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Role of Advanced Glycation End-Products as New Biomarkers in Systemic Lupus Erythematosus

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PHD THESIS

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Department of Medicine
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“The mind that opens to a new idea

never returns to its original size”

–Albert Einstein–

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Os quiero. Nos vemos en la próxima aventura.

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Love you all and see you in the next adventure.

Irene (hopefully soon to be “Doctor Doctor”)

List of abbreviations

- ACR: American College of Rheumatology
- AGEs: Advanced glycation end-products
- AHT: arterial hypertension
- ANA: antinuclear antibodies
- ANCOVA: analysis of covariance
- ANOVA: analysis of Variance
- Anti-dsDNA: anti-double stranded DNA antibodies
- APS: antiphospholipid syndrome
- bDMARDS: biological disease-modifying antirheumatic drugs
- BMI: body mass index
- C_3: complement C_3
- C_4: complement C_4
- CAD: coronary arterial disease
- cDMARD: conventional disease-modifying antirheumatic drugs
- CEIm: Comité de Ética de la Investigación con Medicamentos
- CEL: Nξ-(carboxymthyl)lysine
- CH_{50}: complement CH_{50}
- CI: confidence interval
- CML: Nξ-(carboxymethyl)lysine
- cRAGE: cleaved receptor for advanced glycation end-products
- cRAGE: cleaved receptor for advanced glycation end-products
- CRD: chronic renal disease
- CRP: C-reactive protein
- CVD: cardiovascular disease
- CVE: cardiovascular events
- CVR: cardiovascular risk
- CVRF: cardiovascular risk factors
- DAS28: disease activity score 28 joint
- DLP: dyslipidemia

- DM: diabetes mellitus
- DORIS: definition of remission in systemic lupus erythematosus
- ELISA: enzyme-linked immunosorbent assay
- ESR: erythrocyte sedimentation rate
- esRAGE: endogenous secretory receptor for advanced glycation end-products
- ESRD: end-stage renal disease
- FACIT: functional assessment of chronic illness therapy
- GG: glucocorticoids
- GLM: generalized linear models
- HAQ: health assessment questionnaire
- HC: healthy controls
- IL-6: interleukin 6
- IMT: intima-media thickness
- IS: immunosuppressants
- LDA: low disease activity
- LDL: low-density lipoprotein
- LLDAS: low disease activity score
- LN: lupus nephritis
- MDA: malondialdehyde
- NAD+: nicotinamide adenine dinucleotide
- NF-$\varkappa$B: nuclear factor kappa-B
- NSAIDs: non-steroidal anti-inflammatory drugs
- OLS: ordinary least squares
- PGA: physician global assessment
- PROs: patient reported outcomes
- PtGA: patient global assessment
- *p-val*: *p*-value
- QRISK2: QRESEARCH cardiovascular risk algorithm 2
- QRISK3: QRESEARCH cardiovascular risk algorithm 3
- RA: rheumatoid arthritis

- RAGE: receptor for advanced glycation end-products
- SADs: systemic autoimmune diseases
- SCORE: systematic coronary risk evaluation
- SD: standard deviation
- SDI: Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology (ACR) Damage Index
- SLE: systemic lupus erythematosus
- SLE-DAS: systemic lupus erythematosus disease activity score
- sRAGE: serum receptor for advanced glycation end-products
- SS: Sjögren's syndrome
- SSc: systemic sclerosis
- $STAT_3$: signal transducer and activator of transcription 3
- TET_1: ten-eleven translocation methylcytosine dioxygenase 1
- UPCR: urine protein to creatinine ratio
- VAS: visual analogue scale

Index

Abstract

Abstract

Introduction

It has been postulated that advanced glycation end-products (AGEs) and their soluble receptor (sRAGE) could play a relevant role as inducers in the chronic inflammatory pathway in various conditions; among them, in immune-mediated diseases such as systemic lupus erythematosus (SLE). However, previous studies show conflicting results about their association with SLE characteristics and their usefulness as disease biomarkers.

Objectives

To confirm differences in skin AGEs levels between SLE patients and healthy controls (HC) and to study the association of skin AGEs, serum AGEs and sRAGE levels with various disease parameters to clarify their potential as new biomarkers in SLE.

Methods

Skin AGEs concentrations were measured in 122 SLE patients by skin autofluorescence, and serum pentosidine, CML, CEL, and sRAGE by ELISA in 91-119 patients. Skin AGEs levels of patients and sex- and age-matched HC were compared in a 1:3 proportion through a multiple linear regression model. Associations of skin AGEs levels and pentosidine with demographic and clinical data, indexes of activity, accrual damage, and patient reported outcomes were analyzed through multiple linear regression models, while associations of the rest of AGEs, sRAGE and the ratios AGEs to sRAGE (non-normal) were analyzed using both an OLS regression model and a GML. All analyses were adjusted for confounders.

Results

SLE patients presented significantly higher skin AGEs levels than HC. We found a significant association between skin AGEs and several SLE activity and damage indicators (SLEDAI, SDI, PtGA, PGA, CRP, IL-6, leukocyturia, C_4) and with some disease characteristics (less ANA and anti-Ro60 antibodies and more oral ulcers). Serum AGEs and sRAGE were significantly associated with SLE activity indexes and/or demographic or disease characteristics: pentosidine with pulmonary manifestations; CML with anti-dsDNA antibodies, IL-6, disease duration, and non-Caucasian ethnicities: CEL with anti-dsDNA antibodies, IL-6, and accumulated number of manifestations; and sRAGE with male gender, photosensitivity and being on specific immunosuppressants.

Conclusions

Higher AGEs levels in SLE vs HC support the hypothesis of the connection between AGEs and SLE. The association observed between AGEs and sRAGE levels with SLE activity and damage indexes indicate that the AGEs-RAGE axis seems to have a role as a new management and prognosis biomarker in this disease. Their association with some antibodies, demographics, treatments, and disease manifestations may indicate a specific clinical phenotype related to specific higher/lower AGEs and/or sRAGE levels. The role of the ratios

AGEs/sRAGE, described for the first time in this work, requires further assessment in future studies.

Resumen

Resumen

Introducción

Se ha postulado que los productos finales de la glicación avanzada (AGEs) y su receptor soluble (sRAGE) podrían desempeñar un papel relevante como inductores de la vía inflamatoria crónica en diversas patologías; entre ellos, en enfermedades inmunomediadas como el lupus eritematoso sistémico (LES). Sin embargo, estudios previos muestran resultados contradictorios sobre su asociación con las características del LES y su utilidad como biomarcadores en esta enfermedad.

Objetivos

Confirmar las diferencias en los niveles de AGEs cutáneos entre pacientes con LES y controles sanos (CS) y estudiar la asociación de los niveles de AGEs cutáneos, séricos y sRAGE con diversos parámetros de la enfermedad con la intención de dilucidar su potencial como nuevos biomarcadores en el LES.

Métodos

Las concentraciones de AGEs cutáneos se midieron en 122 pacientes con LES mediante autofluorescencia cutánea, y pentosidina sérica, CML, CEL y sRAGE mediante ELISA en 91-119 pacientes. Se compararon los niveles de AGEs cutáneos de los pacientes y CS emparejados por sexo y edad en una proporción de 1:3 mediante un modelo de regresión lineal múltiple. Las correlaciones de los niveles de AGEs cutáneos y pentosidina con datos demográficos y clínicos, índices de actividad, daño acumulado y *patient reported outcomes* se analizaron mediante modelos de regresión lineal múltiple, mientras que las correlaciones del resto de AGEs, sRAGE y los ratios AGEs/sRAGE (no normales) se analizaron utilizando tanto un modelo de regresión OLS como un GML. Todos los análisis se ajustaron por factores de confusión.

Resultados

Los pacientes con LES presentaron niveles de AGEs cutáneos significativamente más altos que los CS. Encontramos una asociación significativa entre los AGEs cutáneos y varios indicadores de actividad y daño del LES (SLEDAI, SDI, VGP, VGM, PCR, IL-6, leucocituria, C_4) y con algunas características de la enfermedad (menos anticuerpos ANA y anti-Ro60 y más úlceras orales). Los AGEs y sRAGE séricos se asociaron significativamente con índices de actividad del LES y/o características demográficas o de la enfermedad: pentosidina con manifestaciones pulmonares; CML con anticuerpos anti-dsDNA, IL-6, duración de la enfermedad y etnias no caucásicas; CEL con anticuerpos anti-dsDNA, IL-6 y número acumulado de manifestaciones; y sRAGE con sexo masculino, fotosensibilidad y estar tomando determinados inmunosupresores.

Conclusiones

La hipótesis de la asociación entre AGEs y LES se ve confirmada por niveles significativamente más altos de AGEs cutáneos en pacientes con LES frente a CS. La correlación observada entre los niveles de AGEs y sRAGE con marcadores de actividad y daño del LES indica que el eje AGE-sRAGE parece tener un papel como nuevo biomarcador de manejo y pronóstico en esta enfermedad. Su asociación con anticuerpos, datos demográficos, tratamientos y manifestaciones concretas de la enfermedad puede indicar un fenotipo clínico específico relacionado con niveles más altos/bajos de AGEs y/o sRAGE. El papel de los ratios AGEs/sRAGE, descrito por primera vez en este trabajo, requiere mayor evaluación en futuros estudios.

1. Introduction

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a high variety of manifestations, characterized by inflammation and tissue organ damage. Its etiology is complex and not fully understood, being contributing mechanisms genetic, hormonal and environmental factors, the production of pathogenic antibodies, and the deposition of immune complexes (1). It is the prototype of systemic autoimmune diseases (SADs) with a significant disease burden across different populations all around the world. In the most recent systematic analysis of SLE epidemiology, the global SLE incidence and incidence rate were estimated to be 0.40 million people annually and 5.14 (1.4 to 15.13) per 100 000 person-years, respectively. Poland, the USA and Barbados had the highest estimates of SLE incidence (2). Regarding prevalence, the global SLE prevalence and prevalence rate were estimated to be 3.41 million people and 43.7 (15.87 to 108.92) per 100,000 persons, respectively. The United Arab Emirates, Barbados and Brazil had the highest SLE prevalence. Particularly in Spain, the prevalence was found to be 210 cases per 100 000 inhabitants (95% confidence interval (CI): 110, 400) in the EPISER 2016 study (3). It is widely described the remarkably predilection of SLE for women, having females a higher incidence of SLE compared with males in all the studies, the sex ratio ranging from 2:1 (4) to 15:1 (5). In terms of age, studies describe a peak age of incidence before declining. In females, the peak age ranged from the third to seventh decades of life. For males, the peak incidence was usually later, in the fifth to seventh decades (6).

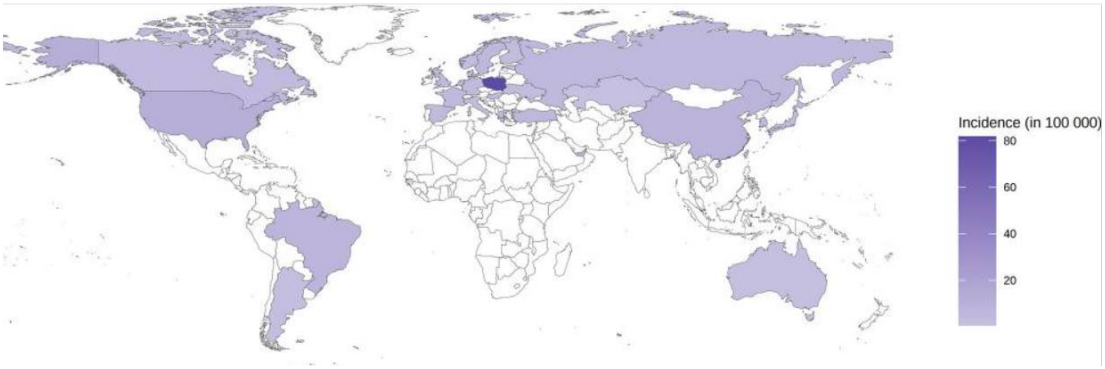

Figure 1 [Extracted from (2)]: Incidence of systemic lupus erythematosus for overall population by country.

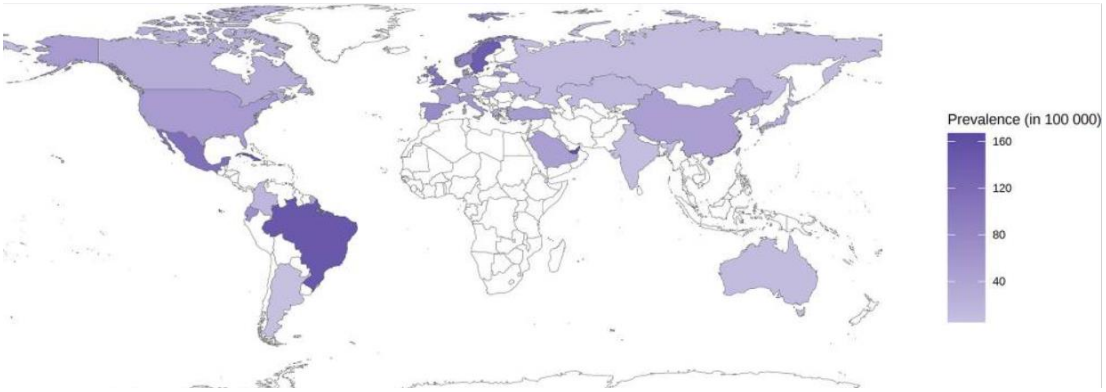

Figure 2 [Extracted from (2)]: One-year period prevalence of systemic lupus erythematosus for overall population by country.

According to this data, SLE is a widespread disease that affects people from young ages, being responsible for high morbidity and mortality. Despite advances in treatment, standardized mortality rates in SLE remain almost three times higher than in the general population, being it increased mainly due to renal disease, cardiovascular disease (CVD) and infections (7). Characteristics such as time from onset to diagnosis > 1-year, renal involvement, high SLE Disease Activity Index (SLEDAI) and severe organ involvement, may be predictors of mortality. African-American and Hispanic-American origin, low socioeconomic status and male sex have also been associated with increased mortality (8).

Besides developing novel treatments, one of the most important things to improve disease management and, consequently, prognosis, is disease assessment. Lupus heterogeneity, the impact of comorbidities like infections and atherosclerosis, as well as subjective symptoms, hampers clear disease evaluation. Because of all of the above, SLE lacks reliable and sensitive gold standard methods for measuring disease activity. Several disease activity indices have been developed over the years, each with their own positive and negative aspects (9), but there is an evident need for new instruments to provide better and improved tools for understanding and managing this disease.

Some of the most frequently used indexes for measuring activity in SLE are the SLEDAI 2000 (SLEDAI-2K) (10), the British Isles Lupus Assessment Group 2004 Index (BILAG) (11), the Lupus Foundation of America Rapid Evaluation of Activity in Lupus (LFA-REAL) (12), the Systemic Lupus Activity Measure-Revised (SLAM-R) (13) and the SLE Disease Activity Score (SLE-DAS) (14).

The SLEDAI-2K contains 24 items: 16 clinical, and 8 based solely on laboratory test results. A manifestation is recorded if it is present in the 10 days previous to the evaluation, regardless of severity or whether it has improved or worsened. The BILAG comprises specific

manifestations across nine organs/systems: constitutional, mucocutaneous, neuropsychiatric, musculoskeletal, cardiorespiratory, gastrointestinal, ophthalmic, renal, and hematological. Each item is assessed with "D = not done, 0 = not present, 1 = improving, 2 = same, 3 = worse, and 4 = new, yes, or no" according to the manifestations present in the last 4 weeks compared with the previous 4 weeks. The SLEDAI is less sensitive to change, is scored based on the 'typical' severity of a symptom, regardless of current severity in an individual patient and cannot record worsening or partial improvement. The BILAG accommodates gradations in severity, but predefined thresholds for change impede its accuracy. Moreover, it compresses different descriptors within each organ, while scoring does not increase when ≥2 descriptors within an organ are equally severe (15). Time burden and the need for special training to the raters are also caveats to the BILAG index.

The LFA-REAL is constructed as an expanded version of the physician global assessment (PGA) and comprises six or more anchored visual analog scales (VAS); the first six assess the most commonly affected organs, and other scales can be added to record features that do not fit the fixed six categories or to separately score each descriptor in organs with two or more manifestations. Although it is possible to detect slight changes in organ-specific activity with this index, these may not reflect clinically significant changes, nor are they necessarily the most discriminatory end points. There also may be measurement bias in using VAS due to potential variations in how physicians interpret these scales.

The SLAM-R includes 23 clinical manifestations in nine organs/systems and seven laboratory features that are to be measured within the previous month. A score of at least 7 is considered clinically important because it is associated with a probability of initiating therapy in more than 50 % of cases. One of its disadvantages is that many of the items are subjective, and a considerable part of the rating depends on symptoms reported by the patients. Also, it may have some difficulty distinguishing a change, in particular when scoring minimally active disease items versus damage (16).

The SLE-DAS appears as an effort to improve SLEDAI, introducing new substantial changes to that index. New items (e.g. haemolytic anaemia) were added and the relative weight of neuropsychiatric SLE was reduced, with a total of 17 items, making possible one more comprehensive and balanced assessment of the disease. As a distinctive feature of SLE-DAS, the value of various items changes according to the severity. It has also been validated against several patient reported outcomes (PROs) (17). The most important limitation would be the need to create more accurate and, perhaps, consensus-derived definitions of the items included.

The physician VAS represents a rating that reflects the clinician's assessment of the overall activity of SLE. It enables a continuous measurement of disease severity, based directly on clinical observations made during scoring. While glossary-based tools may struggle to encompass all possible scoring increments for each clinical observation, VAS offers a potential

solution to this challenge. Moreover, VAS offers the opportunity for research to identify clinically significant changes, rather than relying solely on predetermined glossary-based definitions to determine disease severity milestones (15). Unfortunately, previous studies on VAS in SLE have yielded inconsistent findings, likely due to variations in how clinicians interpret these measurement scales (18).

Apart from activity indexes, big efforts have been made to create standardized definitions to assess low disease activity (LDA)/remission in SLE in order to be able to use a treat-to-target strategy as in other rheumatological diseases. The two definitions more frequently accepted by the medical community are the lupus LDA State (LLDAS) (19), and the task-force for definition of remission in SLE (DORIS) (20), which are described in Figure 3. Both definitions have proven to be associated in different studies with several beneficial endpoints; for example, LLDAS with lower frequency of SLE flare and decreased damage progression (19) and DORIS remission with lower disease damage and hospital admissions (21). However, in the case of LLDAS, it is debated if it represents well patients with LDA because it overlaps with remission but, at the same time, it misses some patients with LDA. In addition, it includes some controversial items such as the use of a binomial disease activity scale that does not align with DORIS definition of remission and two other domains affected by wide variability (PGA and glucocorticoids (GC) dose) (22). Preliminary robust data suggest that SLE-DAS, using its cut-off for LDA, would a better tool to identify patients in true LDA than LLDAS (23). The DORIS clinical remission definition also has some disputable items like allowing low-dose prednisone or other immunosuppressants, not including the duration of remission, accepting serological activity, and the inclusion of the PGA. All those items were included after a discussion at length by the panel with the intent of generating the most useful and practical definition.

LLDAS	DORIS clinical remission on treatment[a]	DORIS complete remission[b]
SLEDAI-2K ≤4, with no activity in major organ systems and no new features of activity compared to previous assessment	Clinical SLEDAI=0	Clinical SLEDAI=0
Serological activity allowed (as long as total SLEDAI-2K ≤4)	Serological activity allowed	No serological activity
SELENA-SLEDAI PGA ≤1 (scale 0–3)	SELENA-SLEDAI PGA ≤0.5 (scale 0–3)	SELENA-SLEDAI PGA ≤0.5 (scale 0–3)
Current prednisolone (or equivalent) dose ≤7.5 mg	Low-dose glucocorticoids (e.g. prednisone ≤5 mg/ day) allowed	No glucocorticoids
Standard maintenance doses of immunosuppressive drugs and approved biological agents, excluding investigational drugs	Maintenance antimalarials, immunosuppressants and/or stable (maintenance) biologics allowed	Maintenance antimalarials allowed, but no immunosuppressants and/or biologics

Figure 3: [Extracted from (24)]: Definitions of the Lupus Low Disease Activity State (LLDAS) and the DORIS definition for systemic lupus erythematosus Serological activity – elevation of antibodies to dsDNA levels above the upper limit of laboratory normal or lowering of complement component 3 and/or 4 levels below the lower limit of laboratory normal. [a]Most attainable of the eight possible definitions of remission. [b]Least attainable of the eight possible definitions of remission. In the LLDAS SLEDAI-2K ≤4 cannot include activity in major organ systems (renal, central nervous system, cardiopulmonary, vasculitis, fever) and no haemolytic anaemia or gastrointestinal activity. *DORIS: definitions of remission in systemic lupus erythematosus; LLDAS: lupus low disease activity state; PGA: physician global assessment; SELENA-SLEDAI: Safety of Estrogen in Lupus National Assessment-SLEDAI; SLEDAI-2K: SLEDAI 2000.*

On a different note, but being part of the “how to measure” dilemma in SLE, conditions as complex as this disease benefit from the use of PROs instruments that validly and precisely measure relevant aspects of health status (e.g., symptoms) and health related quality of life (25). Generic PROs tools have been employed to assess health-related quality of life (HRQOL) in SLE patients, including the Health Assessment Questionnaire (HAQ) and the Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue. The HAQ is a questionnaire, unspecific but commonly used in SLE, that assesses limitations in different aspects of life (26). The FACIT captures multiple aspects of physical and mental fatigue, and their effects on function and daily living and has demonstrated to be a reliable and valid measure of SLE-related fatigue in clinical trials (27). However, generic PROs tools may inadequately assess certain domains of the lupus experience, such as self-image or family planning, or may not be sensitive enough to capture the frequent fluctuations in health status that are seen with SLE. SLE-specific PROs tools address these gaps. Examples of SLE-specific PRO measures include the Lupus PRO (LupusPRO) (28), the Lupus Quality of Life (LupusQoL) (29), the SLE Quality of Life Questionnaire (L-QoL) (30), the SLE-specific Quality of Life Questionnaire (SLEQoL) (31), the Lupus Impact Tracker (LIT) (32), and the Systemic Lupus Activity Questionnaire (SLAQ) (33).

We know SLE is the hallmark of inflammatory diseases, which is clearly supported by looking at the main disease activity scores, where most of the activity assessed is related to inflammation. However, routine laboratory markers of inflammation are another tool that is still limited in its impact. The erythrocyte sedimentation rate (ESR) is the most used, and it is included in three different activity indexes (the European Consensus Lupus Activity Measure (ECLAM), the SLE Index Score (SIS), and the SLAM (34)), but represents a rather crude overall measure. Anemia and reduced serum albumin are other factors to be considered when assessing inflammatory activity. However, both reflect multiple mechanisms, making their association with inflammation complex. C-reactive protein (CRP) is a more reliable marker for infections rather than for SLE activity, with which only shows a limited correlation while procalcitonin is primarily utilized in identifying severe bacterial infections. In urine, proteinuria is essential for assessing kidney involvement, but may also result from damage (35). Several cytokines are correlated with inflammatory disease activity but not currently used for routine purposes, meaning that precise and timely measurement of serological activity markers is still an issue (36).

Apart from the difficulties emerging from trying to assess activity in SLE, another important current topic is prevention of organ damage, especially since there are treatments nowadays that have demonstrated reduction of organ damage when used in the early stages of the disease (37). About 30%-50% of SLE patients will develop organ damage during the first 5 years of the disease (38) and 50% at 10 years (39), being organ damage a key determinant of poor long-term prognosis and early death in SLE patients (40). Assessment of accrual damage in SLE is performed through the Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology (ACR) Damage Index (SDI) which is a validated measure that assesses cumulative damage across multiple organs, regardless of the cause (41). The SDI defines organ damage as irreversible tissue injury occurring after SLE diagnosis; to distinguish from disease activity, most features must persist for ≥6 months, although some items are scored immediately (e.g., stroke or avascular necrosis). The SDI has shown both reliability (42) and validity (43) in the real world and as an end point in clinical trials. As organ damage is per definition irreversible (41) having available tools that could indicate which patients are going to progress to a more extensive organ damage could guide our therapeutic decisions with the aim of preventing irreversible injuries. Some of the caveats of SDI are that component scores, rather than the total score, are more relevant in defining prognosis. The renal component of the SDI has been shown to predict renal failure, and the pulmonary component predicts mortality; hence, instead of a focus on the total score, component scores should be recorded and followed (44). Furthermore, the index does not work as well for children and young adults, in whom different damage aspects as growth retardation should be considered. Due to all these weaknesses, a combined international collaboration between the SLICC, the LFA and the ACR to modernize the SDI is now in progress with four goals: widening the assessment's scope by including organ involvement before SLE diagnosis and reassessing time frames, ensuring

its validity in children, redefining diagnostic criteria with more nuanced definitions and rethinking the scoring system incorporating additional items or weighting others (45).

Taking all of that into account we are encountered with several unmet needs in SLE. The high heterogeneity of the disease and high variability in the evolution and prognosis between individuals creates a need for biomarkers. These biomarkers could help to assess activity, stratify patients in phenotypes and/or serve as predictors of distinctive characteristics of the disease or response to treatment and, ultimately, to prevent organ damage and improve outcomes. For example, it would be highly useful to have markers of an increased probability of developing specific manifestations of the disease which could grant a different follow-up, with more frequent screenings for early diagnosis, or even prevention of the development of that symptom through treatment intensification before its onset. Different approaches to finding new biomarkers are currently trying to be addressed through genetic analysis (46,47), "omics" (48), or the study of other possible serologic markers (49,50).

1.1. Advanced Glycation End-Products

Advanced Glycation End-Products (AGEs) are one type of the endogenous inducers (signals produced by stressed, damaged or otherwise malfunctioning tissues) postulated to have an important role in chronic inflammation (51). AGEs are a set of compounds whose formation is a complicated molecular process resulting from the non-enzymatic interaction of reducing sugars and associated metabolites with peptides, proteins, and amino acids, through the Maillard reaction (52). A nucleophilic addition reaction between a free amino group from a protein and a carbonyl group from a reducing sugar results in the formation of an unstable, freely reversible Schiff base, which is rearranged to a more stable intermediate, an Amadori product (53). Schiff bases and Amadori products are reversible reaction products that can react irreversibly with amino acid residues of peptides or proteins to form protein adducts or protein cross-links that accumulate on proteins (54). The most renown Amadori product in the body is glycated haemoglobin (HbA1c) which is used as an accurate marker of exposition to high persistent levels of glucose.

Despite its ability to react with free amino groups, glucose is a poor glycating agent compared to dicarbonyls such as methylglyoxal (55). The Maillard reaction generates these highly reactive dicarbonyls, also referred to as α-oxoaldehydes, which in addition to methylglyoxal, include glyoxal and 3-deoxyglucosone (56). These molecules can also be generated from glucose autoxidation, lipid peroxidation, and the polyol pathway (57). Dicarbonyls initiate the process of advanced glycation leading to the synthesis of the well characterized AGEs, Nε-(carboxymethyl)lysine (CML) and Nε-(carboxyethyl)lysine (CEL).

Besides the Maillard reaction and the oxidation of glucose, the polyol pathway represents a further mechanism leading to the formation of AGEs. An increase in intracellular glucose levels as a result of hyperglycaemia is toxic, and glucose is subsequently funneled toward the polyol pathway. In the initial step of this pathway, glucose is converted to sorbitol through the action of the enzyme aldose reductase. Subsequently, sorbitol is transformed into fructose by sorbitol dehydrogenase. Hyperactivation of the polyol pathway leads to a decrease in nicotinamide adenine dinucleotide (NAD+), the cofactor for sorbitol dehydrogenase. This depletion inhibits the glycolytic enzyme glyceraldehyde triphosphate dehydrogenase, promoting the accumulation of upstream metabolites, including fructose and triose phosphates. The buildup of these metabolites results in the formation of highly reactive molecules such as fructose 3-phosphate and dicarbonyl derivatives like glyoxal, methylglyoxal, and 3-deoxyglucosone. These reactive molecules interact with intracellular and extracellular proteins, giving rise to AGEs (58).

Lipid peroxidation products also form reactive carbonyls such as malondialdehyde and methylglyoxal, derived from the oxidation of polyunsaturated fatty acids. Additionally, reactive carbonyls are formed from ketones generated by breakdown of amino acids, including the formation of methylglyoxal from threonine catabolism. When the production of these reactive carbonyls produced by normal metabolism surpasses detoxification, AGEs accumulate. The process of AGEs formation can take days or weeks in the body (59,60), with the final concentration depending on the half-life of glycated proteins.

Amino residues such as arginine, lysine, and, to a lesser extent, cysteine, as well as nucleotides like guanosine and deoxyguanosine, are especially susceptible to dicarbonyl modification (61), leading to the formation of AGEs and DNA-AGEs such as N2(1-carboxyethyl)-2′-deoxyguanosine (CEdG).

The Maillard reaction and the alternative pathway for AGEs generation are schematically explained in Figure 4.

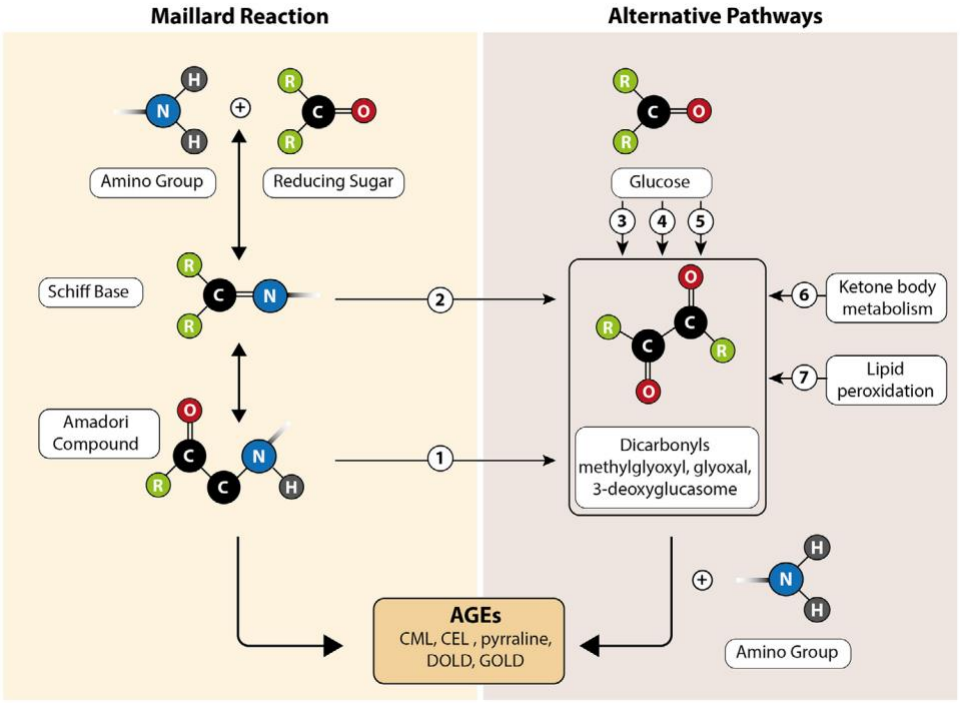

Figure 4 [Extracted from (58)]: Formation of advanced glycation end products (AGEs). Left panel: Maillard reaction. Right panel: Alternative pathways, Hodge pathway: fructosamine, non-oxidative Amadori product cleavage (1); Namiki pathway: cleavage of dicarbonyl compounds from aldimines (2); Wolff pathway: metal catalyzed glucose autoxidation (3); glycolytic pathway intermediates, for example, glyceraldehyde 3 phosphate (4); polyol (sorbitol aldose reductase) pathway (5); amino acid derived ketone body metabolism (6); lipid peroxidation (7). These pathways lead to formation of reactive dicarbonyls, which if not detoxified form AGEs, (e.g., carboxyethyl lysine [CEL], carboxymethyl lysine [CML], glyoxal lysine dimer [GOLD], 3-deoxyglucosone lysine dimer [DOLD], and pyrroline).

The accumulation of AGEs was firstly associated to ageing more than thirty years ago (62), being proposed that a buildup of these compounds may alter the structure and function of proteins, thus affecting several of the hallmarks of aging (63). However, later studies have revealed that AGEs can also accumulate under other conditions such hyperglycaemic and pro-oxidative states, including diabetes mellitus (DM) (64), CVD (65), chronic renal failure (66), and neurological disorders (67). Although this reaction can occur in all proteins, its action is more common in those that present a slow metabolic turnover, such as collagen.

The mechanisms of toxicity of AGEs are mainly related to two facts. On the one hand, glycation favors cross-links between the modified proteins, causing structural alterations and a resulting gradual deterioration in cell and tissue function (68). In addition, these unions decrease the solubility of proteins, making them more resistant to proteolysis and generating new immunological epitopes, having been observed that these oxidative modifications of proteins elicit antibodies in a variety of diseases including SLE (69). On the

other hand, AGEs are recognized by their own receptor (RAGE), a member of the immunoglobulin superfamily, which is expressed in multiple cells like neutrophils, macrophages, T lymphocytes and synovial fibroblasts (70). RAGE is divided into extracellular, transmembrane, and intracellular segments (71). The extracellular region is composed of one V-type and two C-type domains, and the V-type domain is responsible for interaction with multiple RAGE ligands. The transmembrane domain anchors RAGE to the cellular membrane, and signals transduce into the cell via the cytosolic domain. The interaction of AGEs with RAGE can produce reactive oxygen species and activate the downstream nuclear factor kappa-B (NF-κB) signaling pathway and promote the secretion of tumor necrosis factor alpha, interleukin 1, interleukin 6 (IL-6), and other cytokines, contributing to inflammation (72). It has also been described that AGEs can boost their receptor expression through the downstream signaling pathway to facilitate AGE-RAGE interaction, mainly but not exclusively through NF-κB and signal transducer and activator of transcription 3 (STAT3) signaling, the elevation of ten-eleven translocation methylcytosine dioxygenase 1 (TET1) levels and epigenetics (73).

There are three RAGE variants called N-truncated, dominant-negative, and soluble RAGE (sRAGE) (Figure 5). The N-truncated form of RAGE lacks a V-domain so that it cannot interact with ligands, whereas the cytosolic domain is missing in dominant-negative RAGE, which results in no signal transduction, though it can bind to ligands. Both of them are produced by alternative splicing of the RAGE genes (74). sRAGE is a positively charged 48-kDa cleavage product from RAGE that keeps the ligand binding site but loses the other two domains (75). sRAGE binding to ligands terminates intracellular signal transduction due to the loss of the transmembrane and intracellular fragments and inhibits the proinflammatory processes mediated by RAGE and its ligands by acting as a decoy which competitively binds to RAGE ligands (76). The binding of RAGE to its ligand leads to increased RAGE shedding and subsequent production of sRAGE through cleavage, which may reflect the expression of tissue RAGE (77), and indirectly, AGEs levels. Although to what extent AGEs contribute to RAGE and sRAGE expression remains to be clarified (78).

A decrease in sRAGE concentration, normally together with an increase in AGEs levels, is generally postulated to be found in inflammatory conditions. The deficit of sRAGE could be a primary phenomenon, allowing more AGEs to stay unbound in body fluids. It is also possible that the deficit is a secondary phenomenon, as the amount of sRAGE could be depleted by excessively generated AGEs or other ligands of this receptor. It has also been described the presence of autoantibodies specific against RAGE in rheumatoid arthritis (RA) patients. These antibodies can bind to sRAGE and form sRAGE/anti-RAGE antibody complexes and therefore might also influence sRAGE levels (79). Whether these antibodies are also present in the blood of SLE patients has not been established. Regardless of the cause, the deficit of sRAGE might contribute to more frequent interactions between AGEs and transmembrane RAGE (80). Circulating sRAGE exists in two

forms: the most prevalent one, known as cleaved RAGE or cRAGE, is produced by matrix metalloproteinases as a cleavage product of membrane-bound RAGE (81). The second form, known as endogenous secretory RAGE or esRAGE (also referred to as RAGEv1), is generated through alternative splicing of RAGE mRNA (82) (Figure 5).

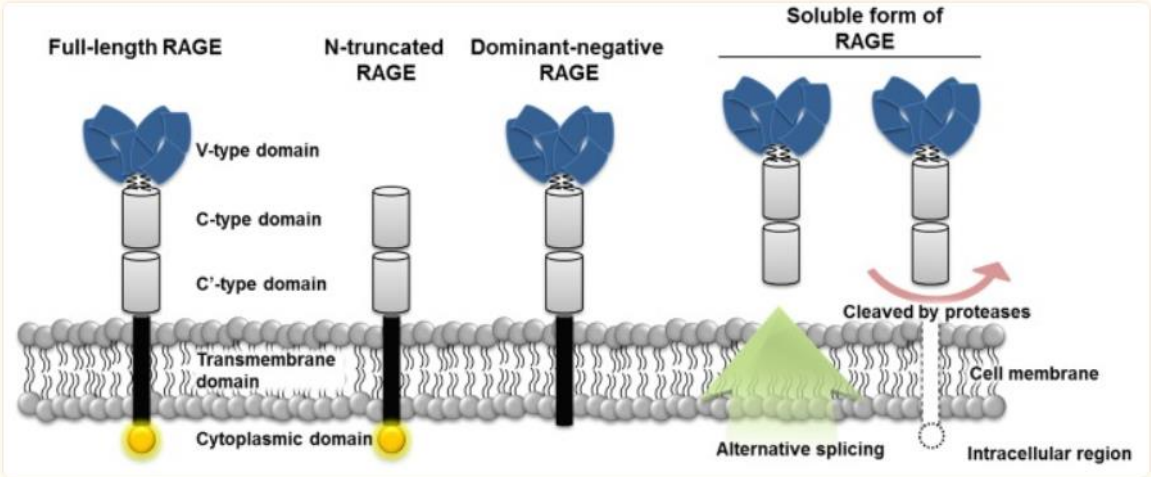

Figure 5 [extracted from (83)]: Structures of receptor for advanced glycation end-products and its three main isoforms. Full-length receptor for advanced glycation end-products (RAGE) has three different extracellular domains (V, C, C') and one cytosolic domain. Multi-ligands bind to the V-type domain and transduce signals through the intracellular domain. The N-truncated isoform lacks the V-type domain, which fails to have receptor-ligand interactions. The dominant-negative form has no cytosolic domain and is not able to transduce signals into the cell. Soluble RAGE is formed either by alternative splicing or protease activity and is secreted and prevents ligands from binding to RAGE. *V: variable domain; C: constant domain.*

As mentioned above, it is commonly proposed that low levels of sRAGE are present in several diseases related to inflammation and generation of oxygen radicals. However, elevated sRAGE levels are also contrarily found in some other diseases like diabetes and chronic renal failure where those elevated levels of sRAGE do not seem to have a protective effect as tissue injury still occurs. It is hypothesized that damage could be due to higher increases in serum levels of AGEs than the increases in the soluble receptors, being sRAGE unable to bind to all AGEs which would, instead, interact with RAGE and activate the inflammatory cascade. In end-stage renal disease (ESRD), it is unclear if high levels of sRAGE could be the result of decreased renal function or whether sRAGE is upregulated to protect against the toxic effects of AGEs. Facing that paradox of opposite sRAGE levels, some authors have studied different AGEs and RAGE parameters trying to find those that could be better indicators of tissue damage and could serve as universal risk markers. Prasad *et al*. found that the ratios of AGEs/sRAGE, AGEs/esRAGE and AGEs/cRAGE were the best biomarkers for ESRD (84). He also defended, in a different work, to use the ratio AGEs/sRAGE to assess CVD in smokers instead of using both variables independently as, similarly to what happens in ESRD and type-2 diabetes, smokers present elevated AGEs levels. Then, it is possible that sRAGE levels are not enough to handle large amounts of AGEs, and sRAGE levels on their own

are not a representative marker of the state of the AGEs-RAGE axis (85). In 2018, the same author exposed the evidence to support why a high ratio AGEs/RAGE could be meant to be a universal biomarker and why AGEs or sRAGE individually could not (86). His workgroup compared levels of AGEs, sRAGE and AGEs/sRAGE in different diseases (non-ST-elevation myocardial infarction, hyperthyroidism, thoracic aortic aneurysm, hypercholesterolemia and ESRD) vs controls. They found that both AGEs and AGEs/sRAGE were elevated in all the diseases, while sRAGE was high in ESRD and low in the rest. This inconsistency excluded the value of sRAGE as a universal marker of disease. As it is known that the AGEs-RAGE axis is composed of three essential elements (AGEs, sRAGE and RAGE) and knowing the role of all of them, they theorized that the optimal biomarker would take all three of them into account as (AGEs+RAGE)/sRAGE. However, RAGE is a cell–bound receptor and hence tissues are required for its measurement, which is not practical, especially in humans. That is why they finally concluded that the ratio of AGE/sRAGE is the best practical universal biomarker/risk marker for diseases associated with the AGE-RAGE axis. Later, Gelžinský *et al*. also studied which of the different AGEs and RAGE parameters held the strongest association with arterial stiffness measured by increased aortic pulse wave velocity, finding them to be skin AGEs and the ratio skin AGEs/sRAGE (87).

AGEs/sRAGE has also been correlated with trimethylamina-N-oxide levels, which is a marker of cardiometabolic disorders (88), with endothelial function measured by flow-mediated vasodilation (89), and with angiographically proven coronary arterial disease (CAD) in asymptomatic patients (90). In none of these works either AGEs or sRAGE values independently were associated with the studied characteristics but the ratio was. AGEs/sRAGE higher ratios have been observed in patients with mild and resistant hypertension vs normotensive patients, suggesting that hypertensive patients are less protected against the side effects of AGEs as a consequence of an insufficient competitive role of sRAGE against the AGEs-RAGE axis (91). It is important to take these observations into account when analyzing AGEs and RAGE on their own and assess if a ratio could constitute a better biomarker.

So far, more than 20 AGEs have been described in tissues. Among them, the most studied are protein adducts, such as CML, CEL or pyrraline; and intra- and intermolecular linkages, including pentosidine (very elevated in uraemia and a good marker of “carbonyl stress”), glucosepane, and imidazolium compounds (92). Due to their stability, the most measured AGEs are CML and pentosidine. Classical AGEs measurement methods include chromatographic techniques associated to mass spectrometry and immunochemical methods, such as enzyme-linked immunosorbent assay (ELISA) (93). However, some AGEs have the characteristic of being fluorescent (for example pentosidine), so it is possible to quantify them in a single measurement using an autofluorescence reader. This technique, developed by Meerwaldt *et al*. in 2004, allows, through a non-invasive method, the measurement of fluorescent AGEs stored in the skin. As it measures long-term tissue

accumulated AGEs, this assessment would be more appropriate to quantify the concentration of AGEs in an individual throughout their life rather than that of a single specific moment in relation to an acute process. So that, skin AGEs may better correlate with SLE control, duration, and complications than serum AGEs, as it has been proposed in other diseases (94). It has been described that this autofluorescent measurement correlates with the concentration of AGEs, both fluorescent and not fluorescent, measured in skin biopsies (95).

Regarding atherosclerosis, it has been observed that AGE-AGE covalent intermolecular unions in collagen I fibers induce an increase in molecular packing, causing an increase in vascular rigidity. In addition, the accumulation of AGEs in the vascular wall induces the adherence of blood cells to the endothelium, capturing immunoglobulins and apoproteins that favor the inflammatory process (96). Moreover, an AGE-modified form of low-density lipoproteins (LDL) has been found to circulate in human plasma, and AGE modifications have been identified as being present on both the apoprotein and the phospholipid components of LDL, converting them to glycated LDL. It has been proposed that those AGE-modified peptides contribute to tissue injury by reattaching to susceptible target proteins both within and outside the vasculature, making them even more atherogenic (97,98). Furthermore, oxidative modification of LDL renders it immunogenic and some autoantibodies to epitopes of oxidized LDL, such as malondialdehyde (MDA) -lysine, are found in serum and recognize the atheromatous tissue (99–102). The presence of these anti-oxidized LDL autoantibodies has been found to be associated with a more rapid progression of atherosclerosis (103).

AGEs production increases with several traditional cardiovascular risk factors (CVRF) as hyperglycemia, aging and smoking; however, there are studies which suggest that AGEs relation to CVD is independent from those CVRF (104,105). It has also been observed that AGEs skin levels are of clinical value for screening for future risk of type 2 diabetes, CVD and mortality at 4 years, independently of glycemic measures and metabolic syndrome (106). AGEs have been considered as major CVRF and proposed to be integrated in risk stratification of patients as well as in treatment decisions due to their pivotal role in the pathogenesis of cardiovascular arterial disease by being directly implicated in vascular stiffness and atherosclerosis as well as in modulation of intracellular signaling with detrimental effects in endothelial cell response, vascular smooth muscle cells function and platelet activity (107). In addition, they also interfere with cardiovascular arterial disease treatment, increasing the risk of stent restenosis (108).

Apart from age and atherosclerosis, some exogenous factors have also been reported to be positively correlated with AGEs levels like smoking status or some foods, mostly baked and roasted.(109–111). Administration of dietary AGEs in mice suggests that AGEs consumed through dry-heat-cooked food could potentially enhance the risk for age-related

diseases (112). Treatment with drugs such as aminoguanidine, vitamins, angiotensin-converting enzyme inhibitors, angiotensin-II receptor blockers, statins, and metformin inhibit AGEs formation while *Alagebrium* breaks their cross-links (113). These medications can help modulate AGE levels and mitigate their detrimental effects. Furthermore, there is evidence to suggest that circulating AGE levels may have a genetic basis. A cohort study conducted on healthy monozygotic and heterozygotic twins demonstrated that the levels of circulating AGEs can be genetically determined (114) while other studies performed in patients with renal disease have also found an association between some genetic variants and AGEs or sRAGE (115). In particular, Martens *et al*. observed that different RAGE polymorphisms are associated with susceptibility to SLE and lupus nephritis (LN), with disease severity and initial response to treatment in LN (116). This highlights the potential role of genetic factors in influencing AGEs metabolism and accumulation in the body. Moreover, other different factors have been associated with sRAGE levels: arterial hypertension (AHT), DM, body mass index (BMI), smoking, treatment duration, kidney function (117), anti-phospholipid syndrome (APS) antibodies or clinical APS (118).

1.2. Advanced Glycation End-Products & Systemic Lupus Erythematosus

SADs like SLE often exhibit elevated formation of AGEs due to the presence of chronic inflammation, which is a characteristic feature of the disease. In SLE, chronic inflammation seems to contribute to an intensified process of glycation and the subsequent formation of AGEs. Interestingly, AGEs also play a role in promoting inflammation and the generation of reactive oxygen species through their pathogenic mechanisms. This creates a cyclic process where AGEs contribute to inflammation which increases the formation of AGEs which, in turn, further exacerbates the generation of inflammation, thus establishing a vicious cycle.

Previous works in other autoimmune diseases, specifically RA, find an association between serum AGEs levels and disease characteristics, as the one by Hein *et al.*, which found that pentosidine levels correlated with interleukin 7, ESR and CRP in the exploratory analysis (119). Knani *et al*. also described that serum CML and pentosidine concentrations were significantly higher in RA patients with high disease activity (120), whereas Kageyama *et al.* detected that serum total and urinary total pentosidine levels correlated with the number of swelling joints and tender joints, and urinary total pentosidine levels correlated with the Disease Activity Score using 28 joints (DAS28) (121). Nevertheless, it is a controversial topic because other studies did show opposite results, as the one by Šenolt *et al.* which observed a correlation only with ESR levels but not with other RA markers (CRP, cartilage oligomeric matrix protein, anti-cyclic citrullinated peptide antibodies, DAS28 or functional status assessed by the HAQ) (122).

In SLE, as well, we find conflicting results in the literature although most of the studies demonstrate higher skin, serum or plasma AGEs levels in individuals with SLE compared to healthy controls (HC) (77,80,82,123,124).

Nonetheless, there are several limitations that make drawing conclusions about the role of AGEs in SLE difficult. In skin AGEs for example, there are only two previous works that study their role in SLE, both of which find higher AGEs levels in SLE patients than in HC (123,124). However, only one of those two studies (124) analyzed their association with disease characteristics, finding an association with age, creatinine, disease duration, the intima-media thickness (IMT) of the common carotid artery, and the SDI in the univariate analysis, and only with age and disease duration in the multivariate one. No other works with bigger sample size and/or more robust statistics have been performed with SLE patients and skin AGEs.

In the case of serum AGEs, scarce previous works have studied the relationship between them and SLE, which are summarized in the Supplementary Table 1. Only two works have studied the concentrations of CML and CEL in SLE (78,80), not finding differences between SLE patients and HC. Nienhius *et al.* also studied CML and CEL relationship with disease activity, without finding an association either (78). Nowak *et al*. studied three serum AGEs (CML, CEL and pentosidine) (80), not finding differences in their levels between SLE and HC but observing statistically significant differences in serum AGEs (as a group) and sRAGE vs HC. That made them conclude that, although SLE patients could be at risk of an intensified glycation process and activation of sRAGE, it is not clear which compounds contribute to the increased concentration of AGEs in the blood, seeing that levels of specific AGEs on their own were not different in patients vs HC. They did not, however, study the associations between the AGEs or sRAGE with disease characteristics. Three other works have studied serum pentosidine levels in SLE patients. Rodríguez-García *et al*. (125) did not find differences between 37 SLE patients as a group and 57 HC, although they observed that some SLE patients had AGEs concentrations up to more than three times those of the mean of the control group. They did not study, however, correlations of AGEs and SLE characteristics. Ene *et al*. (77) explored the association between 38 SLE patients with LN, 44 SLE patients without LN and 40 HC. They found differences in pentosidine and serum AGEs levels between both types of SLE patients and HC but they did not assess disease characteristics either. The work by Nisihara *et al*. did assess the relationship between pentosidine and SLE characteristics, finding lower levels of pentosidine in patients with discoid lesions and photosensitivity while positive direct Coombs test and malar rash were marginally associated ($p=0.09$) with AGEs levels, inversely and directly, respectively (126). Another work examining AGEs in general but this time in plasma, is the one by Chen *et al*. (82) which found differences between SLE and HC and a direct association between SLEDAI and plasma AGEs levels ($p<0.001$).

sRAGE has been studied in deeper detail than serum AGEs, with discrepant results summarized in Supplementary Table 2. It is worth noting that its role in SLE is not clear since, although most works have found lower levels of sRAGE in SLE vs HC (77,80,127–130), and some inconsistent relationship with some SLE characteristics or indexes, three of

them have described opposite results linking higher sRAGE with increased inflammation (78,131,132). Regarding serum sRAGE, Okukyucu *et al.* (131) observed an increase in sRAGE in SLE patients vs HC, without finding an association with disease activity markers like CRP, ESR or SLEDAI. In the same line, Manganelli *et al*. (132) found that 60 APS patients (only 35 with SLE) had higher sRAGE levels than 30 HC, while Nienhius *et al*. found higher sRAGE levels in quiescent SLE patients compared with HC and a further increase during active disease (78).

