for CVD

	Patients (n = 55)		Controls (n = 55)	
	R	p value	R	p value
Age	0.48	<0.001	0.44	0.001
Body mass index	-0.16	0.23	0.15	0.29
Systolic blood pressure	0.18	0.19	0.20	0.17
Diastolic blood pressure	-0.01	0.95	0.07	0.64
Cholesterol	-0.67	0.63	0.03	0.85
HDL	0.07	0.59	-0.03	0.86
LDL	-0.09	0.53	0.01	0.97
SCORE	0.19	0.17	0.10	0.49
Creatinine	0.29	0.03	0.05	0.77
CRP	0.14	0.32	0.29	0.14
Mean IMT	0.35	0.01	0.21	0.21
Duration of disease	0.32	0.02		
SLEDAI	-0.06	0.69		
SLICC/ACR DI	0.29	0.03		
Without damage caused by CVD	0.27	0.04		
Anti ds-DNA	0.05	0.73		
Cumulative prednisolone dose	-0.13	0.35		
Cumulative hydroxychloroquine dose	0.22	0.12		
Cumulative azathioprine dose	-0.05	0.71		

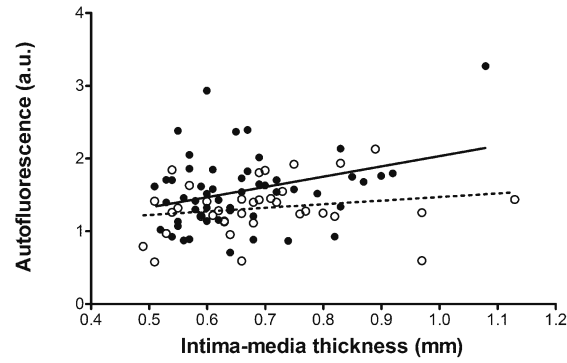


Figure 1. Correlation between autofluorescence and intima-media thickness (IMT) in patients and controls. In SLE patients, IMT is positively correlated with autofluorescence of the skin, as obtained by the Excitation-Emission Matrix Scanner (straight line, $r = 0.35$, $p = 0.01$). No correlation was found in controls (dotted line, $r = 0.21$, $p > 0.05$). Open circles represent controls and closed circles represent SLE patients.

Table 4. Multiple linear regression with AF-EEMS as dependent variable including SLE and CTL ($n = 110$)

Variable	Beta	p value
Constant	1.021	
Age	0.019	<0.001
Gender (men defined as 0)	-0.258	0.09
Mean IMT	-0.541	0.21
Creatinine	-0.001	0.61
Disease duration		
<5 years	0.18	0.33
5-10 years	0.21	0.19
10-15 years	0.33	0.03
>15 years	0.35	0.03
SLICC/ACR DI		
SLICC/ACR DI = 1	-0.057	0.72
SLICC/ACR DI >1	0.056	0.70

DISCUSSION

We hypothesized that AGEs might be increased in SLE, an essentially euglycemic disease with relapsing episodes with systemic inflammation, superposed on chronic low-grade inflammation. As AGEs contribute to atherosclerosis, their presence might be important in SLE, since these patients are prone to develop CVD. Indeed, this study shows for the first time that SLE patients have an increased accumulation of AGEs, which tended to be related to IMT.

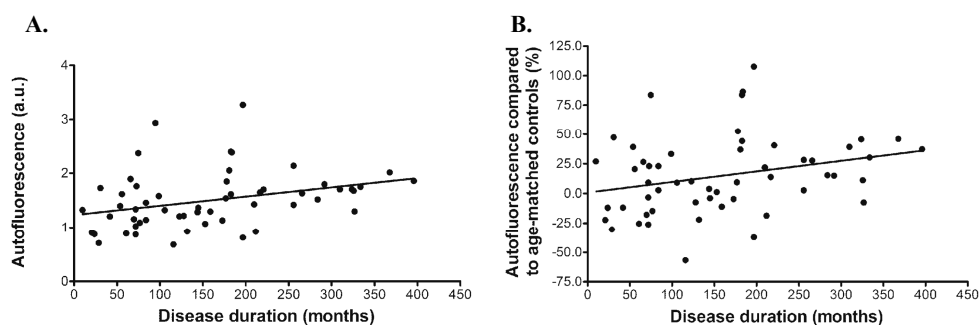


Figure 2. Correlation between autofluorescence and duration of SLE.

A. Autofluorescence, as obtained by the Excitation-Emission Matrix Scanner, is positively correlated with duration of SLE ($r = 0.32$, $p = 0.02$);

B. After correction for age, as determined using the data of the matched controls, this correlation remains significant ($r = 0.28$, $p = 0.04$).