Regarding plasma sRAGE, Bayoumy *et al.* (128) and Ma *et al.* (129) found similar results when studying sRAGE in 120 SLE vs 40 HC, and 105 SLE patients vs 43 HC respectively, observing that sRAGE levels were significantly decreased in SLE patients with respect to HC and in those that had been treated for a longer period of time vs those on a short-period regime. In the Bayoumi *et al*., they also observed a significantly higher plasma levels in patients with a skin rash or serositis and a negative correlation with total white blood cell count, lymphocytes, neutrophils, and monocytes (128). Chen *et al*. (82) also found differences in plasma sRAGE levels between 36 SLE and 16 HC, observing an inverse association between SLEDAI and plasma sRAGE ($p<0.005$). Tang *et al.* (118) did not find differences in plasmatic sRAGE levels between 29 patients with SLE and APS (both APS carriers and with clinical APS) and 10 HC, nor did they found an association with SLEDAI. Yu *et al.* (130) found lower plasmatic sRAGE in 27 SLE patients with LN vs 24 HC, but only in those who did not have a flare. They also found a negative correlation between sRAGE with both esRAGE/sRAGE and C3 levels. A work by Lee *et al*. has investigated the role of infusions of sRAGE (conjugated to the Fc portion of immunoglobulin) in the treatment of LN, finding an efficacy comparable to that of standard induction treatment for LN in lupus-prone mice (133).

In SLE, the presence of accelerated atherosclerosis that cannot be fully explained by traditional risk factors for CVD is a well-recorded phenomenon (134). Despite a growing understanding of the mechanisms involved in atherosclerosis and cardiovascular risk (CVR), the optimal stratification and prevention approach for CVR in patients with SADs is still unknown. Current CVR estimators (Framingham Risk Score, Reynolds Risk Score, Systematic Coronary Risk Evaluation (SCORE and SCORE2)), underestimate this CVR in patients with SADs (135), being particularly notable in young people (136). This poses an additional challenge as SADs are very frequently diagnosed in that age range, being many patients under 40-50 years, while these calculators are not recommended in such cases.

To improve the stratification of CVR in SADs, other specific calculators have been proposed, mainly in RA, which incorporate the measurement of other variables such as the CRP or disease activity. The ones that have received the most scientific attention due to the number of patients in which they were developed have been the Expanded Cardiovascular Risk Prediction Score for RA (ERS-R) (137) and the QRISK2 (138). However,

their effectiveness is limited in cohorts other than those similar to the population included in the initial studies, which is why they have not been incorporated in current guidelines nor has their use been generalized. The most widely used method is to multiply the risk calculated by the score models by a factor of 1.5 (139), but this paradigm negates the heterogeneity of risk in patients with SADs and limits the ability to personalize preventive interventions. This lack of effective algorithms to stratify CVR in patients with SADs hinders their access to intensive interventions in primary prevention (and therefore their potential prognostic benefit) in those patients whose risk is truly increased. In 2017, some recommendations for the management of CVR in SADs were published, but currently there is no consensus, on the management of interventions to be applied to reduce this CVR (140).

In the case of SLE, several general CVR tools like the QRESEARCH cardiovascular risk algorithm 2 and3 (QRISK2 and QRISK3), the Framingham Risk Score and the modified Framingham Risk Score are used to assess CVR but there is a need to develop more accurate calculators in this population because they normally underestimate the risk in SLE patients (141). Recently, a new score that incorporates both specific factors and traditional CVRF has been proposed in SLE; however, its external validity is unknown for now (142).

Some studies have suggested that increased levels of AGEs might contribute to the development of this accelerated atherosclerosis in SLE and, therefore, could be used as early markers for CVD in this pathology. Nienhius *et al*. found a correlation between skin AGEs levels and the small arterial elasticity measured by pulse-wave analysis using tonometric recordings of the radial artery (r = -0.370, P =0.044). However they did not assess the relation with other CVRF (123). Another study by Wang *et al.* found that sRAGE levels were negatively associated with arterial stiffness measured by brachial-ankle pulse wave velocity by an automatic pulse wave analyzer in Chinese female SLE, both in the linear regression and the multivariate logistic regression analysis. In this last work, sRAGE levels were also an independent predictor of arterial stiffness in these patients (143). Levels of serum calgranulins (proteins able to bind and activate RAGE) have been found to be incremented in SLE patients, even in remission, and to possibly be associated with CVD, as well as with severe disease (144). However, De Leeuw *et al*. compared skin AGEs levels between SLE patients with and without manifest CVD (history of ischaemic heart disease, cerebrovascular accidents, or peripheral vascular disease) without being able to find differences between groups. When studying AGEs relation with classical CVRF they only found a correlation with the mean IMT in the univariate analysis, that disappeared after adjusting for age (124). However, besides a small sample size, all the patients included were in remission, which can limit the validity of the study. Consequently, there are some signs, although still unclear, that indicate that AGEs could potentially serve as early markers for CVD in this pathology.

Lately, there has been increased attention on the potential of RAGE and AGEs to target diseases, especially chronic inflammatory diseases such as SLE. Some studies have expounded on their usefulness as biomarkers of SLE monitoring and prognosis, their relationship with accelerated atherosclerosis, as well as their potential place as targets for new treatments. However, it is important to note that there are conflicting results in the existing literature, highlighting the need for further research to fully understand the role of AGEs and sRAGE in SLE. These discrepancies indicate the complexity of their involvement in SLE pathogenesis and underscore the necessity for more comprehensive and rigorous studies to shed light on their precise implications in the disease. Continued investigation will be essential for a better understanding of their potential diagnostic, prognostic, and therapeutic value in the context of SLE.

This current work aims to address this research gap by investigating both skin and serum AGEs levels (CEL, CML and pentosidine), as well as sRAGE, in a multiethnic Spanish cohort of individuals with SLE, trying to answer that unmet need through encompassing several specific goals. First, to describe skin AGEs concentrations in SLE and compare them to age- and sex-matched HC. Secondly, to explore associations between both skin and serum AGEs and sRAGE concentrations and various demographic and SLE characteristics, including specific manifestations, activity or damage indexes, and PROs. Additionally, this research seeks to examine the relationship between AGEs and CVD, as well as CVRF in the SLE population. The ultimate goal is to investigate the potential of AGEs as biomarkers for SLE in routine clinical practice. This includes their possible application for improving the monitoring and prognosis of SLE, as well as their potential as surrogate markers for assessing CVR in individuals with SLE. By addressing these objectives, the study aims to provide valuable insights into the role of the axis AGEs-sRAGE in SLE and their potential clinical utility.

2. Hypothesis

2. Hypothesis

Since SLE occurs in the context of chronic inflammation within an increase in oxidative stress, and oxidative stress is related to AGEs formation, our first hypothesis is that AGEs levels are raised in SLE patients compared to HC.

Our second hypothesis is that, as AGEs seem to be related inflammation, AGEs levels could correlate with SLE activity and damage indexes.

Given that patients suffering from SLE present a high CVR that does not correlate with classical CVRF and, considering the pathogenic role of AGEs in vascular disease (as has been demonstrated in studies in other pathologies), we hypothesize that AGEs and/or sRAGE values could be associated with CVR in SLE.

3. Objectives

3. Objectives

3.1. Primary objective

- To explore if there is an association between the concentrations of AGEs and activity or damage markers of the disease that could support the role of AGEs as a new biomarker in this disease.

3.2. Secondary objectives

- Describe the levels of skin AGEs measured by cutaneous autofluorescence as well as serum AGEs CML, CEL, pentosidine and sRAGE in a Spanish cohort of SLE patients.
- To corroborate the results obtained by other authors in relation to the higher concentration of skin AGEs in SLE patients with respect to that of the general population.
- To investigate associations between skin or serum AGEs or sRAGE with SLE characteristics that could differentiate phenotypes of the disease.
- To assess if AGEs values are related to the presence of cardiovascular events (CVE) in SLE patients and, if so, calculate the relative risk of suffering a CVE according to the concentration of AGEs detected in the patient.

4. Methodology

4. Methodology

4.1. Subjects

Cross-sectional study conducted at the Hospital del Mar where patients of all ages who were visited at the SLE outpatient clinic, met the 1997 ACR (145) or the 2012 SLICC classificatory criteria (146) for SLE, accepted to participate and signed the informed consent were randomly included. The exclusion criteria were pregnancy, DM, treatment with GC at a dose equivalent to prednisone > 20 mg/day, active malignancy, and fibromyalgia.

4.2. Healthy controls

The control population was selected from the ILERVAS cohort (Vascular and Renal Translational Research Group, IRBLleida), which includes HC selected from primary care health centers, with at least one traditional CVRF and aged between 50 and 70 years if women or between 45 and 65 years if men. The traditional CVRF included were: AHT and/or dyslipidemia (DLP) and/or obesity (defined as a BMI >30 Kg/m^2), and/or history in first-degree relatives of premature cardiovascular disease (men before 65-year-old and women before 60 years-old) and/or smokers and former smokers (< 10 years since quitting). Exclusion criteria were: history of cardiovascular disease (angina, myocardial infarction, cerebrovascular accident, peripheral arterial disease, intestinal ischemia or ischemia of some other territory), history of carotid surgery or surgery of arteries from other territories, DM and/or chronic renal disease (CRD), institutionalized population, population on long-term home-care, active neoplastic processes, and life expectancy < 18 months (147). AGEs levels were measured by autofluorescence in all the HC.

4.3. Variables

A blood test was performed at the moment of the AGEs skin measurement. Variables were categorized according to categories already established in the literature (f.i: remission, low activity…), to tertiles, or to individualized categories according to their distributions on our sample. Multiple variables were recorded: demographics, disease characteristics, different indexes for measuring SLE activity and accrual damage, PROs, cardiovascular variables, and AGEs. With respect to the analysis of autoantibodies, antinuclear antibodies (ANA) were determined by indirect immunofluorescence and considered positive if >1:80, anti-Ro60 and anti-Sm antibodies were determined by either multiplex immunoassay, being positive if titers>1 antibody indexes or by blot, and anti-double stranded DNA (anti-dsDNA) antibodies by multiplex immunoassay with titers > 10 UI/mL considered positive. AGEs measurement is specified in Methodology section 4.4 while the other variables and their classifications are detailed in the Supplementary Figure 1.

4.4. Assessment of accumulated AGEs/skin AGEs

In all patients, accumulated AGEs were measured non-invasively in the skin by an auto-fluorescence reader (Age Reader Mu Connect®) as described previously in the literature (95). A light source emitting light at a wavelength of 320 to 400 nm excites fluorescent moieties in compounds in the skin to produce fluorescence at a wavelength of 420 to 600 nm (peak 440 nm). The output represents the ratio between autofluorescence in the range 420 to 600 nm and excitation light in the range 320 to 400 nm and is reported in arbitrary units (AU). Three consecutive AGEs measurements were taken from the ventral (anterior) surface of the forearm of each participant 10 cm below the elbow fold, avoiding any tattoos or heavily pigmented areas of skin. Measurements were performed at room temperature, while patients were in a seated position (148) (See Figure 6). The mean value of the three measures was calculated and compared with AGEs values from age-matched HC obtained from previous works (95). This comparison was visually expressed in five possible categories as < -1 standard deviations (SD), [-1SD-mean), mean, (mean-1SD], >1SD.

Figure 6: Advanced glycation end-products reader and how they were measured in the ventral side of the forearm of subjects.

4.5. Assessment of serum AGEs

The ELISA method was used to evaluate the concentrations of three AGEs (pentosidine, CML and CEL), as well as sRAGE in the serum samples of each patient. During the study, the following ELISA kits were used according to the manufacturer's instructions:

- Human pentosidine Sandwich ELISA Kit (Cusabio Biotech Co. Ltd., CSB-E09415h); sensitivity 7.81 *pmol*/mL; precision measured as coefficient of variation < 8% (intra-assay), < 10% (inter-assay).

- Human Nξ-(carboxymethyl)lysine (CML) Sandwich ELISA Kit (Cusabio Biotech Co. Ltd., CSB-E12798h); sensitivity 15.6 pg/mL; precision measured as co-efficient of variation < 8% (intra-assay), < 10% (inter-assay).

- Human Nξ-(carboxyethyl)lysine (CEL) Sandwich ELISA Kit (Cusabio Biotech Co. Ltd., CSB-EQ027210HU); sensitivity 0.078 nmol/mL; precision measured as coefficient of variation < 8% (intra-assay), < 10% (inter-assay).

- Human receptor for AGEs, (RAGE/AGER) Sandwich ELISA Kit (Cusabio Biotech Co. Ltd., CSB-E09354h); sensitivity 19.5 pg/mL; precision measured as coefficient of variation < 8% (intra-assay), < 10% (inter-assay).

In CEL assessment, some patients could not be included in the analysis due to the use of a different, and not comparable, ELISA kit, due to discontinuation of the original kit used.

4.6. Ethics approval and consent to participate

All patients signed the informed consent form to participate in the study. The protocol for our study was consistent with the provisions of the Declaration of Helsinki and was approved by the Ethics Committee of the Hospital del Mar (CEIm-PSMAR 2018/7907/I).

4.7. Sample size

A random sample of 60 individuals with systemic lupus erythematosus and of 183 healthy controls was calculated to be sufficient to estimate , with 95% confidence, a β risk of 0.2 in a two-sided test, and an accuracy of ± 0.25 units, the population mean of values (with an expected standard deviation of about 0.6 units (124). A flux diagram indicating the sample size used for each analysis is provided in Supplementary Figure 2.

4.8. Statistical methods

In all analysis, categorical data were described with absolute and relative frequencies, whereas continuous variables were displayed as mean (SD), or as median (interquartile range) if non-normally distributed. Regarding the confounding variables, their possible interaction with the main variables was evaluated in the regression models and visualized through graphs. In addition, some continuous variables included in the final models were mean centered to facilitate interpretation. The assumptions of linearity, homoscedasticity and normality of the residuals were verified and the presence of influential points in each

model was evaluated through the Cook's distance. All statistical work was conducted using R version 4.1.2.

4.8.1. Comparison of accumulated AGEs between patients and controls

HC were sex- and age-matched with a factor of approximately 3:1 to each of the SLE patients and selected according to the common variables between both groups. Due to the limited age range of our control group, some of the SLE patients had to be excluded as it was not possible to age-match them with HC. In addition, SLE patients with CVD could not be included in the analysis due to it being an exclusion criterion in the HC sample.

In order to identify potentially confounding variables, in addition to a bibliographic review about previously reported factors related to AGEs, an exploratory analysis was performed splitting the sample into cases and HC, and by tertiles of AGEs. In the case of categorical variables, we employed the Fisher's exact test for variables with small frequencies and the χ^2 test for the rest. For normal continuous variables, the Student's t-test was used when analyzing two groups and the analysis of variance (ANOVA) when there were more than two. For non-normal continuous variables, the test used was the Mann-Whitney U test to compare two groups and the Kruskal-Wallis' test to compare more than two.

Variables with statistically significant differences ($p<0.1$) both between groups and AGE tertiles were considered potential confounding factors and were included in the final multiple linear regression models (fixed-effects analysis of covariance (ANCOVA)) to avoid spurious associations.

4.8.2. Relation between characteristics of SLE and skin AGEs

An exploratory analysis was conducted using ANOVA tests adjusted for both age and current smoking status to investigate the association between SLE patient characteristics and the level of accumulated AGEs, including all patients from the cross-sectional study. For a better analysis, skewed variables of interest were categorized into tertiles or according to non-linear patterns, evaluated with general additive models. Associations with a p value < 0.1 were considered significant and, if consistent, were examined individually. First, the identification of potentially confounding variables was performed as described in the previous analysis (4.8.1). Then, multiple linear regression models studying association between skin AGEs levels and each variable of interest were fitted considering the corresponding confounding factors. Only associations with a *p*-value <0.05 were reported.

If there were any missing data in the study blood test variables or in the retrieved SLE characteristics, the patient was excluded. If there were missing data in other variables, the patient was included assuming the lack of statistical power due to missing data.

4.8.3. Relation between characteristics of SLE and serum AGEs

The initial exploratory analysis using ANOVA tests was performed similarly to what is described in the previous section for skin AGEs (4.8.2). In the case of serum AGEs and sRAGE, however, we did not systematically adjust it for neither smoking status nor age unless the association showed some statistical significance ($p<0.1$) as their contribution to serum levels is not as clearly defined as to skin AGEs levels.

Due to the right-skewed distribution of CEL, CML and sRAGE (Supplementary Figure 3), different multivariate regression models, suitable for log-normal data were investigated with the aim of handling both heteroscedasticity and non-normality and estimating the absolute effect of each predictor. Pentosidine, on the other hand, was analyzed using multiple linear regression models as detailed for skin AGEs in 4.8.2.

Finally, multivariate analysis was performed using both ordinary least squares (OLS) regression model and generalized linear models (GLM) with gamma distribution and the identity link function. The assumptions of both models were evaluated assuming that the OLS model would be heteroskedastic in most of the analyses; therefore, the GLM model was used to verify and provide more evidence to the results obtained in the OLS model.

We also analyzed the associations with the ratios between serum AGEs or skin AGEs and sRAGE, as some authors have defined that the ratios could be better biomarkers than AGEs or RAGEs on their own (See 1.1).

Missing data were treated as described in 4.8.2.

5. Results

5. Results

5.1. Characteristics of patients and controls

The differences between the 189 HC and 62 cases are shown in Table 1. HC had a higher BMI and a higher incidence of dyslipidemia (both in total cholesterol and LDL values), obesity, hypertension, and active smoking. Patients with SLE had higher AGEs values and creatinine concentrations. As all the HC were Caucasian, we performed a sensitivity analysis to assess the influence of ethnicity, testing only Caucasian patients against HC. We did not find any differences, so we kept all the ethnicities in the final analysis.

Variables	Controls	Cases	*p*-value
	N = 189	N = 62	
Ethnicity			**<0.001**
Caucasian	189 (100%)	46 (74.2%)	
Other	0 (0.00%)	16 (25.8%)	
Age	56.0 [52.0;62.0]	55.0 [51.0;61.8]	0.193
Sex: Female	180 (95.2%)	58 (93.5%)	0.748
Hypertension	73 (38.6%)	14 (22.6%)	**0.032**
Obesity	61 (32.3%)	12 (19.4%)	**0.075**
Dyslipidemia	85 (45.0%)	9 (14.5%)	**<0.001**
Smoking			**0.054**
Never	79 (41.8%)	24 (38.7%)	
Former (>1 year)	54 (28.6%)	27 (43.5%)	
Active	56 (29.6%)	11 (17.7%)	
Body mass index	28.9 (5.98)	25.6 (4.65)	**<0.001**
Creatinine	0.70 [0.61;0.77]	0.74 [0.64;0.90]	**0.006**
Uric acid	4.90 (1.27)	4.70 (1.62)	0.365
Cholesterol	210 (37.5)	187 (39.5)	**<0.001**
HDL	61.9 (14.0)	65.9 (15.7)	0.125
LDL	138 (29.3)	112 (34.6)	**<0.001**
Triglycerides	123 [95.8;160]	92.0 [70.0;159]	**0.003**
Dyslipidemia drugs	27 (14.3%)	11 (17.7%)	0.649
Antihypertensives	61 (32.3%)	16 (25.8%)	0.424
AGEs	1.98 (0.45)	2.71 (0.56)	**<0.001**
AGEs in tertiles			**<0.001**
[1.0,1.9)	83 (43.9%)	3 (4.84%)	
[1.9,2.4)	74 (39.2%)	13 (21.0%)	
[2.4,4.2]	32 (16.9%)	46 (74.2%)	

Table 1: Descriptive characteristics of cases and healthy controls and exploratory analysis between both groups. Bold indicates statistically significant variables with *p*-values <0.1. *HDL: High-density lipoprotein; LDL: Low-density lipoprotein; AGEs: advanced glycation end-products.*

5.2. Comparison of skin AGEs in SLE patients vs healthy controls

First of all, in order to evaluate possible confounding factors, we explored the associations between AGEs levels (stratified in tertiles) and data of all the participants of the study (both SLE patients and HC). The exploratory analysis showed a significant positive relationship between smoking and AGEs levels, while creatinine showed a trend in that

same direction. On the contrary, the presence of dyslipidemia was associated with lower values of AGEs (Table 2). Analyzing both groups separately, a significant positive association was found between tertiles of AGEs and both age and smoking, in the two groups. In HC, a significant negative association with dyslipidemia was also found (data not shown).

Variables	[1.0,1.9)	[1.9,2.4)	[2.4,4.2]	Global *p*-value	*p*-value for trend
	N = 86	N = 87	N = 78		
Ethnicity				0.006	0.001
Caucasian	86 (100%)	81 (93.1%)	68 (87.2%)		
Other	0 (0.00%)	6 (6.90%)	10 (12.8%)		
Age	55.0 [51.0;60.0]	56.0 [53.0;63.0]	56.0 [53.0;61.8]	0.112	**0.090**
Sex				0.447	0.363
Men	5 (5.81%)	6 (6.90%)	2 (2.56%)		
Women	81 (94.2%)	81 (93.1%)	76 (97.4%)		
Hypertension	35 (40.7%)	30 (34.5%)	22 (28.2%)	0.244	**0.094**
Obesity	24 (27.9%)	30 (34.5%)	19 (24.4%)	0.344	0.646
Dyslipidemia	42 (48.8%)	34 (39.1%)	18 (23.1%)	**0.003**	**0.001**
Smoking				**0.040**	0.153
Never	34 (39.5%)	46 (52.9%)	23 (29.5%)		
Former (>1 year)	30 (34.9%)	23 (26.4%)	28 (35.9%)		
Active	22 (25.6%)	18 (20.7%)	27 (34.6%)		
Body mass index	28.5 (5.69)	28.4 (6.30)	27.2 (5.45)	0.264	0.147
Creatinine	0.69 [0.59;0.77]	0.71 [0.61;0.79]	0.72 [0.64;0.84]	0.135	**0.046**
Uric acid	4.85 (1.17)	5.02 (1.49)	4.68 (1.42)	0.281	0.454
Cholesterol	214 (40.5)	200 (34.6)	199 (40.8)	**0.021**	**0.014**
HDL	59.4 (12.5)	63.9 (14.5)	66.1 (16.0)	**0.079**	**0.027**
LDL	142 (31.7)	122 (28.3)	121 (36.5)	**0.003**	**0.002**
Triglycerides	134 [93.2;160]	120 [96.0;157]	94.5 [76.2;160]	**0.085**	**0.026**
Dyslipidemia drugs	15 (17.4%)	12 (13.8%)	11 (14.1%)	0.762	0.544
Antihypertensives	27 (31.4%)	27 (31.0%)	23 (29.5%)	0.962	0.794

Table 2: Descriptive table of cases and healthy controls according to tertiles of advanced glycation end-products and exploratory analysis. Bold indicates statistically significant differences indicated by p <0.1. *AGEs: advanced glycation end-products; HDL: High density lipoprotein; LDL: Low density lipoprotein.*

According to these results and the differences found between patients with SLE and HC, age, creatinine, smoking, and dyslipidemia were chosen as confounding variables and evaluated with interaction graphs (Figure 7). We found differences in the slopes of age and dyslipidemia (Figure 7b-c). Furthermore, in the smoking interaction plot (Figure 7a), we observed that the slopes of never smokers and former smokers behaved similarly, with only a slight increase in mean cumulative AGEs in never smokers with SLE, but apparently insignificant, so, to increase statistical power, we unified never smokers and former smokers in the same group compared to active smokers.

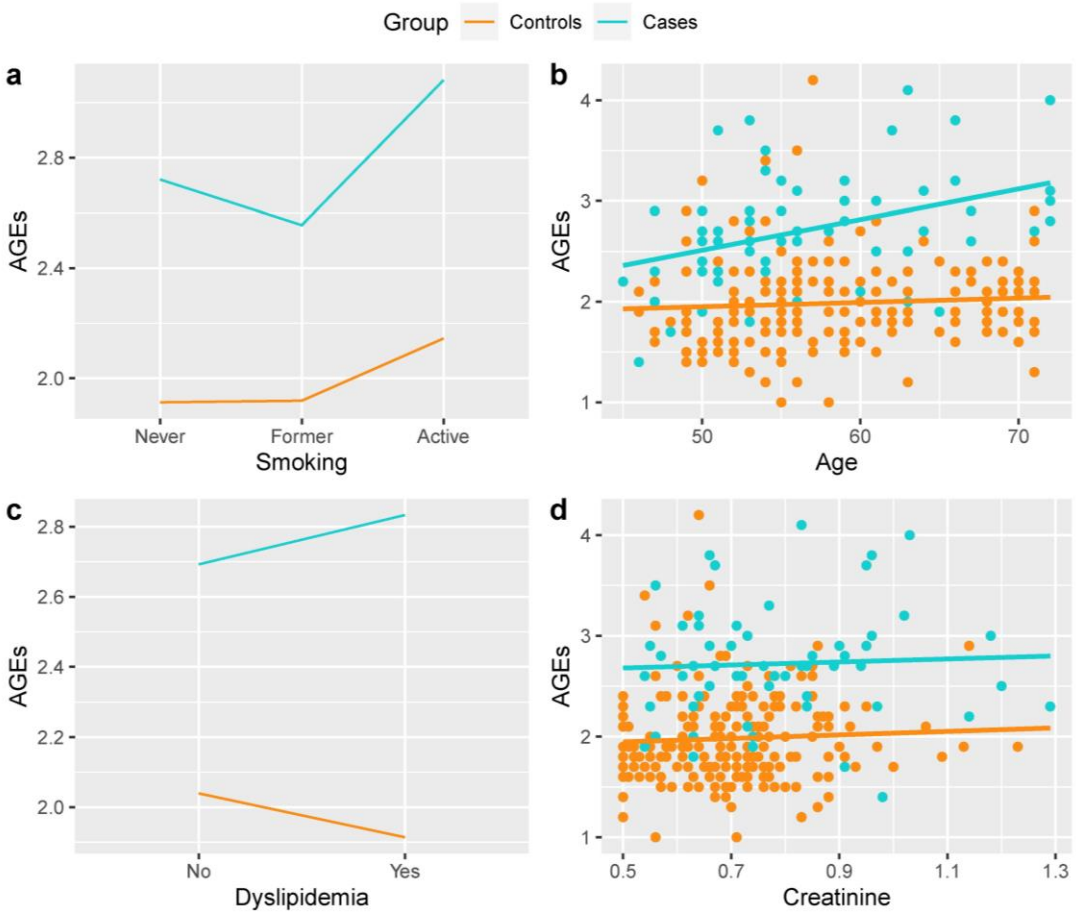

Figure 7: Interaction graphs with skin advanced glycation end-products: a) Smoking; b) Age; c) Dyslipidemia; d) Creatinine.

Finally, according to all the data explored, the multivariate regression model (fixed-effects ANCOVA) was adjusted with age, smoking, dyslipidemia, and creatinine. Interactions that visually seemed significant in Figure 7 were evaluated, but none of them ended up being significant, so they were finally omitted. The final model reported a statistically significant difference between SLE and HC in AGEs values, showing that AGEs values in patients with SLE were 0.745 (95% CI [0.605, 0.885]) units higher than those in HC ($p<0.0001$). See Table 3 for fixed-effects ANCOVA model and Figure 8 for the effects plot.

	Estimate.	2.5%	97.5%	t val.	*p*-value
(Intercept)	1.9331	1.8383	2.0268	40.6324	<0.0001
Group: Cases	0.7450	0.6048	0.8852	10.4667	<0.0001
Age (57.5 years)	0.0172	0.0086	0.0257	3.9666	0.0001
Smoking (Active)	0.3298	0.1983	0.4614	4.9379	<0.0001
Creatinine (0.72 mg/dL)	0.2244	-0.1608	0.6096	1.1474	0.2523
Dyslipidemia	-0.1065	-0.2277	0.0148	-1.7269	0.0850

Table 3: Fixed-effects analysis of covariance (ANCOVA) model to study differences in skin advanced glycation end-products levels between cases and healthy controls adjusted by confounders (in grey).

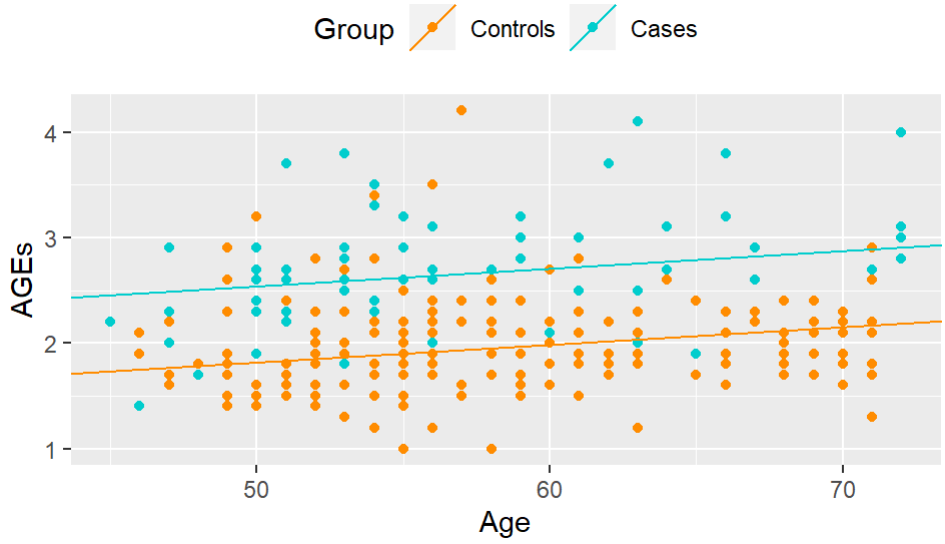

Figure 8: Effects graphic of differences in skin advanced glycation end-products values between cases and healthy controls according to age. *AGEs: advanced glycation end-products.*

5.3. Analysis of skin AGEs and associations with SLE

5.3.1. Characteristics of SLE patients according to skin AGEs levels: exploratory analysis

A total of 122 SLE patients were included. All the variables that showed statistically significant differences according to skin AGEs tertiles in the exploratory analysis are depicted in Table 4, adjusted by age (*p*-value M1) and by both age and smoking (*p*-value M2). The demographic characteristics and other SLE variables of interest are detailed in the Supplementary Table 3.

Variables	All	1st tertile [1.2,2.3)	2nd tertile [2.3,2.8)	3rd tertile [2.8,4.6]	*p-val* M1	*p-val* M2
	N=122	N=44	N=41	N=37		
Age	50.4 (14.9)	41.8 (13.8)	49.9 (12.2)	61.2 (11.9)		**<0.001**
Smoker	32 (26.2%)	10 (22.7%)	11 (26.8%)	11 (29.7%)	**<0.001**	
cDisease duration (years)					**0.082**	**0.090**
0-5	50 (41.0%)	19 (43.2%)	18 (43.9%)	13 (35.1%)		
6-10	16 (13.1%)	7 (15.9%)	6 (14.6%)	3 (8.11%)		
11-20	33 (27.0%)	13 (29.5%)	11 (26.8%)	9 (24.3%)		
>20	23 (18.9%)	5 (11.4%)	6 (14.6%)	12 (32.4%)		
Classificatory Criteria and Other Clinical and Serological Data						
Oral ulcers ever	50 (41.0%)	13 (29.5%)	18 (43.9%)	19 (51.4%)	**0.022**	**0.033**
Arthritis ever	92 (75.4%)	31 (70.5%)	32 (78.0%)	29 (78.4%)	**0.070**	**0.092**
Renal disease ever	8 (6.56%)	2 (4.55%)	1 (2.44%)	5 (13.5%)	**0.067**	**0.054**
cNumber of manifestations					**0.032**	**0.069**
[3, 7)	58 (47.5%)	19 (43.2%)	21 (51.2%)	18 (48.6%)		
7	24 (19.7%)	10 (22.7%)	8 (19.5%)	6 (16.2%)		
[8,12]	40 (32.8%)	15 (34.1%)	12 (29.3%)	13 (35.1%)		
Disease Activity Indexes						
SLEDAI	4.00 [2.00;6.00]	4.00 [0.00;6.00]	4.00 [2.00;6.00]	6.00 [2.00;8.00]	**0.016**	**0.041**
cSLEDAI					**0.003**	**0.008**
Remission/Mild	71 (58.7%)	29 (67.4%)	25 (61.0%)	17 (45.9%)		
Moderate	39 (32.2%)	11 (25.6%)	14 (34.1%)	14 (37.8%)		
Severe	11 (9.09%)	3 (6.98%)	2 (4.88%)	6 (16.2%)		
SDI	0.00 [0.00;1.00]	0.00 [0.00;1.00]	0.00 [0.00;1.00]	1.00 [0.00;2.00]	**0.026**	**0.007**
cSDI_3					**0.052**	**0.017**
0-2	110 (90.9%)	41 (95.3%)	38 (92.7%)	31 (83.8%)		
3-4	8 (6.61%)	2 (4.65%)	2 (4.88%)	4 (10.8%)		
5-6	3 (2.48%)	0 (0.00%)	1 (2.44%)	2 (5.41%)		
PGA	2.00 [1.00;3.00]	1.50 [1.00;2.00]	2.00 [1.00;3.00]	2.00 [1.00;2.00]	**0.083**	**0.051**
cPGA					**0.051**	**0.029**
<1	18 (14.9%)	7 (16.3%)	6 (14.6%)	5 (13.5%)		
1-2	69 (57.0%)	27 (62.8%)	19 (46.3%)	23 (62.2%)		
>2	34 (28.1%)	9 (20.9%)	16 (39.0%)	9 (24.3%)		
Patient Reported Outcomes						
FACIT	17.5 [10.0;27.0]	14.0 [9.00;23.0]	22.0 [13.0;30.0]	18.0 [10.0;28.0]	**0.099**	**0.138**
PtGA	2.75 [1.00;5.00]	2.00 [1.00;3.00]	3.00 [2.00;5.00]	3.00 [1.00;5.00]	**0.028**	**0.042**
cPtGA					**0.112**	**0.121**
[0.0,2.5)	57 (46.7%)	26 (59.1%)	14 (34.1%)	17 (45.9%)		
[2.5,4.5)	28 (23.0%)	9 (20.5%)	12 (29.3%)	7 (18.9%)		
[4.5,8.0]	37 (30.3%)	9 (20.5%)	15 (36.6%)	13 (35.1%)		
Serological Variables						
GPT*	17.0 [13.0;22.0]	16.0 [12.0;22.5]	16.0 [13.0;20.0]	18.0 [15.0;23.0]	**0.095**	**0.068**
Total cholesterol*	181 (37.7)	172 (29.6)	174 (38.0)	201 (39.5)	**0.046**	**0.093**
cCRP*					**0.058**	**0.053**
[0.03,0.12)	45 (37.2%)	24 (55.8%)	8 (19.5%)	13 (35.1%)		
[0.12,0.28)	36 (29.8%)	11 (25.6%)	17 (41.5%)	8 (21.6%)		
[0.28,3.92]	40 (33.1%)	8 (18.6%)	16 (39.0%)	16 (43.2%)		
cIL-6*					**0.049**	**0.025**
[0.63, 1.88)	36 (33.3%)	18 (48.6%)	12 (31.6%)	6 (18.2%)		
[1.88, 3.33)	36 (33.3%)	11 (29.7%)	14 (36.8%)	11 (33.3%)		
[3.33,144.10]	36 (33.3%)	8 (21.6%)	12 (31.6%)	16 (48.5%)		
ANA+*	112 (92.6%)	43 (100%)	38 (92.7%)	31 (83.8%)	**0.027**	**0.036**
Anti-Ro60+*	45 (37.8%)	17 (40.5%)	19 (47.5%)	9 (24.3%)	**0.183**	**0.164**
C4*	19.8 (8.23)	18.5 (7.97)	18.7 (7.09)	22.4 (9.23)	**0.025**	**0.017**
Leukocyturia*	0.00 [0.00;1.00]	0.00 [0.00;0.00]	0.00 [0.00;1.00]	1.00 [0.00;2.00]	**0.004**	**0.001**
Hematuria*	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.00 [0.00;1.00]	**0.031**	**0.067**
cLeukocyturia*					**0.052**	**0.024**
0	72 (60.0%)	33 (78.6%)	24 (58.5%)	15 (40.5%)		

Variables	All	1st tertile [1.2,2.3)	2nd tertile [2.3,2.8)	3rd tertile [2.8,4.6]	*p-val* M1	*p-val* M2
1	25 (20.8%)	6 (14.3%)	11 (26.8%)	8 (21.6%)		
[2,5]	23 (19.2%)	3 (7.14%)	6 (14.6%)	14 (37.8%)		
Treatments						
GC	30 (24.6%)	7 (15.9%)	11 (26.8%)	12 (32.4%)	**0.004**	**<0.001**
Current dose of GC	5.00 [2.50;10.0]	7.50 [3.75;10.0]	5.00 [2.50;12.5]	5.00 [2.50;6.25]	**0.050**	**0.029**
Tacrolimus	1 (0.82%)	0 (0.00%)	0 (0.00%)	1 (2.70%)	0.147	**0.083**
cTreatment					**0.077**	**0.092**
No IS	66 (54.1%)	27 (61.4%)	20 (48.8%)	19 (51.4%)		
IS	56 (45.9%)	17 (38.6%)	21 (51.2%)	18 (48.6%)		

Table 4: Variables that showed statistically significant differences according to skin advanced glycations end-products tertiles in the exploratory analysis. M1: adjusted by age, M2 adjusted by age and smoking. "c" indicates variables which have been categorized as previously stated in the methodology section. Bold indicates *p*-value <0.1 and * indicates values according to the blood test performed in the study. *p-val: p-value; SLEDAI: SLE disease activity index; SDI: Systemic Lupus International Collaborating Clinics /American College of Rheumatology (SLICC/ACR) Damage Index; PGA: Physician global assessment; FACIT: Functional Assessment of Chronic Illness Therapy – Fatigue Scale; PtGA: Patient global assessment; GPT: Glutamic-pyruvic transaminase; CRP: C-reactive protein; IL-6: interleukin-6; ANA: antinuclear antibodies; C4: complement C4; GC glucocorticoids; IS: Immunosuppressants (includes treatment with methotrexate, leflunomide, tacrolimus, mycophenolic acid or mycophenolate mofetil acid, azathioprine, cyclophosphamide, cyclosporine, rituximab or belimumab).*

5.3.2. Associations between skin AGEs and SLE characteristics: multivariate analysis

SLE characteristics that were significant in the exploratory analysis and that might be related to skin AGEs levels were tested in a model adjusted for previously selected confounding variables (see 4.8.2), avoiding spurious associations. After adjustment, several SLE characteristics showed associations with skin AGEs levels.

First of all, two of the most important SLE disease indexes, SLEDAI and SDI, were significantly associated with skin AGEs levels. For the SLEDAI we found a progressive increase in skin AGEs values as the SLEDAI activity escalated, being skin AGEs values in patients with moderate and severe activity 0.2 (95% CI [0.0006; 0.4], p=0.0493) and 0.52 (95% CI [0.177; 0.86], p= 0.003) units higher than in patients in remission/mild activity. Regarding the SDI, we only found differences in the SDI between those with low (0-2) and high scores (5, 6) (AGEs values were 0.717 (95% CI [0.139;1.295], p=0.0156) units higher in the second group). This association of skin AGEs with disease activity is also reflected in the relation found with both the PGA and the patient global assessment (PtGA). In those cases, values > 1 in PGA or > 3 in PtGA were associated with a skin AGEs increase. A PGA score = 1-2 and a PGA score > 2 had AGEs levels 0.033 (95% CI [0.058;0.61], p=0.018) and 0.39 (95% CI [0.094;0.694], p=0.01) units higher, respectively, than patients with a PGA = 0. Patients with a PtGA score > 3 had AGEs levels 0.26 (95% CI [0.063;0.46], p=0.01) units higher than patients with PtGA score ≤ 3. Regarding serum biomarkers, we observed an increment in AGEs levels as CRP and IL-6 increased, but significant differences were only detected between the 3rd and 1st tertile, increasing skin AGEs levels 0.259 (95%CI

[0.035;0.48], p=0,02) units for CRP and 0.352 (95% CI [0.1;0.6], p=0.006) for IL-6. An increase in skin AGEs units in the 3rd tertile was also observed in leukocyturia (0.369 units increase, 95%CI [0.112;0.626], p=0.005) and C4 complement (0.28 units increase (95%CI [0.056;0.514], p=0.015), although in this last one, significant differences with the 2nd tertile were also observed (0.25 units increase (95%CI [0.02;0.48], p=0.0335). With reference to autoantibodies, a negative association was found between skin AGEs levels and both the presence of ANA or anti-Ro60 antibodies in the blood test performed for the study, where skin AGEs values were 0.496 (95%CI [0.937;0.054], p=0.028) and 0.26 (95%CI [0.5;0.017], p=0.035) units lower, respectively, in patients with those antibodies. Finally, patients who had ever presented oral ulcers, a prevalent SLE manifestation, had skin AGEs values 0.216 (95% CI [0.02;0.41], p=0.03) units higher than patients who had never. All these data are depicted, according to the prediction of each model, in Figure 9 and Figure 10, which graphically represent the mean and its corresponding 95% CI of skin AGEs for each category of variables. Also, the linear regression model between skin AGEs and each of the variables are provided in the Supplementary Table 4.

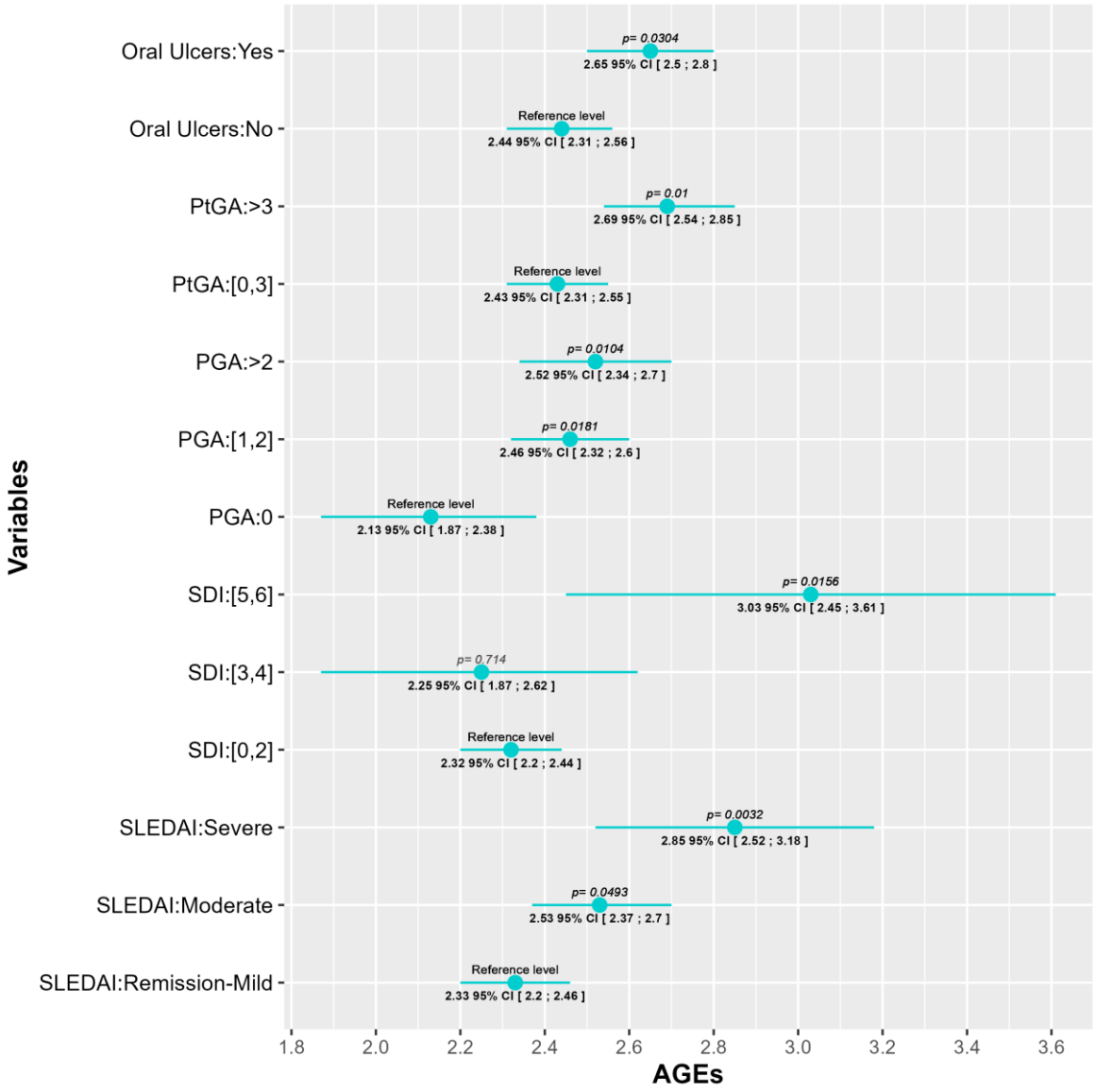

Figure 9: Statistically significant associations between skin advanced glycation end-products (AGEs) levels and systemic lupus erythematosus characteristics and indexes. *p*-values <0.05 (black) indicate significant differences between the categories and the reference level of each variable; *p*-values in gray indicate associations not statistically significant. *PtGA: patient global assessment; PGA: physician global assessment; SDI: SLE damage index; SLEDAI: SLE disease activity index: remission=0, mild [0-4], moderate (4-11], severe >11.*

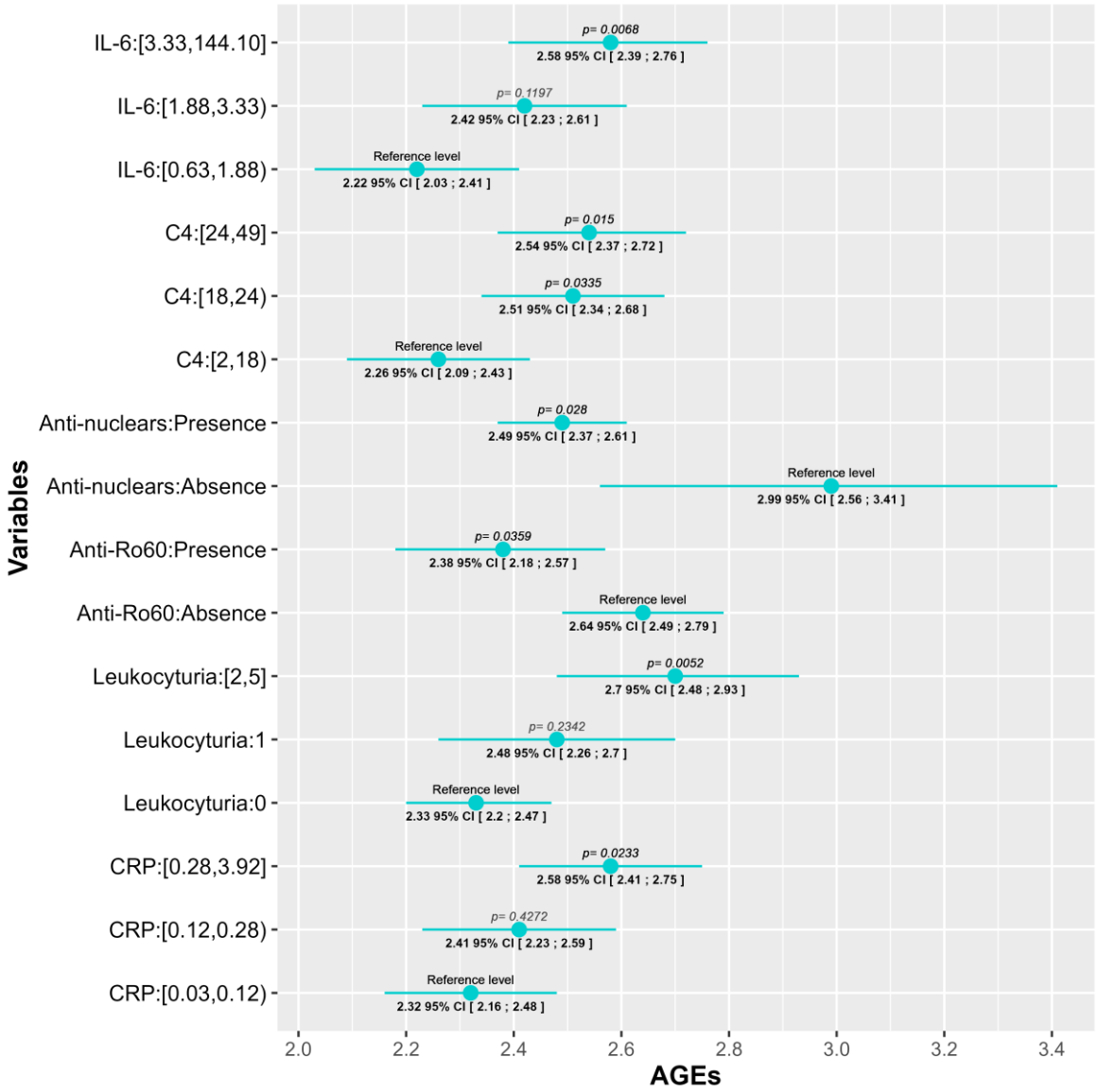

Figure 10: Statistically significant associations between skin advanced glycation end-products (AGEs) levels and systemic lupus erythematosus serological parameters. The change in AGEs values is depicted according to the reference category of each variable. *p*-values <0.05 (black) indicate significant differences between the categories and the reference level of each variable; *p*-values in gray indicate associations not statistically significant. *IL6: interleukin 6; C4: complement C4; CRP: C-reactive protein.*

5.4. Analysis of serum AGEs and association with SLE

5.4.1. Pentosidine

5.4.1.1. Characteristics of SLE patients according to pentosidine levels: exploratory analysis

A total of 117 SLE patients were included. Pentosidine met all the normality premises (Supplementary Figure 3) so the parametric statistical tests defined previously were performed. All the variables that showed statistically significant differences according to pentosidine tertiles in the exploratory analysis are depicted in Table

5. Pentosidine was not found to be influenced by age, smoker status, or any other potential confounding variable. The demographic characteristics and other SLE variables of interest are detailed in the Supplementary Table 5.

Variables	First tertile [0.1180)	Second tertile [1180,1594)	Third tertile [1594,4334]	*p*-value
	N=39	N=39	N=39	
Classificatory Criteria and Other Clinical and Serological Data				
Direct Coombs+ ever	4 (16.7%)	4 (21.1%)	1 (4.17%)	**0.063**
Pulmonary ever	0 (0.00%)	2 (5.13%)	3 (7.69%)	**<0.001**
Disease Activity Indexes				
SLE-DAS	4.18 [1.78;7.28]	1.79 [1.20;6.15]	2.53 [0.82;4.86]	**0.087**
Serological Variables				
Total bilirubin*	0.32 [0.25;0.48]	0.32 [0.26;0.38]	0.35 [0.23;0.41]	**0.097**
Hematuria*	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	**0.027**
UPCR	84.6 [68.5;133]	82.3 [63.5;108]	74.7 [54.9;90.7]	**0.093**
Comorbidities and Cardiovascular Disease				
Densitometric OP	4 (10.3%)	7 (17.9%)	7 (17.9%)	**0.077**
CVE_SDI				**0.091**
0	37 (94.9%)	34 (87.2%)	37 (94.9%)	
1	2 (5.13%)	4 (10.3%)	0 (0.00%)	
2	0 (0.00%)	1 (2.56%)	2 (5.13%)	
Treatments				
Tacrolimus	1 (2.56%)	0 (0.00%)	0 (0.00%)	**0.093**
Other AGEs				
Skin AGEs				**0.065**
<1SD	1 (2.56%)	1 (2.56%)	4 (10.3%)	
1SD-Means	4 (10.3%)	3 (7.69%)	6 (15.4%)	
Means	1 (2.56%)	2 (5.13%)	1 (2.56%)	
Means->1SD	12 (30.8%)	10 (25.6%)	12 (30.8%)	
>1SD	21 (53.8%)	23 (59.0%)	16 (41.0%)	

Table 5: Variables that showed statistically significant differences according to pentosidine tertiles in the exploratory analysis. Bold indicates *p*-value <0.1 and * indicates values according to the blood test performed in the study. *SLE-DAS: SLE disease activity score; UPCR (mg/g): urine protein to creatinine ratio; OP: osteoporosis; CVE_SDI: cardiovascular events assessed in the SLE Damage Index (cerebral vascular accident, pulmonary infarction, angina or coronary bypass, myocardial infarction, venous thrombosis or infarction of the gastrointestinal tract); AGEs: advanced glycation end-products; SD: standard deviation.*

5.4.1.2. Associations between pentosidine and SLE characteristics: multivariate analysis

SLE characteristics that were significant in the exploratory analysis and that might be related to pentosidine levels were assessed in a model adjusted for previously selected confounding variables (see Methodology). In the case of pentosidine, no confounding variables were found to influence the model. Among all the variables studied, only the presence of pulmonary manifestations (lupus pneumonitis and

shrinking lung syndrome) was strongly associated (Figure 1) with pentosidine. Specifically, patients with lung involvement had pentosidine levels 1181.8786 (95% CI [507.4192; 1856.3379], p<0.001) units higher than those without lung involvement. The detailed model is provided in the Supplementary Table 6.

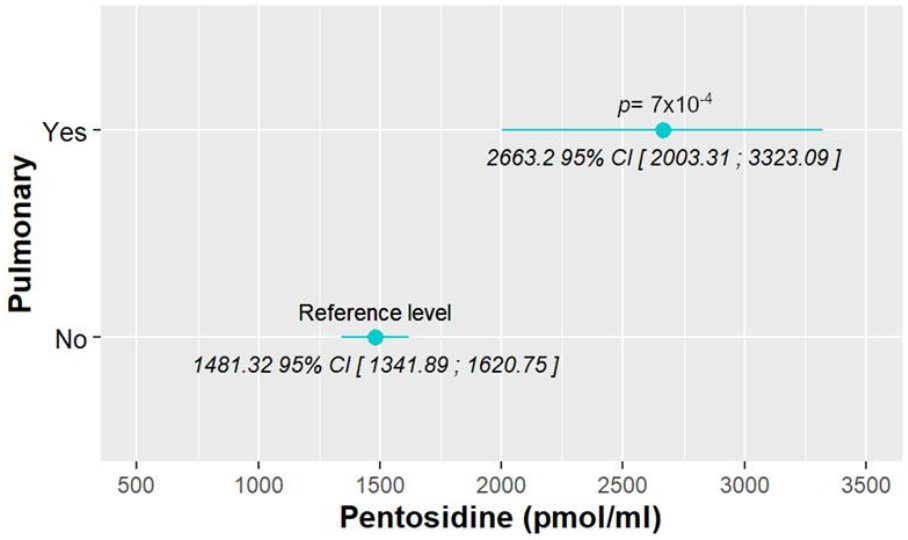

Figure 11: Associations between pentosidine levels and different SLE characteristics, being the pulmonary manifestations the only significant one.

5.4.2. Nξ-(carboxymethyl)lysine (CML)

5.4.2.1. Characteristics of SLE patients according to CML levels: exploratory analysis

A total of 117 SLE patients were included. CML presented a right-skewed distribution (Supplementary Figure 3), so regression models suitable for log-normal data were performed, as defined in the Methodology section. All the variables that showed statistically significant differences according to CML tertiles in the exploratory analysis are depicted in Table 6, not adjusted by any variable. The demographic characteristics and other SLE variables of interest are detailed in the Supplementary Table 7.

Variables	First tertile [57.6, 240)	Second tertile [239.8, 383)	Third tertile [382.9,1555]	*p*-value
	N=39	N=39	N=39	
Demographic Variables				
Ethnicity				**0.023**
Caucasian	30 (76.9%)	29 (74.4%)	20 (51.3%)	
Latin	6 (15.4%)	7 (17.9%)	14 (35.9%)	
Others	3 (7.69%)	3 (7.69%)	5 (12.8%)	
Ethnicity				**0.006**
Caucasian	30 (76.9%)	29 (74.4%)	20 (51.3%)	
Others	9 (23.1%)	10 (25.6%)	19 (48.7%)	
Disease Related Variables				
Years of duration	4.00 [1.00;14.5]	12.0 [4.00;18.5]	12.0 [4.00;21.0]	**0.037**
cYears of duration				**0.088**
0-5	22 (56.4%)	13 (33.3%)	12 (30.8%)	
6-10	5 (12.8%)	6 (15.4%)	5 (12.8%)	
11-20	9 (23.1%)	12 (30.8%)	11 (28.2%)	
>20	3 (7.69%)	8 (20.5%)	11 (28.2%)	
Tertiles years of duration				**0.020**
[0, 5)	21 (53.8%)	11 (28.2%)	10 (25.6%)	
[5,16)	12 (30.8%)	14 (35.9%)	14 (35.9%)	
[16.45]	6 (15.4%)	14 (35.9%)	15 (38.5%)	
Classificatory Criteria and Other Clinical and Serological Data				
Renal disease ever	0 (0.00%)	1 (2.56%)	7 (17.9%)	**0.019**
Disease Activity Indexes				
PGA	1.00 [1.00;2.00]	2.00 [1.00;3.00]	2.00 [1.00;3.00]	**0.094**
Swollen joints	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	**0.093**
Serological Variables				
IL-6 tertiles*				**0.050**
[0.44, 1.88)	15 (40.5%)	12 (30.8%)	11 (28.9%)	
[1.88, 3.24)	13 (35.1%)	18 (46.2%)	7 (18.4%)	
[3.24,39.38]	9 (24.3%)	9 (23.1%)	20 (52.6%)	
Comorbidities and Cardiovascular Disease				
Densitometric OP	5 (12.8%)	4 (10.3%)	9 (23.1%)	**0.034**
Treatments				
Dyslipidemia drugs	4 (10.3%)	1 (2.56%)	9 (23.1%)	**0.004**
Mycophenolic acid	2 (5.13%)	6 (15.4%)	12 (30.8%)	**0.012**
Glucocorticoids	8 (20.5%)	4 (10.3%)	18 (46.2%)	**<0.001**
Other AGEs				
CEL	2.45 [2.09;3.71]	3.17 [2.47;3.66]	3.99 [2.48;4.68]	**0.064**

Table 6: Variables that showed statistically significant differences according to CML tertiles in the exploratory analysis. “c” indicates variables which have been categorized as previously stated in the methodology section. Bold indicates *p*-value <0.1 and * indicates values according to the blood test performed in the study. *PGA: physician global assessment; IL-6: interleukin-6; OP: osteoporosis; AGES; advanced glycation end-products; CEL: Nξ-(carboxyethyl)lysine.*

5.4.2.2. Associations between CML and SLE characteristics: multivariate analysis

SLE characteristics that were significant in the exploratory analysis and that might be related to CML levels were tested in two models adjusted for the previously selected confounding variables (see 4.8.3). After adjustment, we found that CML

levels correlated with longer disease duration, non-Caucasian ethnicity, and positive anti-dsDNA antibodies (≥ 11 IU/mL). These positive associations were significant in both OLS and GLM models: In addition, we also found that the 2nd tertile of anti-dsDNA antibodies (≥ 11 IU/mL) and 3rd tertile of IL-6 values [3.24-39.38] presented higher CML levels than the 1st tertile, but both exclusively in the OLS model (Figure 12). The detailed models and their adjustment by confounding variables are provided in the Supplementary Table 8.

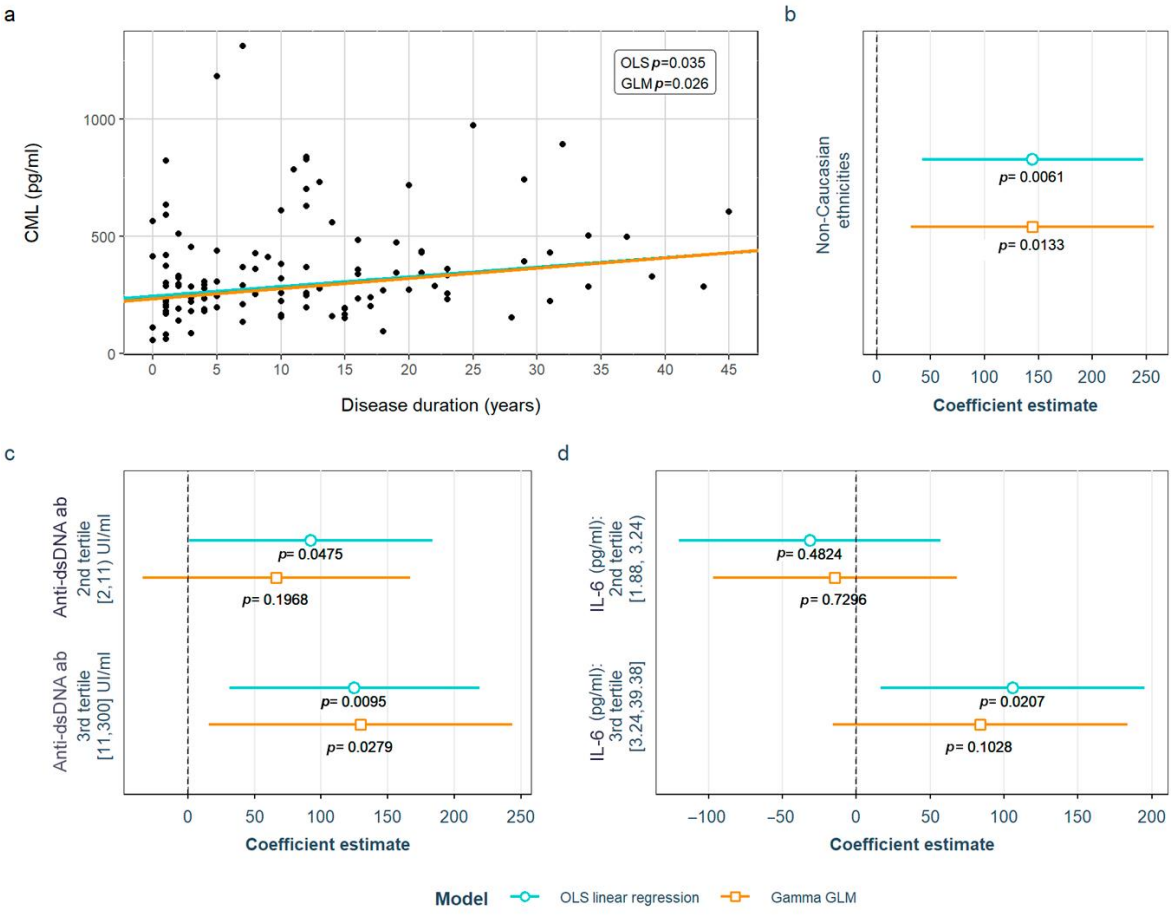

Figure 12: Statistically significant associations between CML and different systemic lupus erythematosus characteristics. *CML: Nξ-(carboxymethyl)lysine; OLS: ordinary least squares; GLM: generalized linear model; IL-6: interleukin 6.*

5.4.3. Nξ-(carboxyethyl)lysine (CEL)

5.4.3.1. Characteristics of SLE patients according to CEL levels: exploratory analysis

A total of 91 SLE patients were included. The distribution of CEL exhibited a right-skewed pattern (Supplementary Figure 3), prompting the utilization of regression models tailored for log-normal data, as outlined in the Methodology section. All the variables that showed statistically significant differences according to CEL tertiles

in the exploratory analysis are depicted in Table 7, not adjusted by any variable. The demographic characteristics and other SLE variables of interest are detailed in the Supplementary Table 9.

Variables	First tertile [0.823, 2.79)	Second tertile [2.793, 4.56)	Third tertile [4.564,31.68]	*p*-value
	N=38	N=37	N=16	
Demographic Variables				
Smoker	3 (7.89%)	8 (21.6%)	8 (50.0%)	**0.087**
Classificatory Criteria and Other Clinical Data				
Constitutional ever	3 (7.89%)	4 (10.8%)	1 (6.25%)	**0.046**
Photosensitivity ever	20 (52.6%)	27 (73.0%)	13 (81.2%)	**0.089**
Manifestations				**0.006**
3	2 (5.26%)	0 (0.00%)	0 (0.00%)	
4	2 (5.26%)	1 (2.70%)	0 (0.00%)	
5	7 (18.4%)	3 (8.11%)	0 (0.00%)	
6	9 (23.7%)	10 (27.0%)	2 (12.5%)	
7	8 (21.1%)	8 (21.6%)	5 (31.2%)	
8	6 (15.8%)	4 (10.8%)	3 (18.8%)	
9	3 (7.89%)	6 (16.2%)	2 (12.5%)	
10	0 (0.00%)	3 (8.11%)	1 (6.25%)	
11	1 (2.63%)	1 (2.70%)	3 (18.8%)	
12	0 (0.00%)	1 (2.70%)	0 (0.00%)	
Disease Activity Indexes				
cSLE-DAS				**0.091**
First tertile [0.82, 1.79)	19 (52.8%)	16 (47.1%)	2 (12.5%)	
Second tertile [1.79, 5.31)	6 (16.7%)	10 (29.4%)	5 (31.2%)	
Third tertile [5.31,23.31]	11 (30.6%)	8 (23.5%)	9 (56.2%)	
Serological Variables				
Glucose*	87.8 (12.4)	82.4 (8.96)	81.2 (7.69)	**0.049**
CRP*	0.12 [0.07;0.28]	0.17 [0.11;0.30]	0.16 [0.07;0.54]	**<0.001**
ESR*	8.00 [4.25;20.0]	10.5 [6.00;15.0]	13.5 [7.00;21.5]	**0.054**
Anti-dsDNA+ ever	23 (60.5%)	26 (70.3%)	14 (87.5%)	**0.025**
Anti-dsDNA+ *	2.50 [1.00;10.8]	5.00 [1.00;13.0]	15.5 [1.75;40.2]	**<0.001**
Anti-dsDNA>RV*	10 (26.3%)	10 (27.8%)	9 (56.2%)	**0.018**
Anti-dsDNA tertiles*				**0.054**
[0, 2)	16 (42.1%)	14 (38.9%)	4 (25.0%)	
[2, 11)	12 (31.6%)	12 (33.3%)	3 (18.8%)	
[11,300]	10 (26.3%)	10 (27.8%)	9 (56.2%)	
Anti-dsDNA presence*	10 (26.3%)	10 (27.8%)	9 (56.2%)	**0.018**
Anti-Ro60+ ever	7 (18.4%)	19 (51.4%)	6 (37.5%)	**0.097**
Anti-Ro60 presence*	7 (18.9%)	17 (47.2%)	6 (37.5%)	**0.086**
Anti-Ro52+ ever	4 (10.5%)	12 (32.4%)	5 (31.2%)	**0.060**
C3*	111 (24.3)	103 (19.2)	98.8 (20.5)	**0.028**
IL-6*	1.98 [1.43;3.77]	2.21 [1.81;2.96]	3.92 [2.99;6.03]	**0.003**
IL-6>RV*	4 (10.8%)	2 (5.41%)	4 (25.0%)	**0.002**
IL-6 tertiles*				**0.019**
[0.44, 1.88)	16 (43.2%)	13 (35.1%)	1 (6.25%)	
[1.88, 3.24)	10 (27.0%)	15 (40.5%)	4 (25.0%)	
[3.24,39.38]	11 (29.7%)	9 (24.3%)	11 (68.8%)	
UPCR*	82.2 [66.2;119]	84.1 [63.0;103]	71.3 [50.1;121]	**0.013**
Treatments				
Mycophenolic acid	4 (10.5%)	8 (21.6%)	5 (31.2%)	**0.007**
NSAIDs	3 (7.89%)	4 (10.8%)	2 (12.5%)	**0.038**
Treatment				**0.030**
Others	6 (15.8%)	3 (8.11%)	0 (0.00%)	
Antimalarials	19 (50.0%)	13 (35.1%)	4 (25.0%)	
IS	13 (34.2%)	21 (56.8%)	12 (75.0%)	
Treatment2				**0.009**

Variables	First tertile [0.823, 2.79)	Second tertile [2.793, 4.56)	Third tertile [4.564,31.68]	*p*-value
Non-IS	25 (65.8%)	16 (43.2%)	4 (25.0%)	
IS	13 (34.2%)	21 (56.8%)	12 (75.0%)	
Other AGEs				
CML	281 [216;374]	302 [248;444]	464 [272;711]	**0.064**

Table 7: Variables that showed statistically significant differences according to CEL tertiles in the exploratory analysis. “c” indicates variables which have been categorized as previously stated in the methodology section. Bold indicates *p*-value <0.1 and * indicates values according to the blood test performed in the study. “Treatment” divides patients into three groups according to the strongest immunosuppression they were taking at the moment of the study (only immunosuppressants, only antimalarials, or neither (Others). “Treatment2” divides patients into two groups: taking or not taking immunosuppressants. *CEL: Nξ-(carboxyethyl)lysine; SLE-DAS: SLE disease activity score; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; RV: reference value according to the laboratory; C3: complement C3; IL-6: interleukin-6; UPCR (mg/g): urine protein to creatinine ratio; NSAIDS: non-steroidal anti-inflammatory drugs; IS: immunosuppressants (includes treatment with methotrexate, leflunomide, tacrolimus, mycophenolic acid or mycophenolate mofetil acid, azathioprine, cyclophosphamide, cyclosporine, rituximab or belimumab); AGEs: advanced glycation end-products; CML: Nξ-(carboxymethyl)lysine.*

5.4.3.2. Associations between CEL and SLE characteristics: multivariate analysis

SLE characteristics that were significant in the exploratory analysis and that might be associated to CEL levels were tested in two models adjusted for the previously selected confounding variables (see 4.8.3). After adjustment, we found that CEL levels correlated with anti-dsDNA antibodies, IL-6 levels and the number of accumulated manifestations throughout the disease (Figure 13Figure 13 d, respectively). Furthermore, patients having ever had positive anti-dsDNA antibodies had significant higher CEL levels (Figure 13c). These associations were found in both models except for one with the anti-dsDNA titers, with was only observed in the OLS linear regression model. Besides, we found a correlation between CEL and CML levels (

Figure 14). The detailed models and their adjustment by confounding variables are provided in the Supplementary Table 10.

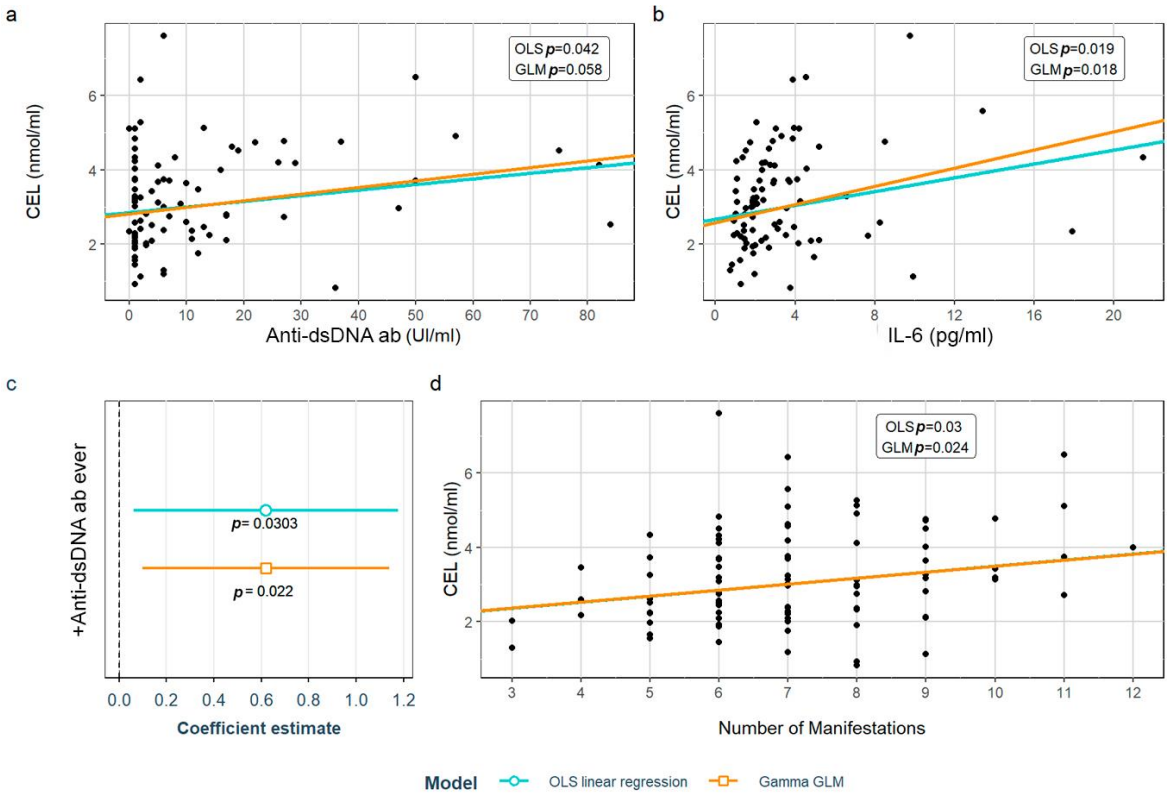

Figure 13: Statistically significant associations between CEL and different systemic lupus erythematosus characteristics. *CEL: Nξ-(carboxyethyl)lysine; OLS: ordinary least squares; GLM: generalized linear model; IL-6: interleukin 6; ab: antibodies.*

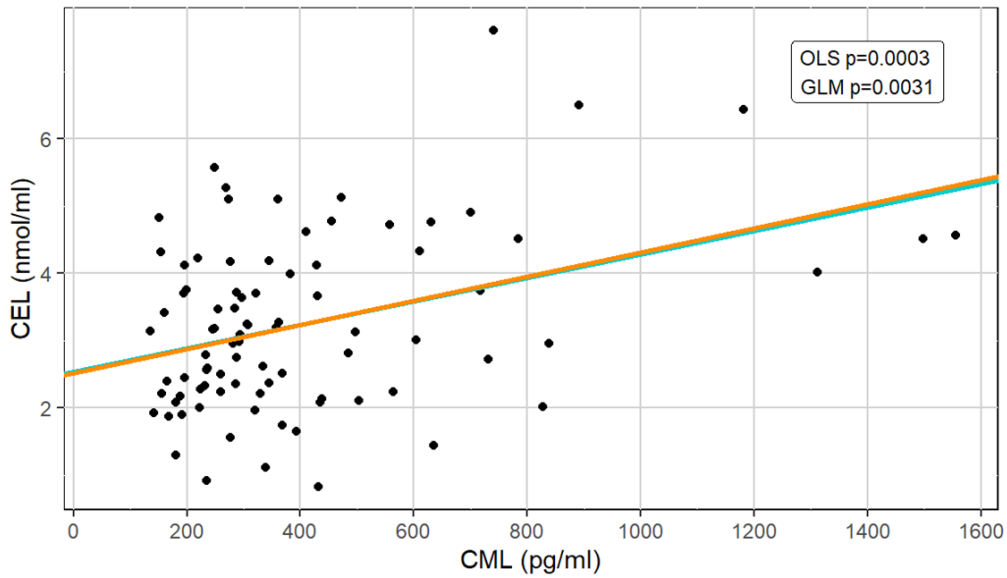

Figure 14: Association between CEL and CML values. CEL: Nξ-(carboxyethyl)lysine; CML: Nξ-(carboxymethyl)lysine; OLS: ordinary least squares; GLM: generalized linear model.

5.4.4. Serum receptor of advanced glycation end-products (sRAGE)

5.4.4.1. Characteristics of SLE patients according to sRAGE levels: exploratory analysis

A total of 119 SLE patients were included. The distribution of sRAGE displayed a right-skewed pattern, as illustrated in the Supplementary Figure 3. Consequently, regression models designed for log-normal data, as described in the Methodology section, were employed to analyze the dataset. All the variables that showed statistically significant differences according to sRAGE tertiles in the exploratory analysis are depicted in Table 8, not adjusted by any variable. The demographic characteristics and other SLE variables of interest are detailed in the Supplementary Table 11.

Variables	First tertile [122, 384)	Second tertile [384, 671)	Third tertile [671,2797]	*p*-value
	N=40	*N=40*	*N=39*	
Demographic Variables				
Gender: Female	35 (87.5%)	37 (92.5%)	39 (100%)	**0.057**
Classificatory Criteria and Other Clinical and Serological Data				
Photosensitivity ever	20 (50.0%)	29 (72.5%)	26 (66.7%)	**0.022**
Disease Activity Indexes				
DAS28	2.16 [1.49;2.58]	2.10 [1.43;3.24]	2.40 [1.57;3.10]	**0.050**
cDAS28				**0.008**
0- Reference	31 (79.5%)	25 (62.5%)	21 (55.3%)	
1- Low Activity	2 (5.13%)	4 (10.0%)	8 (21.1%)	
2- Moderate Activity	4 (10.3%)	9 (22.5%)	7 (18.4%)	
3- High Activity	2 (5.13%)	2 (5.00%)	2 (5.26%)	
Serological Variables				
ESR tertiles*				**0.047**
[2,7)	13 (33.3%)	17 (42.5%)	12 (31.6%)	
[7,17)	12 (30.8%)	10 (25.0%)	15 (39.5%)	
[17.81]	14 (35.9%)	13 (32.5%)	11 (28.9%)	
Leukocyturia*	0.00 [0.00;1.00]	0.00 [0.00;1.00]	0.00 [0.00;1.00]	**0.022**
Patient Reported Outcomes				
Pain VAS	1.50 [0.00;5.00]	2.50 [0.00;6.12]	4.00 [0.00;6.00]	**0.033**
Comorbidities and Cardiovascular Disease				
APS	4 (10.0%)	1 (2.50%)	0 (0.00%)	**0.097**
Pain VAS	1.50 [0.00;5.00]	2.50 [0.00;6.12]	4.00 [0.00;6.00]	**0.033**
Treatments				
Glucocorticoids	13 (32.5%)	12 (30.0%)	5 (12.8%)	**0.053**
bDMARDs	0 (0.00%)	2 (5.00%)	4 (10.3%)	**0.002**
Antimalarials	37 (92.5%)	27 (67.5%)	26 (66.7%)	**0.009**
Mycophenolic acid	7 (17.5%)	5 (12.5%)	8 (20.5%)	**0.016**
Azathioprine	2 (5.00%)	9 (22.5%)	7 (17.9%)	**0.065**
Treatment				**0.016**
Others	1 (2.50%)	6 (15.0%)	7 (17.9%)	
Antimalarials	24 (60.0%)	14 (35.0%)	13 (33.3%)	
IS	15 (37.5%)	20 (50.0%)	19 (48.7%)	
Treatment2				**0.008**
Non-IS	25 (62.5%)	20 (50.0%)	20 (51.3%)	
IS	15 (37.5%)	20 (50.0%)	19 (48.7%)	

Table 8: Variables that showed statistically significant differences according to the serum receptor of advanced glycation end-products tertiles in the exploratory analysis, “c” indicates variables which have been categorized as previously stated in the methodology section. Bold indicates *p*-value <0.1 and * indicates values according to the blood test performed in the study. “Treatment” divides patients into three groups according to the strongest immunosuppression they were taking at the moment of the study (only immunosuppressants, only antimalarials, or neither (Others). “Treatment2” divides patients into two groups: taking or not taking immunosuppressants. *DAS28: Disease Activity Score 28; ESR: erythrocyte sedimentation rate; VAS: visual analogic scale; bDMARDs: biologic disease-modifying antirheumatic drugs, IS: Immunosuppressants (includes treatment with methotrexate, leflunomide, tacrolimus, mycophenolic acid, or mycophenolate mofetil acid, azathioprine, cyclophosphamide, cyclosporine, rituximab or belimumab).*

5.4.4.2. Associations between sRAGE levels and SLE characteristics: multivariate analysis

SLE characteristics that were statistically significant in the exploratory analysis and that might be associated to sRAGE levels were tested in the two models adjusted

for the previously selected confounding variables (see 4.8.3). After adjustment, we found that sRAGE levels were higher in females and in patients having ever had photosensitivity as a SLE symptom, as well as in those on biological disease-modifying antirheumatic drugs (bDMARD) or mycophenolic acid at the moment of the study (Figure 15a, b, c and d, respectively). All the associations were found in both models except for male sex that was only found in the OLS linear regression model. The detailed models and their adjustment by confounding variables are provided in the Supplementary Table 12.

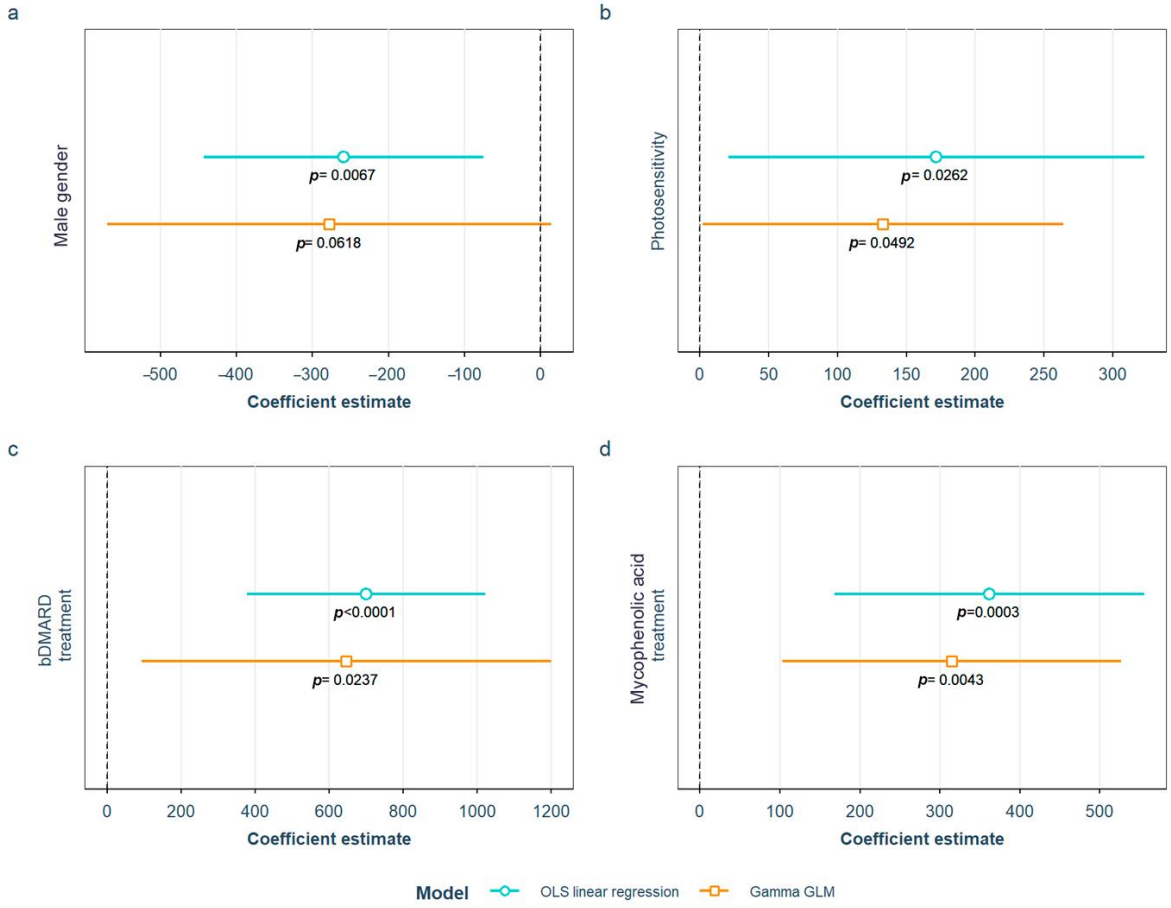

Figure 15: Statistically significant associations between the serum receptor for advanced glycation end-products and different systemic lupus erythematosus characteristics. *OLS: ordinary least squares; GLM: generalized linear model; bDMARD: biological disease-modifying antirheumatic drugs.*

5.4.5. Ratios of advanced glycation end-products/serum soluble receptor from the advanced glycation end-products (AGEs/sRAGE)

5.4.5.1. Characteristics of SLE patients according to skin AGEs/sRAGE or serum AGEs/sRAGE

All the statistically significant associations in the univariate analysis are depicted in the Annexes: Pentosidine/sRAGE in the Supplementary Table 13, CML/sRAGE

in the Supplementary Table 15, CEL/sRAGE in the Supplementary Table 17 and skin AGEs/sRAGE in the Supplementary Table 19.

5.4.5.2. Associations between skin AGEs/sRAGE or serum AGEs/sRAGE and SLE characteristics: multivariate analysis

After adjustment for confounding factors, we found several SLE characteristics that were associated with different AGEs to sRAGE ratios, in one or both models. Pentosidine/sRAGE ratio was higher in those patients not under bDMARD treatment or having ever had anti-Ro52 antibodies (Figure 16). Regarding CML/sRAGE, non-Caucasian patients, as well as patients with a SDI ≥ 2, densitometric osteoporosis or being on dyslipidemia drugs, presented higher ratios (Figure 17). CRP and IL-6 levels had a positive correlation with CEL/sRAGE ratio as continuous variables, with those showing pathological IL-6 values displaying significant higher ratios (Figure 18). Finally, the skin AGEs/RAGE ratio was lower in women and in those patients with disease duration > 16 years (3rd tertile), compared to those with disease duration < 5 years (1st tertile) (Figure 19). The detailed models and their adjustment by confounding variables are provided in the Supplementary Table 14, Supplementary Table 16, Supplementary Table 18 and Supplementary Table 20, respectively.

Some of the associations matched those of the isolated serum AGEs or sRAGE but some were totally new. At this moment, this data are exploratory and merely descriptive, being not possible to interpret them. The role of AGEs to sRAGE ratios need further exploration in general, to confirm if they could be a good, or even better, marker of inflammation than the isolated compounds.

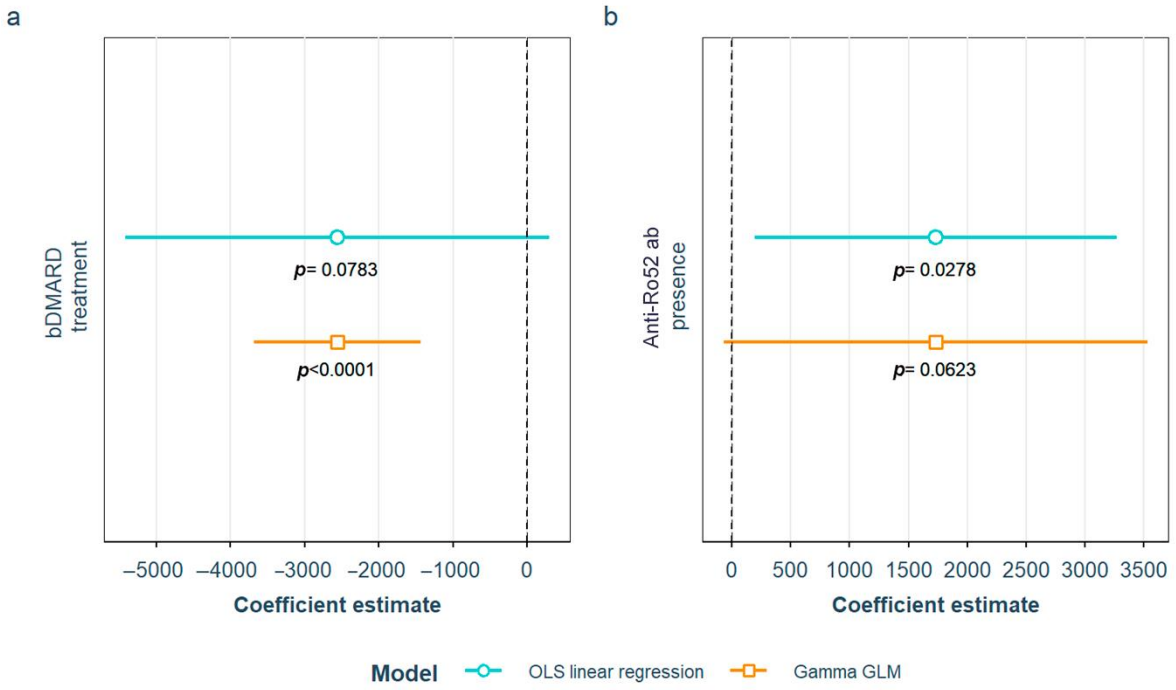

Figure 16: Statistically significant associations between pentosidine/sRAGE and different systemic lupus erythematosus characteristics. *sRAGE: soluble receptor for advanced glycation end-products; OLS: ordinary least squares; GLM: generalized linear model. bDMARD: biological disease-modifying antirheumatic drugs; ab: antibodies.*

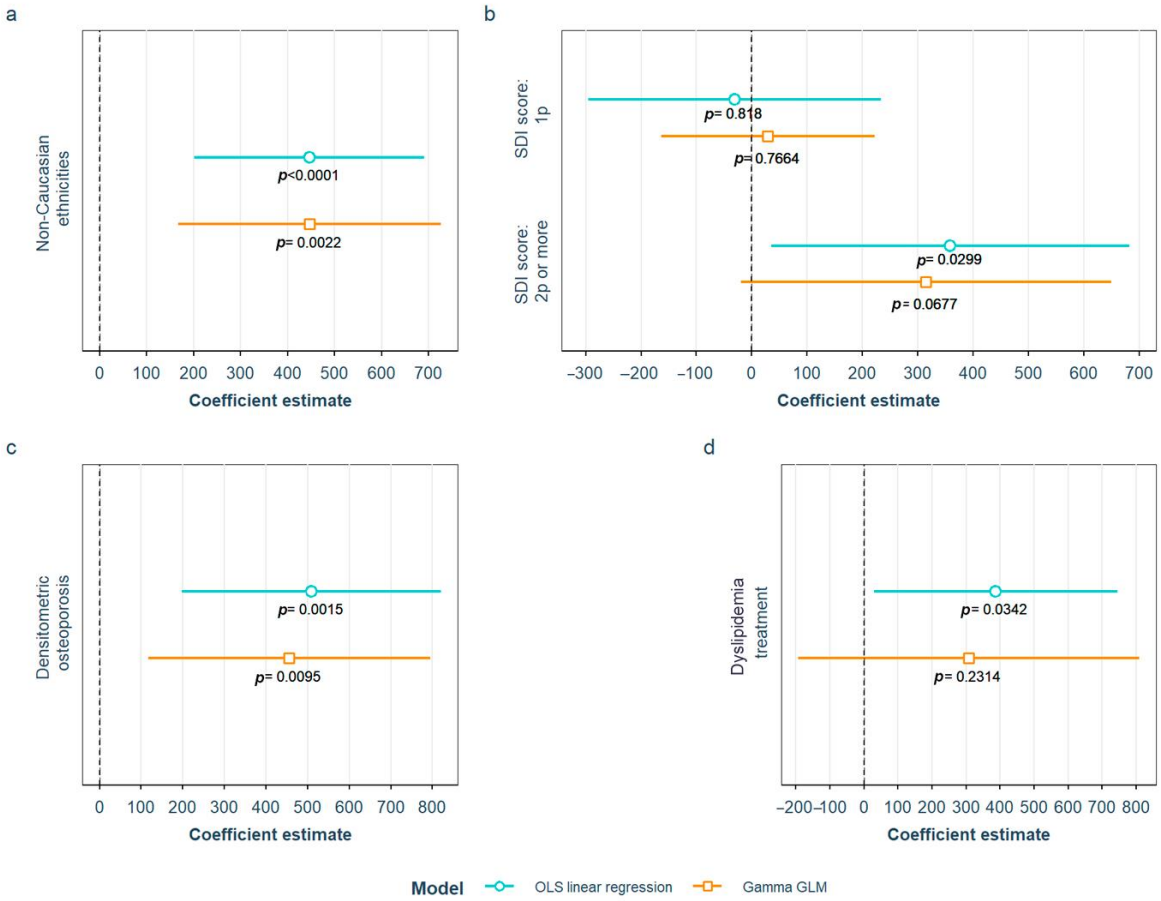

Figure 17: Statistically significant associations between CML/sRAGE and different systemic lupus erythematosus characteristics. *CML: Nξ-(carboxymethyl)lysine; sRAGE: soluble receptor for advanced glycation end-products; OLS: ordinary least squares; GLM: generalized linear model. SDI: systemic lupus erythematosus damage index.*

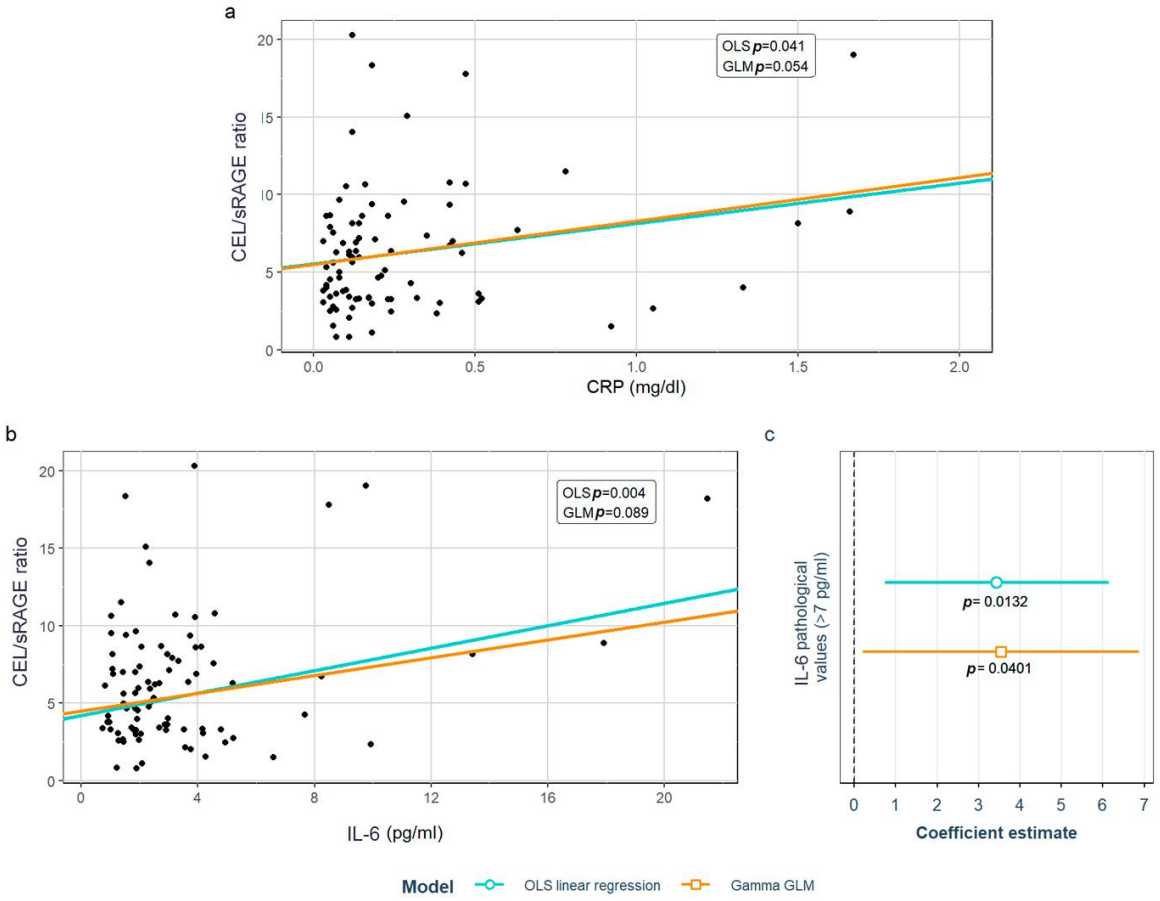

Figure 18: Statistically significant associations between CEL/sRAGE and different systemic lupus erythematosus characteristics. *CEL: Nξ-(carboxyethyl)lysine; sRAGE: soluble receptor for advanced glycation end-products; OLS: ordinary least squares; GLM: generalized linear model. CRP: C-reactive protein; IL-6: interleukin 6.*

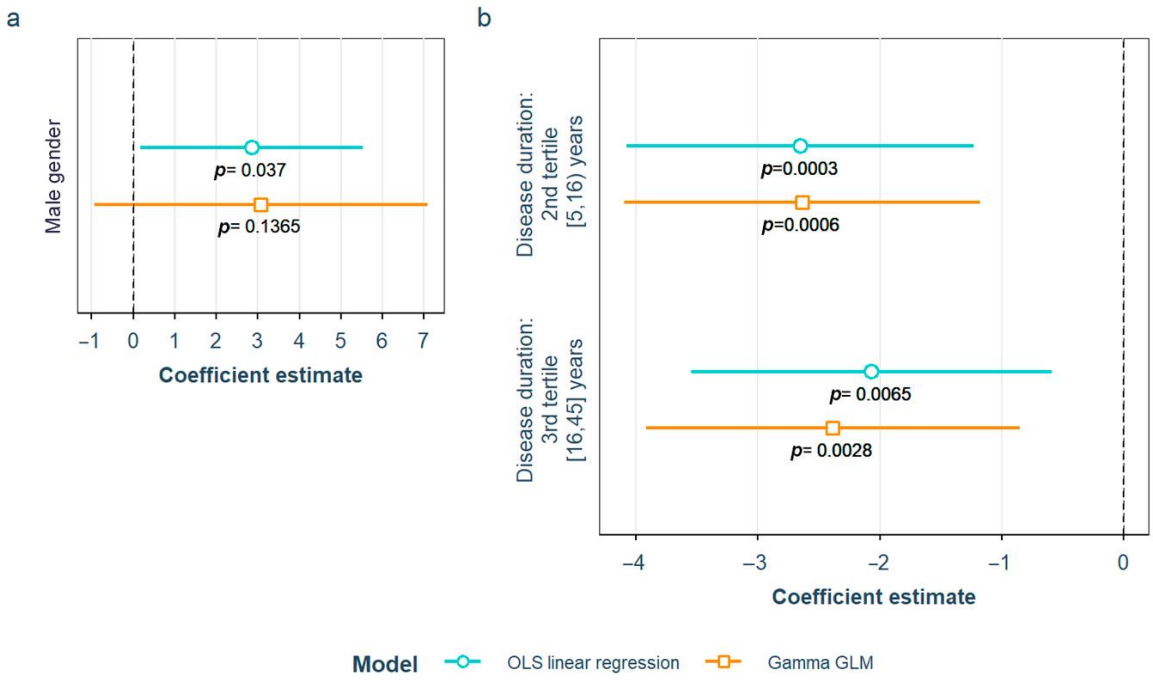

Figure 19: Statistically significant associations between skin AGEs/sRAGE and different systemic lupus erythematosus characteristics. *AGEs: advanced glycation end-products; sRAGE: soluble receptor for advanced glycation end-products OLS: ordinary least squares; GLM: generalized linear model.*

5.5. *Analysis of serum AGEs and sRAGE association with cardiovascular disease*

We did not find any correlation between skin AGEs levels, serum AGEs or sRAGE and either CVRF or CVE.