We determined the accumulation of AGEs by measuring skin AF. This method was validated by Meerwaldt *et al*, who demonstrated that skin AF was strongly associated with AGEs as measured in skin biopsies.^{17,35} This new technique is rather simple, rapid and non-invasive. Skin lesions and skin color may influence the measurement. Recently, new software was developed in our lab to be able to perform AF measurements in more pigmented skin. A pilot study using this new software revealed that even in Fitzpatrick skin type V, AF value can be determined independently of skin pigmentation. We did not measure AGEs blood levels because AGEs in tissue may better reflect the chronic accumulation of AGEs than measuring AGE from serum or plasma.³⁴ Furthermore, there is still a lack of uniformity in assays to determine AGEs in serum or plasma.

In this study, AF-EEMS values were calculated from responses at separate wavelengths as obtained with the EEMS. The relatively equal increases in SLE patients compared to controls for all selected excitation wavelengths seem to suggest that the involved AGEs in SLE are not different from those in controls, but have accumulated more than in the controls.

Furthermore, we determined a mean value by calculating for each excitation step the contribution in excitation light intensity corresponding to that of a conventional AGE-reader lamp intensity spectrum. AF values obtained with this AGE-reader in patients with diabetes and controls (unpublished data) are higher than mean skin AF-EEMS values found in this study. This increase might be explained by slight differences

between both setups. Therefore, these AF-EEMS values cannot be directly compared to data obtained with the AGE-reader.

In previous reports it has been shown that in diabetic patients, the amount of AGEs was related to age, creatinine levels, diabetes duration, mean HbA1c of the previous year, levels of low density lipoproteins, levels of CRP, and the presence of CVD.^{17,39} We confirmed a relation between skin AF-EEMS and age in SLE patients, and between skin AF-EEMS and levels of creatinine. No relation was found between AF value and SCORE, which combines several different cardiovascular risk factors. However, this might be explained by the fact no pre-treatment values of blood pressure were used, resulting in an underestimation of the SCORE in patients. Furthermore, despite the moderate positive correlation between skin AF and IMT, no significant difference was found between patients with and without CVD. This is probably due to the small number of patients having CVD ($n = 13$). Therefore, prospective studies with a larger number of patients should be performed to investigate the relation between skin AF and CVD in SLE.

Interestingly, we also found a moderate, but significant, correlation between skin AF-EEMS and disease duration, which remained significant in multivariate analysis, when disease duration exceeded 10 years. This might suggest that cumulative disease activity itself may play an important role in the increased accumulation of AGEs in patients compared to controls. This suggestion is supported by the fact that also skin AF-EEMS and SLICC/ARC DI tended to be positively correlated ($r = 0.29$, $p = 0.03$).

Several possible mechanisms may explain increased accumulation of AGEs in SLE. First, SLE is characterized by inflammation, as demonstrated by increased levels of CRP. It has been demonstrated that AGEs can be formed in an inflammatory environment. This may explain the increased accumulation of skin AGE during the course of the disease, depicted in figure 2. Indeed, others including larger number of patients than the present study demonstrated a positive correlation between levels of CRP and skin AF.^{17,40}

Secondly, AGEs can be formed in an environment of enhanced oxidative stress, especially lipid derived AGEs, such as N^{ϵ} -(carboxymethyl)lysine. Perhaps enhanced oxidative stress, probably present in such an inflammatory environment as SLE, plays a role in the accumulation of AGEs in this disease. It would be interesting to monitor skin AF repeatedly during and after episodes of active disease. In the presence of active inflammation and oxidative stress skin AF might be even further increased.

Anti-inflammatory drugs, especially corticosteroids, might influence the formation of AGEs, as they might influence atherosclerosis.²⁷ However, it is difficult to interpret data concerning this interaction, as, on the one hand, these drugs may reduce accumulation of AGEs, as they suppress inflammation and oxidative stress, when given in sufficient doses. On the other hand, SLE patients receiving high doses of corticosteroids often have more severe disease, resulting in a more inflammatory environment, and, thus, in an increased accumulation of AGEs. In this study, we did not observe an association between skin AF-EEMS and immunosuppressive treatment. AGEs are involved in the development of atherosclerosis via different pathways.^{3-5;8-11} Through interaction with their major cellular receptor RAGE, AGEs may prime monocytes and endothelial cells, thereby amplifying pro-inflammatory mechanisms in atherosclerotic plaque formation. Therefore, we assessed, besides skin AF-EEMS, also IMT, as a measure of atherosclerosis. Indeed, a moderate, positive correlation was found between skin AF-EEMS and IMT in SLE patients. As accelerated atherosclerosis in SLE cannot be fully explained by traditional risk factors^{19;21-28}, these AGEs may constitute a link between inflammation and accelerated atherosclerosis in SLE. Moreover, when AGEs are indeed playing a pathogenic role in the acceleration of atherosclerosis in SLE, AF values cannot only be used to predict CVD, but also as a monitoring tool for treatment, as AGE accumulation can be reduced using AGE formation inhibitors or breakers, or receptor blockers.²