6. Discussion

6. Discussion

We observed statistically significant differences between skin AGEs values measured by skin autofluorescence in SLE patients vs HC. This difference has only been studied in two previous works (123,124) with small sample sizes (55 and 30 cases respectively, matched 1:1 with HC), having our study several stronger points. First, we have increased the sample size, especially the HC sample, by matching cases with HC in a 1:3 proportion instead of a 1:1 proportion, making the study more robust. Secondly, we selected HC that had at least one CVRF, so they would be more comparable to our patients who at least have one CVRF, being that the disease itself. This is based on the well-reported knowledge that AGEs are related to inflammation and CVR on the one hand and, on the other, on that patients with SADs like RA have an increased risk of CVD that makes necessary to add a fixed multiplier of 1.5 to 2 to the established CVD prediction algorithms for general population in order to adjust for the increased risk due to the disease (139). Nienhuis *et al*. (123) selected a second control population with essential hypertension, apart from the one conformed by HC. They found statistically significant differences in skin AGEs levels between SLE patients and HC but not between the SLE and the essential hypertension cohort, suggesting that finding differences when selecting HC with at least one CVRF could traduce a higher statistical power and a reduced probability of committing a type I error. Furthermore, they selected an SLE population with inactive disease, which might not reflect the reality of SLE patients, in terms of disease characteristics, in the way our patients may, which were included independently of their disease activity.

Additionally, we carefully examined all possible confounding factors to avoid drawing premature conclusions. Two controversial points were raised during the analysis. First, we observed only a positive trend shown by creatinine in the exploratory analysis of skin AGEs levels in the whole sample (both SLE and HC). We discussed if that trend could have a fictitious origin since patients with SLE had higher creatinine levels (although in normal range) and were mostly located in the third skin AGEs tertile, and also because the trend was not observed when we analyzed the two groups separately. However, we finally decided to include creatinine in the model because there is ample evidence of a higher accumulation of AGEs in patients with renal failure (149) and LN (77), and a difference could exist between groups because renal disease was an exclusion criterion in the HC group. Secondly, we found a negative association between dyslipidemia and skin AGEs, which was observed both in the combined analysis of the whole sample and in the HC separately (suggesting that such association came from the HC group). It is reported in the literature the effect of lipid-lowering drugs in reducing AGEs and RAGE levels (150,151). Among the HC of our study, only 27 of the 85 with dyslipidemia (32%) were being treated with lipid-lowering agents so we hypothesized that the rest could be controlling it with a Mediterranean lower-fat diet, which has also been associated with reduced both serum (152) and tissue AGEs

levels (112,153). Hence, the negative association between AGEs and dyslipidemia could be due to the effect of either dyslipidemia treatment and/or diet in AGEs levels.

The only data in the literature that could explain this negative association come from the reported effect of lipid-lowering drugs in reducing AGEs levels (150). Among HC, only 27 of the 85 with dyslipidemia (32%) were being treated with lipid-lowering agents, so we hypothesized that the rest could be controlling it with a lower-fat diet, which has also been associated with reduced AGEs levels (112). Hence, we ended up including dyslipidemia in the model.

As for the interaction term between the main effect and dyslipidemia, although it was not found to be significant in the model, the interaction seemed clear graphically, especially in the group of SLE patients (Figure 7). This could be due to a lack of statistical power, since there were only 8 cases with dyslipidemia in the SLE group, unlike the 85 in the HC one. Therefore, the statistical power to detect this difference was much lower in the patient's group, generating a less precise CI to reject the alternative hypothesis and leading to a lack of significance.

Regarding the study of skin AGEs relationship with SLE characteristics, we have found associations between skin AGEs levels and some disease activity indexes: SLEDAI, PGA, PtGA, CRP, and IL-6. As reflected in the Results section, the increment of skin AGEs levels with the increase of SLEDAI, which is the activity index most frequently used for SLE in clinical practice nowadays, showed a robust correlation. This association was also observed with other markers of activity commonly used to assess the state of the disease: PGA, PtGA and IL-6. PGA is a part of the main indexes used currently to define remission or LDA in SLE. PtGA may be a more subjective parameter which can be influenced by external factors but that is clearly related to quality of life in SLE patients. IL-6 is not used routinely in the follow-up of SLE patients but its role in inflammation in rheumatic diseases, and in SLE in particular, it is widely known (154).

In the case of CRP, a significant association was only found between the upper tertile (0.28-3.92 mg/dL) and the first (<0.12 mg/dL), suggesting that the highest levels of skin AGEs were found among the patients with the highest CRP values, including both normal and abnormal (reference values in our laboratory are those <0.5 mg/dL). However, this correlation was only supported up to CRP values < 0.7 mg/dL ($R2=0.42$, $p<0.0001$), as graphically reflected in the Supplementary Figure 4. No correlation was found with higher CRP levels, which could be justified by a small number of patients with abnormal values.

There was also a positive association with higher C4 levels, which draws attention because low C4 levels are the ones traditionally associated with higher disease activity. However, although a decrease in complement levels is included in SLE classificatory criteria, there is wide controversy in the literature about the limited usefulness of the current techniques and

types of complement measured in SLE and their ability to reflect disease activity (155). Other uncertainties about complement are whether low levels should be persistent or combined (both C_3 and C_4) to be significant (156,157). In our study, C_3 levels showed a statistically significant direct correlation with C_4 values ($p = <0.001$) but not with skin AGEs. There was not an association between having normal C_4 levels at the moment of the study and not having had hypocomplementemia ever: 49% of the patients with current normal C_4 levels had a history of hypocomplementemia and 57% did not, while 77% of the patients with history of low complement had now normal C_4 levels. This could traduce either fluctuant titers or normalized levels of C_4 in response to treatment/lower disease activity and a need for further studies to elucidate the relation between complement and AGEs.

We also found a relationship between skin AGEs and indexes of accrual damage, the SDI. There is only a previous work in the literature that analyzed this association (124). They found a correlation between skin AGEs and SDI in the univariate analysis that was lost both after adjusting for age and in the multivariate analysis. In our case, the association persisted after adjusting for age and smoking status and any other possible confounding factor in the multivariate analysis. Taking into account this association, measuring skin AGEs levels could have a high impact in the prognosis of the disease. Skin AGEs could help identify a subtype of patients with a more serious disease marked by higher accrual damage, which would be susceptible of a stricter follow-up and intensive treatment regimen and, subsequently, allow to improve these patients' outcomes.

Association of skin AGEs with specific manifestations (oral ulcers) or autoantibodies' profile (less frequent anti-Ro60 positive antibodies), could indicate a different clinical phenotype in SLE patients with less inflammation and thus, with lower skin AGEs levels. In clinical practice, it is quite common to find overlaps of autoimmune diseases in the same patient, being especially frequent in SLE its overlap with Sjögren syndrome (SS). It is known that both diseases have different inflammatory profiles (158), which could explain why there could be differences in skin AGEs levels between patients anti-Ro60 positive and negative. AGEs concentrations have been scarcely studied in SS and efforts have not been directed to skin AGEs but RAGE and sRAGE with conflicting results (159–161). So that, more studies are needed to investigate AGEs levels in SS and their differences both with anti-Ro positive SLE patients and with patients with a SLE-SS overlap. Unfortunately, we could not validate this hypothesis about the influence of SS in our study as its presence was collected together with other autoimmune diseases as "presence of autoimmune overlaps" in general, making not possible to study the association only in SS. Furthermore, some patients had ongoing diagnostic SS tests at the moment of our work. Similarly, oral ulcers are much more frequent in SLE than other autoimmune diseases, potentially traducing a more typical SLE disease than in those without, which might justify differences in skin AGEs levels.

With respect to the negative relation found between skin AGEs and ANA antibodies, all our patients were ANA positive at SLE diagnosis but 10 of them (8.2%) converted during disease

follow-up and were ANA negative at the moment of the study. It has been reported that the reduction of ANA responses might reflect the natural history of the disease as well as the effects of therapy (162). Accordingly, these patients could have increased skin AGEs levels due to longer disease duration or more intense need for therapy due to more severe disease, and consequent more accrual damage and potentially higher skin AGEs levels. In our cohort, current ANA negative patients showed higher disease duration (15 vs 10 years) and higher SDI (same levels of p25 and p50 but differences in p75: 1.56 vs 0.68), although the differences were not statistically significant, probably due to lack of statistical power on account of the small sample size, also shown by the wide CI of this variable in the Supplementary Table 4. We did not observe differences on immunosuppressant treatment in the moment of the study between ANA positive and ANA negative patients, but we did not retrieve data of the therapeutic history of the patients, so we cannot rule out differences in the number of immunosuppressants or time on therapy between both groups.

Only one of the two previous works studying skin AGEs in SLE have analyzed their association with disease characteristics, finding an association with age, creatinine, disease duration, the IMT of the common carotid artery, and the SDI in the univariate analysis, and only with age and disease duration in the multivariate one (124). Our work has conducted a much more extensive analysis taking into account a great number of demographic and clinical variables and performing a more complex statistical analysis considering all possible confounding factors. This provides a much deeper knowledge into these relationships and opens the door to the feasibility of using skin AGEs as a clinical tool for SLE management and prognosis.

The second part of our work was to study serum AGEs, in which have performed different studies. First, we analyzed different serum AGEs on their own (pentosidine, CML and CEL), although we did not measure the total amount of serum AGEs. Secondly, sRAGE. And third, the ratios between the serum AGEs or skin AGEs and sRAGE.

Concerning pentosidine, we have only found one significant association; a nearly 80% increase in pentosidine levels were observed in patients with SLE pulmonary manifestations, which in our cohort were only comprised of shrinking lung syndrome and lupus pneumonitis while pleuritis was considered inside the serositis term. Only one previous work has studied the relationship between pentosidine and SLE characteristics, but they did not assess pulmonary manifestations as they only collected the ones included in SLE classificatory criteria. They found, however, lower levels of pentosidine in patients with discoid lesions and photosensitivity that we could not confirm in our cohort (126). Nevertheless, several characteristics were very different in their cohort compared with ours: 37% of their patients were from African descent while, in ours, < 10% were from an ethnicity different from Caucasian or Hispanic, overlap with other inflammatory conditions was an exclusion criterion in theirs, and the mean disease duration of their cohort was 24 months while ours had a remarkably longer disease duration (only 41% of patients with a disease duration < 5 years). It is known

that RAGE is constitutively highly expressed in the lung (163,164) and it has been demonstrated to be importantly linked to lung inflammation in several lung diseases (165). Pentosidine specifically has been associated with the progression to metastases in lung cancer (166) and with asthma (measured in sputum) (167), where its role as a biomarker of a reduce response to bronchodilator treatment has been proposed (168). Based on that physiological link and on the statistically strong association with these specific pulmonary symptoms in our study, pentosidine could represent a strong predictor of these, infrequent but serious, manifestations and a useful tool in their monitoring.

When analyzing CML and CEL, we found an association with different SLE characteristics and indexes. It makes sense that both of these serum AGEs show similar results seeing that we found a positive correlation between their levels, as has been reported on a previous work performed in HC (169). Levels of both CML and CEL showed a positive correlation with anti-dsDNA antibodies and IL-6 levels (evaluated as tertiles, in the case of CML, or as continuous variables in the case of CEL). We consider that the results found in relation with CEL are more consistent with the clinical practice, as the CEL increase depended directly on the titers of anti-dsDNA antibodies and IL-6. In the case of CML, the tertiles did not match values considered positive (for anti-dsDNA antibodies) or pathogenic (for IL-6). We found an association with the 3rd tertile of anti-dsDNA antibodies (those with positive titers) and the 2nd tertile (those with what are considered negative titers but higher than undetectable); while in the case of IL-6 levels, we only saw an association between CML and the 3rd tertile [3,24-39.28 pg/mL]. Having into account that normal IL-6 values are considered < 7pg/mL, and that that 3rd tertile includes both normal and abnormal values, we reassessed the association splitting the sample into those with high values of IL-6 (> 7pmg/L) vs normal (< 7 mg/dL) but we did not find differences between groups, which makes the association difficult to interpret.

In addition, other associations with CML were also found. For each year of disease duration, CML levels increased a 1.7%, non-Caucasian patients showed CML levels almost 50% higher than the Caucasian ones, and patients suffering from densitometric osteoporosis, not associated to GC' intake, also showed increased CML (34.2%). Regarding CEL, for each new manifestation that the patient presented throughout the course of the disease (evaluated according to the ones included in either the ACR or the SLICC SLE classificatory criteria) we found CEL increases of 8.2%, while patients that had ever presented positive anti-dsDNA also had higher CEL levels (23.8%). There is only one previous work in the literature that studied the relation between SLE characteristics and CML or CEL without finding any association with disease indexes or characteristics or the number of accumulated manifestations according to the 1990 ACR classificatory criteria (78). Nevertheless, the study was done in a very small sample size (10 SLE patients and 10 HC) and both AGEs were determined through mass spectrometry and not ELISA, which makes it non-comparable to our work.

All the above characteristics are known to be correlated with disease indexes. For example, anti-dsDNA antibodies' (170) and IL-6 titers –despite the failure of IL-6 blockade therapies to the date– are correlated with disease activity (154), the number of manifestations with activity and, possibly with organ damage, disease duration with organ damage (171), and non-Caucasian ethnicities, particularly African-American and Caribbean ones, with both activity and organ damage (172). The fact that both CML and/or CEL correlate with all those indexes opens the door to their use as a novel activity/damage/prognosis biomarker in SLE.

With regards to sRAGE, we found a negative association with male gender, showing almost a 40% less sRAGE levels than females. On the other hand, patients having ever had photosensitivity or being on treatment with bDMARD or with mycophenolic acid at the moment of the study presented higher sRAGE levels (corresponding to an increase of 31.3%, 111.3%, and 59.8%, respectively). As stated in the Introduction section, there is still much to elucidate about which sRAGE levels (high or low) are associated with deleterious effects because there is evidence for both, making interpretation of the results conflicting. Assuming the mainstream theory that supports low sRAGE levels as the ones associated with inflammation in SLE, the fact that we found lower levels in males, which are known to have a more severe disease both extrarenal and renal (173), would be consequent. In the case of the positive association with photosensitivity, we have several hypothesis: First, that patients who are photosensitive tend to protect themselves more from ultraviolet radiation, a notorious trigger for both cutaneous and systemic flares in SLE (174). Secondly, that photosensitive patients are normally treated with drugs that are photoprotective like hydroxychloroquine, known to absorb ultraviolet light in the skin in a concentration-dependent manner and which has demonstrated to reduce mortality in SLE (175) by preventing flares and organ damage and also by having an effect in other comorbidities as thrombosis or bone destruction (176).

Looking at previous evidence published in the topic, there is a lack of consistent results regarding sRAGE in SLE. Ene *et al.* did not study the association between sRAGE levels and disease characteristics, but found that sRAGE decreased a 7.6% in the non-LN group ($p < 0.001$), a 5.8% in the LN group ($p < 0.001$), and a 5.5% in the type IV LN ($p < 0.001$), when compared with HC (77). Lan *et al.* observed that sRAGE was decreased in the proliferative types of LN (III and IV) and in patients with bad response to treatment (those who did not achieve partial or complete renal remission with cyclophosphamide and GC therapy) (177). The authors discussed that although the reason why lower AGEs levels are related to poor response to treatment is unknown, it had most likely to do with the NF-κB pathway, which is activated by AGEs and blocked by both GC (178) and cyclophosphamide (179). However, they did not find an association between sRAGE and activity measured by SLEDAI ($r = 0.12$ (95% CI: -0.02454 to 0.2653, $p = 0.11$) or the activity or damage index in the kidney biopsy. We have not specifically studied associations with types of LN individually, the renal response to treatment, or indexes in the renal biopsy as we had a small sample size of patients with LN (8 patients) which probably prevented us from finding any

associations with it. Other authors, like Bobek *et al*., have found a correlation of sRAGE levels with C4 concentrations in 37 children with SLE, although not with other indirect parameters of activity like the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) or anti-dsDNA titers (127). Nowak *et al.* did not find either an association between sRAGE and disease characteristics or SLEDAI-2K in 31 SLE vs 26 HC (80). Discrepancies between their cohorts and ours (for example, children vs adults) and the small sample size in most of these previous works could explain the differences in the associations found regarding our work.

Concerning the association also found between sRAGE and taking bDMARD and/or mycophenolic acid, there is scarce literature about the effect of immunomodulatory/immunosuppressant drugs in sRAGE. There is one study that observed a decrease in serum levels of sRAGE and esRAGE by 32.4% ($p = 0.004$) when treating patients with multiple sclerosis with fingolimod for 12 months. They also observed a decrease in pentosidine serum levels by 41.3% (although not significant) together with a decrease (although not significant) of clinical relapses (180). In another study performed by Gross *et al.* in renal transplant recipients, sRAGE levels were statistically significantly inversely associated in the multivariate linear regression analysis with treatment with mycophenolate mofetilo ($\beta st = -0.21$, $p < 0.001$) (181). Low sRAGE levels were also associated with a 2-3 higher risk of mortality ($p = 0.006$). Azathioprine, on the other hand, was associated with higher levels of sRAGE ($p=0.02$), despite azathioprine being also associated with taking mycophenolate mofetil ($r = -0.58$, $p < 0.001$). The authors conclude that the relationship between mycophenolate mofetil and sRAGE requires further investigation, affirmation that we fully support.

Despite these limited data showing a trend towards an association between lower sRAGE and treatment with immunosuppressants, we found that patients being treated with specific immunosuppressants showed an increase in sRAGE. Our hypothesis for explaining these results would be that patients treated with bDMARD or mycophenolate mofetil have less inflammation, as these treatments are more potent inhibitors of the inflammatory pathways than other treatments used for less severe disease. This would be supported by the previously mentioned study that found an association between higher AGEs and azathioprine (181). This association of sRAGE with certain immunosuppressants could have therapeutic implications as those treatment could be used to modulate sRAGE levels and, with, them, inflammation. However, in the current study we could not assess in more depth the relationship between immunosuppressants and SLE or check our hypothesis, so future studies should be designed for this specific purpose.

On a different note, this is the first work in the literature to study the ratios between specific serum or skin AGEs and sRAGE in SLE. We found a significant relationship between the ratios and several variables. A statistically significant positive association was observed with the presence of anti-Ro52 antibodies in the study blood test, non-Caucasian ethnicities, the SDI (only in the OLS model), densitometric osteoporosis, taking dyslipidemia drugs, CRP

and IL-6 values, IL-6 pathological values (> 7 pg/mL), and male sex. A negative association was found with being on bDMARD treatment and disease duration. Due to the novelty of theses analyses and, until further validation of these results, the purpose of this part of our study was solely exploratory, taking into account that some authors defend the highest suitability as biomarkers of the ratios over the molecules on their own (86).

Despite the known relationship between AGEs and atherosclerosis, we did not find any association between skin AGEs levels, serum AGEs or sRAGE and either CVRF or CVE. However, the *p*-value in some of the exploratory analysis was < 0.1 and, considering that we have a small number of patients with CVE (N=9), it is likely that our results are limited by a lack of statistical power which prevents us from drawing conclusions about the role of AGEs or sRAGE in CVR. Furthermore, we assessed CVD only through traditional CVRF or CVE and did not perform additional tests like the IMT of the common carotid artery measured by ultrasound (124) or the small artery elasticity measured by pulse-wave analysis using tonometric recordings of the radial artery (123), both of which have been associated with skin AGEs levels in previous works. Nowak *et al.* (80) did not find either that serum CEL, CML or sRAGE levels influenced the presence of CVD in their work, but it is necessary to point out that 80.65% of SLE patients had CVD in their cohort and the sample size was small (n=31), which could have influenced the ability to find differences between groups. No other works have studied the association between serum AGEs or sRAGE with CVD, and even, in some, it was an exclusion criteria (77). We also reassessed the correlation between skin AGEs and SDI excluding all variables related to CVD (expressed as CVE in our study) as De Leeuw *et al*. do in their work (124). They found a correlation in the exploratory analysis between skin AGEs and SDI, also after correction for the damage caused by CVD although the association was lost after adjusting for age or in the multivariate analysis. In our cohort, this new analysis did not alter the statistical correlation between SDI and AGEs or sRAGE, indicating that the association is not attributable to them being associated with CV damage.

Our work presents several limitations. Firstly, due to the retrospective nature of the study, some data could not be retrieved like the cumulative dose of GC taken throughout the disease, being only able to assess the impact of GC through the dose taken at the moment of the study. Likewise, the design makes it impossible to assess causality, which warrants future prospective studies. Secondly, and in order to clarify the effect of longstanding disease and therapy in AGEs levels, studies in newly diagnosed patients with short disease duration and naïve to treatments should be performed. Another limitation is that we did not measure the serum total AGEs levels but some specific AGEs on their own. The fact that some characteristics occurred at a low frequency could also have had an influence on the statistical power. Additionally, we did not check for all the factors that have been described to influence AGEs levels such as diet.

Our work represents a pioneer study that analyzes, in a deep and methodical way, the AGEs-RAGE axis in SLE, comparing it to HC and to a vast array of demographic and clinical

characteristics. There is scarce literature on this area, having our work several strengths. We would like to remark the larger sample size compared to other published previously, the 1:3 proportion when comparing SLE vs HC, the selection of HC with at least one CVRF and of SLE patients that reflect those found on real-life clinical practice, multiple and detailed data retrieved, complex statistics and a comprehensive analysis encompassing skin AGEs, serum individual AGEs, sRAGE, as well as their ratios. To our knowledge this is the first work to find an association between SLE activity parameters and some accrual damage indexes with skin AGEs, CML, CEL and sRAGE. Also, it is the first report of the ratios skin AGEs or serum AGEs to sRAGE in SLE. Furthermore, we have described, for the first time, AGEs (pentosidine, CML and CEL) and sRAGE associations with specific serological and clinical parameters that could define more precisely a specific phenotype of patients in whom these molecules could have a particularly meaningful contribution. Therefore, our results are innovative and indicative of the promising role of AGEs and sRAGE as a tool to be implemented in daily clinical practice as a real-time noninvasive (skin AGEs) and quickly available low-invasive (serum AGEs) surrogate biomarkers of SLE disease activity, damage, and specific manifestations.

7. Conclusions

7. Conclusions

- SLE patients present higher skin AGEs levels than HC, even after adjusting for confounders in the multivariate analysis and selecting a control population with one CVRF to be more comparable to the increased CVR of the disease itself in SLE patients. These results confirm, using a larger sample size and more elaborate statistics, the findings of previous works and support the hypothesis of the association between AGEs and SLE.
- The correlation observed between skin AGEs, some serum AGEs and sRAGE with SLE activity and/or damage markers suggests that the AGEs-sRAGE axis has a role as a new biomarker in this disease related to management and prognosis, which would have enormous implications in a field currently uncovered in SLE.
- The association of AGEs or sRAGE with specific antibodies and disease may indicate a particular clinical phenotype related to specific higher/lower AGEs and/or sRAGE levels, unveiling another potential clinical use of these products. The association of sRAGE with certain immunosuppressants could have therapeutic implications, as it could reveal a possible way of modulating sRAGE levels and, with them, inflammation. However, it needs further assessment to clarify contradictory results.
- We could not find an association between AGEs or sRAGE and CVD or CVRF, but our sample did present a small number of CVE and specific tests for CV assessment, detection of subclinical atherosclerosis or undiagnosed CVRF were not performed. Subsequent studies designed to focus on these aspects should be conducted to explore this relationship.
- We found that different ratios skin AGEs or serum AGES to sRAGE, proposed by some authors as better universal markers than the individual components, were associated with activity, damage and severity markers, antibodies, treatments, and comorbidities. However, the role of the ratios in SLE, described for the first time in this work, requires further assessment in future studies.

8. Future lines

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We currently have the following projects already active or pending initiation, which will continue with the line of research initiated in this work:

- We are currently in the last stages of a longitudinal study in patients with recently diagnosed SLE (< 1 year) and who can only have been exposed, previously to their inclusion in the study, to hydroxychloroquine or prednisone at doses < 20mg/d, nonsteroidal anti-inflammatory drugs, or analgesics within the therapeutic arsenal used in SLE. Pathologies such as SLE suffer a clear diagnostic delay due to unspecific manifestations, the infrequency of the disease, lack of doctors expertise or subclinical involvement, all of which make diagnosis complex (182,183). Our hypothesis is that the values of AGEs or sRAGE at the onset of the disease, in a “pure” measurement, unaffected by SLE treatment, could be a marker of damage accumulated before diagnosis and could have prognostic implications: the higher the values at disease onset, more accrual damage and worse prognosis. In order to assess prognosis, the study design is longitudinal with an 18-month follow-up and determinations of cutaneous and serum AGEs and sRAGE, as well as recollection of demographic and disease characteristics at 0, 9 and 18 months since inclusion.
- Another currently active project is to measure AGEs in other SADs such as RA, to compare them with the healthy population and to correlate them with disease characteristics. Subsequently, we will compare the levels of AGEs between SLE and RA adjusted for a series of factors such as disease activity. Another SAD in which we are measuring AGEs is in systemic sclerosis (SSc) and we will also study their associations with different parameters and subtypes of the disease as part of a project financed by La Marató de TV3 called "Design of an integrative patients’ stratification approach for the systemic sclerosis management”. There are few previous works that study the role of AGEs in SSc, having found an association between AGEs and the early nailfold capillaroscopic changes of SSc (184), between AGEs skin deposition in limited SSc and calcinosis (185), as well as a positive association with the modified Rodnan skin score and disease severity and a negative one with pulmonary function tests (186). The intention of measuring AGEs in other SADs is to carry out high-quality studies that can help us elucidate the role of these molecules in SADs.
- One future line of research following this study is to re-explore the relationship between skin AGEs and CVR in two projects:

  The first one is entitled “Estrategia para la evaluación y prevención personalizada del riesgo de enfermedad cardiovascular en pacientes con enfermedad autoinmune sistémica (PRECVEAS)” which has received funding from a “Fondo de Investigaciones Sanitarias” (FIS) of the “Instituto the Salud Carlos III”. People with SADs have an increased CVR. The tools for CVR assessment often underestimate CVR in SADs patients, limiting their access to timely preventive interventions. The main objective of this project is to

identify key CVRF, associated with the occurrence of atherosclerotic CVE. These CVRF will include both classical variables and others identified as potential predictors of CVR in inflammatory diseases, with special attention to the role of AGEs. The presence of subclinical coronary atherosclerosis, a powerful marker of increased CVR, will be evaluated using multi-slice computed tomography. The CVRF will be firstly assessed in a large population-based database, and subsequently in our own prospective clinical cohort. This is a large and extensively phenotyped cohort, which will undergo coronary computed tomography to assess their burden of coronary plaque. Next, we will combine the most robust predictors into a score that may be clinically used in patients with SADs to estimate their odds of having subclinical coronary plaque. There is very limited guidance as to which interventions should be performed for CVR reduction in patients with SADs and high CVR, and their effectiveness is unknown. Our goal is to fill this evidence gap by conducting a multidisciplinary CVR reduction intervention in patients identified as high CVR and evaluate its effectiveness at 6 months of follow-up.

The second one entitled “Assessment of biomarkers of venous thromboembolism in patients with granulomatosis with polyangiitis and Behçet’s disease” aims to study the relationship between diverse compounds of innate immunity and inflammatory molecules, among them the high-mobility group box 1 (HMGB1), which links to and activates RAGE, and cardiovascular disease. The work intends to study the mechanisms contributing to the increased cardiovascular morbidity and mortality seen in patients with inflammatory rheumatic diseases, using the study of venous and arterial thromboembolism as the hallmark of CVD in two profoundly inflammatory vasculopathic diseases, granulomatosis with polyangiitis and Behçet’s disease, and compared them with HC, and before and after treatment with B-cell depleting drugs.

9. Bibliography

9. Bibliography

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10. Annexes

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10.1. Published paper 1

Carrión-Barberà I, Triginer L, Tío L, Pérez-García C, Ribes A, Abad V, et al. Role of Advanced Glycation End Products as New Biomarkers in Systemic Lupus Erythematosus. Int J Mol Sci. 2024;25(5).

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Role of Advanced Glycation End Products as New Biomarkers in Systemic Lupus Erythematosus

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Abstract: Advanced glycation end-products (AGEs) may play a relevant role as inducers in the chronic inflammatory pathway present in immune-mediated diseases, such as systemic lupus erythematosus (SLE). AGEs concentrations have been associated, with discrepant results to date, with some parameters such as disease activity or accrual damage, suggesting their potential usefulness as biomarkers of the disease. Our objectives are to confirm differences in AGEs levels measured by cutaneous autofluorescence between SLE patients and healthy controls (HC) and to study their correlation with various disease parameters. Cross-sectional study, where AGEs levels were measured by skin autofluorescence, and SLE patients' data were compared with those of sex- and age-matched HC in a 1:3 proportion through a multiple linear regression model. Associations of AGEs levels with demographic and clinical data were analyzed through ANOVA tests. Both analyses were adjusted for confounders. AGEs levels in SLE patients were significantly higher than in HC ($p < 0.001$). We found statistically significant positive associations with SLE disease activity index (SLEDAI) and damage index (SDI), physician and patient global assessment, C-reactive protein, leukocyturia, complement C4, IL-6 and oral ulcers. We also found a negative statistically significant association with current positivity of anti-nuclear and anti-Ro60 antibodies. AGEs seem to have a contribution in LES pathophysiology, being associated with activity and damage and having a role as a new management and prognosis biomarker in this disease. The association with specific antibodies and disease manifestations may indicate a specific clinical phenotype related to higher or lower AGEs levels.

Keywords: systemic lupus erythematosus; advanced glycation end products; cardiovascular disease; biomarkers

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1. Background

Advanced glycation end-products (AGEs) are a set of compounds whose formation is a complicated molecular process resulting from the non-enzymatic interaction of reducing sugars and associated metabolites with peptides, proteins, and amino acids [1]. AGEs can

accumulate under hyperglycaemic and pro-oxidative conditions, and it has been postulated that they have a role in inflammation.

The mechanisms of toxicity of AGEs are mainly related to two facts. On the one hand, glycation favors cross-links between the modified proteins, causing structural alterations and resulting in gradual deterioration in cell and tissue function and the generation of new immunological epitopes [2]. On the other hand, AGEs are recognized by their own receptor (RAGE), which is expressed in multiple cells from the immune system [3]. RAGE is divided into extracellular, transmembrane, and intracellular segments [4]. The interaction of AGEs with RAGE can activate the downstream nuclear factor kappa-B (NF-κB) signaling pathway and promote the secretion of several cytokines.

Soluble RAGE (sRAGE is variant of RAGE, a positively charged 48-kDa cleavage product from RAGE that keeps the ligand binding site but loses the other two domains [5]. sRAGE binding to ligands terminates intracellular signal transduction due to the loss of the transmembrane and intracellular fragments and inhibits the proinflammatory processes mediated by RAGE and its ligands by acting as a decoy which competitively binds to RAGE ligands [6]. sRAGE and not RAGE levels have been studied and linked to inflammation [7] as sRAGE is soluble and easy measurable, while RAGE is a cell–bound receptor and hence tissues are required for its measurement.

So far, more than 20 AGEs have been described in tissues [8]. Due to their stability, the most measured AGEs are serum or plasmatic Nε-(carboxymethyl)lysine (CML) and pentosidine. However, a part of the AGEs has the characteristic of being fluorescent, so it is possible to quantify them in a single measurement using an autofluorescence reader. This technique that measures accumulated AGEs in the skin, makes this assessment more appropriate to quantify the concentration of AGEs in an individual throughout their life than that of a single specific moment in relation to an acute process. So that, skin AGEs may better correlate with disease control, duration, and complications than serum AGEs [9]. As a validation method, it has been described that this autofluorescent measurement correlates with the concentration of AGEs, both fluorescent and not fluorescent, measured in skin biopsies [10]. Some of the advantages of measuring skin AGEs vs serum or plasmatic ones consist of having non-invasive, real-time data, easily available and affordable.

In systemic autoimmune diseases, such as systemic lupus erythematosus (SLE), increased AGEs formation can be expected, as inflammation is one of the hallmarks of the disease. Chronic inflammation in SLE appears to be associated with an intensified glycation process and the formation of AGEs, having higher values compared to healthy controls (HC) been demonstrated in some studies [11–15]. At the same time, AGEs are also involved in the generation of more inflammation and reactive oxygen species, creating positive feedback that enhances inflammation and AGEs levels.

Regarding atherosclerosis, AGEs have been linked to increased vascular rigidity and atherosclerosis [16–18]. In SLE, the presence of accelerated atherosclerosis that cannot be fully explained by traditional risk factors for cardiovascular disease is a well-recorded phenomenon [19]. Some studies have suggested that increased levels of AGEs might contribute to the development of this accelerated atherosclerosis in SLE and, therefore, could be used as early markers for cardiovascular disease in this pathology [14,15].

Lately, there has been increased attention on the potential of RAGE and AGEs to target chronic inflammatory diseases such as SLE. Some studies have expounded on their usefulness as biomarkers of SLE diagnosis and prognosis, their relationship with accelerated atherosclerosis, as well as their potential place as targets for new treatments. However, we find some controversial results in the literature, showing that more and better studies are needed to fully elucidate their role in SLE.

Taking into account that the relation between skin AGEs and SLE has only been reported in one previous paper, the purpose of this work is to try to elucidate the role of AGEs in SLE as potential biomarkers of the disease, as well as their application in routine clinical practice as a tool for improving the diagnosis, monitoring, and/or prognosis of the disease, or as surrogate markers for the assessment of cardiovascular risk in this

population. Our study involved describing AGEs concentrations in SLE and comparing them to age- and sex-matched HC; searching for correlations between AGEs concentrations and SLE characteristics such as specific manifestations, indexes of activity or accrual damage, or patient reported outcomes (PROs); and finally, exploring AGEs relationship with cardiovascular disease and cardiovascular risk factors (CVRF).

2. Results

2.1. Characteristics of Patients and Controls

The differences between the 189 HC and 62 cases are shown in Table 1: HC had a higher BMI and a higher incidence of dyslipidemia (both in total cholesterol and low-density lipoprotein values), obesity, hypertension, and active smoking. Patients with SLE had higher AGEs values and creatinine concentrations.

Table 1. Descriptive characteristics of cases and healthy controls and bivariate analysis between both groups. As we are exploring confounding variables *p*-value was widened and considered statistically significant if <0.1 (highlighted in bold in the text). AGEs: advanced glycation end products; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

	Controls	**Cases**	***p*-Value**
	N = 189	**N = 62**	
Ethnicity			**<0.001**
Caucasian	189 (100%)	46 (74.2%)	
Other	0 (0.00%)	16 (25.8%)	
Age	56.0 [52.0; 62.0]	55.0 [51.0; 61.8]	0.193
Sex: Female	180 (95.2%)	58 (93.5%)	0.748
Hypertension	73 (38.6%)	14 (22.6%)	**0.032**
Obesity	61 (32.3%)	12 (19.4%)	**0.075**
Dyslipidemia	85 (45.0%)	9 (14.5%)	**<0.001**
Smoking			**0.054**
Never	79 (41.8%)	24 (38.7%)	
Former (>1 year)	54 (28.6%)	27 (43.5%)	
Active	56 (29.6%)	11 (17.7%)	
Body mass index	28.9 (5.98)	25.6 (4.65)	**<0.001**
Creatinine	0.70 [0.61; 0.77]	0.74 [0.64; 0.90]	**0.006**
Uric acid	4.90 (1.27)	4.70 (1.62)	0.365
Cholesterol	210 (37.5)	187 (39.5)	**<0.001**
HDL	61.9 (14.0)	65.9 (15.7)	0.125
LDL	138 (29.3)	112 (34.6)	**<0.001**
Triglycerides	123 [95.8; 160]	92.0 [70.0; 159]	**0.003**
Antidyslipidemics	27 (14.3%)	11 (17.7%)	0.649
Antihypertensives	61 (32.3%)	16 (25.8%)	0.424
AGEs	1.98 (0.45)	2.71 (0.56)	**<0.001**
AGEs in tertiles			**<0.001**
[1.0, 1.9)	83 (43.9%)	3 (4.84%)	
[1.9, 2.4)	74 (39.2%)	13 (21.0%)	
[2.4, 4.2]	32 (16.9%)	46 (74.2%)	

2.2. Comparison of AGEs in SLE Patients vs. Healthy Controls

According to all of the data explored, the multivariate model was adjusted with age, smoking, dyslipidemia, creatinine. The model reported a statistically significant difference between SLE and HC in AGEs values, showing that AGEs values in SLE patients were 0.721

(95% confidence interval (CI) [0.566; 0.876]) units higher ($p < 0.001$) than HC. See Table 2 for the analysis of covariance of fixed effects and Supplementary Figure S3 for the effects graphic.

Table 2. Fixed-effects analysis of covariance (ANCOVA) model to study differences in AGEs levels between cases and healthy controls. y: years.

	Est.	2.5%	97.5%	t Val.	*p*-Value
Intercept	1.9418	1.8450	2.0385	39.5252	<0.0001
Group: Cases	0.7210	0.5660	0.8759	9.1645	<0.0001
Age (57.5 years)	0.0168	0.0081	0.0254	3.8359	0.0002
Smoking (Yes)	0.3265	0.1945	0.4585	4.8724	<0.0001
Creatinine (0.72 mg/dL)	0.2110	−0.1763	0.5983	1.0732	0.2843
Dyslipidemia (Yes)	−0.1240	−0.2544	0.0065	−1.8720	0.0624
(Group: Cases) + (Dyslipidemia (Yes))	0.1286	−0.2227	0.4799	0.7211	0.4715

2.3. Characteristics of SLE Patients According to AGEs Levels: Bivariate Analysis

A total of 122 SLE patients were included. All of the variables that showed statistically significant differences according to AGEs tertiles in the bivariate analysis are depicted in Table 3, adjusted by age (*p*-value M1) and by both age and smoking (*p*-value M2). The demographic characteristics and other SLE variables of interest are detailed in Supplementary Table S3.

Table 3. Variables that showed statistically significant differences according to AGEs tertiles in the bivariate analysis. M1: adjusted by age, M2: adjusted by age and smoking. "c" indicates variables which have been categorized as stated in Section 4. Bold indicates *p*-value < 0.1 and * indicates values according to the blood test performed in the study. *p*-val: *p*-value; SLEDAI: SLE disease activity index; SDI: systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index; PGA: Physician global assessment; FACIT: Functional Assessment of Chronic Illness Therapy—Fatigue Scale; PtGA: Patient global assessment; GPT: Glutamic-pyruvic transaminase; CRP: C-reactive protein; IL-6: interleukin-6; ANA: antinuclear antibodies; C4: complement C4; GC: glucocorticoids; IS: Immunosuppressants (includes treatment with methotrexate, leflunomide, tacrolimus, mycophenolic acid or mycophenolate mofetil, azathioprine, cyclophosphamide, cyclosporine, rituximab or belimumab).

Variables	All	1st Tertile [1.2, 2.3)	2nd Tertile [2.3, 2.8)	3rd Tertile [2.8, 4.6]	*p*-Val M1	*p*-Val M2
	N = 122	N = 44	N = 41	N = 37		
Age	50.4 (14.9)	41.8 (13.8)	49.9 (12.2)	61.2 (11.9)		**<0.001**
Smoker	32 (26.2%)	10 (22.7%)	11 (26.8%)	11 (29.7%)	**<0.001**	
cDisease duration (years)					**0.082**	**0.090**
0–5	50 (41.0%)	19 (43.2%)	18 (43.9%)	13 (35.1%)		
6–10	16 (13.1%)	7 (15.9%)	6 (14.6%)	3 (8.11%)		
11–20	33 (27.0%)	13 (29.5%)	11 (26.8%)	9 (24.3%)		
>20	23 (18.9%)	5 (11.4%)	6 (14.6%)	12 (32.4%)		
Classificatory Criteria and Other Clinical and Serological Data						
Oral ulcers ever	50 (41.0%)	13 (29.5%)	18 (43.9%)	19 (51.4%)	**0.022**	**0.033**
Arthritis ever	92 (75.4%)	31 (70.5%)	32 (78.0%)	29 (78.4%)	**0.070**	**0.092**
Renal disease ever	8 (6.56%)	2 (4.55%)	1 (2.44%)	5 (13.5%)	**0.067**	**0.054**
cNumber of manifestations					**0.032**	**0.069**
[3, 7)	58 (47.5%)	19 (43.2%)	21 (51.2%)	18 (48.6%)		
7	24 (19.7%)	10 (22.7%)	8 (19.5%)	6 (16.2%)		
[8, 12]	40 (32.8%)	15 (34.1%)	12 (29.3%)	13 (35.1%)		
Disease Activity Indexes						
SLEDAI	4.00 [2.00; 6.00]	4.00 [0.00; 6.00]	4.00 [2.00; 6.00]	6.00 [2.00; 8.00]	**0.016**	**0.041**

Table 3. *Cont.*

Variables	All	1st Tertile [1.2, 2.3)	2nd Tertile [2.3, 2.8)	3rd Tertile [2.8, 4.6]	*p*-Val M1	*p*-Val M2
	N = 122	N = 44	N = 41	N = 37		
cSLEDAI					**0.003**	**0.008**
Remission/Mild	71 (58.7%)	29 (67.4%)	25 (61.0%)	17 (45.9%)		
Moderate	39 (32.2%)	11 (25.6%)	14 (34.1%)	14 (37.8%)		
Severe	11 (9.09%)	3 (6.98%)	2 (4.88%)	6 (16.2%)		
SDI	0.00 [0.00; 1.00]	0.00 [0.00; 1.00]	0.00 [0.00; 1.00]	1.00 [0.00; 2.00]	**0.026**	**0.007**
cSDI_3					**0.052**	**0.017**
0–2	110 (90.9%)	41 (95.3%)	38 (92.7%)	31 (83.8%)		
3–4	8 (6.61%)	2 (4.65%)	2 (4.88%)	4 (10.8%)		
5–6	3 (2.48%)	0 (0.00%)	1 (2.44%)	2 (5.41%)		
PGA	2.00 [1.00; 3.00]	1.50 [1.00; 2.00]	2.00 [1.00; 3.00]	2.00 [1.00; 2.00]	**0.083**	**0.051**
cPGA					**0.051**	**0.029**
<1	18 (14.9%)	7 (16.3%)	6 (14.6%)	5 (13.5%)		
1–2	69 (57.0%)	27 (62.8%)	19 (46.3%)	23 (62.2%)		
>2	34 (28.1%)	9 (20.9%)	16 (39.0%)	9 (24.3%)		
Patient Reported Outcomes						
FACIT	17.5 [10.0; 27.0]	14.0 [9.00; 23.0]	22.0 [13.0; 30.0]	18.0 [10.0; 28.0]	**0.099**	**0.138**
PtGA	2.75 [1.00; 5.00]	2.00 [1.00; 3.00]	3.00 [2.00; 5.00]	3.00 [1.00; 5.00]	**0.028**	**0.042**
cPtGA					**0.112**	**0.121**
[0.0, 2.5)	57 (46.7%)	26 (59.1%)	14 (34.1%)	17 (45.9%)		
[2.5, 4.5)	28 (23.0%)	9 (20.5%)	12 (29.3%)	7 (18.9%)		
[4.5, 8.0]	37 (30.3%)	9 (20.5%)	15 (36.6%)	13 (35.1%)		
Serological variables						
GPT *	17.0 [13.0; 22.0]	16.0 [12.0; 22.5]	16.0 [13.0; 20.0]	18.0 [15.0; 23.0]	**0.095**	**0.068**
Total cholesterol *	181 (37.7)	172 (29.6)	174 (38.0)	201 (39.5)	**0.046**	**0.093**
cCRP *					**0.058**	**0.053**
[0.03, 0.12)	45 (37.2%)	24 (55.8%)	8 (19.5%)	13 (35.1%)		
[0.12, 0.28)	36 (29.8%)	11 (25.6%)	17 (41.5%)	8 (21.6%)		
[0.28, 3.92]	40 (33.1%)	8 (18.6%)	16 (39.0%)	16 (43.2%)		
cIL-6 *					**0.049**	**0.025**
[0.63, 1.88)	36 (33.3%)	18 (48.6%)	12 (31.6%)	6 (18.2%)		
[1.88, 3.33)	36 (33.3%)	11 (29.7%)	14 (36.8%)	11 (33.3%)		
[3.33, 144.10]	36 (33.3%)	8 (21.6%)	12 (31.6%)	16 (48.5%)		
ANA+ *	112 (92.6%)	43 (100%)	38 (92.7%)	31 (83.8%)	**0.027**	**0.036**
Anti-Ro60+ *	45 (37.8%)	17 (40.5%)	19 (47.5%)	9 (24.3%)	**0.183**	**0.164**
C4 *	19.8 (8.23)	18.5 (7.97)	18.7 (7.09)	22.4 (9.23)	**0.025**	**0.017**
Leukocyturia *	0.00 [0.00; 1.00]	0.00 [0.00; 0.00]	0.00 [0.00; 1.00]	1.00 [0.00; 2.00]	**0.004**	**0.001**
Hematuria *	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.00 [0.00; 1.00]	**0.031**	**0.067**
cLeukocyturia *					**0.052**	**0.024**
0	72 (60.0%)	33 (78.6%)	24 (58.5%)	15 (40.5%)		
1	25 (20.8%)	6 (14.3%)	11 (26.8%)	8 (21.6%)		
[2, 5]	23 (19.2%)	3 (7.14%)	6 (14.6%)	14 (37.8%)		
Treatments						
GC	30 (24.6%)	7 (15.9%)	11 (26.8%)	12 (32.4%)	**0.004**	**<0.001**
Current dose of GC	5.00 [2.50; 10.0]	7.50 [3.75; 10.0]	5.00 [2.50; 12.5]	5.00 [2.50; 6.25]	**0.050**	**0.029**
Tacrolimus	1 (0.82%)	0 (0.00%)	0 (0.00%)	1 (2.70%)	0.147	**0.083**
cTreatment2					**0.077**	**0.092**
No IS	66 (54.1%)	27 (61.4%)	20 (48.8%)	19 (51.4%)		
IS	56 (45.9%)	17 (38.6%)	21 (51.2%)	18 (48.6%)		

2.4. Correlations between AGEs and SLE Characteristics: Multivariate Analysis

After adjustment for confounding variables, several SLE characteristics showed associations with AGEs levels. First of all, two of the most important SLE disease indexes, SLE disease activity index (SLEDAI) and SLE damage index (SDI), were significantly associated with AGEs levels. While for the SLEDAI we found a progressive increase in AGEs values as the SLEDAI activity escalated (AGEs values in patients with moderate and severe activity were 0.2 (95% CI [0.0006; 0.4], $p = 0.0493$) and 0.52 (95% CI [0.177; 0.86], $p = 0.003$) units higher than patients in remission/mild, respectively, we only found differences in SDI between those with low (0–2) and high scores (5, 6) (AGEs values 0.717 (95% CI [0.139; 1.295], $p = 0.0156$) units higher). This association with disease activity is also reflected in both the physician global assessment (PGA) and the patient global assessment (PtGA). In those cases, values higher than 1 (PGA) or 3 (PtGA) were associated with an AGEs increase. PGA score of 1–2 and a PGA score higher than 2 had AGEs levels 0.033 (95% CI [0.058; 0.61], $p = 0.018$) and 0.39 (95% CI [0.094; 0.694], $p = 0.01$) units higher than patients with a PGA of 0, respectively; and patients with a PtGA score >3 had AGEs levels 0.26 (95% CI [0.063; 0.46], $p = 0.01$) units higher than patients with PtGA score $\leq$3.

Regarding serum biomarkers, we observed an increment in AGEs levels as C-reactive protein (CRP) and IL-6 increased, but significant differences were only detected between the 3rd and 1st tertile: 0.259 (95% CI [0.035; 0.48], $p = 0.02$) units higher for CRP and 0.352 (95% CI [0.1; 0.6], $p = 0.006$) for IL-6. The same tendency was observed in the level of leukocyturia (0.369, 95% CI [0.112; 0.626], $p = 0.005$) and C4 complement, although in this last one, significant differences with the 2nd tertile were also observed (0.25 (95% CI [0.02; 0.48], $p = 0.0335$) units higher for the 2nd tertile; and 0.28 (95% CI [0.056; 0.514], $p = 0.015$) for the 3rd one).

With reference to autoantibodies, a negative association was found between AGEs levels and both the presence of ANA or anti-Ro60 antibodies in the blood test performed for the study, where AGEs values were 0.496 (95% CI [0.937; 0.054], $p = 0.028$) and 0.26 (95% CI [0.5; 0.017], $p = 0.035$) units lower, respectively.

Finally, patients which had ever presented oral ulcers, a prevalent SLE manifestation, had AGEs values 0.216 (95% CI [0.02; 0.41], $p = 0.03$) units higher than patients who had never. All of these data are depicted, according to the prediction of each model, in Figures 1 and 2 which graphically represent the mean and its corresponding 95% CI of AGEs for each category of variables. p-values < 0.05 indicate significant differences between the categories and the reference level of each variable. Also, the fixed-effects ANCOVA model between AGEs and each of the variables are provided Supplementary Table S4.

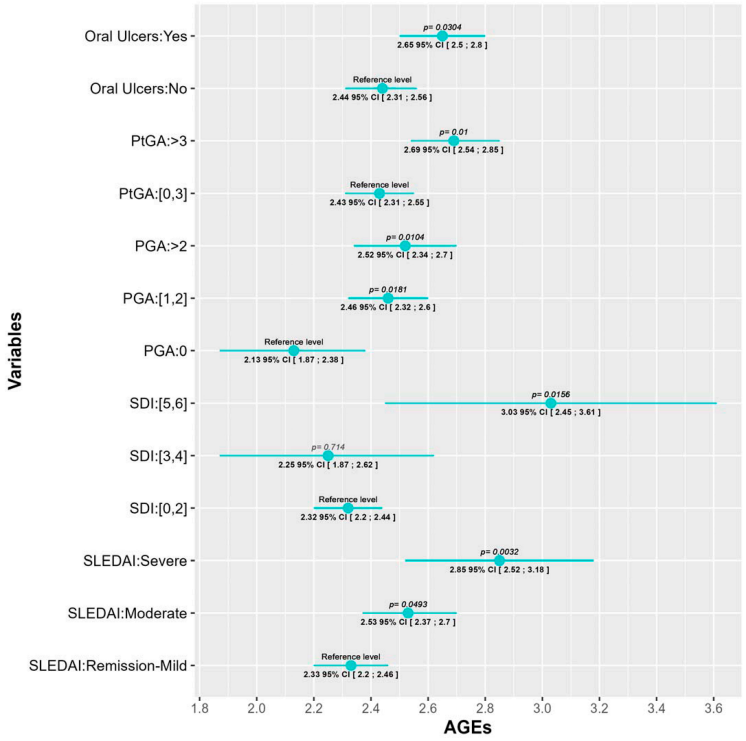

Figure 1. Statistically significant associations between AGEs levels and SLE characteristics and indexes. *p*-values < 0.05 (bold) indicate significant differences between the categories and the reference level of each variable; *p*-values not in bold indicate associations not statistically significant. PtGA: patient global assessment; PGA: physician global assessment; SDI: SLE damage index; SLEDAI: SLE disease activity index.

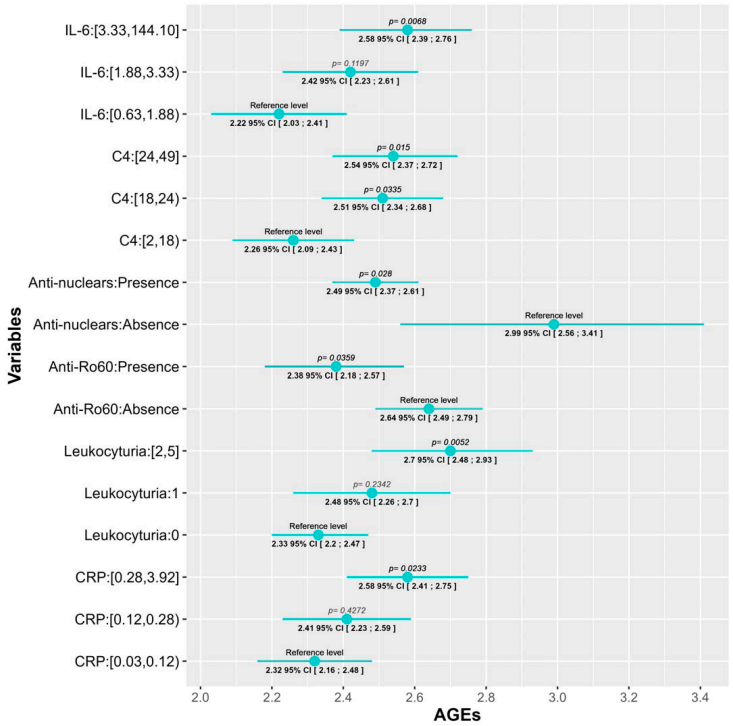

Figure 2. Statistically significant correlations between AGEs levels and SLE serological parameters. The change in AGEs values is depicted according to the reference category of each variable. *p*-value is considered significant if <0.05 (bold). IL-6: interleukin 6 (pg/mL); C4: complement 4 (mg/dL); CRP: C-reactive protein (mg/dL).

3. Discussion

We observed statistically significant differences between AGEs values measured by skin autofluorescence in SLE patients vs. HC. This difference has only been studied in two previous works [14,15] with small sample sizes (55 and 30 cases respectively, matched 1:1 with HC), and our research builds upon these studies in the following ways. First, we have increased the sample size, especially the HC sample, by matching cases with HC in a 1:3 proportion instead of a 1:1 proportion, making the study more robust. Secondly, we selected HC that had at least one CVRF, so they would be more comparable to our patients who at least have one CVRF, being that the disease itself. This is based on the well-reported knowledge that AGEs are related to inflammation and cardiovascular risk on the one hand and, on the other, that patients with autoimmune diseases such as rheumatoid arthritis, have an increased risk of cardiovascular disease that makes necessary to add a fixed multiplier of 1.5 to 2 to the established cardiovascular disease prediction general algorithms in order to adjust for the increased risk due to the disease [20]. Nienhuis et al. [14] selected a second control population with essential hypertension (EH), apart from the one conformed by HC. They found statistically significant differences in AGEs levels between SLE patients and HC but not between the SLE and the EH cohort, suggesting that finding differences when selecting HC with at least one CVRF could traduce a higher statistical power and a reduced probability of committing a type I error. Furthermore, they selected an SLE population with inactive disease, which might not reflect the reality of SLE patients in terms of disease characteristics in the way our patients might, which were included independently of their disease activity.

Additionally, we carefully examined all possible confounding factors to avoid drawing premature conclusions. Two controversial points were raised during the analysis. First, we observed only a positive trend shown by creatinine in the bivariate analysis of AGEs levels in the whole sample [21]. We discussed if that trend could have a fictitious origin since patients with SLE had higher creatinine levels (although in normal range) and were mostly located in the third AGEs tertile, and also since the trend was not observed when we analyzed the two groups separately. However, we finally decided to include creatinine in the model since there is ample evidence of a higher accumulation of AGEs in patients with renal failure [22] and lupus nephritis [12], and a difference could exist between groups since renal disease was an exclusion criterion in the HC group. Secondly, we found a negative association between dyslipidemia and AGEs, which was observed both in the combined analysis of the whole sample and in the HC separately (suggesting that such association comes from the HC group). The only data in the literature that could explain this negative association comes from the reported effect of lipid-lowering drugs in reducing AGEs levels [23]. Among HC, only 27 of the 85 with dyslipidemia (32%) were being treated with lipid-lowering agents, so we hypothesized that the rest could be controlling it with a lower-fat diet, which has also been associated with reduced AGEs levels [24]. Hence, we ended up including dyslipidemia in the model.

As for the interaction term between the main effect and dyslipidemia, although it was not found to be significant in the model, graphically the interaction seemed clear, especially in the group of SLE patients (Supplementary Figure S2). This could be due to a lack of statistical power, since in the group of SLE patients there were only 8 dyslipidemic cases, unlike the 85 dyslipidemic HC. Therefore, the statistical power to detect this difference was much lower in the patient group, generating a less precise CI to reject the alternative hypothesis and leading to a lack of significance.

Regarding the study of AGEs relationship with SLE characteristics, we have found associations between AGEs levels and some disease activity indexes: SLEDAI, PGA, PtGA, CRP, and IL-6. As reflected in Section 2, the rise of AGEs levels with the increase of SLEDAI, which is the activity index most frequently used for SLE in clinical practice nowadays, showed a robust correlation. This association was also observed with other markers of activity commonly used to assess the disease state: PGA, PtGA, and IL-6. PGA is a part of the main indexes used currently to define remission or low disease activity in SLE. PtGA

may be a more subjective parameter which can be influenced by external factors but that is clearly related to quality of life in SLE patients. IL-6 is not used routinely in the follow-up of SLE patients but its role in inflammation it is widely known generally and in rheumatic diseases in particular.

In the case of CRP, a significant association was only found between the upper tertile (0.28–3.92 mg/dL) and the first (<0.12), suggesting that the highest levels of AGEs were found among the patients with higher CRP values, both with values considered normal and abnormal (normal reference values in our laboratory <0.5 mg/dL). However, this correlation is only supported up to CRP values < 0.7 (R2 = 0.42, $p < 0.0001$), as graphically reflected in Supplementary Figure S4. No correlation was found with higher CRP levels, which could be justified by a small number of patients with abnormal CRP levels. There was also a positive association with higher C4 levels, which draws attention since low C4 levels are the ones traditionally associated with high disease activity. However, although a decrease in complement levels is included in SLE classificatory criteria, there is wide controversy in the literature about the limited usefulness of the current techniques and types of complement measured in SLE and their ability to reflect disease activity [25]. Other uncertainties about complement are whether low levels should be persistent or combined (both C3 and C4) to be significant [26,27]. In our study, C3 levels showed a statistically significant direct correlation with C4 values ($p \leq 0.001$) but not with AGEs levels. There was no association between having normal C4 levels at the moment of the study and not having had hypocomplementemia ever: 43% of the patients with current normal C4 levels had history of hypocomplementemia and 57% did not, while 77% of the patients with history of low C4 had now normal levels. This could traduce either fluctuant titers or normalized levels of C4 in response to treatment/lower disease activity and a need for further studies to elucidate the relation between complement and AGEs.

We also found a relationship between AGEs and indexes of accrual damage, the SDI. There is only a previous work in the literature that analyzed this association [15]. They found a correlation between AGEs and SDI in the univariate analysis that was lost after adjusting for age as well as in the multivariate analysis. In our case, the association persisted after adjusting for age and smoking status and any other possible confounding factor in the multivariate analysis. Considering this association, measuring AGEs levels could have a high impact in the prognosis of the disease helping to identify a subtype of patients with a more serious disease marked by higher accrual damage, which would be susceptible of a stricter follow-up and intensive treatment regimen, and subsequently allowing to improve these patients' outcomes.

Specific manifestations (oral ulcers) or autoantibodies profile (less frequent anti-Ro60+ antibodies), could indicate a different clinical phenotype in SLE patients with less inflammation and thus, with lower AGEs levels. In clinical practice, it is very common to find overlaps of autoimmune diseases in the same patient, being especially frequent in SLE its overlap with Sjögren syndrome (SjS). It is known that both diseases have different inflammatory profiles [28], which could explain why there could be differences in AGEs levels between patients anti-Ro60 positive and negative. AGEs concentrations have been scarcely studied in SjS and efforts have not been directed to skin AGEs but RAGE and sRAGE with conflicting results [29–31], so more studies are needed to investigate AGEs levels in SjS and their differences both with SLE patients and with patients with a SLE-SjS overlap. Unfortunately, we could not validate this hypothesis in our study as the presence of SjS was recorded together with other autoimmune diseases as presence of overlapping syndrome in general, making studying the association only in SjS not possible. Furthermore, some patients had ongoing diagnostic SjS tests at the moment of our work. Similarly, oral ulcers are much more frequent in SLE than other autoimmune disease, potentially traducing a more typical SLE disease than in those without, which might justify differences in AGEs levels.

Regarding the negative relation found between AGEs and ANA antibodies, all patients were ANA+ at SLE diagnosis but 10 of them (8.2%) converted during disease follow-up

and were ANA− at the moment of the study. It has been reported that the reduction of ANA responses might reflect the natural history of the disease as well as the effects of therapy [32]. Accordingly, these patients could have increased AGEs levels due to longer disease duration or more intense need for therapy due to more severe disease, and consequent more accrual damage and potentially higher AGEs levels. In our cohort, currently ANA− patients showed higher disease duration (15 vs. 10 years) and higher SDI (same levels of p25 and p50 but differences in p75: 1.56 vs. 0.68) although the differences were not statistically significant, probably due to lack of statistical power on account of the small sample size, also shown by the wide CI of this variable Supplementary Table S4. We didn't observe differences in terms of taking immunosuppressants in the moment of the study between ANA+ and ANA− patients, but we did not retrieve data of the therapy history of patients, so we cannot rule out differences in the number of immunosuppressants or time taking therapy between both groups.

Despite the known relationship between AGEs and atherosclerosis, we did not find any correlation between AGEs levels and either CVRF or cardiovascular events (CVE). However, the *p*-value in the bivariate analysis was <0.1 and, considering that we have a small number of patients with CVE (N = 9), it is likely that our results are limited by a lack of statistical power which prevents us from drawing conclusions about the role of AGEs in cardiovascular risk. Furthermore, we assessed cardiovascular disease only through traditional CVRF or CVE and did not perform additional tests such as the intima-media thickness of the common carotid artery measured by ultrasound [15] or the small artery elasticity measured by pulse-wave analysis using tonometric recordings of the radial artery [14], both of which have been associated with AGEs levels in previous works. We also reassessed the correlation between AGEs and SDI excluding all variables related to cardiovascular disease (expressed as CVE in our study) as De Leeuw et al. do in their work [15]. They found a correlation in the bivariate analysis between skin AGEs and SDI, also after correction for the damage caused by CV disease. This association was not seen after adjusting for age or in the multivariate analysis. In our cohort, this new analysis did not alter the statistical correlation between SDI and AGEs, indicating that the association is not attributable to AGEs being associated to CV damage.

Only one of the two previous works studying skin AGEs in SLE have analyzed their association with disease characteristics, finding an association with age, creatinine, disease duration, the intima-media thickness of the common carotid artery, and the SDI in the univariate analysis, and only with age and disease duration in the multivariate one [15]. Our work has carried out a much more extensive analysis considering a great amount of demographic and clinical variables and performing a more complex statistical analysis considering all possible confounding factors, which provides a much deeper knowledge into these relationships and opens the door to the feasibility of using AGEs as a clinical tool for SLE management and prognosis.

Our study presents several limitations. Firstly, due to the retrospective nature of the study some data could not be retrieved such as the cumulative glucocorticoid (GC) dose that the patients had taken throughout the disease, and we could only assess the impact of GC through the current dose at the moment of the study. Likewise, the design makes it impossible to assess causality, which warrants future prospective studies. Secondly, and in order to clarify the effect on longstanding disease and therapy in AGEs levels, studies in newly diagnosed patients should be performed. Another limitation is that we did not check for all of the factors that have been described to influence AGEs levels such as diet [24].

To our knowledge, this is the second work to study and the first to find an association between SLE activity parameters and skin AGEs. We have found a correlation with, not one, but several SLE activity biomarkers and, also, with damage indexes. Furthermore, we have described, for the first time, skin AGEs associations with specific serological and clinical parameters that could define more precisely a specific type of patients in whom AGEs could have a particularly meaningful contribution. Therefore, our results are innovative and indicative of the promising role of AGEs and the AGEs skin reader as a tool to be

implemented in daily clinical practice as a noninvasive, fast, real-time surrogate biomarker of SLE disease activity, damage, and specific manifestations.

4. Methodology

4.1. Subjects

This was a cross-sectional study conducted at the Hospital del Mar where patients of all ages who were visited at the SLE outpatient clinic, met the 1997 American College of Rheumatology (ACR) [33] or the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classificatory criteria [34] for SLE, accepted to participate and signed the informed consent were randomly included. The exclusion criteria were pregnancy, diabetes mellitus (DM), treatment with corticosteroids at a dose equivalent to prednisone >20 mg/day, active malignancy, and fibromyalgia. Patients and the public were not involved in the design, conduct, reporting, or dissemination of this work.

4.2. Healthy Controls

The control population was selected from the ILERVAS cohorts (Vascular and Renal Translational Research Group, IRBLleida), which includes HC selected from primary care health centers, with at least one traditional CVRF and aged between 50 and 70 years if women or between 45 and 65 years if men. The traditional CVRF included were arterial hypertension (AHT) and/or dyslipidemia (DLP) and/or obesity (defined as a body mass index (BMI) > 30 kg/m^2), and/or history in first-degree relatives of premature cardiovascular disease (men before 65-year-old and women before 60 years-old) and/or smokers and former smokers (<10 years since quitting). Exclusion criteria were as follows: history of cardiovascular disease (angina, myocardial infarction, cerebrovascular accident, peripheral arterial disease, intestinal ischemia or ischemia of some other territory), history of carotid surgery or surgery of arteries from other territories, DM and/or chronic renal disease (CRD), institutionalized population, population on long-term home-care, active neoplastic processes, life expectancy < 18 months [35]. AGEs levels were measured by autofluorescence in all of the HC.

4.3. Assessment of AGES Accumulation

In all patients, accumulated AGEs were measured non-invasively in the skin by an autofluorescence reader (Age Reader Mu Connect®, DiagnOptics Technologies BV, Groningen, The Netherlands) as described previously in the literature [10]. A light source emitting light at a wavelength of 320 to 400 nm excites fluorescent moieties in compounds in the skin to produce fluorescence at a wavelength of 420 to 600 nm (peak 440 nm). The output represents the ratio between autofluorescence in the range 420 to 600 nm and excitation light in the range 320 to 400 nm and is reported in arbitrary units (AU). Three consecutive AGEs measurements were taken from the ventral (anterior) surface of the forearm of each participant 10 cm below the elbow fold, avoiding any tattoos or heavily pigmented areas of skin. Measurements were performed at room temperature, while patients were in a seated position [36] (see Supplementary Figure S1). The mean value of the three measures was calculated and compared with AGEs values from age-matched HC obtained from previous works [10].

4.4. Statistical Methods

4.4.1. Comparison of Accumulated AGEs between Patients and Controls

A random sample of 60 individuals with systemic lupus erythematosus and of 183 healthy controls was calculated to be sufficient to estimate, with 95% confidence, a beta risk of 0.2 in a two-sided test, and an accuracy of $\pm$0.25 units, the population mean of values (with an expected standard deviation of about 0.6 units [15]). HC were sex- and age-matched with a factor of approximately 3:1 to each of the SLE patients and selected according to the common variables between both groups. Due to the limited age range of our control group, some of the SLE patients had to be excluded as it was not possible

to age-match them with HC. In addition, SLE patients with cardiovascular disease could not be included in the analysis due to it being an exclusion criterion in the HC sample. Difference of AGEs between SLE cases and HC was assessed through a fixed-effects analysis of covariance (ANCOVA) model adjusted for the confounding factors.

In order to identify potentially confounding variables, in addition to a bibliographic review about previously reported factors related to AGEs, a bivariate analysis was performed separating by cases and HC, and by tertiles of AGEs. Categorical data were described with absolute and relative frequencies, whereas continuous variables were displayed as mean (standard deviation), or as median (interquartile range) if non-normally distributed. In the case of categorical variables, we employed the Fisher's exact test for variables with small frequencies and the χ^2 test for the rest. For normal continuous variables, the Student's *t*-test was used when analyzing two groups and the analysis of variance (ANOVA) when there were more than two. For non-normal continuous variables, the test used was the Mann-Whitney U test to compare two groups and the Kruskal-Wallis' test to compare more than two. The significance level for these explorative analyses of confounding variables was taken to be <0.1.

Variables with statistically significant differences both between groups and with the AGEs response variable were considered potential confounders and were examined through interaction graphs before including them in the final model.

In the specific case of comparing AGEs levels between cases and controls and, as all of the HC were Caucasian, we performed a sensitivity analysis to assess the influence of ethnicity, testing only Caucasian patients against HC. We did not find any differences, so we kept all of the ethnicities in the final analysis.

Later on, we explored the associations between AGEs levels (stratified in tertiles) and data of all of the participants of the study (both SLE patients and HC), in order to evaluate possible confounding factors. The bivariate analysis showed a significant positive relationship between smoking and AGEs levels, while creatinine showed a trend in that same direction. On the contrary, the presence of dyslipidemia was associated with lower values of AGEs (Supplementary Table S1).

According to these results and the differences found between SLE patients and HC, interaction graphs were created to visually assess smoking, age, dyslipidemia, and creatinine as cofounding variables. We found differences in the slopes of age and dyslipidemia (Supplementary Figure S2) which were then evaluated in the fixed-effects analysis of covariance model (Supplementary Figure S3). Smoking was also added to the model due to extensive literature linking it to AGEs values. Furthermore, in the smoking interaction graph we observed that the slopes of non-smokers and former smokers behaved similarly, with only a slight increase in mean cumulative AGEs in non-smokers with SLE, but apparently insignificant, so we unified non-smokers and former smokers in the same group vs. active smokers to increase statistical power (Supplementary Figure S2a).

According to all of the data explored, the multivariate model was adjusted with age, smoking, dyslipidemia, creatinine, and the interaction terms. None of the interaction terms were statistically significant so they were finally removed from the model except for the interaction between dyslipidemia and group (SLE or HC). This one, was not omitted since it allowed us to observe the effect ($p = 0.062$) of dyslipidemia, granting a better estimation of the AGEs value (Table 2). This was verified by adjusting it without the interaction, where the main effect of dyslipidemia was lost. Dyslipidemia was also adjusted for age and smoking (since HC with dyslipidemia were younger and smoked less), and its effect remained unchanged, ruling out that it was confused by other variables (Supplementary Table S2).

4.4.2. Relation between Characteristics of SLE and Accumulated AGEs

An exploratory analysis was conducted using ANOVA tests adjusted for both age and current smoking status to investigate the association between SLE patient characteristics and the level of accumulated AGEs, including all patients from the cross-sectional study.

For a better analysis, skewed variables of interest were categorized into tertiles or according to non-linear patterns, evaluated with general additive models. Associations with a p value < 0.1 were considered significant and, if consistent, were examined individually. First of all, the identification of potentially confounding variables was performed as described in the previous analysis (D.1.). Then multiple lineal regression models studying association between AGEs levels and each variable of interest were fitted considering the corresponding confounding factors, to avoid spurious associations. In this case, the significance level was taken to be <0.05.

In both analysis, continuous variables included in the final models were mean centered to facilitate interpretation. The assumptions of linearity, homoscedasticity and normality of the residuals were verified and the presence of influential points in each model was evaluated. All statistical work was carried out4 using R version 4.1.2.

5. Conclusions

SLE patients present higher skin AGEs levels than HC, supporting the hypothesis of the association between AGEs and SLE. Furthermore, the correlation observed between skin AGEs levels and SLE activity and damage markers indicate that AGEs seem to have a role as a new biomarker in this disease related to management and prognosis, which would have enormous implications in a field currently uncovered in SLE. The association with specific antibodies and disease manifestations may indicate a particular clinical phenotype related to higher AGEs levels, unveiling another potential clinical use of these products.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ongoing research analysis.

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Abbreviations

AGEs	Advanced glycation end-products
ANA	antinuclear antibodies
ANCOVA	analysis of covariance
BMI	body mass index
CVRF	cardiovascular risk factors
HC	healthy controls
LDL	low-density lipoproteins
PGA	physician global assessment
PROs	patient reported outcomes
PtGA	patient global assessment
RAGE	receptor for advanced glycation end-products
SDI	systemic lupus erythematosus damage index
SjS	Sjögren syndrome
SLE	systemic lupus erythematosus
SLEDAI	systemic lupus erythematosus disease activity index
SLICC	Systemic Lupus International Collaborating Clinics

Appendix A

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10.2. Published paper 2

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Article

Serum Advanced Glycation End Products and Their Soluble Receptor as New Biomarkers in Systemic Lupus Erythematosus

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Abstract: It has been postulated that advanced glycation end products (AGEs) and their soluble receptor (sRAGE) may play a relevant role as inducers in the chronic inflammatory pathway in various conditions, among them, in immune-mediated diseases such as systemic lupus erythematosus (SLE). However, previous studies show conflicting results about their association with SLE characteristics and their usefulness as disease biomarkers. We aimed to study the association of specific serum AGEs (pentosidine, Nξ-(carboxymethyl)lysine (CML), Nξ-(carboxyethyl)lysine (CEL)), sRAGE levels and AGEs (specific serum AGEs and skin AGEs) to sRAGE ratios with various disease parameters, in order to clarify their potential as new biomarkers in SLE and to study their relationship with cardiovascular disease (CVD). To this aim, serum pentosidine, CML, CEL and sRAGE were measured via ELISA, and skin AGEs levels were measured by skin autofluorescence. Correlations of pentosidine levels with demographic and clinical data, indexes of activity, accrual damage and patient-reported outcomes were analyzed through multiple linear regression models, while correlations of the rest of the AGEs, sRAGE and AGE to sRAGE ratios (non-normal) were analyzed using both an OLS regression model and a GML. All of the analyses were adjusted for confounders. A total of 119 SLE patients were recruited. Serum AGEs and sRAGEs were significantly associated with SLE activity indexes and/or demographic or disease characteristics: pentosidine with pulmonary manifestations; CML with anti-dsDNA antibodies, IL-6, disease duration and non-Caucasian ethnicities; CEL with anti-dsDNA antibodies, IL-6 and accumulated number of manifestations; and sRAGE with male gender, photosensitivity and being on specific immunosuppressants. These results suggest that the AGE–sRAGE axis may serve as a novel biomarker for managing and prognosticating this disease. Its correlation with certain antibodies, demographics and disease presentations may indicate a distinct clinical phenotype associated with varying levels of AGEs and/or sRAGE. The significance of specific AGE/sRAGE ratios, introduced in this study for the first time, warrants additional investigation in forthcoming research. Our study did not confirm the link between serum AGEs and CVD, which merits further exploration through studies designed for this specific purpose.

Keywords: advanced glycation end products; systemic lupus erythematosus; cardiovascular disease; activity index

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Advanced glycation end products (AGEs) have been postulated to be pivotal participants in chronic inflammation [2]. AGEs, a diverse range of compounds, undergo intricate molecular processes resulting from the non-enzymatic interaction between reducing sugars, associated metabolites, peptides, proteins and amino acids. Under conditions such as aging, hyperglycemia and pro-oxidative states (e.g., diabetes mellitus, cardiovascular disease (CVD), chronic renal failure and neurological disorders), this interaction is enhanced, leading to the formation of protein adducts or cross-links [3], therefore increasing AGEs' propensity to accumulate [4–7]. Systemic autoimmune diseases like SLE, which are characterized by inflammation as the hallmark of the disease, are among the factors that could potentially promote AGE formation.

More than 20 AGEs have been identified in tissues, with Nξ-(carboxymethyl)lysine (CML) and pentosidine being the most studied due to their stability. Classical measurement methods involve chromatographic techniques and immunochemical methods such as enzyme-linked immunosorbent assay (ELISA) [8,9]. AGEs actively participate in promoting inflammation and reactive oxygen species generation through two primary mechanisms. First and foremost, glycation induces cross-links between modified proteins, leading to structural alterations and gradual dysfunction of cells and tissues. Additionally, AGEs interact with their receptors (RAGEs) expressed in various cells, including neutrophils, macrophages and T lymphocytes, triggering reactive oxygen species production and activating the NF-ϰB signaling pathway, ultimately contributing to inflammation [10–13].

RAGEs can be also found as a soluble form (sRAGE), acting as a decoy when binding to its ligands, which competitively bind to AGEs, inhibiting the proinflammatory processes mediated by the intracellular signal transduction of RAGEs [14,15]. While low sRAGE levels are commonly associated with inflammatory conditions, the paradoxical finding of elevated sRAGE levels in diseases like diabetes and chronic renal failure raises questions about their protective effect. Some authors propose exploring ratios between AGEs and sRAGE as potential universal risk markers for tissue damage, with better performance than AGE or sRAGE levels on their own [16–19].

Scarce previous research, with small sample sizes and simple statistics, have studied the relationship between AGEs and SLE, showing conflicting results (Supplementary Table S1). In terms of the association of AGEs with SLE characteristics, only three investigations have studied it: one finding no association with CML and Nξ-(carboxyethyl)lysine (CEL) [20]; the second one finding lower levels of pentosidine in patients with discoid lesions and photosensitivity, while positive direct Coombs test and malar rash were marginally associated ($p = 0.09$) with AGEs levels, inversely and directly, respectively [21]; and the third one reporting a direct association between the SLE disease activity index (SLEDAI) and AGE levels, but measured in plasma [22].

Serum sRAGE have been studied in deeper detail than serum AGEs, but also show discrepant results, as summarized in Supplementary Table S2. It is worth noting that the role of sRAGE in SLE is not clear, since although most studies have found lower sRAGE levels in SLE vs. healthy controls (HC) [23–28], as well as some inconsistent relationships with some SLE characteristics or indexes, three studies have described opposite results linking higher sRAGE levels with increased inflammation [20,29,30].

In SLE, the presence of accelerated atherosclerosis that cannot be fully explained by traditional risk factors for cardiovascular disease (CVD) is a well-recorded phenomenon [31]. Likewise, the role of AGE–RAGE has been suggested in atherosclerosis, with an increase in AGE production in the presence of several traditional cardiovascular risk factors (CVRFs) as hyperglycemia, aging and smoking, and some studies that additionally suggest that AGEs' relation to CVD is independent from CVRFs [20,21]. Based on that, AGEs have been considered as a major CVRF, and have been proposed to be integrated into risk stratification of patients as well as in treatment decisions due to their pivotal role in the pathogenesis of cardiovascular arterial disease [22]. Some studies have suggested that increased levels of AGEs may contribute to the development of accelerated atherosclerosis in SLE, and therefore could be used as early markers for CVD in this pathology [32–35]. However,

despite the association of AGEs or sRAGE with several CV factors in SLE, their role as early markers for CVD in this pathology is still unclear.

This current study aims to address this research gap by investigating both serum AGE levels (CEL, CML and pentosidine), as well as sRAGE, in a multiethnic Spanish cohort of individuals with SLE. We try to answer some of the unmet needs through encompassing several specific goals; firstly, to explore correlations between these specific serum AGEs, sRAGE and the ratio between both serum and skin AGE to sRAGE concentrations and various demographic and SLE characteristics, including specific manifestations, activity or damage indexes, and patient-reported outcomes (PROs). Additionally, this research seeks to examine the association between AGEs and CVD, as well as CVRFs in the SLE population. The ultimate goal is to investigate the potential of AGEs as biomarkers for SLE in routine clinical practice. This includes their possible application for improving the monitoring and prognosis of SLE, as well as their potential as surrogate markers for assessing CVR in individuals with SLE. By addressing these objectives, the study aims to provide valuable insights into the role of the AGE–sRAGE axis in SLE and its potential clinical utility.

2. Materials and Methods

2.1. Subjects

This cross-sectional study was conducted at the Hospital del Mar. Patients of all ages who visited the SLE outpatient clinic were randomly included. The selected patients met the 1997 American College of Rheumatology (ACR) [36] or the 2012 SLE International Collaborating Clinics (SLICC) classificatory criteria [37] for SLE, and accepted participation by signing informed consent. The exclusion criteria were pregnancy, diabetes mellitus (DM), treatment with glucocorticoids (GC) at a dose equivalent to prednisone > 20 mg/day, active malignancy and fibromyalgia.

It was estimated that a random sample of 97 individuals with SLE is sufficient to assess, with a 95% confidence interval (CI) and an accuracy of ± 0.1 units, with the AGEs population mean values expected to have a standard deviation (SD) of about 0.5 units [35]. A diagram indicating the sample size used for each analysis is provided in Supplementary Figure S1.

All of the patients signed the informed consent form to participate in the study. The protocol for our study was consistent with the provisions of the Declaration of Helsinki, and was approved by the ethics committee of the Hospital del Mar (CEIm-PSMAR 2018/7907/I).

2.2. Variables

A specific clinical visit was performed for this study. In this visit, demographic and clinical data were recorded, including indexes of activity, accrual damage and patient-reported outcomes (PROs) assessed through the recommended guidelines for each measurement. Furthermore, accumulated skin AGEs were also measured non-invasively in the skin with an autofluorescence reader (Age Reader Mu Connect® DiagnOptics Technologies BV, Groningen, The Netherlands), as described previously in literature [38]. Briefly, the mean value was recorded from three consecutive AGE measurements taken from the ventral (anterior) surface of the forearm of each participant 10 cm below the elbow fold. The ratio between autofluorescence (measured between 420 to 600 nm) and the excitation light (emitted by a light source within the wavelength range of 320 to 400 nm) was recorded and expressed in arbitrary units (AU). Finally, a blood extraction was performed at the visit to determine the presence of autoantibodies, other biochemical compounds and specific serum AGEs and sRAGE. Antinuclear antibodies (ANAs) were determined by indirect immunofluorescence and considered positive if >1:80; anti-Ro60 and anti-Sm antibodies were determined by either multiplex immunoassay, being positive if titers > 1 antibody indexes or by blot, and considered positive for anti-double-stranded DNA (anti-dsDNA) antibodies by multiplex immunoassay with titers > 10 UI/mL. The measurement of serum AGEs is specified in Materials and Methods Section 2.3, while the other variables and their classifications are detailed in Supplementary Figure S2.

2.3. Assessment of Specific Serum AGEs

The ELISA method was used to evaluate the concentrations of three AGEs (pentosidine, CML and CEL) and sRAGE in the serum samples of each patient. During the study, the following ELISA kits were used according to the manufacturer's instructions:

- Human pentosidine sandwich ELISA kit (Cusabio Biotech Co., Ltd. Wuhan, China, CSB-E09415h); sensitivity 7.81 pmol/mL; precision measured as coefficient of variation < 8% (intra-assay), <10% (inter-assay).
- Human CML sandwich ELISA kit (Cusabio Biotech Co., Wuhan, China Ltd., CSB-E12798h); sensitivity 15.6 pg/mL; precision measured as co-efficient of variation < 8% (intra-assay), <10% (inter-assay).
- Human CEL sandwich ELISA kit (Cusabio Biotech Co., Ltd., Wuhan, China CSB-EQ027210HU); sensitivity 0.078 nmol/mL; precision measured as coefficient of variation < 8% (intra-assay), <10% (inter-assay).
- Human receptor for AGEs, (RAGE/AGER) sandwich ELISA kit (Cusabio Biotech Co., Ltd., Wuhan, China CSB-E09354h); sensitivity 19.5 pg/mL; precision measured as coefficient of variation < 8% (intra-assay), <10% (inter-assay).

In the CEL assessment, some patients could not be included in the analysis due to the use of a different and not comparable ELISA kit, which has been discontinued.

2.4. Statistical Methods

The categorical data were described with absolute and relative frequencies, and continuous variables were displayed in terms of the mean (SD) or median (interquartile range) if non-normally distributed. Some continuous variables included in the final models were mean centered to facilitate interpretation. The assumptions of linearity, homoscedasticity and normality of the residuals were evaluated. If these premises were met, ANCOVA (analysis of covariance) multiple linear regression models were performed; however, when the assumptions could not be verified, mainly due to the right-skewed distribution of some variables, different multivariate regression models suitable for log-normal data were investigated with the aim of handling both heteroscedasticity and non-normality, and for estimating the absolute effect of each predictor. Finally, these multivariate analyses were performed using both ordinary least squares (OLS) regression models and generalized linear models (GLM) with gamma distribution and the identity link function. The assumptions of both models were evaluated assuming that the OLS model would be heteroskedastic in most of the analyses; therefore, the GLM model was used to verify and provide more evidence to the results obtained in the OLS model. The presence of influential points was also evaluated in each model through the Cook's distance. All statistical analyses were carried out using R version 4.1.2.

In order to identify potentially confounding variables, in addition to a bibliographic review about previously reported factors related to AGEs, an exploratory analysis was performed using tertiles of the AGEs. This exploratory analysis was conducted using ANOVA tables, not only for the detection of confounding variables, but also to investigate associations between SLE patient characteristics and the level of each soluble AGE and sRAGE. For a better analysis, skewed variables of interest were categorized into tertiles or according to non-linear patterns and evaluated with general additive models.

Associations with a p-value < 0.1 were considered significant and, if consistent, were examined individually. On the other hand, potentially confounding variables with statistically significant differences ($p < 0.1$), both between groups (characteristic yes/no) and AGE tertiles, were included in the final models to avoid spurious associations.

We also analyzed the associations with the ratios between specific serum AGEs or skin AGEs and sRAGE, as some authors determined that the ratios could be better biomarkers than AGE or RAGE levels on their own [18].

3. Results

The characteristics of the cohort of SLE patients are depicted in Table 1. Most of the patients were women (93.4%), mostly of Caucasian or Latin ethnicities, with low disease activity (65% in remission according to SLEDAI) and low damage (91% with SDI $\leq$ 2), and with a low number of CVRFs (61.5% with none) or CVEs (7.39% with $\geq$1 CVE).

Table 1. Demographic and disease characteristics of the SLE cohort. ESR: erythrocite sedimentation rate; CRP: C-reactive; protein; IL-6: antileukin 6; ANA: antinuclear antibodies; anti-dsDNA: anti-double-stranded; CH50, C3 and C4: complement CH50, C3 and C4; DAS28: disease activity score 28 joints; SLEDAI: SLE disease activity index; SDI: SLE damage index; PGA: physician global assessment; HAQ: health assessment questionnaire; VAS: visual analogue scale; FACIT: functional assessment of chronic illness therapy–fatigue scale; PtGA: patient global assessment; CVRF: cardiovascular risk factors (obesity = IMC > 30 kg/m^2, arterial hypertension, dyslipidemia, chronic renal disease or hyperuricemia); CVE: cardiovascular events (angina, myocardial infarction, cerebrovascular accident, peripheral arterial disease, intestinal ischemia or ischemia of some other region); GC: glucocorticoids; cDMARD: disease-modifying antirheumatic drugs; bDMARD: biological DMARD. IS: immunosuppressants (includes treatment with methotrexate, leflunomide, tacrolimus, mycophenolic acid or mycophenolate mofetil acid, azathioprine, cyclophosphamide, cyclosporine, rituximab or belimumab).

Variables	**All**
	N = 122
Gender: Female	114 (93.4%)
Body mass index	25.4 (4.74)
Ethnicity	
Caucasian	81 (66.4%)
Latin	29 (23.8%)
Other	12 (9.84%)
Age	50.4 (14.9)
Smoker	32 (26.2%)
cDisease duration (years)	
0–5	50 (41.0%)
6–10	16 (13.1%)
11–20	33 (27.0%)
>20	23 (18.9%)
Serological Variables	
ESR *	11.0 [5.00; 20.0]
cCRP *	
[0.03, 0.12)	45 (37.2%)
[0.12, 0.28)	36 (29.8%)
[0.28, 3.92]	40 (33.1%)
cIL-6 *	
[0.63, 1.88)	36 (33.3%)
[1.88, 3.33)	36 (33.3%)
[3.33, 144.10]	36 (33.3%)
ANA+ *	112 (92.6%)
Anti-dsDNA+ *	4.00 [1.00; 13.0]
Anti-Ro52+ *	26 (21.8%)

Table 1. *Cont.*

Variables	All
Anti-Ro60+ *	45 (37.8%)
CH50 *	60.3 [51.8; 70.9]
C3 *	106 (22.3)
C4 *	19.8 (8.23)
SLE Activity Indexes	
cDAS28	
0—Remission	78 (65.0%)
1—Low activity	15 (12.5%)
2—Moderate activity	21 (17.5%)
3—High activity	6 (5.00%)
cSLEDAI	
Remission/Mild	71 (58.7%)
Moderate	39 (32.2%)
Severe	11 (9.09%)
SDI	0.00 [0.00; 1.00]
cSDI_3	
0–2	110 (90.9%)
3–4	8 (6.61%)
5–6	3 (2.48%)
PGA	2.00 [1.00; 3.00]
Patient-Reported Outcomes	
HAQ	0.38 [0.00; 0.88]
Patient pain VAS	2.00 [0.00; 6.00]
FACIT	17.5 [10.0; 27.0]
PtGA	2.75 [1.00; 5.00]
Comorbidities and Cardiovascular Disease	
Hypertension	26 (21.3%)
Dyslipidemia	12 (9.84%)
Cardiovascular disease	5 (4.10%)
Chronic renal disease	3 (2.46%)
Hyperuricemia	2 (1.64%)
Obesity	22 (18.0%)
CVRF > 0	47 (38.5%)
CVE	9 (7.38%)
CVRF and CVE > 0	48 (39.3%)
Treatments	
GC	30 (24.6%)
Current dose of GC	5.00 [2.50; 10.0]
Antimalarials	93 (76.2%)
cDMARD	19 (15.6%)
bDMARD	6 (4.92%)
Azathioprine	19 (15.6%)
Mycophenolic acid	20 (16.4%)
Tacrolimus	1 (0.82%)

Table 1. *Cont.*

Variables	All
cTreatment	
No IS	66 (54.1%)
IS	56 (45.9%)

* Indicates values according to the blood test performed in the study.

3.1. Pentosidine

3.1.1. Characteristics of SLE Patients According to Pentosidine Levels: Exploratory Analysis

A total of 117 SLE patients were included. Pentosidine met the normality and homoscedasticity premises (Supplementary Figure S3), so the parametric statistical tests defined previously were performed. All of the variables that showed statistically significant differences according to pentosidine tertiles in the exploratory analysis are depicted in Table 2. Pentosidine was not found to be influenced by age or smoker status, so the analyses were not adjusted by any variable. The demographic characteristics and other SLE variables of interest are detailed in Supplementary Table S3.

Table 2. Variables that showed statistically significant differences (p-value < 0.1) according to pentosidine tertiles in the exploratory analysis. * Indicates values according to the blood test performed in the study. SLE-DAS: SLE disease activity score; UPCR (mg/g): urine protein to creatinine ratio; OP: osteoporosis; CVE_SDI: cardiovascular events assessed in the SLE damage index (cerebral vascular accident, pulmonary infarction, angina or coronary bypass, myocardial infarction, venous thrombosis or infarction of the gastrointestinal tract); AGEs: advanced glycation end products; SD: standard deviation.

Variables	First Tertile [0, 1180)	Second Tertile [1180, 1594)	Third Tertile [1594, 4334]	*p*-Value
	N = 39	N = 39	N = 39	
Classificatory Criteria and Other Clinical and Serological Data				
Direct Coombs+ ever	4 (16.7%)	4 (21.1%)	1 (4.17%)	0.063
Pulmonary ever	0 (0.00%)	2 (5.13%)	3 (7.69%)	<0.001
Disease Activity Indexes				
SLE-DAS	4.18 [1.78; 7.28]	1.79 [1.20; 6.15]	2.53 [0.82; 4.86]	0.087
Serological Variables				
Total bilirubin *	0.32 [0.25; 0.48]	0.32 [0.26; 0.38]	0.35 [0.23; 0.41]	0.097
Hematuria *	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.027
UPCR	84.6 [68.5; 133]	82.3 [63.5; 108]	74.7 [54.9; 90.7]	0.093
Comorbidities and Cardiovascular Disease				
Densitometric OP	4 (10.3%)	7 (17.9%)	7 (17.9%)	0.077
CVE_SDI				0.091
0	37 (94.9%)	34 (87.2%)	37 (94.9%)	
1	2 (5.13%)	4 (10.3%)	0 (0.00%)	
2	0 (0.00%)	1 (2.56%)	2 (5.13%)	
Treatments				
Tacrolimus	1 (2.56%)	0 (0.00%)	0 (0.00%)	0.093
Other AGEs				
Skin AGEs				0.065
<1SD	1 (2.56%)	1 (2.56%)	4 (10.3%)	
1SD-Means	4 (10.3%)	3 (7.69%)	6 (15.4%)	
Means	1 (2.56%)	2 (5.13%)	1 (2.56%)	
Means–>1SD	12 (30.8%)	10 (25.6%)	12 (30.8%)	
>1SD	21 (53.8%)	23 (59.0%)	16 (41.0%)	

3.1.2. Correlations between Pentosidine and SLE Characteristics: Multivariate Analysis

SLE characteristics that were significant in the exploratory analysis and possibly related to pentosidine levels were tested in a model adjusted for previously selected confounding variables (see Materials and Methods). After adjustment, only the presence of pulmonary manifestations (lupus pneumonitis and shrinking lung syndrome) was strongly associated (Figure 1). Specifically, patients with lung involvement had pentosidine levels that were 1181.8786 (95% CI [507.4192; 1856.3379], $p < 0.001$) units higher than those without lung involvement. The model, which does not have any confounding factors, is provided in Supplementary Table S4.

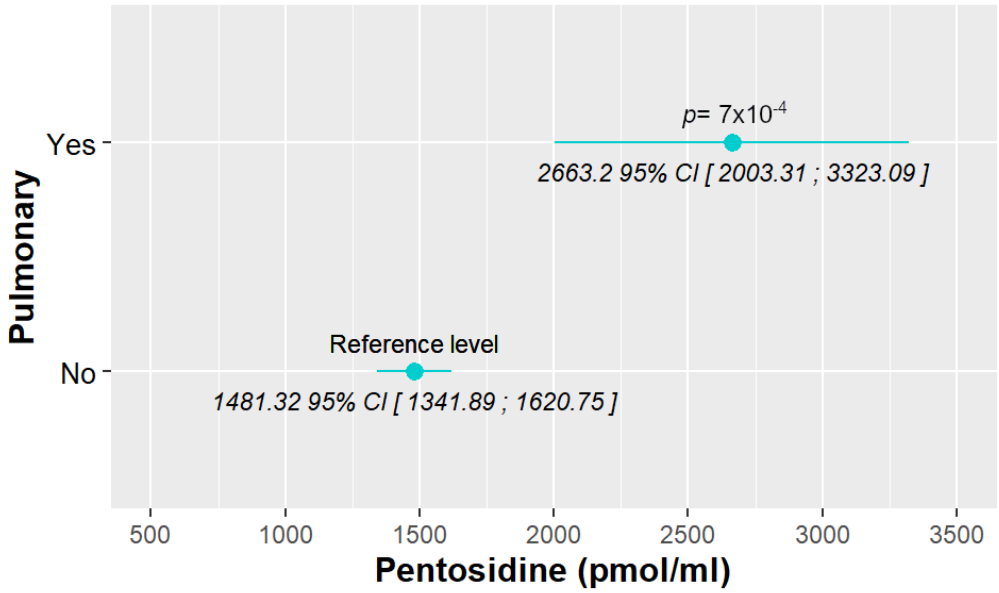

Figure 1. Associations between pentosidine levels and different SLE characteristics, with only pulmonary manifestations being significant.

3.2. CML

3.2.1. Characteristics of SLE Patients According to CML Levels: Exploratory Analysis

A total of 117 SLE patients were included. CML presented a right-skewed distribution (Supplementary Figure S3), so regression models suitable for log-normal data were performed, as defined in Materials and Methods. All of the variables that showed statistically significant differences according to CML tertiles in the exploratory analysis are depicted in Table 3. The demographic characteristics and other SLE variables of interest are detailed in Supplementary Table S5.

Table 3. Variables that showed statistically significant differences (p-value < 0.1) according to CML tertiles in the exploratory analysis. "c" indicates variables that were categorized as previously stated in Section 2. * Indicates values according to the blood test performed in the study. PGA: physician global assessment; IL-6: interleukin-6; OP: osteoporosis; AGEs; advanced glycation end products; CEL: Nξ-(carboxyethyl)lysine.

Variables	First Tertile [57.6, 240)	Second Tertile [239.8, 383)	Third Tertile [382.9, 1555]	*p*-Value
	N = 39	N = 39	N = 39	
Demographic variables				
Ethnicity 3 categories				0.023
Caucasian	30 (76.9%)	29 (74.4%)	20 (51.3%)	
Latin	6 (15.4%)	7 (17.9%)	14 (35.9%)	

Table 3. *Cont.*

Variables	First Tertile [57.6, 240)	Second Tertile [239.8, 383)	Third Tertile [382.9, 1555]	*p*-Value
	N = 39	N = 39	N = 39	
Others	3 (7.69%)	3 (7.69%)	5 (12.8%)	
Ethnicity 2 categories				0.006
Caucasian	30 (76.9%)	29 (74.4%)	20 (51.3%)	
Others	9 (23.1%)	10 (25.6%)	19 (48.7%)	
Disease-related variables				
Years of duration	4.00 [1.00; 14.5]	12.0 [4.00; 18.5]	12.0 [4.00; 21.0]	0.037
cYears of duration				0.088
0–5	22 (56.4%)	13 (33.3%)	12 (30.8%)	
6–10	5 (12.8%)	6 (15.4%)	5 (12.8%)	
11–20	9 (23.1%)	12 (30.8%)	11 (28.2%)	
>20	3 (7.69%)	8 (20.5%)	11 (28.2%)	
Tertiles years of duration				0.020
[0, 5)	21 (53.8%)	11 (28.2%)	10 (25.6%)	
[5, 16)	12 (30.8%)	14 (35.9%)	14 (35.9%)	
[16, 45]	6 (15.4%)	14 (35.9%)	15 (38.5%)	
Classificatory Criteria and Other Clinical and Serological Data				
Renal disease ever	0 (0.00%)	1 (2.56%)	7 (17.9%)	0.019
Disease Activity Indexes				
PGA	1.00 [1.00; 2.00]	2.00 [1.00; 3.00]	2.00 [1.00; 3.00]	0.094
Swollen joints	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.093
Serological variables				
IL-6 tertiles *				0.050
[0.44, 1.88)	15 (40.5%)	12 (30.8%)	11 (28.9%)	
[1.88, 3.24)	13 (35.1%)	18 (46.2%)	7 (18.4%)	
[3.24, 39.38]	9 (24.3%)	9 (23.1%)	20 (52.6%)	
Comorbidities and Cardiovascular Disease				
Densitometric OP	5 (12.8%)	4 (10.3%)	9 (23.1%)	0.034
Treatments				
Dyslipidemia drugs	4 (10.3%)	1 (2.56%)	9 (23.1%)	0.004
Mycophenolic acid	2 (5.13%)	6 (15.4%)	12 (30.8%)	0.012
Glucocorticoids	8 (20.5%)	4 (10.3%)	18 (46.2%)	<0.001
Other AGEs				
CEL	2.45 [2.09; 3.71]	3.17 [2.47; 3.66]	3.99 [2.48; 4.68]	0.064

3.2.2. Correlations between CML and SLE Characteristics: Multivariate Analysis

SLE characteristics that were significant in the exploratory analysis and possibly related to CML levels were tested in two models adjusted for the previously selected confounding variables (see Section 2). After adjustment, we found that non-Caucasian patients present higher values than Caucasian ones, and those positive for anti-dsDNA antibodies (≥11 IU/mL) also have increased CML levels. Finally, they also correlated with longer disease duration. These positive associations were significant in both OLS and GLM models. In addition, we also found that the 2nd tertile of anti-dsDNA antibodies (≥2 IU/mL) and 3rd tertile of IL-6 values [>3.24 pg/mL) presented higher CML levels than the 1st tertile, but both exclusively in the OLS model (Figure 2). The detailed models and their adjustments by confounding variables are provided in Supplementary Table S6.