In conclusion, SLE patients have increased levels of AGEs, which are moderately associated with disease duration and IMT. Our data suggest that the longer the disease is present, the more AGEs have accumulated, resulting in accelerated development of atherosclerosis. Therefore, new strategies to prevent acceleration of atherosclerosis in SLE patients could aim at reduction of AGEs formation. Furthermore, studies should be performed to investigate whether this simple, rapid and non-invasive method can be used to predict cardiovascular mortality in SLE.

Conflict of Interest

Dr. R. Graaff and dr. A.J. Smit are founders of Diagnostics BV, The Netherlands, manufacturing autofluorescence readers (www.diagnostics.com).

REFERENCES

- 1 Stitt AW, He C, Friedman S et al. Elevated AGE-modified ApoB in sera of euglycemic, normolipidemic patients with atherosclerosis: relationship to tissue AGEs. *Mol.Med.* 1997; 3: 617-27.
- 2 Smit AJ, Lutgers HL. The clinical relevance of advanced glycation endproducts (AGE) and recent developments in pharmaceuticals to reduce AGE accumulation. *Curr.Med.Chem.* 2004; 11: 2767-84.
- 3 Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. *Cardiovasc.Res.* 2004; 63: 582-92.
- 4 Schmidt AM, Yan SD, Wautier JL, Stern D. Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. *Circ.Res.* 1999; 84: 489-97.
- 5 Sims TJ, Rasmussen LM, Oxlund H, Bailey AJ. The role of glycation cross-links in diabetic vascular stiffening. *Diabetologia* 1996; 39: 946-51.
- 6 Anderson MM, Requena JR, Crowley JR, Thorpe SR, Heinecke JW. The myeloperoxidase system of human phagocytes generates Nepsilon-(carboxymethyl)lysine on proteins: a mechanism for producing advanced glycation end products at sites of inflammation. *J.Clin.Invest* 1999; 104: 103-13.
- 7 Weiss MF, Erhard P, Kader-Attia FA et al. Mechanisms for the formation of glycoxidation products in end-stage renal disease. *Kidney Int.* 2000; 57: 2571-85.
- 8 Wautier JL, Schmidt AM. Protein glycation: a firm link to endothelial cell dysfunction. *Circ.Res.* 2004; 95: 233-8.
- 9 Wendt T, Harja E, Bucciarelli L et al. RAGE modulates vascular inflammation and atherosclerosis in a murine model of type 2 diabetes. *Atherosclerosis* 2006; 185: 70-7.
- 10 Yan SD, Schmidt AM, Anderson GM et al. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J.Biol.Chem.* 1994; 269: 9889-97.
- 11 Basta G, Lazzarini G, Massaro M et al. Advanced glycation end products activate endothelium through signal-transduction receptor RAGE: a mechanism for amplification of inflammatory responses. *Circulation* 2002; 105: 816-22.
- 12 Bucala R, Tracey KJ, Cerami A. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. *J.Clin.Invest* 1991; 87: 432-8.
- 13 Quehenberger P, Bierhaus A, Fasching P et al. Endothelin 1 transcription is controlled by nuclear factor-kappaB in AGE-stimulated cultured endothelial cells. *Diabetes* 2000; 49: 1561-70.
- 14 Park L, Raman KG, Lee KJ et al. Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. *Nat.Med.* 1998; 4: 1025-31.
- 15 Genuth S, Sun W, Cleary P et al. Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. *Diabetes* 2005; 54: 3103-11.
- 16 Kilhovd BK, Juutilainen A, Lehto S et al. High serum levels of advanced glycation end products predict increased coronary heart disease mortality in nondiabetic women but not in nondiabetic men: a population-based 18-year follow-up study. *Arterioscler.Thromb.Vasc.Biol.* 2005; 25: 815-20.
- 17 Meerwaldt R, Hartog JW, Graaff R et al. Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. *J.Am.Soc.Nephrol.* 2005; 16: 3687-93.
- 18 Meerwaldt R, Lutgers HL, Links TP et al. Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. *Diabetes Care* 2007; 30: 107-12.
- 19 Esdaile JM, Abrahamowicz M, Grodzicky T et al. Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. *Arthritis Rheum.* 2001; 44: 2331-7.
- 20 Leeuw K de, Freire B, Smit AJ, Bootsma H, Kallenberg CG, Bijl M. Traditional and non-traditional risk factors contribute to the development of accelerated atherosclerosis in patients with systemic lupus erythematosus. *Lupus* 2006; 15: 675-82.
- 21 Jimenez S, Garcia-Criado MA, Tassies D et al. Preclinical vascular disease in systemic lupus erythematosus and primary antiphospholipid syndrome. *Rheumatology.(Oxford)* 2005; 44: 756-61.
- 22 Selzer F, Sutton-Tyrrell K, Fitzgerald SG et al. Comparison of risk factors for vascular disease in the carotid artery and aorta in women with systemic lupus erythematosus. *Arthritis Rheum.* 2004; 50: 151-9.
- 23 Manzi S, Selzer F, Sutton-Tyrrell K et al. Prevalence and risk factors of carotid plaque in women with systemic lupus erythematosus. *Arthritis Rheum.* 1999; 42: 51-60.