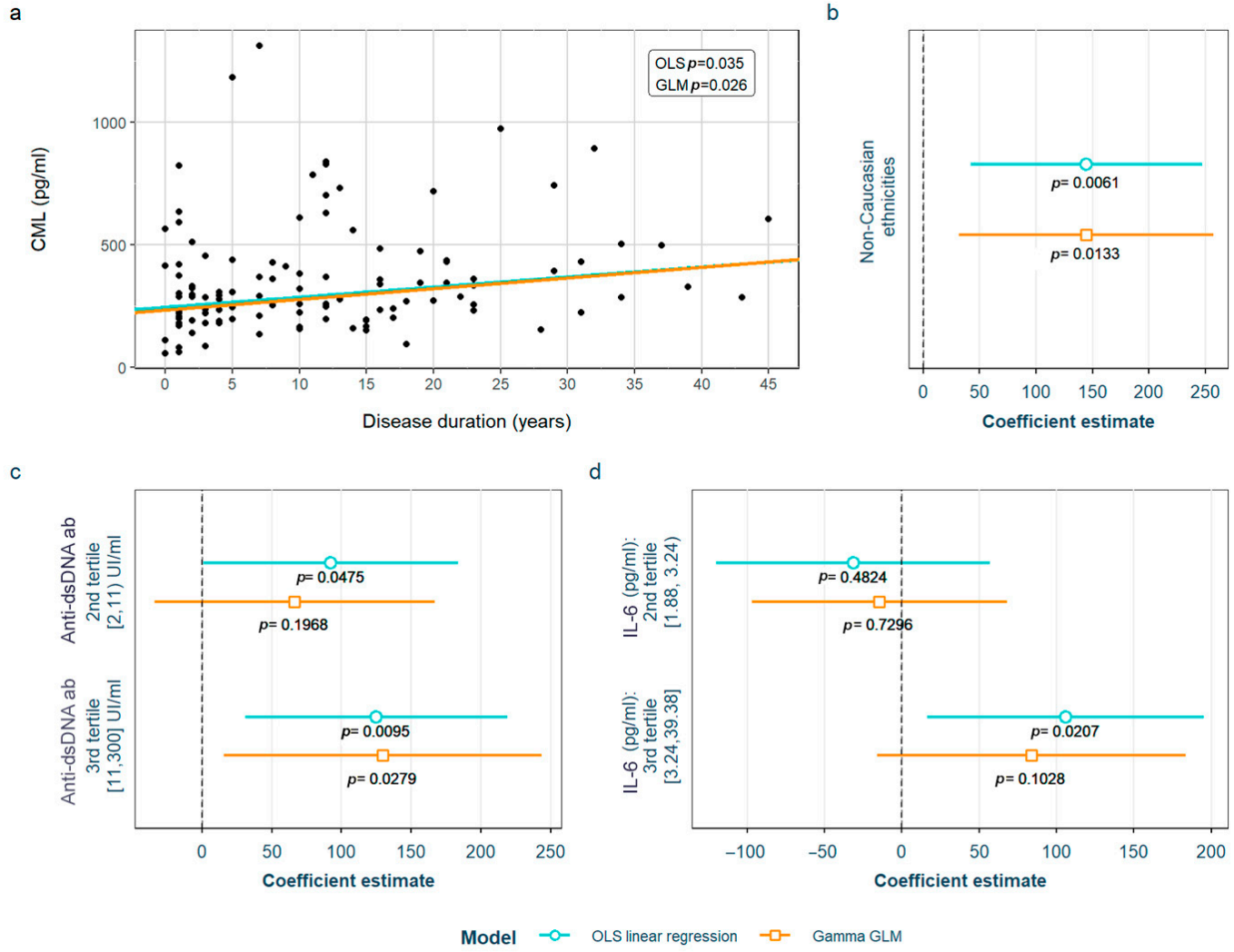

Figure 2. Statistically significant associations between CML and different systemic lupus erythematosus characteristics: (**a**) disease duration; (**b**) non-Caucasian ethnicities; (**c**) anti-dsDNA values; (**d**) IL-6 values. CML Nξ-(carboxymethyl)lysine; OLS: ordinary least squares; GLM: generalized linear model; IL-6: interleukin 6.

3.3. CEL

3.3.1. Characteristics of SLE Patients According to CEL Levels: Exploratory Analysis

A total of 91 SLE patients were included. The distribution of CML exhibited a right-skewed pattern (Supplementary Figure S3), prompting the utilization of regression models tailored for log-normal data, as outlined in the Section 2. All of the variables that showed statistically significant differences according to CEL tertiles in the exploratory analysis are depicted in Table 4, not adjusted by any variable (*p*-value). The demographic characteristics and other SLE variables of interest are detailed in Supplementary Table S7.

Table 4. Variables that showed statistically significant differences (p-value < 0.1) according to CEL tertiles in the exploratory analysis. "c" indicates variables that were categorized as previously stated in Section 2. * Indicates values according to the blood test performed in the study. "Treatment" divides patients into three groups according to the strongest immunosuppression they were taking at the moment of the study (only immunosuppressants, only antimalarials or neither (others)). "Treatment2" divides patients into two groups: taking or not taking immunosuppressants. CEL: Nξ-(carboxyethyl)lysine; SLE-DAS: SLE disease activity score; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; RV: reference value according to the laboratory; C3: complement C3; IL-6: interleukin-6; UPCR (mg/g): urine protein to creatinine ratio; IS: immunosuppressants (includes treatment with methotrexate, leflunomide, tacrolimus, mycophenolic acid or mycophenolate mofetil acid, azathioprine, cyclophosphamide, cyclosporine, rituximab or belimumab); "Treatment" divides treatment into three groups according to the strongest immunosuppression used (taking immunosuppressants, taking only antimalarials or not taking either (others)). AGEs: advanced glycation end products; CML: Nξ-(carboxymethyl)lysine.

Variables	First Tertile [0.823, 2.79)	Second Tertile [2.793, 4.56)	Third Tertile [4.564, 31.68]	p-Value
	N = 38	N = 37	N = 16	
Demographic variables				
Smoker	3 (7.89%)	8 (21.6%)	8 (50.0%)	0.087
Classificatory Criteria and Other Clinical Data				
Constitutional ever	3 (7.89%)	4 (10.8%)	1 (6.25%)	0.046
Photosensitivity ever	20 (52.6%)	27 (73.0%)	13 (81.2%)	0.089
Manifestations				0.006
3	2 (5.26%)	0 (0.00%)	0 (0.00%)	
4	2 (5.26%)	1 (2.70%)	0 (0.00%)	
5	7 (18.4%)	3 (8.11%)	0 (0.00%)	
6	9 (23.7%)	10 (27.0%)	2 (12.5%)	
7	8 (21.1%)	8 (21.6%)	5 (31.2%)	
8	6 (15.8%)	4 (10.8%)	3 (18.8%)	
9	3 (7.89%)	6 (16.2%)	2 (12.5%)	
10	0 (0.00%)	3 (8.11%)	1 (6.25%)	
11	1 (2.63%)	1 (2.70%)	3 (18.8%)	
12	0 (0.00%)	1 (2.70%)	0 (0.00%)	
Disease Activity Indexes				
cSLE-DAS				0.091
First tertile [0.82, 1.79)	19 (52.8%)	16 (47.1%)	2 (12.5%)	
Second tertile [1.79, 5.31)	6 (16.7%)	10 (29.4%)	5 (31.2%)	
Third tertile [5.31, 23.31]	11 (30.6%)	8 (23.5%)	9 (56.2%)	
Serological variables				
Glucose *	87.8 (12.4)	82.4 (8.96)	81.2 (7.69)	0.049
CRP *	0.12 [0.07; 0.28]	0.17 [0.11; 0.30]	0.16 [0.07; 0.54]	<0.001
ESR *	8.00 [4.25; 20.0]	10.5 [6.00; 15.0]	13.5 [7.00; 21.5]	0.054
Anti-dsDNA+ ever	23 (60.5%)	26 (70.3%)	14 (87.5%)	0.025
Anti-dsDNA+ *	2.50 [1.00;10.8]	5.00 [1.00; 13.0]	15.5 [1.75; 40.2]	<0.001
Anti-dsDNA > RV *	10 (26.3%)	10 (27.8%)	9 (56.2%)	0.018

Table 4. *Cont.*

Variables	First Tertile [0.823, 2.79)	Second Tertile [2.793, 4.56)	Third Tertile [4.564, 31.68]	*p*-Value
	N = 38	N = 37	N = 16	
Anti-dsDNA tertiles *				0.054
[0, 2)	16 (42.1%)	14 (38.9%)	4 (25.0%)	
[2, 11)	12 (31.6%)	12 (33.3%)	3 (18.8%)	
[11, 300]	10 (26.3%)	10 (27.8%)	9 (56.2%)	
Anti-dsDNA presence *	10 (26.3%)	10 (27.8%)	9 (56.2%)	0.018
Anti-Ro60+ ever	7 (18.4%)	19 (51.4%)	6 (37.5%)	0.097
Anti-Ro60 presence *	7 (18.9%)	17 (47.2%)	6 (37.5%)	0.086
Anti-Ro52+ ever	4 (10.5%)	12 (32.4%)	5 (31.2%)	0.060
C3 *	111 (24.3)	103 (19.2)	98.8 (20.5)	0.028
IL-6 *	1.98 [1.43; 3.77]	2.21 [1.81; 2.96]	3.92 [2.99; 6.03]	0.003
IL-6 > RV *	4 (10.8%)	2 (5.41%)	4 (25.0%)	0.002
IL-6 tertiles *				0.019
[0.44, 1.88)	16 (43.2%)	13 (35.1%)	1 (6.25%)	
[1.88, 3.24)	10 (27.0%)	15 (40.5%)	4 (25.0%)	
[3.24, 39.38]	11 (29.7%)	9 (24.3%)	11 (68.8%)	
UPCR *	82.2 [66.2; 119]	84.1 [63.0; 103]	71.3 [50.1; 121]	0.013
Treatments				
Mycophenolic acid	4 (10.5%)	8 (21.6%)	5 (31.2%)	0.007
NSAIDs	3 (7.89%)	4 (10.8%)	2 (12.5%)	0.038
Treatment				0.030
Others	6 (15.8%)	3 (8.11%)	0 (0.00%)	
Antimalarials	19 (50.0%)	13 (35.1%)	4 (25.0%)	
IS	13 (34.2%)	21 (56.8%)	12 (75.0%)	
Treatment2				0.009
Non-IS	25 (65.8%)	16 (43.2%)	4 (25.0%)	
IS	13 (34.2%)	21 (56.8%)	12 (75.0%)	
Other AGEs				
CML	281 [216; 374]	302 [248; 444]	464 [272; 711]	0.064

3.3.2. Correlations between CEL and SLE Characteristics: Multivariate Analysis

SLE characteristics that were significant in the exploratory analysis and possibly associated to CEL levels were tested in two models adjusted for the previously selected confounding variables (see Section 2). After adjustment, we found that the CEL levels correlated with anti-dsDNA antibodies, IL-6 levels and the number of accumulated manifestations throughout the disease (Figure 3a, Figure 3b, and Figure 3d, respectively). Furthermore, patients having ever had positive anti-dsDNA antibodies had significantly higher CEL levels (Figure 3c). These associations were found in both models except for one with the anti-dsDNA titers, which was only observed in the OLS linear regression model. Moreover, we found a correlation between CEL and CML levels (Supplementary Figure S4). The detailed models and their adjustments by confounding variables are provided in Supplementary Table S8.

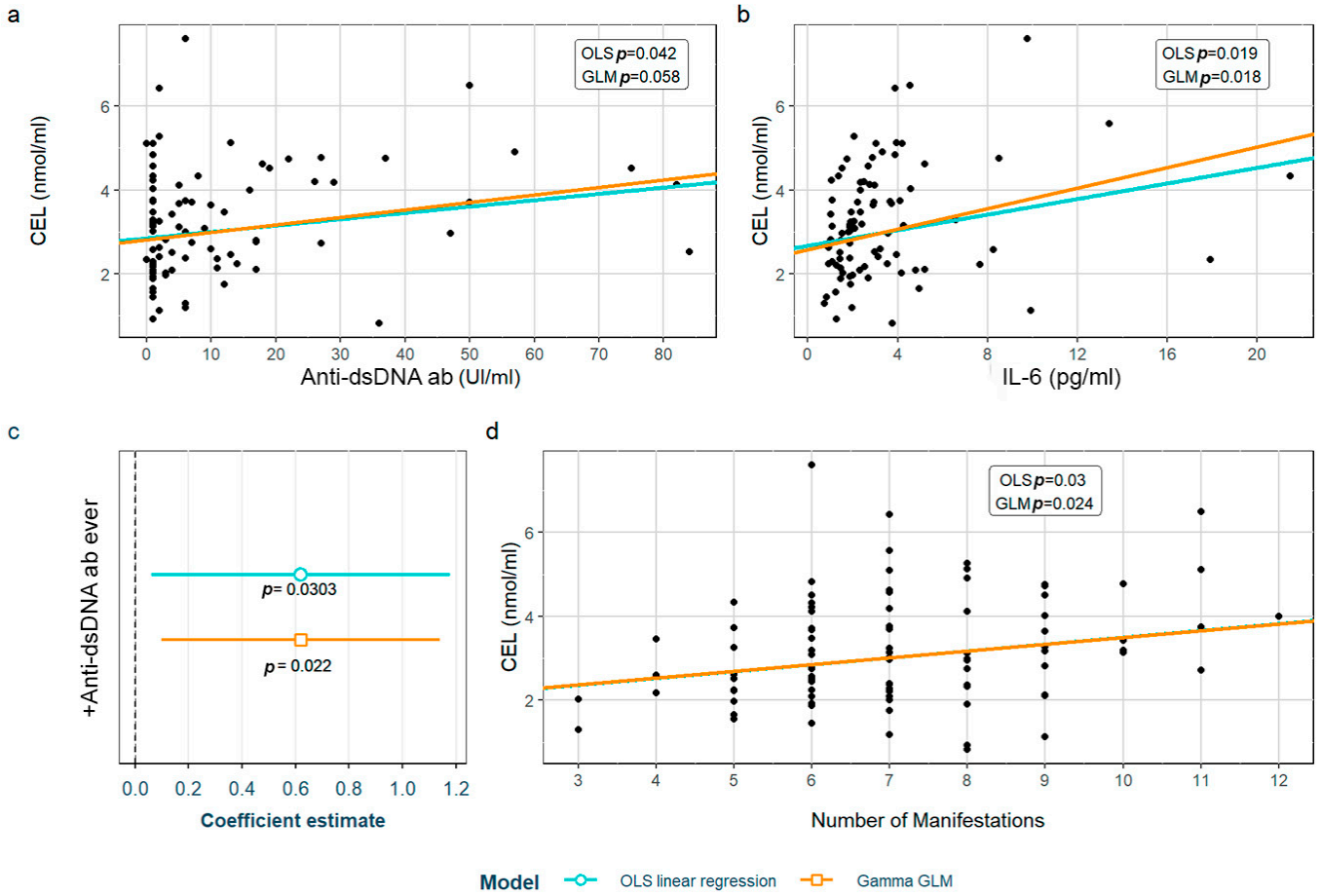

Figure 3. Statistically significant associations between CEL and different systemic lupus erythematosus characteristics: (**a**) anti-dsDNA values; (**b**) IL-6 values; (**c**) positivity of anti-dsDNA antiboides; (**d**) number of accumulated SLE manifestations throughout the disease. CEL: Nξ-(carboxyethyl)lysine; OLS: ordinary least squares; GLM: generalized linear model; IL-6: interleukin 6.

3.4. Serum Receptor for Advanced Glycation End Products (sRAGE)

3.4.1. Characteristics of SLE Patients According to sRAGE Levels: Exploratory Analysis

A total of 119 SLE patients were included. The sRAGE distribution displayed a right-skewed pattern, as illustrated in Supplementary Figure S3. Consequently, regression models designed for log-normal data, as described in the Section 2, were employed to analyze the dataset. All of the variables that showed statistically significant differences according to sRAGE tertiles in the exploratory analysis are depicted in Table 5, not adjusted by any variable. The demographic characteristics and other SLE variables of interest are detailed in Supplementary Table S9.

3.4.2. Correlations between sRAGE Levels and SLE Characteristics: Multivariate Analysis

SLE characteristics that were statistically significant in the exploratory analysis and possibly associated with sRAGE levels were tested in the two models adjusted for the previously selected confounding variables (see Materials and Methods). After adjustment, we found that sRAGE levels were higher in women and in patients having ever had photosensitivity as an SLE symptom, as well as in those on biological disease-modifying antirheumatic drugs (bDMARD), which in our cohort included rituximab or belimumab, or mycophenolic acid (Figure 4). All of the associations were found in both models except for male gender, which was only found in the OLS linear regression model. The detailed models and their adjustments by confounding variables are provided in Supplementary Table S10.

Table 5. Variables that showed statistically significant differences (p-value < 0.1) according to the serum receptor of advanced glycation end products tertiles in the exploratory analysis. "c" indicates variables that were categorized as previously stated in the Section 2. * Indicates values according to the blood test performed in the study. "Treatment" divides patients into three groups according to the strongest immunosuppression they were taking at the moment of the study (only immunosuppressants, only antimalarials or neither (others)). "Treatment2" divides patients into two groups: taking or not taking immunosuppressants. DAS28: disease activity score 28; ESR: erythrocyte sedimentation rate; VAS: visual analogic scale; bDMARDs: biologic disease-modifying antirheumatic drugs, IS: immunosuppressants (includes treatment with methotrexate, leflunomide, tacrolimus, mycophenolic acid, or mycophenolate mofetil acid, azathioprine, cyclophosphamide, cyclosporine, rituximab or belimumab).

Variables	First Tertile [122, 384)	Second Tertile [384, 671)	Third Tertile [671, 2797]	p-Value
	N = 40	N = 40	N = 39	
Demographic variables				
Gender: Female	35 (87.5%)	37 (92.5%)	39 (100%)	0.057
Classificatory Criteria and Other Clinical and Serological Data				
Photosensitivity ever	20 (50.0%)	29 (72.5%)	26 (66.7%)	0.022
Disease Activity Indexes				
DAS28	2.16 [1.49; 2.58]	2.10 [1.43; 3.24]	2.40 [1.57; 3.10]	0.050
cDAS28				0.008
0—Reference	31 (79.5%)	25 (62.5%)	21 (55.3%)	
1—Low Activity	2 (5.13%)	4 (10.0%)	8 (21.1%)	
2—Moderate Activity	4 (10.3%)	9 (22.5%)	7 (18.4%)	
3—High Activity	2 (5.13%)	2 (5.00%)	2 (5.26%)	
Serological variables				
ESR tertiles *				0.047
[2, 7)	13 (33.3%)	17 (42.5%)	12 (31.6%)	
[7, 17)	12 (30.8%)	10 (25.0%)	15 (39.5%)	
[17, 81]	14 (35.9%)	13 (32.5%)	11 (28.9%)	
Leukocyturia *	0.00 [0.00; 1.00]	0.00 [0.00; 1.00]	0.00 [0.00; 1.00]	0.022
Patient-Reported Outcomes				
Pain VAS	1.50 [0.00;5.00]	2.50 [0.00;6.12]	4.00 [0.00;6.00]	0.033
Comorbidities and Cardiovascular Disease				
APS	4 (10.0%)	1 (2.50%)	0 (0.00%)	0.097
Pain VAS	1.50 [0.00; 5.00]	2.50 [0.00; 6.12]	4.00 [0.00; 6.00]	0.033
Treatments				
bDMARDs	0 (0.00%)	2 (5.00%)	4 (10.3%)	0.002
Antimalarials	37 (92.5%)	27 (67.5%)	26 (66.7%)	0.009
Mycophenolic acid	7 (17.5%)	5 (12.5%)	8 (20.5%)	0.016
Azathioprine	2 (5.00%)	9 (22.5%)	7 (17.9%)	0.065
Glucocorticoids	13 (32.5%)	12 (30.0%)	5 (12.8%)	0.053
Treatment				0.016
Others	1 (2.50%)	6 (15.0%)	7 (17.9%)	
Antimalarials	24 (60.0%)	14 (35.0%)	13 (33.3%)	
IS	15 (37.5%)	20 (50.0%)	19 (48.7%)	
Treatment2				0.008
Non-IS	25 (62.5%)	20 (50.0%)	20 (51.3%)	
IS	15 (37.5%)	20 (50.0%)	19 (48.7%)	

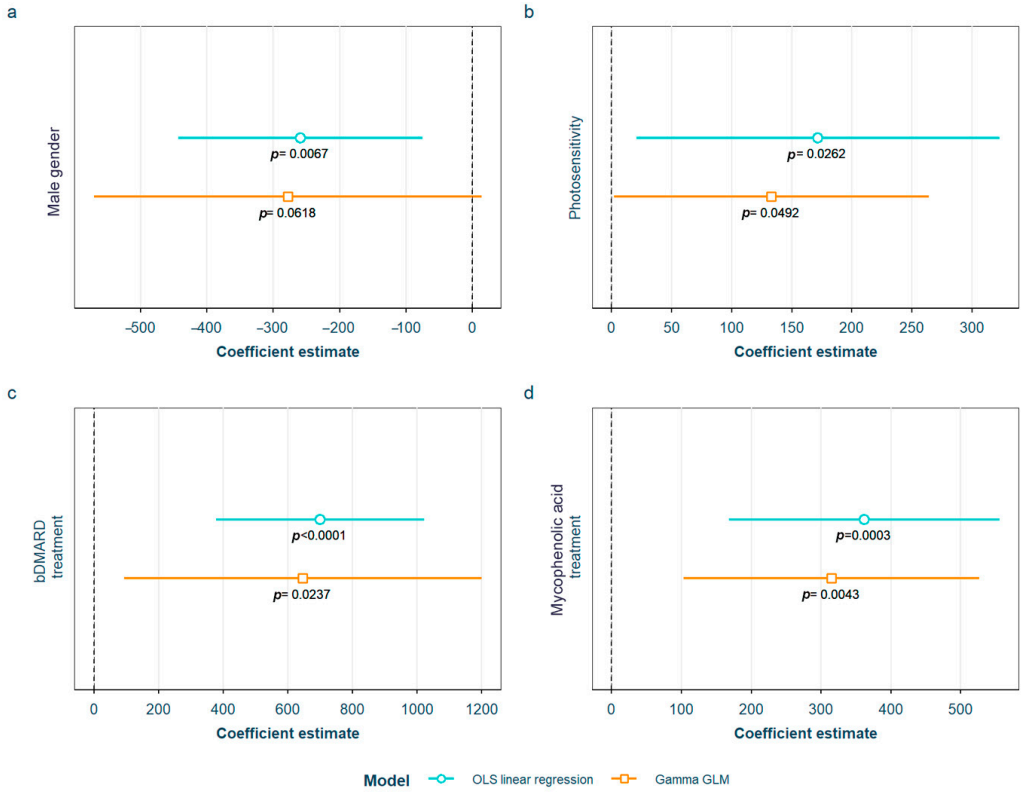

Figure 4. Statistically significant associations between the serum receptor for advanced glycation end products and different systemic lupus erythematosus characteristics: (**a**) male gender; (**b**) having ever had photosensitivity; (**c**) current treatment with bDMARD; (**d**) current treatment with mycophenolic acid OLS: ordinary least squares; GLM: generalized linear model; bDMARD: biological disease-modifying antirheumatic drugs.

3.5. Ratios of Advanced Glycation End Products/Serum Soluble Receptor for Advanced Glycation End Products (AGEs/sRAGE)

3.5.1. Characteristics of SLE Patients According to Skin AGEs/sRAGE or Specific Serum AGEs/sRAGE

All of the statistically significant associations in the univariate analysis are depicted in the Supplementary Materials: Pentosidine/sRAGE in Supplementary Table S11, CML/sRAGE in Supplementary Table S13, CEL/sRAGE in Supplementary Table S15 and skin AGEs/sRAGE in Supplementary Table S17.

3.5.2. Correlations between Skin AGEs/sRAGE or Specific Serum AGEs/sRAGE and SLE Characteristics: Multivariate Analysis

After adjustment for confounding factors, we found several SLE characteristics that were associated with different serum AGE to sRAGE ratios, in one or both of the models. The pentosidine/sRAGE ratio was higher in those patients not following bDMARD treatment or having ever had anti-Ro52 antibodies (Figure 5). Regarding CML/sRAGE, non-Caucasian patients as well as patients showing SLICC/ACR damage index (SDI) $\geq$ 2 densitometric osteoporosis, or those on dyslipidemia drugs, presented higher ratios (Figure 6). CRP and IL-6 levels had a positive correlation with the CEL/sRAGE ratio, with those showing pathological IL-6 values displaying significantly higher ratios (Figure 7). Finally, the skin AGEs/RAGE ratio was lower in women and in those patients with disease duration > 16 years (3rd tertile) compared to those with disease duration < 5 years (1st tertile) (Figure 8). The detailed models and their adjustments by confounding variables are provided in Supplementary Table S12 (pentosidine/sRAGE), Supplementary Table S14 (CML/sRAGE), Supplementary Table S16 CEL/sRAGE and Supplementary Table S18 (skin AGEs/sRAGE).

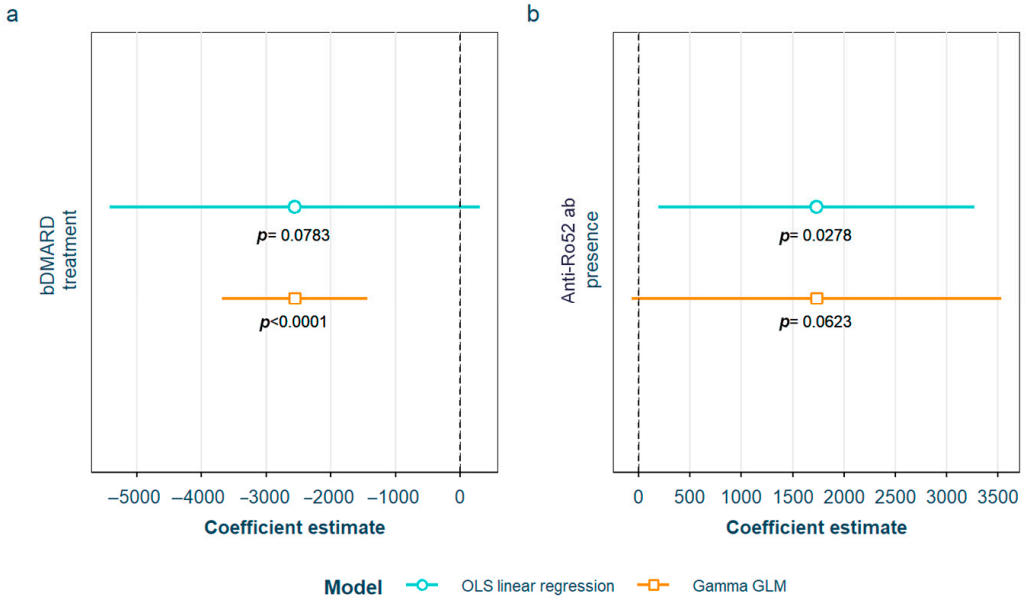

Figure 5. Statistically significant associations between pentosidine/sRAGE and different systemic lupus erythematosus characteristics: (**a**) current treatment with bDMARD; (**b**) positive anti-Ro52 antibodies sRAGE: soluble receptor for advanced glycation end products; OLS: ordinary least squares; GLM: generalized linear model. bDMARD: biological disease-modifying antirheumatic drugs.

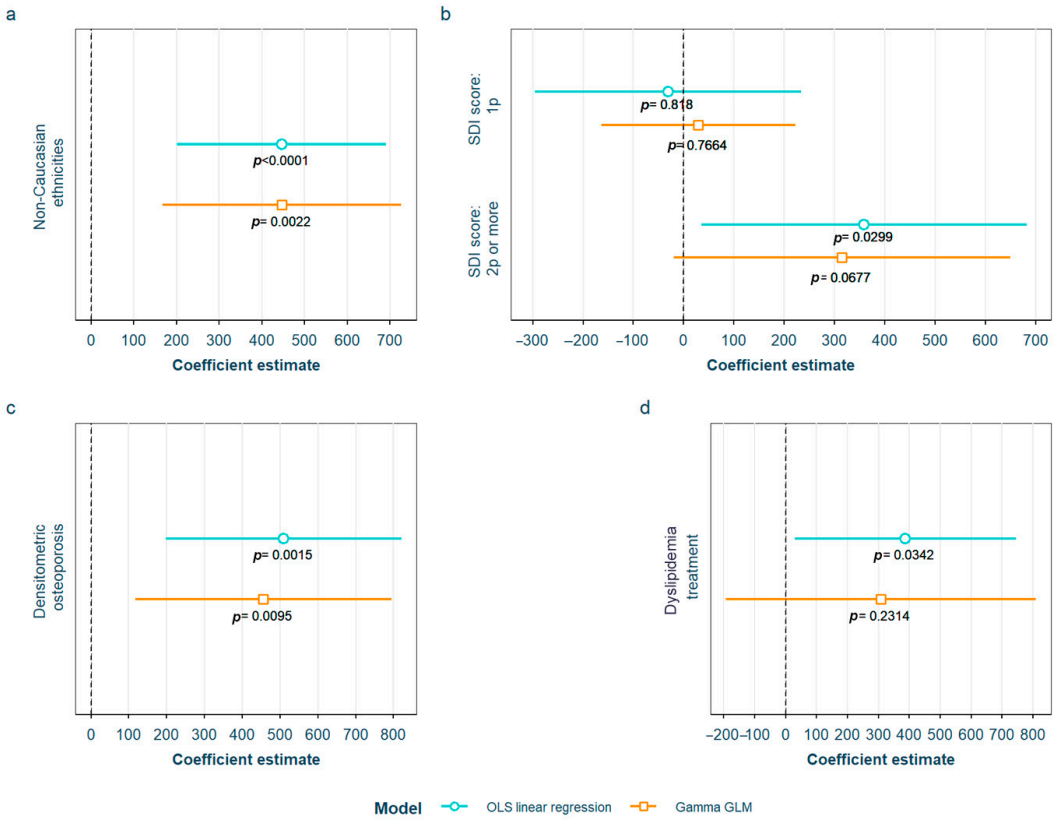

Figure 6. Statistically significant associations between CML/sRAGE and different systemic lupus erythematosus characteristics: (**a**) non-Caucasian ethnicities; (**b**) SDI score; (**c**) densitometric osteoporosis; (**d**) dyslipidemia drugs treatment CML: Nε-(carboxymethyl)lysine; sRAGE: soluble receptor for advanced glycation end products; OLS: ordinary least squares; GLM: generalized linear model. SDI: systemic lupus erythematosus damage index.

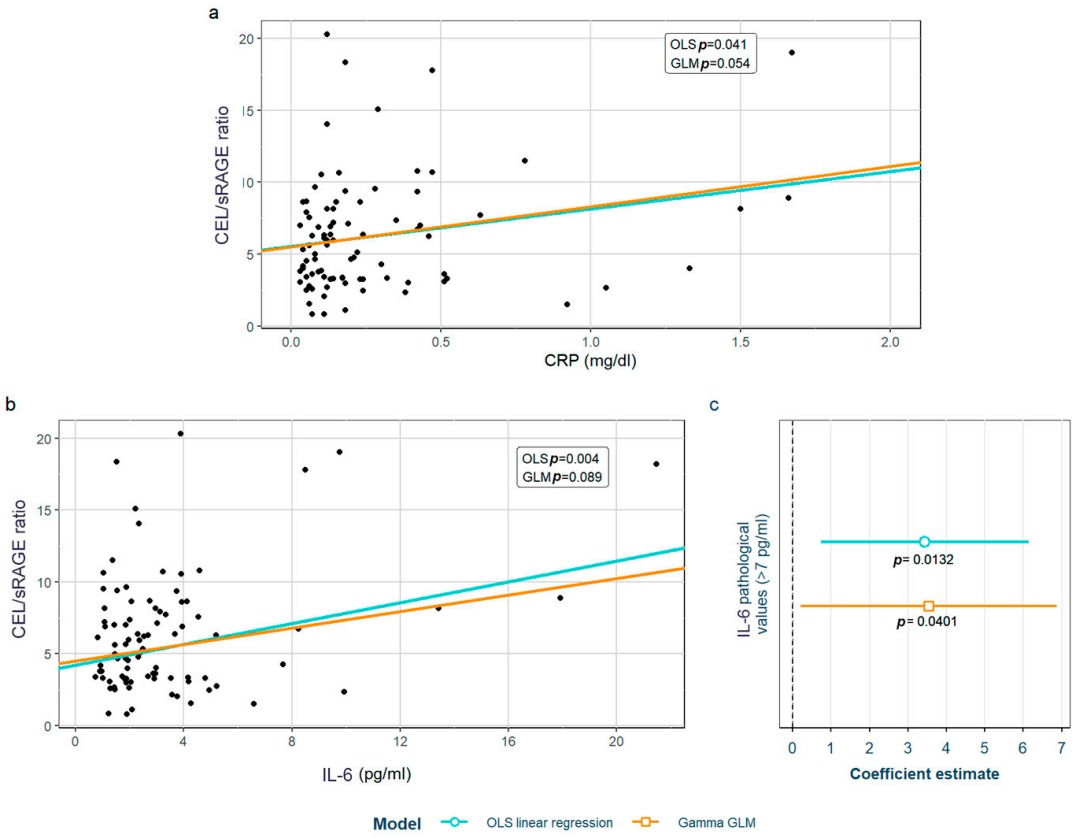

Figure 7. Statistically significant associations between CEL/sRAGE and different systemic lupus erythematosus characteristics: (**a**) CRP values, (**b**) IL-6 values; (**c**) pathological (>7 pg/mL) IL-6 values. CEL: Nξ-(carboxyethyl)lysine; sRAGE: soluble receptor for advanced glycation end products; OLS: ordinary least squares; GLM: generalized linear model. CRP: C-reactive protein; IL-6: interleukin 6.

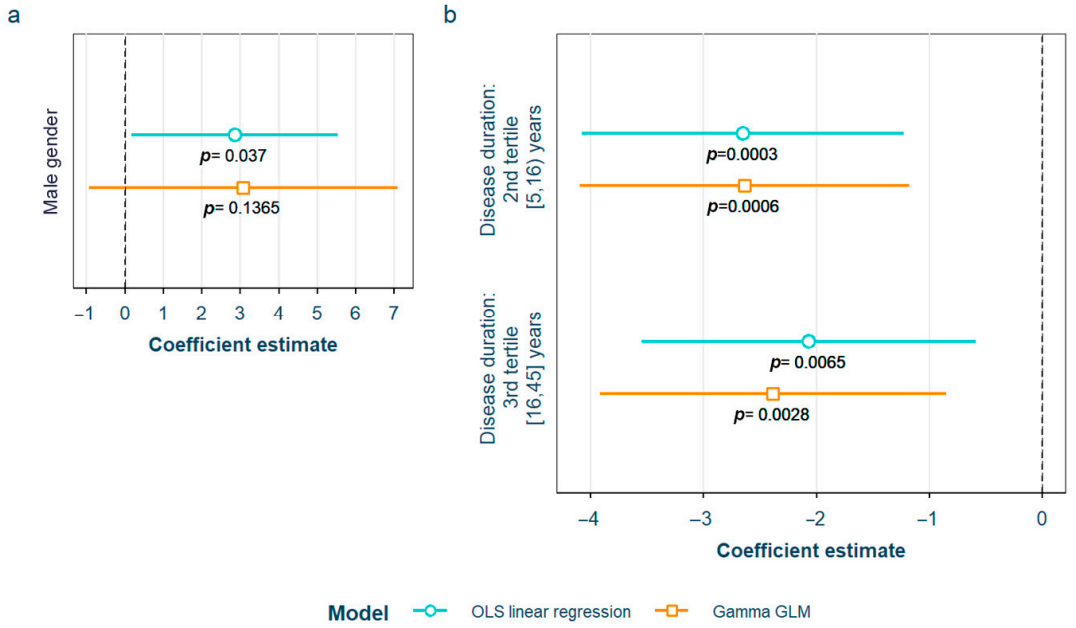

Figure 8. Statistically significant associations between skin AGEs/sRAGE and different systemic lupus erythematosus characteristics: (**a**) male gender; (**b**) disease duration in tertiles AGEs: advanced glycation end products; sRAGE: soluble receptor for advanced glycation end products OLS: ordinary least squares; GLM: generalized linear model.

4. Discussion

In this study, we observed that both the studied serum AGEs, including pentosidine, CEL and CML, along with their receptor, sRAGE, and the calculated ratios involving the latter, present associations with relevant clinical characteristics and indexes in SLE.

Concerning pentosidine, we only found one significant association; a nearly 80% increase in pentosidine levels were observed in patients with SLE pulmonary manifestations, which in our cohort only comprised shrinking lung syndrome and lupus pneumonitis, while pleuritis was considered inside the serositis term. Only one previous study analyzed the relationship between pentosidine and SLE characteristics, but they did not assess pulmonary manifestations because they only collected the ones included in the SLE classificatory criteria [21]. They found, however, lower levels of pentosidine in patients with discoid lesions and photosensitivity that we could not confirm in our cohort. Nevertheless, several characteristics were very different: in their cohort compared with ours, 37% of their patients were from African descent, while in ours < 10% were from an ethnicity different from Caucasian or Hispanic, overlapping with other inflammatory conditions, which was an exclusion criterion in theirs. The mean disease duration of their cohort was 24 months, while ours had a remarkably longer disease duration (only 41% of patients had a disease duration under 5 years). RAGE has been described to be constitutively highly expressed in the lung [39,40], and importantly linked to lung inflammation in several lung diseases [41]. Specifically, pentosidine has been associated with the progression to metastases in lung cancer and with asthma (measured in sputum) [42], where its role as a biomarker of a reduced response to bronchodilator treatment has been proposed [43]. Based on that physiological link and on the statistically strong association with these specific pulmonary symptoms in our study, pentosidine may represent a strong predictor of these infrequent but serious manifestations, and a useful tool in their monitoring.

When analyzing CML and CEL, we found similar associations with different SLE serological characteristics. These common results make sense, since we found a positive correlation between their levels, as has been reported in a previous study performed in HC [44]. Levels of both showed a positive correlation with anti-dsDNA antibodies and IL-6 (evaluated as tertiles in the case of CML, or as continuous variables in the case of CEL). We consider that the results in relation with CEL levels are more consistent with the cut-off used in clinical practice, as the CEL increase depends directly on anti-dsDNA antibodies and IL-6. In the case of CML, the tertiles did not match values considered positive (for anti-dsDNA antibodies) or pathogenic (for IL-6). Having taken into account that normal IL-6 values are considered < 7 pg/mL, and that the 3rd tertile includes both normal and abnormal values, we reassessed the association, splitting the sample into those with high values of IL-6 (>7 pmg/L) vs. normal (<7 mg/dL) values; however, we did not find differences between the groups, which makes the association difficult to interpret. Something similar happens in the case of anti-dsDNA antibodies, where both pathological values (>10 IU/mL, 3rd tertile) and the highest values in the non-pathological range [2–10 IU/mL, 2nd tertile] were associated with increased CML levels—the latter only according to the OLS model. Even though CML values included in the 2nd tertile are not considered positive, they are closer to being pathological if we consider anti-dsDNA values as a continuum, perhaps initiating a rise in their levels, a fact that has been associated with an increased risk of flares [45]. In addition, other associations with CML were also found. For each year of disease duration, CML levels increased by 1.7%; non-Caucasian patients showed CML levels almost 50% higher than Caucasian patients, and patients suffering from densitometric osteoporosis not associated to GC' intake also showed increased CML levels (34.2%). Regarding CEL, for each new manifestation that the patient presented throughout the course of the disease (evaluated according to the symptoms included in either the ACR or the SLICC SLE classificatory criteria) we found CEL increases of 8.2%, while patients that had ever presented positive anti-dsDNA also had higher CEL levels (23.8%). There is only one previous study in the literature that studied the relation between SLE characteristics and CML or CEL without finding any association with disease indexes or characteristics

or the number of accumulated manifestations according to the 1990 ACR classificatory criteria [20]. Nevertheless, the study was conducted with a very small sample size (10 SLE patients and 10 HC), and both AGEs were determined through mass spectrometry and not ELISA, which makes it non-comparable to our research.

All of the above characteristics are known to be correlated with disease indexes. For example, anti-dsDNA antibodies [46] and IL-6 titers are correlated with disease activity, despite the current failure of IL-6 blockade therapies [47]. The number of manifestations is also correlated with activity and possibly with organ damage, while disease duration is associated with organ damage [48] and non-Caucasian ethnicities, particularly African American and Caribbean ethnicities, with both activity and organ damage [49]. The fact that CML and/or CEL correlate with all those indexes opens the door to their use as a new activity/damage/prognosis biomarker in SLE.

With regards to sRAGE, we found a negative association with male gender, showing almost 40% lower sRAGE levels than females. On the other hand, patients having ever had photosensitivity or being on treatment with bDMARD or with mycophenolic acid at the time of the study presented higher sRAGE levels (corresponding to increases of 31.3%, 111.3% and 59.8%, respectively). As stated in the Section 1, there is still much to elucidate about which sRAGE levels (high or low) are associated with inflammation because there is evidence for both, making interpretation of the results conflicting. Assuming the mainstream theory that supports low sRAGE levels as being deleterious in SLE, the fact that we found lower levels in males is consequential, since males are known to have more severe extrarenal and renal diseases [50]. In the case of the positive association with photosensitivity, we have several hypotheses: Firstly, patients who are photosensitive tend to protect themselves more from ultraviolet radiation, a notorious trigger for both cutaneous and systemic flares in SLE [51]. Secondly, photosensitive patients are normally treated with drugs that are photoprotective like hydroxychloroquine, which is known to absorb ultraviolet light in the skin in a concentration-dependent manner; it has also been demonstrated to reduce mortality in SLE [52] by preventing flares and organ damage, and by having an effect in other comorbidities such as thrombosis or bone destruction [53].

Looking at previous evidence published on the topic, there is a lack of consistent results regarding sRAGE in SLE. Ene et al. did not study the association between sRAGE levels and disease characteristics, but when compared with HC, found that sRAGE decreased by 7.6% in a non-lupus nephritis (LN) group ($p < 0.001$), by 5.8% in an LN group ($p < 0.001$) and by 5.5% in a type IV LN ($p < 0.001$) group [24]. Lan et al. observed that sRAGE decreased in the proliferative types of LN (III and IV) and in patients with poor response to treatment (those who did not achieve partial or complete renal remission with cyclophosphamide and GC therapy) [54]. The authors reported that although the reason why lower AGEs levels are related to poor response to treatment is unknown, it has most likely to do with the NF-κB pathway, which is activated by AGEs and blocked by both GC [55] and cyclophosphamide [56]. However, they did not find an association between sRAGE and activity measured by SLEDAI (r = 0.12 (95% CI: −0.02454 to 0.2653, $p = 0.11$) or the activity or damage index in kidney biopsies. We did not specifically study associations with types of LN individually, the renal response to treatment or indexes in the renal biopsy, as we had a small sample size of patients with LN (8 patients), which probably prevented us from finding any associations with it. Other authors, like Bobek et al., found a correlation of sRAGE levels with C4 concentrations in 37 children with SLE, although not with other indirect parameters of activity like the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) or anti-dsDNA titers [25]. Nowak et al. did not find either an association between sRAGE and disease characteristics or SLEDAI-2K in 31 SLE vs. 26 HC cases [23]. Discrepancies between their cohorts and ours (for example, children vs. adults) and the small sample sizes in most of these previous studies could explain the differences in the associations found regarding our research.

Concerning the association of sRAGE with taking bDMARD and/or mycophenolic acid, there is scarce literature about the effect of immunomodulatory/immunosuppressant

drugs in sRAGE. There is one study that observed a decrease in serum levels of sRAGE and esRAGE (endogenous secretory receptor for AGEs, generated through alternative splicing of RAGE mRNA) by 32.4% (p = 0.004) when treating patients with multiple sclerosis with fingolimod for 12 months. They also observed a decrease in pentosidine serum levels by 41.3% (although not significant), together with a decrease (although not significant) in clinical relapses [57]. In another study performed by Gross et al. in renal transplant recipients, sRAGE levels were statistically significantly inversely associated in the multivariate linear regression analysis with treatment with mycophenolate mofetil ($\beta st = -0.21$, $p < 0.001$) [58]. Low sRAGE levels were also associated with a 2–3 times higher risk of mortality ($p = 0.006$). Azathioprine, on the other hand, was associated with higher levels of sRAGE ($p = 0.02$), despite azathioprine also being associated with taking mycophenolate mofetil ($r = -0.58$, $p < 0.001$). The authors concluded that the relationship between mycophenolate mofetil and sRAGE requires further investigation, an affirmation that we fully support.

Despite these limited data showing an association between lower sRAGE and treatment with immunosuppressants, we found that patients being treated with specific immunosuppressants showed an increase in sRAGE. Our hypothesis for explaining these results is that patients treated with bDMARD or mycophenolate mofetil have less inflammation, as these treatments are more potent inhibitors of inflammatory pathways than other treatments used for less severe disease. This would be supported by the previously mentioned study that found an association between higher AGEs and azathioprine [58]. This association of sRAGE with certain immunosuppressants could have therapeutic implications, as those treatments could be used to modulate sRAGE levels as well as inflammation. However, in the current study, we could not assess in more depth the relationship between immunosuppressants and SLE or check our hypothesis, so future studies should be designed for this specific purpose.

On a different note, this is the first study in the literature to analyze the ratios between specific serum or skin AGEs and sRAGE in SLE. We found a significant relationship between these ratios and several variables. A statistically significant positive association was observed with the presence of anti-Ro52 antibodies in the blood test results, in non-Caucasian ethnicities, in the SDI (only in the OLS model), as well as densitometric osteoporosis, taking dyslipidemia drugs, CRP and IL-6 values, IL-6 pathological values (>7 pg/mL), and male sex. A negative association was found with being on bDMARD treatment and disease duration. Due to the novelty of these analyses and, until further validation of these results, the purpose of this part of our study was solely exploratory, taking into account that some authors defend the highest suitability as biomarkers of the ratios over the molecules on their own [18].

Despite the known relationship between AGEs and atherosclerosis, we did not find any correlation between serum AGEs, sRAGE or the ratios and either CVRFs or cardiovascular events (CVEs). However, the p-values in some of the exploratory analyses were <0.1 and, considering that we have a small number of patients with CVE (N = 9), it is likely that our results are limited by a lack of statistical power, thus preventing us from drawing conclusions about the role of AGEs or sRAGE in CVR. Furthermore, we only assessed CVD through traditional CVRF or CVE, and did not perform additional tests like the intima–media thickness (IMT) of the common carotid artery measured by ultrasound [35], or the small artery elasticity measured via pulse-wave analysis using tonometric recordings of the radial artery [32]; both of these tests have been associated with skin AGEs levels in previous research. Nowak et al. [23] did not find either that serum CEL, CML or sRAGE levels influenced the presence of CVD in their analyses, but it is necessary to point out that 80.65% of SLE patients had CVD in their cohort and the sample size was small (n = 31), which could have influenced the ability to find differences between groups. No other research studied the association between serum AGEs or sRAGE with CVD; in some studies, it was even used as an exclusion criterion [24].

Our study presents several limitations. Firstly, due to the retrospective nature of the study, some data could not be retrieved, such as the cumulative dose of GC taken throughout the disease; thus, we were only able to assess the impact of GC through the dose taken at the time of the study. Likewise, the design makes it impossible to assess causality, which warrants future prospective studies. Secondly, and to clarify the effect of longstanding disease and therapy in AGEs levels, studies should be performed in newly diagnosed patients with short disease duration who are naïve to treatments. Other limitations are that we did not measure the total serum AGE levels but some specific AGEs on their own. The fact that some characteristics occurred at low frequencies may also have had an influence on the statistical power.

Our research represents a pioneering study that analyzed, in a deep and methodical way, the AGEs–RAGE axis in SLE, associating it with a vast array of demographic and clinical characteristics. There is scarce literature on this area, and our research has several strengths like the large sample size compared to other previously published studies; multiple and detailed data retrieved; complex statistics; and a comprehensive analysis encompassing serum individual AGEs, sRAGE, as well as their ratios. To our knowledge, this is the first study to find an association between SLE activity parameters and some accrual damage indexes with CML, CEL and sRAGE. Also, it is the first to report the ratios of skin AGEs or serum AGEs to sRAGE in SLE. Furthermore, we described, for the first time, AGE (pentosidine, CML and CEL) and sRAGE associations with specific serological and clinical parameters that could define more precisely a specific phenotype of patients in whom these molecules may have a particularly meaningful contribution. Therefore, our results are innovative and indicative of the promising role of AGEs and sRAGE as low-invasive surrogate biomarkers of SLE disease activity, damage and specific manifestations.

The next steps in continuing to determine the role of AGEs or sRAGE as biomarkers of the disease would include validation of our findings in independent cohorts; determination of clinically useful cut-off values; assessment of the performance of the biomarkers; and, finally, validation in external cohorts. Prospective studies are necessary to be able to establish causality and temporality, reduce selection and recall biases, analyze changes in the biomarkers throughout the course of the disease, and to improve the identification and control of confounders. Studies should be designed for specific purposes, such as assessing AGEs' role in cardiovascular disease, and be sufficiently powered to find statistically significant differences in each case.

5. Conclusions

The correlation observed between some serum AGEs and sRAGE with SLE activity and/or damage markers suggests that the AGEs–sRAGE axis has a role as a new biomarker in this disease related to management and prognosis, which would have enormous implications in a field where knowledge of SLE is currently lacking. Furthermore, the association of AGEs or sRAGE with specific antibodies and disease manifestations may indicate a particular clinical phenotype related to specific higher/lower AGEs and/or sRAGE levels, unveiling another potential clinical use of these products.

AGEs/RAGE ratios have been proposed by some authors as better universal markers than their individual components. In this research, we described, for the first time in SLE, skin AGEs and serum AGE to sRAGE ratios and their association with activity, damage and severity markers, antibodies, treatments and comorbidities. However, the role of these ratios in SLE requires further assessment in future studies.