- 24 Bruce IN, Urowitz MB, Gladman DD, Ibanez D, Steiner G. Risk factors for coronary heart disease in women with systemic lupus erythematosus: the Toronto Risk Factor Study. *Arthritis Rheum.* 2003; 48: 3159-67.
- 25 Svenungsson E, Jensen-Urstad K, Heimbürger M et al. Risk factors for cardiovascular disease in systemic lupus erythematosus. *Circulation* 2001; 104: 1887-93.
- 26 Doria A, Shoenfeld Y, Wu R et al. Risk factors for subclinical atherosclerosis in a prospective cohort of patients with systemic lupus erythematosus. *Ann.Rheum.Dis.* 2003; 62: 1071-7.
- 27 Roman MJ, Shanker BA, Davis A et al. Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. *N.Engl.J.Med.* 2003; 349: 2399-406.
- 28 Vlachoyiannopoulos PG, Kanellopoulos PG, Ioannidis JP, Tektonidou MG, Mastorakou I, Moutsopoulos HM. Atherosclerosis in premenopausal women with antiphospholipid syndrome and systemic lupus erythematosus: a controlled study. *Rheumatology.(Oxford)* 2003; 42: 645-51.
- 29 Tan EM, Cohen AS, Fries JF et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1982; 25: 1271-7.
- 30 Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum.* 1992; 35: 630-40.
- 31 Brevetti G, Silvestro A, Schiano V, Chiariello M. Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: additive value of flow-mediated dilation to ankle-brachial pressure index. *Circulation* 2003; 108: 2093-8.
- 32 Conroy RM, Pyörälä K, Fitzgerald AP et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur.Heart J.* 2003; 24: 987-1003.
- 33 Gladman D, Ginzler E, Goldsmith C et al. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. *Arthritis Rheum.* 1996; 39: 363-9.
- 34 Mulder DJ, Water TV, Lutgers HL et al. Skin autofluorescence, a novel marker for glycemic and oxidative stress-derived advanced glycation endproducts: an overview of current clinical studies, evidence, and limitations. *Diabetes Technol.Ther.* 2006; 8: 523-35.
- 35 Meerwaldt R, Graaff R, Oomen PH et al. Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia* 2004; 47: 1324-30.
- 36 Graaff R, Meerwaldt R, Lutgers HL et al. Instrumentation for the measurement of autofluorescence in human skin. *Proc.SPIE Int.Soc.Opt.Eng.* 2005; 5692: 111-8.
- 37 Leeuw K de, Sanders JS, Stegeman C, Smit A, Kallenberg CG, Bijl M. Accelerated atherosclerosis in patients with Wegener's granulomatosis. *Ann.Rheum.Dis.* 2005; 64: 753-9.
- 38 Brouwer JL, Bijl M, Veeger NJ, Kluin-Nelemans HC, Meer J van der. The contribution of inherited and acquired thrombophilic defects, alone or combined with antiphospholipid antibodies, to venous and arterial thromboembolism in patients with systemic lupus erythematosus. *Blood* 2004; 104: 143-8.
- 39 Meerwaldt R, Links TP, Graaff R et al. Increased accumulation of skin advanced glycation end-products precedes and correlates with clinical manifestation of diabetic neuropathy. *Diabetologia* 2005; 48: 1637-44.
- 40 Hartog JW, de Vries AP, Bakker SJ et al. Risk factors for chronic transplant dysfunction and cardiovascular disease are related to accumulation of advanced glycation end-products in renal transplant recipients. *Nephrol.Dial.Transplant.* 2006; 21: 2263-9.