Finally, we could not find an association between AGEs or sRAGE and CVD or CVRF, but our sample did present a low number of CVEs and specific tests for CV assessment, and detections of subclinical atherosclerosis or undiagnosed CVRF were not performed. Subsequent studies designed to focus on these aspects should be carried out to explore this relationship.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biomedicines12030610/s1, Table S1: Previous works in the literature studying advanced glycation end-products (AGEs) in systemic lupus erythematosus (SLE) and their observations [59]; Table S2: Previous works in the literature studying the soluble receptor for advanced glycation end-products (sRAGE) in systemic lupus erythematosus (SLE) and their observations [60]; Table S3: Non-significant (p-value > 0.1) demographic and disease characteristics of systemic lupus erythematosus patients and their distribution according to pentosidine tertiles in the exploratory analysis; Table S4: Linear regression model showing associations between pentosidine levels and systemic lupus erythematosus variables; Table S5: Non-significant (p-value > 0.1) demographic and disease characteristics of systemic lupus erythematosus patients and their distribution according to CML tertiles in the exploratory analysis; Table S6: Ordinary least squares linear regression and gamma generalized linear model showing associations found between CML and systemic lupus erythematosus characteristics adjusted by their confounders; Table S7: Non-significant (p-value > 0.1) demographic and disease characteristics of systemic lupus erythematosus patients and their distribution according to CEL tertiles in the exploratory analysis; Table S8: Ordinary least squares linear regression and gamma generalized linear model showing associations found between CEL and systemic lupus erythematosus characteristics adjusted by their confounders; Table S9: Non-significant (p-value > 0.1) demographic and disease characteristics of systemic lupus erythematosus patients and their distribution according to sRAGE tertiles in the exploratory analysis; Table S10: Ordinary least squares linear regression and gamma generalized linear model showing associations found between sRAGE and systemic lupus erythematosus characteristics adjusted by their confounders; Table S11: Variables that showed statistically significant (p-value < 0.1) differences according to pentosidine/sRAGE tertiles in the exploratory analysis; Table S12: Ordinary least squares linear regression and gamma generalized linear model showing associations found between pentosidine/sRAGE and systemic lupus erythematosus characteristics adjusted by their confounders; Table S13: Variables that showed statistically significant (p-value < 0.1) differences according to CML/sRAGE tertiles in the exploratory analysis; Table S14: Ordinary least squares linear regression and gamma generalized linear model showing associations found between CML/sRAGE and systemic lupus erythematosus characteristics adjusted by their confounders; Table S15: Variables that showed statistically significant (p-value < 0.1) differences according to CEL/sRAGE tertiles in the exploratory analysis; Table S16: Ordinary least squares linear regression and gamma generalized linear model showing associations found between CEL/sRAGE and systemic lupus erythematosus characteristics adjusted by their confounders; Table S17: Variables that showed statistically significant (p-value < 0.1) differences according to skin AGEs/sRAGE tertiles in the exploratory analysis; Table S18: Ordinary least squares linear regression and gamma generalized linear model showing associations found between skin AGEs/sRAGE and systemic lupus erythematosus characteristics adjusted by their confounders; Figure S1: Sample size for each of the analysis; Figure S2: Variables collected and their classification; Figure S3: Density graphics of serum advanced glycation end-products, their soluble receptor and their ratios showing their right-skewed distribution; Figure S4: Association between CEL and CML values.

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10.3. Supplementary tables and figures

Study	SLE:HC	SLE vs HC	Type of AGE	AGEs vs SLE characteristics
Skin AGEs				
De Leeuw2007 (124)	30:30	**Higher in SLE**		**Age, creatinine, disease duration, IMT common carotid artery and SDI in univariate** **Age and disease duration in multivariate**
Nienhuis2010 (123)	55:55	**Higher in SLE**		–
Serum AGEs				
Rodríguez-García1998 (125)	37:57	**No**	Pentosidine	–
Nienhuis2008 (78)	10:10	**No**	CEL, CML	No differences
Nowak2021 (80)	31:26	**Only as a group**	CEL, CML, pentosidine	–
Ene2021 (77)	38 LN 44 SLE non-LN 40 HC	**Higher in SLE**	Pentosidine	–
Nisihara2021 (126)	79 SLE No HC	–	Pentosidine	**Lower pentosidine in skin discoid lesions and photosensitivity** No differences in SLEDAI or SDI
Plasma AGEs				
Chen2015 (82)	36:16	**Higher in SLE**	AGEs	**Positive correlation with SLEDAI**

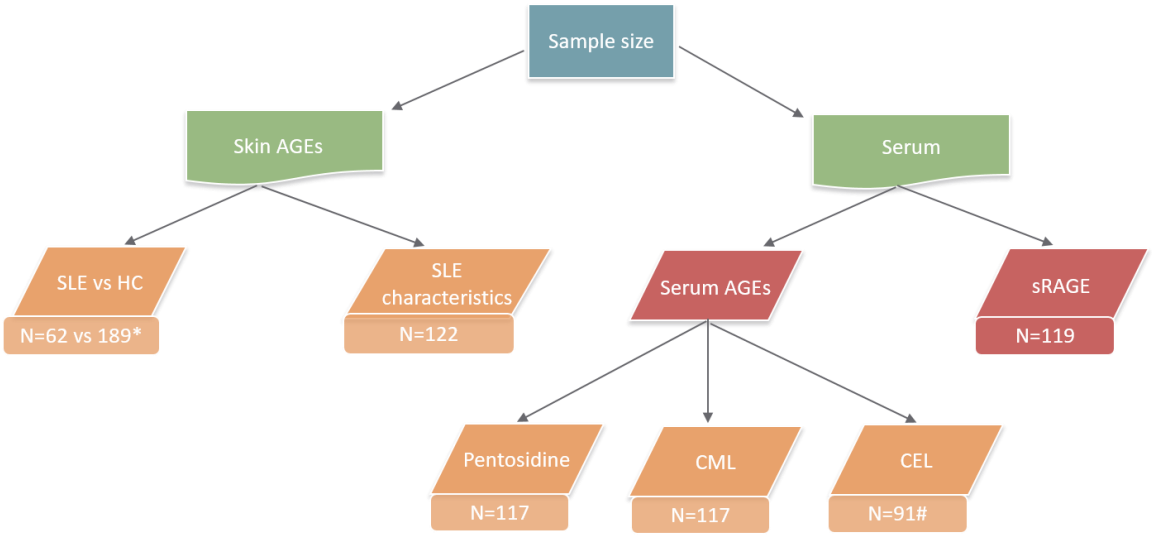
Supplementary Table 1: Previous works in the literature studying advanced glycation end-products (AGEs) in systemic lupus erythematosus (SLE) and their observations. *HC: healthy controls; IMT: intimate media thickness; CEL: Nξ-(carboxyethyl)lysine; CML: Nξ-(carboxymethyl)lysine; SLEDAI: SLE disease activity index; LN: lupus nephritis; SDI: SLE damage index*

Study	SLE:HC	SLE vs HC	sRAGE vs SLE characteristics
Serum sRAGE			
Nienhuis2008 (78)	10:10	**Higher in quiescent SLE vs HC**	**Higher in active disease**
Lee2013 (133)	60 APS (35 SLE)	**Higher in APS**	–
Bayoumy2013 (128)	82 proliferative LN 53 non-proliferative LN 43 mixed LN No HC	–	**Lower in patients with poor response to therapy**
Bobek2014 (127)	19:28	**Lower in SLE**	**Positive correlation with C4**
Manganelli2019 (132)	60:22	**Higher in SLE**	No difference in activity markers
Ene2021 (77)	38 LN 44 SLE non-LN 40 HC	**Lower in SLE**	–
Plasma sRAGE			
Ma2012 (129)	120:40	**Lower in SLE**	**Higher in patients with skin rash, serositis or longer treatment** **Negative correlation with WBC, lymphocytes, and neutrophils**
Yu2015 (130)	105:43	**Lower in SLE**	**Higher in patients with longer treatment**
Okuyucu2022 (131)	27:24	**Lower in SLE**	**Negative correlation with SLEDAI and lower patients with flare**

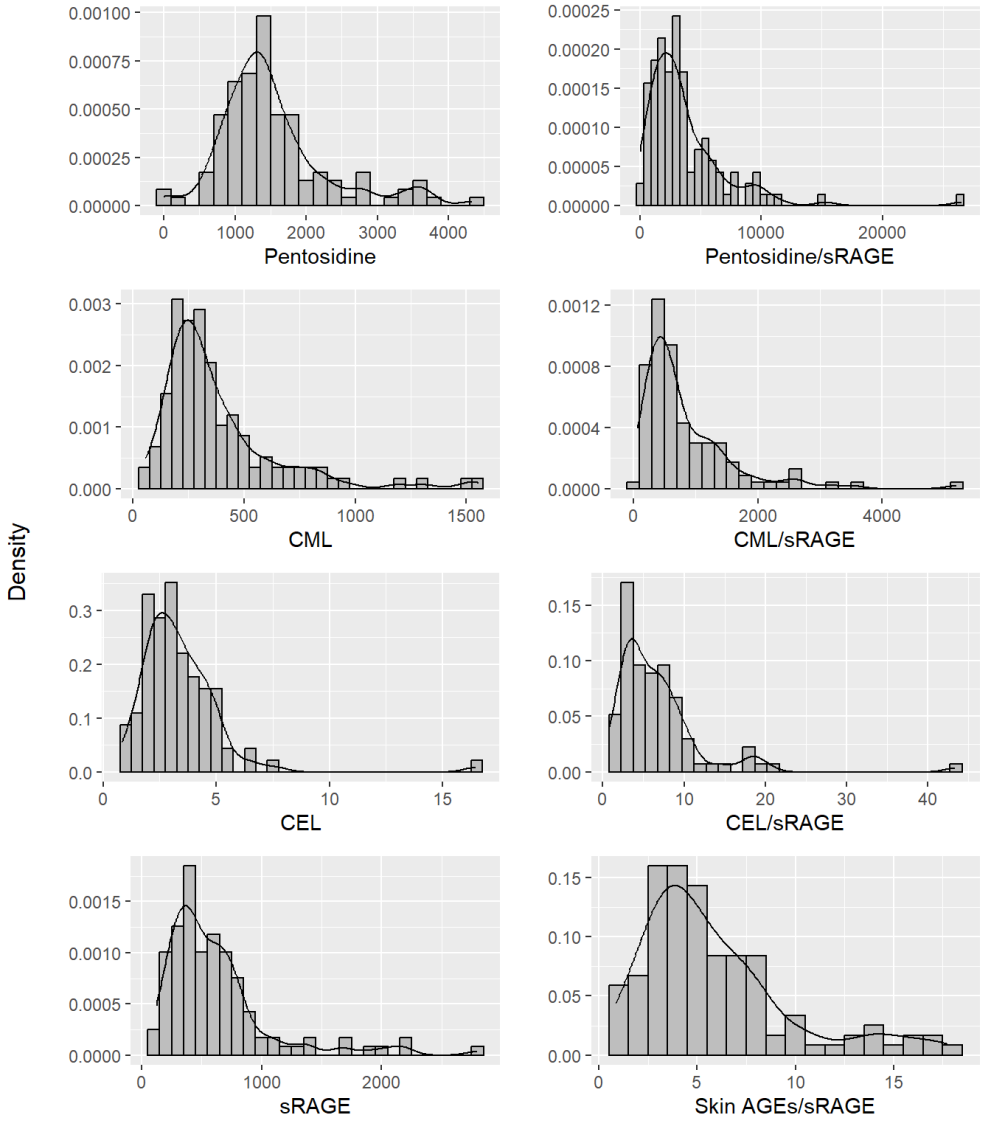
Supplementary Table 2: Previous works in the literature studying the soluble receptor for advanced glycation end-products (sRAGE) in systemic lupus erythematosus (SLE) and their observations. *HC: healthy controls; APS: antiphospholipid syndrome; LN: lupus nephritis; WBC: white blood count; SLEDAI: SLE disease activity index.*

- Demographics like age, gender, and ethnicity.
- Disease characteristics: All the criteria gathered in the 1997 ACR and 2012 SLICC SLE classificatory criteria were recorded, as well as other characteristics such as different ab or overlap with other SADs.
    - Disease duration was assessed as a continuous variable and divided into 4 groups: 0-5, 6-10, 11-20 and ≥ 20 years since SLE diagnosis.
    - Leukocyturia and hematuria were divided into 5 categories according to number of leukocytes/red blood cells detected per field (S0: none, S1: 0-5, S2: 5-10, S3: 10-20, S4: 20-50, S5: >50/camp).
    - Number of manifestations ever: the accumulated number of manifestations according to those included in the ACR or SLICC SLE classificatory criteria.
- Treatment: all SLE treatments that patients were receiving at the moment of inclusion were recorded. We created 3 groups with treatment regimens progressively more intense: receiving no treatment or only glucocorticoids vs on antimalarials ± glucocorticoids vs on immunosuppressants/biological drugs ± antimalarials ± glucocorticoids. We also recorded treatments for CVRF like antihypertensive or dyslipidemia drugs, antiplatelet or anticoagulant therapy.
- Different indexes for measuring SLE activity and accrual damage:
    - Physician global assessment (PGA): divided into <1, [1-3], >3 according to the sample's distribution.
    - Patient global assessment (PtGA) ≤ 3 vs >3, categorized according to the nonlinear association observed in the scatter plot (see Supplementary Figure 5).
    - Disease Activity Score 28 (DAS28): remission ≤ 2.6, low (2.6-3.2], moderate (3.2-5.1] and high activity >5.1.
    - SLE disease activity index 2000 (SLEDAI 2-K): remission=0, mild [0-4], moderate (4-11], severe >11. For statistical purposes we grouped patients in remission and with mild activity.
    - SLE disease activity score (SLE-DAS) as a continuous variable. Remission ≤2.08; mild activity, (2.08-7.64]; moderate/severe activity>7.64.
    - SLICC/American College of Rheumatology (SLICC/ACR) Damage Index (SDI), analyzed both as a quantitative variable, categorized in two groups (SDI 0 vs ≥ 1), and in three groups (0 vs 1 vs ≥ 1).
    - IL-6 was measured using a Sandwich ELISA Kit provided by R&D Systems (Human IL-6 Quantikine, D6050); sensitivity 0.70 pg/mL; precision measured as coefficient of variation < 8% (intra-assay), < 10% (inter-assay).
- PROs like the Health Assessment Questionnaire (HAQ) (divided into normal<0.3, mild [0.3-1.3], moderate (1.3-1.8], severe >1.8), the Functional Assessment of Chronic Illness Therapy – Fatigue Scale (FACIT), and patient global assessment by a visual analogic scale (PtGA).
- Cardiovascular variables:
    - CVRF: presence of at least one: obesity (BMI > 30 Kg/m^2), AHT, DLP, CRD or hyperuricaemia. The smoking status was considered as a separate variable due to its high association with AGEs levels.
    - Cardiovascular events (CVE): angina, myocardial infarction, cerebrovascular accident, peripheral arterial disease, intestinal ischemia or ischemia of some other territory). CVRF&CVE indicates the presence of either/both CVRF or CVE (CVRF&CVE>0) or the sum (CVRF&CVE).
    - CVE_SDI: cardiovascular events assessed in the SDI (cerebral vascular accident, pulmonary infarction, angina or coronary bypass, myocardial infarction, venous thrombosis, or infarction of the gastrointestinal tract).

Supplementary Figure 1: Variables collected and their classification. *ACR: American College of Rheumatology; SLE: systemic lupus erythematosus, SLICC: systemic lupus International Collaborating Clinics, CVRF: cardiovascular risk factors; ab: antibodies; SADs: systemic autoimmune diseases; BMI: body mass index; AHT: arterial hypertensio; DLP: dislipidemia; CRD: chronic renal disease; AGEs: advance glycation end-products.*



Supplementary Figure 2: Flux diagram showing the sample size for each of the analysis. *For the comparison between patients with SLE and HC only 62 SLE patients could be included due to a younger mean age than HC and impossibility to age-match a higher number. Also, SLE patients with cardiovascular risk factors had to be excluded due to it being a criterion of exclusion in the HC. #: The sample size for analyzing CEL is reduced due to a shortage of the same ELISA kit. *AGEs: advanced glycation end-products; SLE: systemic lupus erythematosus; HC: healthy controls; sRAGE: soluble receptor for advanced glycation end-products; CML: Nξ-(carboxymethyl)lysine; CEL: Nξ-(carboxyethyl)lysine.*



Supplementary Figure 3: Density graphics of serum advanced glycation end-products, their soluble receptor and their ratios showing their right-skewed distribution. *CML: Nξ-(carboxymethyl)lysine; CEL: Nξ-(carboxyethyl)lysine; sRAGE: soluble receptor for advanced glycation end-products.*

Variables	All	1st tertile [1.2,2.3)	2nd tertile [2.3,2.8)	3rd tertile [2.8,4.6]	*p-val* M1	*p-val* M2
	N=122	N=44	N=41	N=37		
Gender: Female	114 (93.4%)	40 (90.9%)	38 (92.7%)	36 (97.3%)	0.429	0.333
Body mass index	25.4 (4.74)	24.7 (5.00)	24.9 (3.21)	26.8 (5.58)	0.341	0.132
Ethnicity					0.614	0.747
Caucasian	81 (66.4%)	25 (56.8%)	25 (61.0%)	31 (83.8%)		
Latin	29 (23.8%)	16 (36.4%)	9 (22.0%)	4 (10.8%)		
Other	12 (9.84%)	3 (6.82%)	7 (17.1%)	2 (5.41%)		
Classificatory Criteria and Other Clinical and Serological Data						
Constitutional symptoms	11 (9.02%)	8 (18.2%)	1 (2.44%)	2 (5.41%)	0.520	0.578
Cutaneous	91 (74.6%)	32 (72.7%)	28 (68.3%)	31 (83.8%)	0.348	0.361
Photosensitivity	74 (60.7%)	25 (56.8%)	27 (65.9%)	22 (59.5%)	0.446	0.402
Alopecia	55 (45.1%)	22 (50.0%)	15 (36.6%)	18 (48.6%)	0.715	0.963
Serositis	10 (8.20%)	4 (9.09%)	5 (12.2%)	1 (2.70%)	0.951	0.988
Neurological	11 (9.02%)	5 (11.4%)	2 (4.88%)	4 (10.8%)	0.974	0.853
Hematological	87 (71.3%)	32 (72.7%)	26 (63.4%)	29 (78.4%)	0.716	0.344
ANA+ ever	122 (100%)	44 (100%)	41 (100%)	37 (100%)		
Anti-dsDNA+ ever	77 (63.1%)	25 (56.8%)	25 (61.0%)	27 (73.0%)	0.471	0.585
Anti-Sm+ ever	22 (18.0%)	11 (25.0%)	5 (12.2%)	6 (16.2%)	0.974	0.867
Low complement	64 (52.5%)	25 (56.8%)	24 (58.5%)	15 (40.5%)	0.733	0.540
Direct Coombs+	10 (14.1%)	3 (12.0%)	3 (13.6%)	4 (16.7%)	0.103	0.179
Anti-Ro60+ ever	49 (40.2%)	20 (45.5%)	20 (48.8%)	9 (24.3%)	0.212	0.242
Anti-Ro52+ ever	28 (23.0%)	11 (25.0%)	9 (22.0%)	8 (21.6%)	0.542	0.482
Pulmonary	5 (4.10%)	4 (9.09%)	0 (0.00%)	1 (2.70%)	0.367	0.650
Cardiac	4 (3.28%)	0 (0.00%)	2 (4.88%)	2 (5.41%)	0.258	0.244
Raynaud	39 (32.2%)	18 (40.9%)	10 (24.4%)	11 (30.6%)	0.886	0.827
APS antibodies carrier	29 (23.8%)	6 (13.6%)	14 (34.1%)	9 (24.3%)	0.560	0.681
APS	5 (4.10%)	2 (4.55%)	2 (4.88%)	1 (2.70%)	0.553	0.288
Other SADs	77 (63.1%)	33 (75.0%)	20 (48.8%)	24 (64.9%)	0.223	0.315
Serological Variables						
ESR*	11.0 [5.00;20.0]	8.00 [2.50;15.0]	11.0 [4.00;21.0]	14.0 [7.50;20.2]	0.271	0.213
Anti-dsDNA+*	4.00 [1.00;13.0]	6.00 [1.00;16.5]	3.00 [1.00;9.00]	4.00 [1.00;14.0]	0.443	0.538
Anti-Ro52+*	26 (21.8%)	10 (23.8%)	9 (22.5%)	7 (18.9%)	0.359	0.368
CH50*	60.3 [51.8;70.9]	57.6 [44.1;68.9]	62.7 [54.7;70.8]	65.5 [54.7;72.1]	0.806	0.779
C3*	106 (22.3)	104 (25.3)	103 (18.2)	111 (22.6)	0.254	0.164
SLE Activity Indexes						
cDAS28					0.668	0.606
0- Remission	78 (65.0%)	29 (67.4%)	26 (63.4%)	23 (63.9%)		
1- Low activity	15 (12.5%)	5 (11.6%)	5 (12.2%)	5 (13.9%)		
2- Moderate activity	21 (17.5%)	8 (18.6%)	8 (19.5%)	5 (13.9%)		
3- High activity	6 (5.00%)	1 (2.33%)	2 (4.88%)	3 (8.33%)		
Patient Reported Outcomes						
HAQ	0.38 [0.00;0.88]	0.31 [0.00;0.75]	0.31 [0.00;0.91]	0.38 [0.12;1.00]	0.800	0.746
Patient pain VAS	2.00 [0.00;6.00]	1.00 [0.00;5.00]	4.00 [0.00;6.50]	2.00 [0.00;5.00]	0.660	0.607
Comorbidities and Cardiovascular Disease						
Hypertension	26 (21.3%)	3 (6.82%)	10 (24.4%)	13 (35.1%)	0.946	0.673
Dyslipidemia	12 (9.84%)	1 (2.27%)	5 (12.2%)	6 (16.2%)	0.401	0.505
Cardiovascular disease	5 (4.10%)	0 (0.00%)	3 (7.32%)	2 (5.41%)	0.817	0.846
Chronic renal disease	3 (2.46%)	1 (2.27%)	1 (2.44%)	1 (2.70%)	0.331	0.364
Hyperuricemia	2 (1.64%)	1 (2.27%)	1 (2.44%)	0 (0.00%)	0.616	0.805
Obesity	22 (18.0%)	8 (18.2%)	3 (7.32%)	11 (29.7%)	0.746	0.505
CVRF >0	47 (38.5%)	11 (25.0%)	16 (39.0%)	20 (54.1%)	0.465	0.592
cCVRF					0.498	0.703

Variables	All	1st tertile [1.2,2.3)	2nd tertile [2.3,2.8)	3rd tertile [2.8,4.6]	*p-val* M1	*p-val* M2
0	75 (61.5%)	33 (75.0%)	25 (61.0%)	17 (45.9%)		
1	33 (27.0%)	8 (18.2%)	13 (31.7%)	12 (32.4%)		
>1	14 (11.5%)	3 (6.82%)	3 (7.32%)	8 (21.6%)		
CVE	9 (7.38%)	3 (6.82%)	3 (7.32%)	3 (8.11%)	0.216	0.095
CVRF&CVE>0	48 (39.3%)	12 (27.3%)	16 (39.0%)	20 (54.1%)	0.495	0.590
CVRF&CVE					0.869	0.840
2	74 (60.7%)	32 (72.7%)	25 (61.0%)	17 (45.9%)		
3	27 (22.1%)	7 (15.9%)	11 (26.8%)	9 (24.3%)		
4	13 (10.7%)	4 (9.09%)	2 (4.88%)	7 (18.9%)		
5	8 (6.56%)	1 (2.27%)	3 (7.32%)	4 (10.8%)		
Treatments						
Dyslipidemia drugs	14 (11.5%)	2 (4.55%)	6 (14.6%)	6 (16.2%)	0.729	0.942
Antihypertensives	28 (23.0%)	4 (9.09%)	10 (24.4%)	14 (37.8%)	0.970	0.758
Antimalarials	93 (76.2%)	34 (77.3%)	33 (80.5%)	26 (70.3%)	0.211	0.215
cDMARD	19 (15.6%)	4 (9.09%)	9 (22.0%)	6 (16.2%)	0.163	0.536
bDMARD	6 (4.92%)	2 (4.55%)	2 (4.88%)	2 (5.41%)	0.156	0.260
Mycophenolic acid	20 (16.4%)	7 (15.9%)	6 (14.6%)	7 (18.9%)	0.739	0.617
Azathioprine	19 (15.6%)	6 (13.6%)	8 (19.5%)	5 (13.5%)	0.396	0.263

Supplementary Table 3: Other demographic and disease characteristics of systemic lupus erythematosus patients and their distribution according to advanced glycation end-products tertiles in the exploratory analysis. M1: adjusted by age, M2 adjusted by age and smoking. "c" indicates variables which have been categorized as previously stated in the methodology section. Bold indicates *p*-value <0.1 and * indicates values according to the blood test performed in the study. *p-val: p-value; ANA: antinuclear antibodies; APL: antiphospholipid; APS: antiphospholipid syndrome; SADs: systemic autoimmune diseases; ESR: erythrocyte sedimentation rate; CH50 and C3: Complement CH50 and C3; DAS28: disease activity score 28 remission ≤ 2.6, low (2.6-3.2], moderate (3.2-5.1] and high activity >5.1; HAQ: health assessment questionnaire disability index; VAS: visual analogic scale; CVRF: cardiovascular risk factors (obesity = IMC> 30 Kg/m2, arterial hypertension, dyslipidemia, chronic renal disease or hyperuricaemia); CVE: cardiovascular events (angina, myocardial infarction, cerebrovascular accident, peripheral arterial disease, intestinal ischemia or ischemia of some other territory); cDMARD; disease-modifying antirheumatic drugs; bDMARD: biological DMARD.*

Variable		Estimate	2.5%	97.5%	t-value	*p*-value
SLEDAI: Remission or Mild [0-4]	**(Intercept)**	2.3371	2.2066	2.4676	35.4786	<0.0001
	cSLEDAI3: Moderate (4-11]	0.2001	0.0006	0.3995	1.9867	**0.0493**
	cSLEDAI3: Severe >11	0.5188	0.1774	0.8601	3.0106	**0.0032**
	Age	0.0268	0.0204	0.0332	8.3580	<0.0001
	Smoking	0.3341	0.1168	0.5515	3.0452	0.0029
SDI [0-2]	**(Intercept)**	2.3158	2.1955	2.4361	38.1426	<0.0001
	SDI [3,4]	-0.0684	-0.4372	0.3004	-0.3673	0.7140
	SDI [5,6]	0.7169	0.1386	1.2951	2.4557	**0.0156**
	Age	0.0273	0.0211	0.0334	8.7537	<0.0001
	Glucocorticoids	0.3392	0.1250	0.5534	3.1362	0.0022
	Smoking	0.4315	0.2227	0.6404	4.0937	0.0001
PGA < 1	**(Intercept)**	2.1256	1.8742	2.3770	16.7452	<0.0001
	PGA [1,2]	0.3335	0.0580	0.6090	2.3975	**0.0181**
	PGA >2	0.3942	0.0943	0.6941	2.6031	**0.0104**
	Age	0.0291	0.0226	0.0357	8.7863	<0.0001
PtGA ≤ 3	**(Intercept)**	2.4297	2.3092	2.5503	39.9056	<0.0001
	PtGA>3	0.2622	0.0639	0.4605	2.6189	**0.0100**
	Age	0.0236	0.0172	0.0301	7.2499	<0.0001
CRP < 0.12 mg/dL	**(Intercept)**	2.3191	2.1560	2.4822	28.1631	<0.0001
	CRP [0.12,0.28)	0.0925	-0.1374	0.3224	0.7968	0.4272
	CRP [0.28,3.92]	0.2594	0.0359	0.4830	2.2988	**0.0233**
	Age	0.0267	0.0202	0.0332	8.1774	<0.0001
	Smoking	0.3627	0.1444	0.5809	3.2918	0.0013
Oral Ulcers	**(Intercept)**	2.4379	2.3124	2.5635	38.4462	<0.0001
	Oral Ulcers	0.2162	0.0209	0.4116	2.1918	**0.0304**
	Age	0.0245	0.0180	0.0309	7.4832	<0.0001
Leukocyturia 0	**(Intercept)**	2.3324	2.1981	2.4667	34.4129	<0.0001
	Leukocyturia 1	0.1460	-0.0958	0.3878	1.1960	0.2342
	Leukocyturia [2,5]	0.3695	0.1128	0.6261	2.8520	**0.0052**
	Age	0.0242	0.0174	0.0309	7.0873	<0.0001
	Smoking	0.3732	0.1566	0.5898	3.4131	0.0009
Anti-Ro60+	**(Intercept)**	2.6356	2.4858	2.7855	34.8364	<0.0001
	Anti-Ro60 presence	-0.2601	-0.5027	-0.0174	-2.1227	**0.0359**
ANA+	**(Intercept)**	2.9889	2.5642	3.4136	13.9370	<0.0001
	ANA presence	-0.4961	-0.9377	-0.0545	-2.2248	0.0280
C4 <18 mg/dL	**(Intercept)**	2.2585	2.0866	2.4305	26.0264	<0.0001
	C4 [18,24)	0.2503	0.0200	0.4806	2.1530	**0.0335**
	C4 [24,49]	0.2854	0.0566	0.5143	2.4710	**0.0150**
	Age	0.0251	0.0182	0.0320	7.1965	<0.0001
	Total cholesterol	0.0019	-0.0008	0.0046	1.3890	0.1676
	Smoking	0.3799	0.1554	0.6044	3.3533	0.0011
IL-6 < 1.88 pg/mL	**(Intercept)**	2.2279	2.0384	2.4174	23.3132	<0.0001
	cIL-6 [1.88, 3.33)	0.1972	-0.0521	0.4465	1.5691	0.1197
	cIL-6 [3.33,144.10]	0.3524	0.0995	0.6053	2.7631	**0.0068**
	Age	0.0228	0.0155	0.0301	6.1716	<0.0001
	Smoking	0.3967	0.1678	0.6257	3.4370	0.0008

Supplementary Table 4: Linear regression model between advanced glycation end-products and statistically significant variables (*p*-value <0.05) adjusted according to confounders (in grey). “c” indicates categorized variables according to previous categories explained in the Methodology section. *SLEDAI: SLE disease activity index; SDI: SLE disease damage index (SDI); PGA: Physician global assessment visual analogic scale 0-10; PtGA: Patient global assessment visual analogic scale 0-10; CRP: C-reactive protein; Leukocyturia defined as 0-5 according to number of leucocytes in urine per field; C4: Complement 4 levels (reference levels 10-40 mg/dL); IL-6: interleukin 6; Anti-Ro60+: positivity of anti-Ro60 antibodies in the study blood test; ANA+: positivity of anti-nuclear antibodies in the study blood test.*

Variables	First tertile [0.1180)	Second tertile [1180,1594)	Third tertile [1594,4334]	*p*-value
	N=39	*N=39*	*N=39*	
Gender: Female	36 (92.3%)	36 (92.3%)	37 (94.9%)	0.815
Age	52.1 (14.8)	49.5 (15.5)	51.1 (14.9)	0.453
Body mass index	25.7 (5.03)	25.9 (4.54)	24.9 (4.87)	0.474
Ethnicity				0.992
Caucasian	29 (74.4%)	25 (64.1%)	25 (64.1%)	
Latin	8 (20.5%)	8 (20.5%)	11 (28.2%)	
Other	2 (5.13%)	6 (15.4%)	3 (7.69%)	
Years of duration	8.00 [4.00;13.5]	10.0 [2.50;17.5]	10.0 [1.00;18.5]	0.994
Smoker	11 (28.2%)	9 (23.1%)	10 (25.6%)	0.723
Classificatory Criteria and Other Clinical and Serological Data				
Constitutional symptoms	4 (10.3%)	4 (10.3%)	3 (7.69%)	0.207
Cutaneous	25 (64.1%)	34 (87.2%)	28 (71.8%)	0.725
Photosensitivity	21 (53.8%)	25 (64.1%)	25 (64.1%)	0.305
Oral ulcer	17 (43.6%)	17 (43.6%)	14 (35.9%)	0.160
Alopecia	13 (33.3%)	20 (51.3%)	19 (48.7%)	0.883
Arthritis	28 (71.8%)	32 (82.1%)	29 (74.4%)	0.346
Serositis	5 (12.8%)	4 (10.3%)	1 (2.56%)	0.593
Renal	3 (7.69%)	4 (10.3%)	1 (2.56%)	0.228
Neurological	4 (10.3%)	3 (7.69%)	3 (7.69%)	0.258
Hematological	33 (84.6%)	26 (66.7%)	24 (61.5%)	0.333
ANA+ ever	39 (100%)	39 (100%)	39 (100%)	
Anti-dsDNA+ ever	23 (59.0%)	26 (66.7%)	25 (64.1%)	0.179
Anti-Sm+ ever	8 (20.5%)	10 (25.6%)	3 (7.69%)	0.199
Anti-Ro60+ ever	16 (41.0%)	19 (48.7%)	13 (33.3%)	0.899
Anti-Ro52+ ever	11 (28.2%)	9 (23.1%)	7 (17.9%)	0.186
Low complement	20 (51.3%)	22 (56.4%)	20 (51.3%)	0.210
Cardiac	1 (2.56%)	1 (2.56%)	2 (5.13%)	0.110
Raynaud	14 (35.9%)	12 (31.6%)	13 (33.3%)	0.417
APL antibodies carrier	8 (20.5%)	10 (25.6%)	9 (23.1%)	0.305
APS	1 (2.56%)	3 (7.69%)	1 (2.56%)	0.733
Other SADs	24 (61.5%)	23 (59%)	28 (71.8%)	0.100
Serological Variables				
CRP*	0.14 [0.08;0.32]	0.18 [0.08;0.42]	0.13 [0.08;0.31]	0.869
ESR*	13.0 [7.00;21.0]	10.0 [5.00;18.0]	9.00 [2.00;18.5]	0.448
Anti-dsDNA+*	4.00 [2.00;15.8]	5.00 [1.00;12.0]	3.00 [1.00;12.5]	0.551
CH50*	58.7 [42.0;67.5]	60.3 [48.2;72.8]	62.2 [56.4;70.9]	0.109
C3*	105 (22.6)	105 (22.4)	109 (23.5)	0.671
C4*	18.8 (8.18)	20.4 (9.80)	20.5 (6.99)	0.349
IL-6*	2.90 [1.93;4.66]	1.98 [1.55;3.53]	2.32 [1.40;3.63]	0.598
SLE Activity and Damage Indexes				
DAS28	2.36 [1.68;3.11]	2.02 [1.37;2.76]	2.10 [1.33;3.25]	0.698
SLEDAI	4.00 [2.00;6.00]	4.00 [2.00;6.00]	4.00 [2.00;7.00]	0.255
SLE-DAS	4.18 [1.78;7.28]	1.79 [1.20;6.15]	2.53 [0.82;4.86]	0.087
SDI	0.00 [0.00;1.00]	1.00 [0.00;1.00]	0.00 [0.00;1.00]	0.587
PGA	2.00 [1.00;3.00]	2.00 [1.00;2.50]	2.00 [1.00;3.00]	0.565
Patient Reported Outcomes				
FACIT	18.0 [13.0;28.0]	18.0 [10.0;27.0]	16.0 [9.50;26.0]	0.386
HAQ	0.50 [0.00;0.94]	0.38 [0.00;0.88]	0.31 [0.00;0.72]	0.795
PtGA	2.50 [1.00;5.00]	3.00 [1.00;4.50]	3.00 [1.00;4.75]	0.368
Pain VAS	2.00 [0.00;6.25]	2.00 [0.00;5.00]	3.00 [0.00;6.00]	0.926
Comorbidities and Cardiovascular Disease				
Hypertension	9 (23.1%)	9 (23.1%)	8 (20.5%)	0.929
Dyslipidemia	6 (15.4%)	3 (7.69%)	3 (7.69%)	0.850
Cardiovascular disease	1 (2.56%)	3 (7.69%)	1 (2.56%)	0.706
Chronic renal disease	1 (2.56%)	1 (2.56%)	1 (2.56%)	0.129
Hyperuricemia	0 (0.00%)	2 (5.13%)	0 (0.00%)	0.887
Obesity	9 (23.1%)	8 (20.5%)	5 (12.8%)	0.360

Variables	First tertile [0.1180)	Second tertile [1180,1594)	Third tertile [1594,4334]	*p*-value
CVRF >0	15 (38.5%)	17 (43.6%)	15 (38.5%)	0.816
CVRF				0.579
0	24 (61.5%)	22 (56.4%)	24 (61.5%)	
1	8 (20.5%)	12 (30.8%)	13 (33.3%)	
2	4 (10.3%)	4 (10.3%)	2 (5.13%)	
3	3 (7.69%)	1 (2.56%)	0 (0.00%)	
CVE				0.249
0	36 (92.3%)	33 (84.6%)	37 (94.9%)	
1	3 (7.69%)	4 (10.3%)	0 (0.00%)	
2	0 (0.00%)	1 (2.56%)	1 (2.56%)	
3	0 (0.00%)	1 (2.56%)	1 (2.56%)	
CVE_SDI_presence	2 (5.13%)	5 (12.8%)	2 (5.13%)	0.944
CVRF&CVE >0	15 (38.5%)	17 (43.6%)	16 (41.0%)	0.518
CVRF & CVE				0.795
0	24 (61.5%)	22 (56.4%)	23 (59.0%)	
1	12 (30.8%)	11 (28.2%)	14 (35.9%)	
2	3 (7.69%)	4 (10.3%)	1 (2.56%)	
3	0 (0.00%)	1 (2.56%)	0 (0.00%)	
4	0 (0.00%)	1 (2.56%)	1 (2.56%)	
Treatments				
Dyslipidemia drugs	5 (12.8%)	5 (12.8%)	4 (10.3%)	0.749
Antihypertensives	9 (23.1%)	10 (25.6%)	9 (23.1%)	0.976
Antimalarials	28 (71.8%)	32 (82.1%)	29 (74.4%)	0.493
cDMARD	6 (15.4%)	6 (15.4%)	4 (10.3%)	0.591
bDMARD	2 (5.13%)	3 (7.69%)	1 (2.56%)	0.432
Mycophenolic acid	9 (23.1%)	6 (15.4%)	4 (10.3%)	0.493
Cyclosporine: 0	39 (100%)	39 (100%)	39 (100%)	
Azathioprine	8 (20.5%)	3 (7.69%)	7 (17.9%)	0.933
Cyclophosphamide: 0	39 (100%)	39 (100%)	39 (100%)	
Treatment				0.494
Others	3 (7.69%)	5 (12.8%)	6 (15.4%)	
Antimalarials	14 (35.9%)	19 (48.7%)	18 (46.2%)	
Immunosuppressants	22 (56.4%)	15 (38.5%)	15 (38.5%)	
AGEs				
Skin AGEs	2.61 (0.61)	2.55 (0.69)	2.43 (0.66)	0.214
CML	277 [199;365]	320 [194;497]	294 [234;460]	0.372
CEL	3.14 [2.33;4.18]	3.16 [2.04;3.73]	2.82 [2.38;4.33]	0.485
sRAGE	505 [369;790]	447 [317;751]	556 [341;703]	0.342

Supplementary Table 5: Other non-significant (*p*-value >0.1) demographic and disease characteristics of systemic lupus erythematosus patients and their distribution according to pentosidine tertiles in the exploratory analysis. “c” indicates variables which have been categorized as previously stated in the methodology section. *Indicates values according to the blood test performed in the study. *ANA: antinuclear antibodies; APL: antiphospholipid; APS; antiphospholipid syndrome; SADs: systemic autoimmune diseases; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; CH50, C3 and C4: Complement CH50, C3 and C4; IL-6: interleukin 6; DAS28: disease activity score 28; SLEDAI: systemic lupus erythematosus disease activity index; SLE-DAS: systemic lupus erythematosus disease activity score; SDI: systemic lupus erythematosus damage index; PGA: physician global assessment; FACIT: functional assessment of chronic illness therapy; HAQ: health assessment questionnaire disability index; PtGA: patient global assessment; VAS: visual analogic scale; CVRF: cardiovascular risk factors (obesity = IMC> 30 Kg/m2, arterial hypertension, dyslipidemia, chronic renal disease or hyperuricaemia); CVE: cardiovascular events (angina, myocardial infarction, cerebrovascular accident, peripheral arterial disease, intestinal ischemia or ischemia of some other territory); CVE_SDI: cardiovascular events assessed in the SLE damage index (cerebral vascular accident, pulmonary infarction, angina or coronary bypass, myocardial infarction, venous thrombosis or infarction of the gastrointestinal tract); cDMARD; Disease-modifying antirheumatic drugs; bDMARD: biological DMARD; AGEs: advanced glycation end products; CML: Nξ-(carboxymethyl)lysine; CEL: Nξ-(carboxyethyl)lysine; sRAGE: receptor for advanced glycation end-products.*

	Estimate	2.5%	97.5%	t-value	*p*-value
(Intercept)	1481.3214	1341.8941	1620.7487	21.0447	0.0000
Pulmonary	1181.8786	507.4192	1856.3379	3.4710	**0.0007**

Supplementary Table 6: Linear regression model showing associations between pentosidine levels and systemic lupus erythematosus variables. We only show the results that were statistically significant indicated by p<0.05 (bold).

Variables	First tertile [57.6, 240)	Second tertile [239.8, 383)	Third tertile [382.9,1555]	*p*-value
	N=39	*N=39*	*N=39*	
Gender: Female	34 (87.2%)	37 (94.9%)	38 (97.4%)	0.417
Age	50.3 (16.2)	52.0 (14.3)	50.3 (14.7)	0.496
Body mass index	25.5 (5.23)	25.9 (5.01)	24.8 (4.20)	0.311
Smoker	11 (28.2%)	12 (30.8%)	8 (20.5%)	0.127
Classificatory Criteria and Other Clinical and Serological Data				
Constitutional symptoms	3 (7.69%)	3 (7.69%)	4 (10.3%)	0.564
Cutaneous	32 (82.1%)	24 (61.5%)	32 (82.1%)	0.566
Photosensitivity	22 (56.4%)	25 (64.1%)	27 (69.2%)	0.883
Oral ulcers	20 (51.3%)	12 (30.8%)	17 (43.6%)	0.281
Alopecia	17 (43.6%)	13 (33.3%)	24 (61.5%)	0.926
Arthritis	27 (69.2%)	29 (74.4%)	33 (84.6%)	0.459
Serositis	3 (7.69%)	4 (10.3%)	2 (5.13%)	0.554
Neurological	3 (7.69%)	4 (10.3%)	3 (7.69%)	0.899
Hematological	24 (61.5%)	31 (79.5%)	28 (71.8%)	0.480
ANA+ ever	39 (100%)	39 (100%)	39 (100%)	
Anti-dsDNA+ ever	19 (48.7%)	28 (71.8%)	26 (66.7%)	0.587
Anti-Sm+ ever	7 (17.9%)	4 (10.3%)	10 (25.6%)	0.205
Anti-Ro60+ ever	16 (41.0%)	13 (33.3%)	19 (48.7%)	0.106
Anti-Ro52+ ever	10 (25.6%)	7 (17.9%)	10 (25.6%)	0.208
Low complement	21 (53.8%)	21 (53.8%)	19 (48.7%)	0.939
Direct Coombs +	1 (4.55%)	4 (16.7%)	4 (19.0%)	0.242
Pulmonary	0 (0.00%)	2 (5.13%)	2 (5.13%)	0.587
Cardiac	0 (0.00%)	3 (7.69%)	1 (2.56%)	0.981
Raynaud	13 (33.3%)	14 (35.9%)	12 (30.8%)	0.286
APL antibodies carrier	10 (25.6%)	11 (28.2%)	8 (20.5%)	0.594
APS	2 (5.13%)	1 (2.56%)	2 (5.13%)	0.887
Other SADs	24 (61.5%)	26 (66.7%)	23 (59.0%)	0.713
Serological Variables				
CRP*	0.14 [0.08;0.38]	0.16 [0.07;0.30]	0.12 [0.08;0.34]	0.497
ESR*	9.00 [4.25;20.0]	9.00 [5.00;16.8]	13.0 [7.50;20.5]	0.120
Anti-dsDNA+*	2.00 [1.00;9.00]	3.00 [1.00;11.8]	6.00 [2.00;20.5]	0.675
Anti-dsDNA>RV*	7 (17.9%)	11 (28.9%)	18 (46.2%)	0.135
CH50*	67.2 [53.0;72.2]	60.6 [53.1;68.2]	58.6 [43.2;66.3]	0.366
C3*	107 (22.2)	108 (21.3)	104 (24.7)	0.216
C4*	19.5 (8.43)	20.3 (7.18)	19.8 (9.47)	0.848
IL-6*	2.28 [1.29;3.23]	2.08 [1.84;3.01]	3.43 [1.86;4.69]	0.176
SLE Activity and Damage Indexes				
DAS28	2.11 [1.28;3.09]	2.10 [1.47;2.85]	2.43 [1.74;3.31]	0.270
SLEDAI	4.00 [2.00;6.00]	4.00 [2.00;6.00]	5.00 [2.00;7.00]	0.393
SLE-DAS	2.55 [1.20;6.19]	3.55 [1.01;5.59]	2.53 [1.78;7.18]	0.470
SDI	0.00 [0.00;1.00]	0.00 [0.00;1.00]	0.00 [0.00;1.00]	0.297
Patient Reported Outcomes				
FACIT	15.0 [9.50;26.5]	20.0 [12.5;30.5]	18.0 [10.0;25.5]	0.930
HAQ	0.25 [0.00;1.00]	0.50 [0.00;0.94]	0.31 [0.03;0.59]	0.870
PtGA	2.50 [1.00;5.00]	3.00 [0.00;5.00]	3.00 [1.50;5.00]	0.974
Pain VAS	2.00 [0.00;6.25]	3.00 [0.00;6.00]	2.00 [0.00;5.50]	0.810
Comorbidities and Cardiovascular Disease				
Hypertension	7 (17.9%)	10 (25.6%)	9 (23.1%)	0.536
Dyslipidemia	4 (10.3%)	2 (5.13%)	6 (15.4%)	0.136
Cardiovascular disease	2 (5.13%)	1 (2.56%)	2 (5.13%)	0.879
Chronic renal disease	1 (2.56%)	1 (2.56%)	1 (2.56%)	0.953
Hyperuricemia	0 (0.00%)	1 (2.56%)	1 (2.56%)	0.609
Obesity	7 (17.9%)	9 (23.1%)	6 (15.4%)	0.421
CVRF >0	12 (30.8%)	18 (46.2%)	17 (43.6%)	0.568
CVRF				0.312
0	27 (69.2%)	21 (53.8%)	22 (56.4%)	
1	6 (15.4%)	14 (35.9%)	13 (33.3%)	

Variables	First tertile [57.6, 240)	Second tertile [239.8, 383)	Third tertile [382.9,1555]	*p*-value
2	5 (12.8%)	3 (7.69%)	2 (5.13%)	
3	1 (2.56%)	1 (2.56%)	2 (5.13%)	
CVE				0.160
0	35 (89.7%)	37 (94.9%)	34 (87.2%)	
1	4 (10.3%)	1 (2.56%)	2 (5.13%)	
2	0 (0.00%)	0 (0.00%)	2 (5.13%)	
3	0 (0.00%)	1 (2.56%)	1 (2.56%)	
CVE_SDI_presence	2 (5.13%)	2 (5.13%)	5 (12.8%)	0.848
CVRF&CVE >0	12 (30.8%)	18 (46.2%)	18 (46.2%)	0.417
CVRF&CVE				0.512
0	27 (69.2%)	21 (53.8%)	21 (53.8%)	
1	8 (20.5%)	16 (41.0%)	13 (33.3%)	
2	4 (10.3%)	1 (2.56%)	3 (7.69%)	
3	0 (0.00%)	0 (0.00%)	1 (2.56%)	
4	0 (0.00%)	1 (2.56%)	1 (2.56%)	
Treatments				
Antihypertensives	7 (17.9%)	10 (25.6%)	11 (28.2%)	0.380
cDMARD	8 (20.5%)	7 (17.9%)	3 (7.69%)	0.104
bDMARD	1 (2.56%)	2 (5.13%)	3 (7.69%)	0.548
Antimalarials	33 (84.6%)	26 (66.7%)	29 (74.4%)	0.459
Tacrolimus	0 (0.00%)	0 (0.00%)	1 (2.56%)	0.849
Cyclosporine: 0	39 (100%)	39 (100%)	39 (100%)	
Azathioprine	3 (7.69%)	8 (20.5%)	7 (17.9%)	0.615
Cyclophosphamide: 0	39 (100%)	39 (100%)	39 (100%)	
Treatment				0.257
Non-IS	26 (66.7%)	20 (51.3%)	17 (43.6%)	
IS	13 (33.3%)	19 (48.7%)	22 (56.4%)	
AGEs				
Skin AGEs	2.41 (0.59)	2.57 (0.57)	2.65 (0.76)	0.242
Skin AGEs assessment				0.233
<1SD	3 (7.69%)	2 (5.13%)	0 (0.00%)	
1SD-Mean	6 (15.4%)	4 (10.3%)	3 (7.69%)	
Mean	1 (2.56%)	2 (5.13%)	1 (2.56%)	
Mean->1SD	13 (33.3%)	9 (23.1%)	12 (30.8%)	
>1SD	16 (41.0%)	22 (56.4%)	23 (59.0%)	
Pentosidine	1304 [970;1721]	1372 [1047;1797]	1408 [1182;1871]	0.372
sRAGE	359 [245;514]	716 [534;847]	476 [354;676]	0.859

Supplementary Table 7: Non-significant (*p*-value >0.1) demographic and disease characteristics of systemic lupus erythematosus patients and their distribution according to CML tertiles in the exploratory analysis. “c” indicates variables which have been categorized as previously stated in the methodology section. *Indicates values according to the blood test performed in the study. *ANA: antinuclear antibodies; APL: antiphospholipid; APS; antiphospholipid syndrome; SADs: systemic autoimmune diseases; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; RV: reference value; CH50, C3 and C4: Complement CH50, C3 and C4; IL-6: interleukin 6; DAS28: Disease activity score 28; SLEDAI: systemic lupus erythematosus disease activity index; SLE-DAS: systemic lupus erythematosus disease activity score; SDI: systemic lupus erythematosus damage index; PGA: physician global assessment; FACIT: functional assessment of chronic illness therapy; HAQ: health assessment questionnaire disability index; PtGA: patient global assessment; VAS: Visual analogic scale; CVRF: cardiovascular risk factors (obesity = IMC> 30 Kg/m2, arterial hypertension, dyslipidemia, chronic renal disease or hyperuricaemia); CVE: cardiovascular events (angina, myocardial infarction, cerebrovascular accident, peripheral arterial disease, intestinal ischemia or ischemia of some other territory); CVE_SDI: cardiovascular events assessed in the SLE damage index (cerebral vascular accident, pulmonary infarction, angina or coronary bypass, myocardial infarction, venous thrombosis or infarction of the gastrointestinal tract); cDMARD; Disease-modifying antirheumatic drugs; bDMARD: biological DMARD; IS: immunosuppressants; AGEs: advanced glycation end products; SD: standard deviation; sRAGE: receptor for advanced glycation end-products;*

	OLS linear regression	Gamma GLM
(Intercept)	332.806 ***	332.806 ***
	(CI=[274.336, 391.276],p = 0.000)	(CI=[283.766, 381.845],p = 0.000)
Non-Caucasian ethnicities	144.618 **	144.618 *
	(CI=[42.021, 247.215],**p = 0.006**)	(CI=[31.953, 257.283],**p = 0.013**)
(Intercept)	244.217 ***	234.488 ***
	(CI=[174.929, 313.505],p = 0.000)	(CI=[181.650, 287.326],p = 0.000)
Disease duration	4.102 *	4.347 *
	(CI=[0.290, 7.914],**p = 0.035**)	(CI=[0.558, 8.135],**p = 0.026**)
Non-Caucasian ethnicities	100.640 *	128.964 **
	(CI=[14.939, 186.340],p = 0.022)	(CI=[39.714, 218.213],p = 0.005)
Glucocorticoids	153.386 **	202.328 **
	(CI=[62.856, 243.915],p = 0.001)	(CI=[84.720, 319.937],p = 0.001)
(Intercept)	281.586 ***	307.334 ***
	(CI=[217.110, 346.062],p = 0.000)	(CI=[244.084, 370.584],p = 0.000)
Anti-dsDNA 2nd tertile [2, 11)	92.224 *	66.477
	(CI=[1.042, 183.407],**p = 0.047**)	(CI=[-33.876, 166.829],p = 0.197)
Anti-dsDNA 3r tertile [11,300]	124.908 **	129.480 *
	(CI=[31.157, 218.660],**p = 0.009**)	(CI=[15.550, 243.410],**p = 0.028**)
(Intercept)	298.664 ***	300.312 ***
	(CI=[234.713, 362.615],p = 0.000)	(CI=[240.613, 360.011],p = 0.000)
IL-6 2nd tertile [1.88, 3.24)	-31.427	-14.590
	(CI=[-119.805, 56.951],p = 0.482)	(CI=[-97.111, 67.930],p = 0.730)
IL-6 3d tertile [3.24,39.38]	105.876 *	83.828
	(CI=[16.522, 195.231],**p = 0.021**)	(CI=[-16.035, 183.690],p = 0.103)
Glucocorticoids	129.405 **	231.524 ***
	(CI=[43.234, 215.577],p = 0.004)	(CI=[106.488, 356.559],p = 0.000)
(Intercept)	302.059 ***	299.238 ***
	(CI=[255.371, 348.747],p = 0.000)	(CI=[257.970, 340.505],p = 0.000)
Densitometric osteoporosis	103.270 *	136.359 *
	(CI=[0.093, 206.447],**p = 0.050**)	(CI=[11.680, 261.037],**p = 0.034**)
Non-Caucasian ethnicities	88.636 *	102.513 *
	(CI=[10.842, 166.431],p = 0.026)	(CI=[17.808, 187.218],p = 0.019)

Supplementary Table 8: Ordinary least squares linear regression and gamma generalized linear model showing associations found between CML and systemic lupus erythematosus characteristics adjusted by their confounders(in grey). We only show the results that were statistically significant. Bold indicates those *p*-values significant ($p<0.05$). *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. *Anti-dsDNA: anti-dsDNA antibodies; IL-6: interleukin 6.*

Variables	First tertile [0.823, 2.79)	Second tertile [2.793, 4.56)	Third tertile [4.564,31.68]	*p*-value
	N=38	N=37	N=16	
Gender: Female	35 (92.1%)	35 (94.6%)	16 (100%)	0.636
Age	53.2 (14.1)	52.4 (15.0)	51.6 (11.8)	0.702
BMI	26.3 (5.74)	24.9 (3.77)	25.3 (3.51)	0.413
Ethnicity				0.210
Caucasian	23 (60.5%)	26 (70.3%)	13 (81.2%)	
Other	15 (39.5%)	11 (29.7%)	3 (18.8%)	
Years of duration tertiles				0.144
[0, 5)	11 (28.9%)	8 (21.6%)	1 (6.25%)	
[5,16)	15 (39.5%)	17 (45.9%)	7 (43.8%)	
[16.45]	12 (31.6%)	12 (32.4%)	8 (50.0%)	
Classificatory Criteria and Other Clinical and Serological Data				
Constitutional symptoms	3 (7.89%)	4 (10.8%)	1 (6.25%)	0.046
Cutaneous	29 (76.3%)	28 (75.7%)	14 (87.5%)	0.714
Oral ulcer	18 (47.4%)	12 (32.4%)	10 (62.5%)	0.604
Alopecia	17 (44.7%)	18 (48.6%)	6 (37.5%)	0.513
Arthritis	30 (78.9%)	29 (78.4%)	14 (87.5%)	0.415
Serositis	2 (5.26%)	4 (10.8%)	2 (12.5%)	0.954
Renal	4 (10.5%)	2 (5.41%)	2 (12.5%)	0.881
Neurological	4 (10.5%)	4 (10.8%)	1 (6.25%)	0.576
Hematological	30 (78.9%)	28 (75.7%)	13 (81.2%)	0.625
ANA+ ever	38 (100%)	37 (100%)	16 (100%)	
Anti-dsDNA+ ever	23 (60.5%)	26 (70.3%)	14 (87.5%)	0.025
Anti-Sm+ ever	5 (13.2%)	6 (16.2%)	6 (37.5%)	0.142
Low complement	19 (50.0%)	19 (51.4%)	9 (56.2%)	0.475
Direct Coombs+	1 (5.26%)	3 (13.6%)	3 (21.4%)	0.853
Pulmonary	2 (5.26%)	1 (2.70%)	0 (0.00%)	0.403
Cardiac	0 (0.00%)	2 (5.41%)	1 (6.25%)	0.667
Raynaud	13 (34.2%)	14 (38.9%)	6 (37.5%)	0.434
APL antibodies carrier	9 (23.7%)	7 (18.9%)	6 (37.5%)	0.862
APS	2 (5.26%)	2 (5.41%)	0 (0.00%)	0.845
Other SADs	25 (65.8%)	23 (62.2%)	8 (50.0%)	0.663
Serological Variables				
CH50*	59.8 [52.4;73.6]	62.8 [57.4;69.0]	61.4 [55.4;70.8]	0.765
C4*	20.5 (8.69)	20.5 (7.51)	17.9 (7.15)	0.204
SLE Activity and Damage Indexes				
DAS28	2.11 [1.27;3.05]	2.25 [1.68;2.73]	2.39 [1.68;2.89]	0.345
SLEDAI	4.00 [0.00;7.50]	5.00 [2.00;6.00]	5.00 [3.50;9.00]	0.291
SLE-DAS	1.78 [0.82;5.84]	2.16 [0.82;4.93]	6.05 [3.60;7.94]	0.253
SDI	0.00 [0.00;1.00]	0.00 [0.00;1.00]	1.00 [0.00;1.25]	0.876
PGA	1.50 [1.00;2.00]	2.00 [1.00;3.00]	2.00 [1.00;3.00]	0.591
Patient Reported Outcomes				
FACIT	14.5 [10.0;23.8]	20.0 [16.0;29.0]	16.5 [10.8;26.8]	0.963
HAQ	0.25 [0.00;0.72]	0.62 [0.12;1.00]	0.38 [0.22;0.47]	0.937
PtGA	2.00 [1.00;4.75]	2.50 [1.00;5.00]	3.25 [2.00;5.00]	0.984
Pain VAS	2.00 [0.00;6.00]	3.00 [0.00;6.50]	3.00 [0.00;5.25]	0.526
Comorbidities and Cardiovascular Disease				
Hypertension	10 (26.3%)	8 (21.6%)	3 (18.8%)	0.547
Dyslipidemia	3 (7.89%)	4 (10.8%)	3 (18.8%)	0.669
Cardiovascular disease	2 (5.26%)	2 (5.41%)	1 (6.25%)	0.837
Chronic renal disease	0 (0.00%)	1 (2.70%)	0 (0.00%)	0.762
Hyperuricemia	2 (5.26%)	0 (0.00%)	0 (0.00%)	0.213
Obesity	10 (26.3%)	4 (10.8%)	2 (12.5%)	0.387
CVRF >0	16 (42.1%)	15 (40.5%)	6 (37.5%)	0.448
CVRF				0.383
0	22 (57.9%)	22 (59.5%)	10 (62.5%)	
1	8 (21.1%)	13 (35.1%)	5 (31.2%)	
2	7 (18.4%)	2 (5.41%)	0 (0.00%)	

Variables	First tertile [0.823, 2.79)	Second tertile [2.793, 4.56)	Third tertile [4.564,31.68]	*p*-value
3	1 (2.63%)	0 (0.00%)	1 (6.25%)	
CVE				0.673
0	33 (86.8%)	33 (89.2%)	15 (93.8%)	
1	4 (10.5%)	2 (5.41%)	0 (0.00%)	
2	1 (2.63%)	1 (2.70%)	0 (0.00%)	
3	0 (0.00%)	1 (2.70%)	1 (6.25%)	
CVE_SDI_presence	3 (7.89%)	4 (10.8%)	1 (6.25%)	0.823
CVRF&CVE >0	17 (44.7%)	15 (40.5%)	6 (37.5%)	0.407
CVRF&CVE				0.731
0	21 (55.3%)	22 (59.5%)	10 (62.5%)	
1	12 (31.6%)	11 (29.7%)	5 (31.2%)	
2	5 (13.2%)	2 (5.41%)	0 (0.00%)	
3	0 (0.00%)	1 (2.70%)	0 (0.00%)	
4	0 (0.00%)	1 (2.70%)	1 (6.25%)	
Treatments				
Dyslipidemia drugs	3 (7.89%)	6 (16.2%)	4 (25.0%)	0.332
Antihypertensives	10 (26.3%)	10 (27.0%)	3 (18.8%)	0.510
cDMARD	3 (7.89%)	8 (21.6%)	3 (18.8%)	0.550
bDMARD	0 (0.00%)	3 (8.11%)	3 (18.8%)	0.180
Antimalarials	29 (76.3%)	25 (67.6%)	14 (87.5%)	0.256
Tacrolimus	1 (2.63%)	0 (0.00%)	0 (0.00%)	0.179
Cyclosporine: 0	38 (100%)	37 (100%)	16 (100%)	
Azathioprine	6 (15.8%)	7 (18.9%)	4 (25.0%)	0.954
Cyclophosphamide: 0	38 (100%)	37 (100%)	16 (100%)	
AGEs				
Skin AGEs	2.46 (0.77)	2.60 (0.53)	2.74 (0.65)	0.877
Skin AGEs assessment				0.388
<1SD	5 (13.2%)	1 (2.70%)	1 (6.25%)	
1SD-Mean	5 (13.2%)	3 (8.11%)	1 (6.25%)	
Mean	2 (5.26%)	1 (2.70%)	0 (0.00%)	
Mean->1SD	13 (34.2%)	11 (29.7%)	3 (18.8%)	
>1SD	13 (34.2%)	21 (56.8%)	11 (68.8%)	
Pentosidine	1337 [1029;1740]	1363 [1146;1580]	1343 [1042;2536]	0.485
sRAGE	448 [335;671]	562 [433;852]	660 [546;977]	0.919

Supplementary Table 9: Non-significant (*p*-value >0.1) demographic and disease characteristics of systemic lupus erythematosus patients and their distribution according to CEL tertiles in the exploratory analysis. “c” indicates variables which have been categorized as previously stated in the methodology section. *Indicates values according to the blood test performed in the study. *BMI: body mass index; ANA: antinuclear antibodies; APL: antiphospholipid; APS; antiphospholipid syndrome; SADs: systemic autoimmune diseases; CH50 and C4: complement CH50 and C4; DAS28: disease activity score 28; SLEDAI: systemic lupus erythematosus disease activity index; SLE-DAS: systemic lupus erythematosus disease activity score; SDI: systemic lupus erythematosus damage index; PGA: physician global assessment; FACIT: functional assessment of chronic illness therapy; HAQ: health assessment questionnaire disability index; PtGA: patient global assessment; VAS: visual analogic scale; CVRF: cardiovascular risk factors (obesity = IMC> 30 Kg/m2, arterial hypertension, dyslipidemia, chronic renal disease or hyperuricaemia); CVE: cardiovascular events (angina, myocardial infarction, cerebrovascular accident, peripheral arterial disease, intestinal ischemia or ischemia of some other territory); CVE_SDI: cardiovascular events assessed in the SLE damage index (cerebral vascular accident, pulmonary infarction, angina or coronary bypass, myocardial infarction, venous thrombosis or infarction of the gastrointestinal tract); cDMARD; disease-modifying antirheumatic drugs; bDMARD: biological DMARD; AGEs: advanced glycation end products; SD: standard deviation; sRAGE: receptor for advanced glycation end-products.*

	OLS linear regression	Gamma GLM
(Intercept)	1.873 ***	1.891 ***
	(CI=[0.851, 2.894],p = 0.000)	(CI=[0.908, 2.874],p = 0.000)
Number of manifestations ever	0.162 *	0.161 *
	(CI=[0.022, 0.302],**p = 0.024**)	(CI=[0.018, 0.303],**p = 0.030**)
Smoker	0.940 **	0.905 *
	(CI=[0.313, 1.568],p = 0.004)	(CI=[0.145, 1.664],p = 0.022)
(Intercept)	2.847 ***	2.813 ***
	(CI=[2.517, 3.176],p = 0.000)	(CI=[2.500, 3.127],p = 0.000)
Anti-dsDNA ab titer (IU/mL)	0.015 *	0.018
	(CI=[0.001, 0.030],**p = 0.042**)	(CI=[-0.000, 0.036],p = 0.058)
Smoker	0.886 **	0.907 *
	(CI=[0.242, 1.531],p = 0.008)	(CI=[0.136, 1.678],p = 0.024)
(Intercept)	2.606 ***	2.606 ***
	(CI=[2.144, 3.069],p = 0.000)	(CI=[2.213, 2.999],p = 0.000)
Positive anti-dsDNA ever	0.619 *	0.620 *
	(CI=[0.060, 1.178],**p = 0.030**)	(CI=[0.099, 1.141],**p = 0.022**)
Smoker	0.905 **	0.906 *
	(CI=[0.271, 1.539],p = 0.006)	(CI=[0.134, 1.678],p = 0.024)
(Intercept)	2.666 ***	2.576 ***
	(CI=[2.269, 3.062],p = 0.000)	(CI=[2.189, 2.963],p = 0.000)
IL-6 (pg/mL)	0.093 *	0.122 *
	(CI=[0.016, 0.170],**p = 0.019**)	(CI=[0.023, 0.222],**p = 0.018**)
Smoker	1.084 ***	1.059 **
	(CI=[0.458, 1.711],p = 0.001)	(CI=[0.334, 1.783],p = 0.005)
(Intercept)	2.543 ***	2.523 ***
	(CI=[2.085, 3.001],p = 0.000)	(CI=[2.052, 2.995],p = 0.000)
CML (pg/mL)	0.002 ***	0.002 **
	(CI=[0.001, 0.003],**p = 0.000**)	(CI=[0.001, 0.003],**p = 0.003**)

Supplementary Table 10: Ordinary least squares linear regression and gamma generalized linear model showing associations found between CEL and systemic lupus erythematosus characteristics adjusted by their confounders (in grey). We only show the results that were statistically significant. Bold indicates those *p*-values significant (p<0.05). *** p < 0.001; ** p < 0.01; * p < 0.05. *ab: antibodies; IL-6: interleukin 6; CML: Nξ-(carboxymethyl)lysine.*

Variables	First tertile [122, 384) *N=40*	Second tertile [384, 671) *N=40*	Third tertile [671,2797] *N=39*	*p*-value
Age	48.0 (14.5)	52.8 (16.1)	51.4 (13.8)	0.515
Body mass index	25.6 (5.67)	25.0 (3.67)	25.8 (4.87)	0.401
Ethnicity				0.494
Caucasian	24 (60.0%)	30 (75.0%)	27 (69.2%)	
Latin	10 (25.0%)	7 (17.5%)	10 (25.6%)	
Other	6 (15.0%)	3 (7.50%)	2 (5.13%)	
Years of duration tertiles				0.266
[0, 5)	20 (50.0%)	12 (30.0%)	10 (25.6%)	
[5,16)	14 (35.0%)	12 (30.0%)	15 (38.5%)	
[16.45]	6 (15.0%)	16 (40.0%)	14 (35.9%)	
Smoker	9 (22.5%)	11 (27.5%)	12 (30.8%)	0.782
Classificatory Criteria and Other Clinical and Serological Data				
Constitutional symptoms	3 (7.50%)	5 (12.5%)	3 (7.69%)	0.526
Cutaneous	31 (77.5%)	32 (80.0%)	26 (66.7%)	0.383
Oral ulcers	15 (37.5%)	19 (47.5%)	16 (41.0%)	0.624
Alopecia	18 (45.0%)	22 (55.0%)	14 (35.9%)	0.358
Arthritis	28 (70.0%)	30 (75.0%)	33 (84.6%)	0.205
Serositis	5 (12.5%)	1 (2.50%)	4 (10.3%)	0.509
Renal	3 (7.50%)	1 (2.50%)	4 (10.3%)	0.866
Neurological	4 (10.0%)	4 (10.0%)	2 (5.13%)	0.666
Hematological	29 (72.5%)	27 (67.5%)	29 (74.4%)	0.583
ANA+ ever	40 (100%)	40 (100%)	39 (100%)	
Anti-dsDNA+ ever	25 (62.5%)	21 (52.5%)	29 (74.4%)	0.171
Anti-Sm+ ever	8 (20.0%)	5 (12.5%)	8 (20.5%)	0.970
Anti-Ro60+ ever	18 (45.0%)	15 (37.5%)	15 (38.5%)	0.704
Anti-Ro52+ ever	11 (27.5%)	7 (17.5%)	9 (23.1%)	0.998
Low complement	21 (52.5%)	22 (55.0%)	20 (51.3%)	0.483
Direct Coombs+	2 (9.52%)	3 (10.7%)	4 (20.0%)	0.105
Pulmonary	2 (5.00%)	2 (5.00%)	1 (2.56%)	0.342
Cardiac	0 (0.00%)	2 (5.00%)	2 (5.13%)	0.164
Raynaud	14 (35.0%)	13 (33.3%)	12 (30.8%)	0.357
APL antibodies carrier	9 (22.5%)	13 (32.5%)	7 (17.9%)	0.628
Other SADs	22 (55.0%)	24 (60.0%)	29 (74.4%)	0.840
Serological Variables				
CRP*	0.14 [0.08;0.46]	0.16 [0.10;0.30]	0.12 [0.06;0.27]	0.209
AntidsDNA>RV*	11 (27.5%)	10 (25.0%)	15 (39.5%)	0.393
CH50*	58.2 [44.0;71.3]	60.3 [54.6;71.0]	62.2 [53.5;70.4]	0.167
C3*	106 (23.0)	104 (18.9)	108 (26.0)	0.563
C4*	19.4 (9.43)	19.5 (7.32)	20.6 (8.20)	0.904
IL-6>RV*	7 (18.4%)	4 (10.0%)	3 (7.89%)	0.198
SLE Activity and Damage Indexes				
SLEDAI	4.00 [1.50;6.00]	4.00 [2.00;6.00]	4.00 [2.00;7.00]	0.255
SLE-DAS	2.53 [1.20;6.85]	3.59 [1.57;5.10]	3.50 [1.57;5.66]	0.616
SDI	0.00 [0.00;1.00]	0.00 [0.00;1.00]	0.00 [0.00;1.00]	0.421
PGA	2.00 [1.00;2.00]	2.00 [1.00;3.00]	2.00 [1.00;3.00]	0.928
Patient Reported Outcomes				
FACIT	16.0 [10.0;27.0]	19.0 [11.5;28.0]	15.0 [10.5;25.5]	0.342
cHAQ				0.105
Normal (<0.3)	23 (57.5%)	18 (45.0%)	16 (42.1%)	
Mild (<1.3)	12 (30.0%)	17 (42.5%)	21 (55.3%)	
Moderate (<1.8)	4 (10.0%)	1 (2.50%)	1 (2.63%)	
Serious	1 (2.50%)	4 (10.0%)	0 (0.00%)	
PtGA	2.50 [1.00;4.25]	2.75 [1.00;5.00]	3.00 [1.00;5.00]	0.771
Comorbidities and Cardiovascular Disease				
Hypertension	7 (17.5%)	9 (22.5%)	10 (25.6%)	0.777
Dyslipidemia	3 (7.50%)	5 (12.5%)	4 (10.3%)	0.466
Cardiovascular disease	0 (0.00%)	4 (10.0%)	1 (2.56%)	0.777

Variables	First tertile [122, 384)	Second tertile [384, 671)	Third tertile [671,2797]	*p*-value
Chronic renal disease	1 (2.50%)	1 (2.50%)	1 (2.56%)	0.782
Hyperuricemia	2 (5.00%)	0 (0.00%)	0 (0.00%)	0.247
Obesity	9 (22.5%)	6 (15.0%)	7 (17.9%)	0.352
CVRF >0	16 (40.0%)	15 (37.5%)	16 (41.0%)	0.964
CVRF				0.947
0	24 (60.0%)	25 (62.5%)	23 (59.0%)	
1	11 (27.5%)	11 (27.5%)	11 (28.2%)	
2	4 (10.0%)	2 (5.00%)	4 (10.3%)	
3	1 (2.50%)	2 (5.00%)	1 (2.56%)	
CVE				0.407
0	35 (87.5%)	35 (87.5%)	38 (97.4%)	
1	4 (10.0%)	3 (7.50%)	0 (0.00%)	
2	1 (2.50%)	1 (2.50%)	0 (0.00%)	
3	0 (0.00%)	1 (2.50%)	1 (2.56%)	
CVRF&CVE >0	17 (42.5%)	15 (37.5%)	16 (41.0%)	0.854
CVRF&CVE				0.537
0	23 (57.5%)	25 (62.5%)	23 (59.0%)	
1	12 (30.0%)	10 (25.0%)	15 (38.5%)	
2	5 (12.5%)	3 (7.50%)	0 (0.00%)	
3	0 (0.00%)	1 (2.50%)	0 (0.00%)	
4	0 (0.00%)	1 (2.50%)	1 (2.56%)	
Treatments				
Dyslipidemia drugs	5 (12.5%)	6 (15.0%)	3 (7.69%)	0.163
Antihypertensives	7 (17.5%)	11 (27.5%)	10 (25.6%)	0.834
cDMARD	6 (15.0%)	7 (17.5%)	5 (12.8%)	0.386
Tacrolimus	0 (0.00%)	1 (2.50%)	0 (0.00%)	0.630
Cyclosporine: 0	40 (100%)	40 (100%)	39 (100%)	
Cyclophosphamide: 0	40 (100%)	40 (100%)	39 (100%)	
AGEs				
Skin AGEs	2.42 (0.67)	2.57 (0.61)	2.63 (0.67)	0.867
Skin AGEs assessment				0.574
<1SD	2 (5.00%)	3 (7.50%)	1 (2.56%)	
1SD-Mean	5 (12.5%)	3 (7.50%)	5 (12.8%)	
Mean	2 (5.00%)	0 (0.00%)	2 (5.13%)	
Mean->1SD	12 (30.0%)	13 (32.5%)	9 (23.1%)	
>1SD	19 (47.5%)	21 (52.5%)	22 (56.4%)	
CML	233 [175;435]	335 [225;462]	301 [277;402]	0.859
CEL	2.57 [2.17;3.99]	3.15 [2.07;4.12]	3.25 [2.62;4.51]	0.919
Pentosidine	1452 [1029;1737]	1368 [1040;1934]	1304 [1090;1710]	0.342

Supplementary Table 11: Non-significant (*p*-value >0.1) demographic and disease characteristics of systemic lupus erythematosus patients and their distribution according to sRAGE tertiles in the exploratory analysis. “c” indicates variables which have been categorized as previously stated in the methodology section. *Indicates values according to the blood test performed in the study. *ANA: antinuclear antibodies; APL: antiphospholipid; SADs: systemic autoimmune diseases; CRP: C-reactive protein; RV: reference value; CH50, C3 and C4: Complement CH50, C3 and C4; IL-6: interleukin 6; DAS28: Disease activity score 28; SLEDAI: systemic lupus erythematosus disease activity index; SLE-DAS: systemic lupus erythematosus disease activity score; SDI: systemic lupus erythematosus damage index; PGA: physician global assessment; FACIT: functional assessment of chronic illness therapy; HAQ: health assessment questionnaire disability index; PtGA: patient global assessment; VAS: visual analogic scale; CVRF: cardiovascular risk factors (obesity = IMC> 30 Kg/m2, arterial hypertension, dyslipidemia, chronic renal disease or hyperuricaemia); CVE: cardiovascular events (angina, myocardial infarction, cerebrovascular accident, peripheral arterial disease, intestinal ischemia or ischemia of some other territory); CVE_SDI: cardiovascular events assessed in the SLE Damage Index (cerebral vascular accident, pulmonary infarction, angina or coronary bypass, myocardial infarction, venous thrombosis or infarction of the gastrointestinal tract); cDMARD; disease-modifying antirheumatic drugs; CML: Nξ-(carboxymethyl)lysine;*

CEL: Nξ-(carboxyethyl)lysine; AGEs: advanced glycation end products; SD: standard deviation; sRAGE: receptor for advanced glycation end-products.

	OLS linear regression	Gamma GLM
(Intercept)	675.659 ***	661.469 ***
	(CI=[580.002, 771.315],p = 0.000)	(CI=[575.059, 747.880],p = 0.000)
Male gender	-258.777 **	-278.148
	(CI=[-442.601, -74.952],**p = 0.007**)	(CI=[-570.268, 13.973],p = 0.062)
Glucocorticoids	-146.875	-149.658
	(CI=[-297.280, 3.529],p = 0.058)	(CI=[-318.314, 18.998],p = 0.081)
(Intercept)	549.351 ***	566.784 ***
	(CI=[420.658, 678.043],p = 0.000)	(CI=[458.581, 674.987],p = 0.000)
Photosensitivity ever	171.764 *	133.005 *
	(CI=[20.756, 322.772],p = 0.026)	(CI=[1.860, 264.150],p = 0.049)
Male gender	-194.269	-223.367 **
	(CI=[-483.549, 95.012],**p = 0.186**)	(CI=[-385.526, -61.209],**p = 0.008**)
Glucocorticoids	-188.862 *	-124.170
	(CI=[-354.068, -23.655],p = 0.025)	(CI=[-255.604, 7.263],p = 0.067)
(Intercept)	629.742 ***	624.089 ***
	(CI=[549.521, 709.964],p = 0.000)	(CI=[543.477, 704.702],p = 0.000)
bDMARD	700.624 ***	646.896 *
	(CI=[378.761, 1022.487],**p = 0.000**)	(CI=[93.627, 1200.166],**p = 0.024**)
Glucocorticoids	-239.162 **	-210.665 **
	(CI=[-401.550, -76.774],p = 0.004)	(CI=[-336.505, -84.826],p = 0.001)
(Intercept)	604.578 ***	599.723 ***
	(CI=[520.249, 688.908],p = 0.000)	(CI=[524.638, 674.807],p = 0.000)
Mycophenolic acid	361.566 ***	314.785 **
	(CI=[167.792, 555.340],**p = 0.000**)	(CI=[103.233, 526.337],**p = 0.004**)
Glucocorticoids	-241.104 **	-198.645 **
	(CI=[-408.067, -74.140],p = 0.005)	(CI=[-319.486, -77.805],p = 0.002)

Supplementary Table 12: Ordinary least squares linear regression and gamma generalized linear model showing associations found between sRAGE and systemic lupus erythematosus characteristics adjusted by their confounders (in grey). We only show the results that were statistically significant. Bold indicates those *p*-values significant (p<0.05). *** p < 0.001; ** p < 0.01; * p < 0.05. *bDMARD: biological disease-modifying antirheumatic drugs.*

Variables	First tertile [0, 1878)	Second tertile [1878, 3673)	Third tertile [3673,26506]	*p*-value
	N=39	*N=39*	*N=39*	
Years of duration	11.0 [5.00;15.0]	15.0 [3.00;21.0]	3.00 [1.00;12.0]	**0.096**
Anti-Ro52+ ever	8 (20.5%)	9 (23.1%)	10 (25.6%)	**0.062**
Pulmonary	0 (0.00%)	1 (2.56%)	4 (10.3%)	**0.048**
cTertile manifestations				**0.082**
[3, 7)	14 (35.9%)	15 (38.5%)	26 (66.7%)	
7	10 (25.6%)	9 (23.1%)	4 (10.3%)	
[8,12]	15 (38.5%)	15 (38.5%)	9 (23.1%)	
Anti-Ro52+*	6 (15.8%)	9 (23.1%)	10 (26.3%)	**0.028**
bDMARD	5 (12.8%)	1 (2.56%)	0 (0.00%)	**0.078**
AGEs	2.60 [2.15;2.90]	2.60 [2.05;3.05]	2.40 [1.95;2.60]	**0.099**
Pentosidine	1092 [833;1295]	1337 [1034;1640]	1875 [1480;2794]	**<0.001**
sRAGE	812 [660;1249]	456 [381;637]	315 [238;392]	**<0.001**

Supplementary Table 13: Variables that showed statistically significant differences (*p*-value <0.1) according to pentosidine/sRAGE tertiles in the exploratory analysis. “c” indicates variables which have been categorized as previously stated in the methodology section. *Indicates values according to the blood test performed in the study. *b-DMARD: biologic disease modifying antirheumatic-drugs; AGEs: advance glycation endo-products; sRAGE: receptor for advanced glycation end-products.*

	OLS linear regression	Gamma GLM
(Intercept)	3833.516 ***	3833.516 ***
	(CI=[3186.695, 4480.337],p = 0.000)	(CI=[3190.114, 4476.919],p = 0.000)
On bDMARD	-2561.386	-2561.386 ***
	(CI=[-5417.671, 294.899],p = 0.078)	(CI=[-3682.685, -1440.086],**p = 0.000)**
(Intercept)	3350.850 ***	3350.850 ***
	(CI=[2633.920, 4067.780],p = 0.000)	(CI=[2759.641, 3942.059],p = 0.000)
Anti-Ro52+*	1729.880 *	1729.880
	(CI=[192.236, 3267.524],**p = 0.028)**	(CI=[-70.782, 3530.542],p = 0.062)

Supplementary Table 14: Ordinary least squares linear regression and gamma generalized linear model showing associations found between pentosidine/sRAGE and systemic lupus erythematosus characteristics adjusted by their confounders (in grey). We only show the results that were statistically significant. Bold indicates those *p*-values significant (p<0.05). *** p < 0.001; ** p < 0.01; * p < 0.05. *Indicates values according to the blood test performed in the study. *bDMARD: biological disease-modifying antirheumatic drugs.*

Variables	First tertile [66.5, 432)	Second tertile [432.1, 827)	Third tertile [826.9,5194]	*p*-value
	N=39	*N=39*	*N=39*	
Ethnicity				**0.029**
Caucasian	30 (76.9%)	29 (74.4%)	20 (51.3%)	
Latin	7 (17.9%)	7 (17.9%)	13 (33.3%)	
Other	2 (5.13%)	3 (7.69%)	6 (15.4%)	
Ethnicity2				**0.010**
Caucasian	30 (76.9%)	29 (74.4%)	20 (51.3%)	
Other	9 (23.1%)	10 (25.6%)	19 (48.7%)	
Anti-Ro60+ ever	16 (41.0%)	12 (30.8%)	20 (51.3%)	**0.018**
Anti-Ro52+ ever	7 (17.9%)	9 (23.1%)	11 (28.2%)	**0.026**
Anti-Ro52+*	5 (13.2%)	9 (23.7%)	11 (28.2%)	**0.011**
Hyperuricemia	0 (0.00%)	0 (0.00%)	2 (5.13%)	**0.041**
Densitometric OP	4 (10.3%)	4 (10.3%)	10 (25.6%)	**0.095**
Dyslipidemia drugs	2 (5.13%)	3 (7.69%)	9 (23.1%)	**0.021**
GC	3 (7.69%)	8 (20.5%)	19 (48.7%)	**<0.001**
GC dosage	10.0 [8.75;11.2]	5.00 [4.38;10.0]	5.00 [2.50;8.75]	**0.091**
Anticoagulants	0 (0.00%)	0 (0.00%)	3 (7.69%)	**0.025**
CML	273 [185;295]	260 [205;345]	510 [379;736]	**<0.001**
sRAGE	778 [614;1220]	462 [358;626]	372 [265;466]	**<0.001**

Supplementary Table 15: Variables that showed statistically significant (*p*-value <0.1) differences according to CML/sRAGE tertiles in the exploratory analysis. “c” indicates variables which have been categorized as previously stated in the methodology section. *Indicates values according to the blood test performed in the study. *OP: osteoporosis; GC: glucocorticoids; CML: Nξ-(carboxymethyl)lysine; sRAGE: receptor for advanced glycation end-products.*

	OLS linear regression	Gamma GLM
(Intercept)	652.287 ***	652.287 ***
	(CI=[512.290, 792.283],p = 0.000)	(CI=[545.362, 759.212],p = 0.000)
Ethnicity: non-Caucasian	446.441 ***	446.441 **
	(CI=[201.842, 691.040],**p = 0.000**)	(CI=[167.125, 725.758],**p = 0.002**)
(Intercept): SDI (sum=0)	503.773 ***	510.213 ***
	(CI=[333.758, 673.787],p = 0.000)	(CI=[404.232, 616.195],p = 0.000)
cSDI (sum=1)	-30.764	29.278
	(CI=[-294.986, 233.458],p = 0.818)	(CI=[-163.422, 221.978],p = 0.766)
cSDI (sum>1)	358.614 *	314.662
	(CI=[35.570, 681.658],**p = 0.030**)	(CI=[-19.602, 648.926],p = 0.068)
Glucocorticoids	381.902 **	396.364 *
	(CI=[117.503, 646.301],p = 0.005)	(CI=[91.044, 701.684],p = 0.012)
Non-Caucasian ethnicities	451.710 ***	390.731 **
	(CI=[214.220, 689.201],p = 0.000)	(CI=[154.994, 626.467],p = 0.002)
(Intercept)	547.907 ***	564.080 ***
	(CI=[399.062, 696.751],p = 0.000)	(CI=[469.952, 658.209],p = 0.000)
Densitometric osteoporosis	508.853 **	456.089 **
	(CI=[198.100, 819.607],**p = 0.002**)	(CI=[117.263, 794.915],**p = 0.010**)
Non-Caucasian ethnicities	524.040 ***	492.828 ***
	(CI=[284.307, 763.772],p = 0.000)	(CI=[246.113, 739.544],p = 0.000)
(Intercept)	642.088 ***	653.954 ***
	(CI=[510.359, 773.817],p = 0.000)	(CI=[541.291, 766.617],p = 0.000)
Dyslipidemia drugs	387.215 *	307.681
	(CI=[29.425, 745.004],**p = 0.034**)	(CI=[-193.526, 808.888],p = 0.231)
Glucocorticoids	438.857 **	419.836 *
	(CI=[169.683, 708.031],p = 0.002)	(CI=[63.517, 776.155],p = 0.023)

Supplementary Table 16: Ordinary least squares linear regression and gamma generalized linear model showing associations found between CML/sRAGE and systemic lupus erythematosus characteristics adjusted by their confounders (in grey). We only show the results that were statistically significant. Bold indicates those *p*-values significant (p<0.05). *** p < 0.001; ** p < 0.01; * p < 0.05. *SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index; CML: Nξ-(carboxymethyl)lysine; sRAGE: receptor for advanced glycation end-products.*

Variables	First tertile [0.806, 3.77)	Second tertile [3.768, 7.20)	Third tertile [7.195,43.31]	*p*-value
	N=30	*N=30*	*N=30*	
Constitutional symptoms ever	1 (3.33%)	4 (13.3%)	3 (10.0%)	0.043
Anti-Ro52+ ever	5 (16.7%)	5 (16.7%)	11 (36.7%)	0.017
CRP	0.17 [0.07;0.32]	0.11 [0.07;0.21]	0.21 [0.12;0.47]	<0.001
CRP tertiles				0.015
[0.03,0.12)	11 (37.9%)	15 (50.0%)	6 (20.0%)	
[0.12,0.28)	10 (34.5%)	10 (33.3%)	10 (33.3%)	
[0.28,3.92]	8 (27.6%)	5 (16.7%)	14 (46.7%)	
ESR	10.0 [5.00;18.0]	8.00 [5.00;13.0]	13.0 [6.25;20.8]	0.045
Anti-dsDNA titers*	4.00 [1.00;11.0]	1.00 [1.00;12.8]	6.00 [2.00;34.5]	<0.001
AntiRo52+ *	4 (13.8%)	5 (17.2%)	10 (33.3%)	0.011
IL-6*	2.38 [1.75;4.06]	2.13 [1.48;2.91]	3.17 [1.90;4.43]	0.009
IL-6>RV*	1 (3.33%)	3 (10.0%)	6 (20.0%)	<0.001
Glucocorticoids	5 (16.7%)	5 (16.7%)	16 (53.3%)	0.036
Antimalarials	20 (66.7%)	21 (70.0%)	26 (86.7%)	0.036
NSAIDs	4 (13.3%)	2 (6.67%)	3 (10.0%)	0.042
CEL	2.30 [1.78;3.22]	2.90 [2.29;3.41]	4.12 [3.48;4.89]	<0.001
sRAGE	870 [614;1382]	561 [394;713]	378 [271;499]	<0.001

Supplementary Table 17: Variables that showed statistically significant (*p*-value <0.1) differences according to CEL/sRAGE tertiles in the exploratory analysis. "c" indicates variables which have been categorized as previously stated in the methodology section. *Indicates values according to the blood test performed in the study. *CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IL-6: interleukin 6; RV: reference value; NSAIDs: non-steroid anti-inflammatory drugs; CEL: Nξ-(carboxyethyl)lysine; sRAGE: receptor for advanced glycation end-products.*

	OLS linear regression	Gamma GLM
(Intercept)	5.551 ***	5.514 ***
	(CI=[4.465, 6.638],p = 0.000)	(CI=[4.513, 6.516],p = 0.000)
CRP	2.607 *	2.800
	(CI=[0.113, 5.101],**p = 0.041**)	(CI=[-0.006, 5.606],p = 0.054)
(Intercept)	4.221 ***	4.485 ***
	(CI=[3.007, 5.436],p = 0.000)	(CI=[3.337, 5.633],p = 0.000)
IL-6*	0.362 **	0.287
	(CI=[0.120, 0.604],**p = 0.004**)	(CI=[-0.040, 0.614],p = 0.089)
Glucocorticoids	3.274 ***	3.176 **
	(CI=[1.489, 5.059],p = 0.000)	(CI=[1.001, 5.351],p = 0.005)
(Intercept)	5.090 ***	4.842 ***
	(CI=[4.105, 6.075],p = 0.000)	(CI=[4.183, 5.501],p = 0.000)
IL-6>RV*	3.440 *	3.544 *
	(CI=[0.739, 6.140],**p = 0.013**)	(CI=[0.212, 6.875],**p = 0.040**)
Glucocorticoids	3.121 ***	3.336 ***
	(CI=[1.331, 4.912],p = 0.001)	(CI=[1.485, 5.187],p = 0.001)

Supplementary Table 18: Ordinary least squares linear regression and gamma generalized linear model showing associations found between CEL/sRAGE and systemic lupus erythematosus characteristics adjusted by their confounders (in grey). We only show the results that were statistically significant. Bold indicates those *p*-values significant (p<0.05). *** p < 0.001; ** p < 0.01; * p < 0.05. *Indicates values according to the blood test performed in the study. *CRP: C-reactive protein; IL-6: interleukin 6; RV: reference value; CEL: Nξ-(carboxyethyl)lysine; sRAGE: receptor for advanced glycation end-products.*

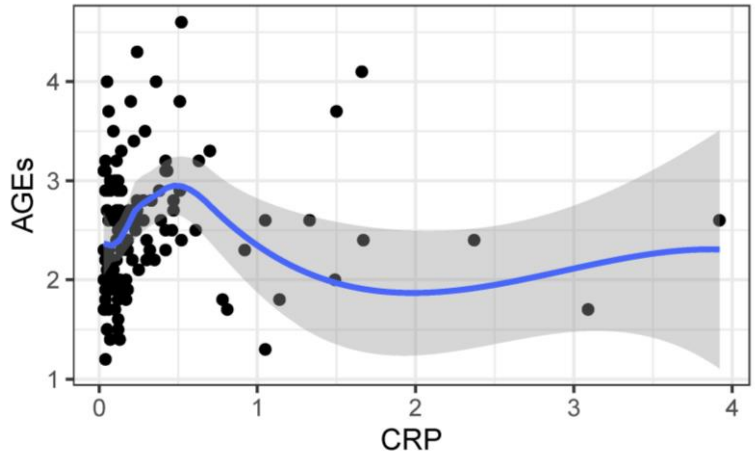
Variables	First tertile [0.858, 3.76)	Second tertile [3.761, 6.55)	Third tertile [6.550,17.74]	*p*-value
	N=40	*N=40*	*N=39*	
Gender: Female	39 (97.5%)	38 (95.0%)	34 (87.2%)	**0.027**
Age				0.114
<40	12 (30.0%)	11 (27.5%)	9 (23.1%)	
40-60	20 (50.0%)	21 (52.5%)	15 (38.5%)	
≥ 60	8 (20.0%)	8 (20.0%)	15 (38.5%)	
Years of duration	11.5 [4.00;16.0]	12.0 [4.75;20.0]	4.00 [1.00;13.5]	**0.088**
cYears duration				**0.059**
0-5	12 (30.0%)	14 (35.0%)	21 (53.8%)	
6-10	7 (17.5%)	5 (12.5%)	4 (10.3%)	
11-20	14 (35.0%)	12 (30.0%)	7 (17.9%)	
>20	7 (17.5%)	9 (22.5%)	7 (17.9%)	
Years of duration tertiles				**0.009**
[0, 5)	11 (27.5%)	10 (25.0%)	21 (53.8%)	
[5,16)	17 (42.5%)	14 (35.0%)	10 (25.6%)	
[16.45]	12 (30.0%)	16 (40.0%)	8 (20.5%)	
Photosensitivity ever	27 (67.5%)	26 (65.0%)	19 (48.7%)	**0.011**
SDI	0.00 [0.00;1.00]	0.00 [0.00;1.00]	0.00 [0.00;1.00]	**0.023**
APS	0 (0.00%)	1 (2.50%)	4 (10.3%)	**0.020**
APS or APL antibodies+	0.00 [0.00;0.00]	0.00 [0.00;1.00]	0.00 [0.00;1.00]	**0.069**
CRP	0.11 [0.07;0.26]	0.15 [0.12;0.26]	0.18 [0.08;0.44]	**0.078**
CRP tertiles				**0.086**
[0.03,0.12)	20 (51.3%)	9 (22.5%)	15 (38.5%)	
[0.12,0.28)	9 (23.1%)	21 (52.5%)	6 (15.4%)	
[0.28,3.92]	10 (25.6%)	10 (25.0%)	18 (46.2%)	
ANA+*	37 (94.9%)	37 (92.5%)	35 (89.7%)	**0.062**
IL-6>RV	2 (5.00%)	6 (15.4%)	6 (16.2%)	**0.049**
cHAQ				**0.006**
Normal (<0.3)	16 (40.0%)	19 (48.7%)	22 (56.4%)	
Mild (<1.3)	23 (57.5%)	16 (41.0%)	11 (28.2%)	
Moderate (<1.8)	1 (2.50%)	1 (2.56%)	4 (10.3%)	
Serious	0 (0.00%)	3 (7.69%)	2 (5.13%)	
CVE_SDI				**0.097**
0	40 (100%)	37 (92.5%)	33 (84.6%)	
1	0 (0.00%)	2 (5.00%)	4 (10.3%)	
2	0 (0.00%)	1 (2.50%)	2 (5.13%)	
CVE_SDI presence	0 (0.00%)	3 (7.50%)	6 (15.4%)	**0.059**
Glucocorticoids	3 (7.50%)	13 (32.5%)	14 (35.9%)	**0.033**
bDMARD	4 (10.0%)	2 (5.00%)	0 (0.00%)	**0.047**
Antimalarials	24 (60.0%)	32 (80.0%)	34 (87.2%)	**0.020**
Antiplatelet drugs	6 (15.0%)	15 (37.5%)	12 (30.8%)	**0.061**
Azathioprine	7 (17.5%)	9 (22.5%)	2 (5.13%)	**0.060**
Skin AGEs	2.28 (0.50)	2.53 (0.68)	2.82 (0.66)	**<0.001**
Skin AGEs assessment				**0.045**
<1SD	3 (7.50%)	2 (5.00%)	1 (2.56%)	
1SD-Mean	7 (17.5%)	5 (12.5%)	1 (2.56%)	
Mean	2 (5.00%)	1 (2.50%)	1 (2.56%)	
Mean->1SD	12 (30.0%)	13 (32.5%)	9 (23.1%)	
>1SD	16 (40.0%)	19 (47.5%)	27 (69.2%)	
sRAGE	807 [701;1210]	521 [383;625]	301 [238;372]	**<0.001**

Supplementary Table 19: Variables that showed statistically significant (*p*-value <0.1) differences according to skin AGEs/sRAGE tertiles in the exploratory analysis. “c” indicates variables which have been categorized as

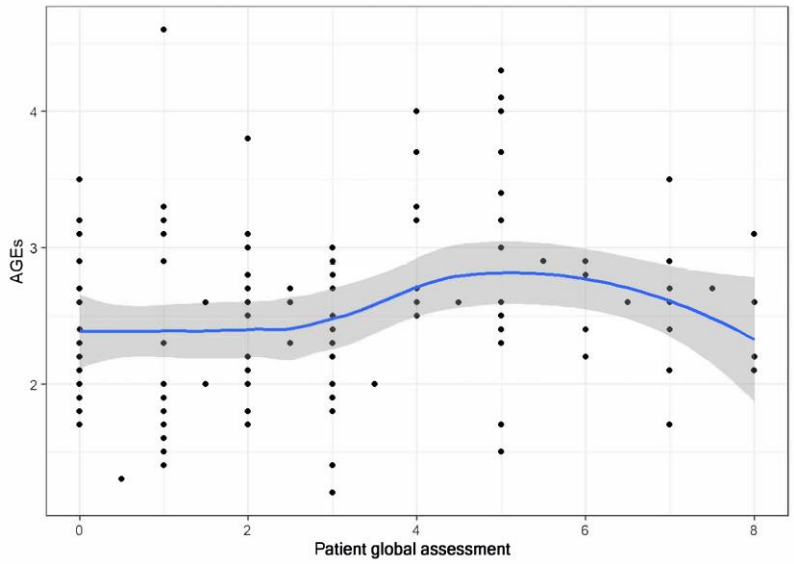
previously stated in the methodology section. *Indicates values according to the blood test performed in the study. *SDI: systemic lupus erythematosus damage index; APS: antiphospholipid syndrome; APL: antiphospholipid antibodies; CRP: C-reactive protein; ANA: antinuclear antibodies; RV: reference value; HAQ: health assessment questionnaire; CVE_SDI: cardiovascular events assessed in the SLE damage index (cerebral vascular accident, pulmonary infarction, angina or coronary bypass, myocardial infarction, venous thrombosis or infarction of the gastrointestinal tract); bDMARD: biologic disease-modifying antirheumatic drugs; AGEs: advanced glycation end-products; SD: standard deviation; sRAGE: receptor for advanced glycation end-products.*

	OLS linear regression	**Gamma GLM**
(Intercept)	5.286 ***	5.247 ***
	(CI=[4.498, 6.074],p = 0.000)	(CI=[4.527, 5.968],p = 0.000)
Gender: Male	2.854 *	3.069
	(CI=[0.176, 5.532],**p = 0.037**)	(CI=[-0.943, 7.082],p = 0.136)
Glucocorticoids	1.574 *	1.690
	(CI=[0.029, 3.118],p = 0.046)	(CI=[-0.105, 3.484],p = 0.068)
(Intercept)	6.6433 ***	6.8620 ***
	(CI=[5.621, 7.666],p = 0.0000)	(CI=[5.633, 8.0910],p = 0.0000)
Years of duration 2nd tertile [5,16)	-2.652 ***	-2.6390 ***
	(CI=[-4.075, -1.229],**p = 0.0003**)	(CI=[-4.099, -1.178],**p = 0.0006**)
Years of duration 3rd tertile [16,45]	-2.0700 **	-2.3861 **
	(CI=[-3.549, -0.591],**p = 0.0065**)	(CI=[-3.918, -0.854],**p = 0.0028)**
Glucocorticoids	2.0268 **	2.1690 **
	(CI=[0.647, 3.406],p = 0.0043)	(CI=[0.647, 3.691],p = 0.0061)

Supplementary Table 20: Ordinary least squares linear regression and gamma generalized linear model showing associations found between skin AGEs/sRAGE and systemic lupus erythematosus characteristics adjusted by their confounders (in grey). We only show the results that were statistically significant. Bold indicates those *p*-values significant (p<0.05). *** p < 0.001; ** p < 0.01; * p < 0.05. *RAGE: receptor for advanced glycation end-products.*



Supplementary Figure 4: C-reactive protein (CRP) scatter plot. *AGES: advanced-glycation end-products*.



Supplementary Figure 5: Scatter plot to evaluate the nonlinear association of the patient global assessment and advanced glycation end-products (AGEs).

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