



HAL
open science

Are lupus patients characterized by a phenomenon of accelerated aging? Analysis of literature and pilot study on epigenetic biomarkers of age.

Noémie Gensous

► To cite this version:

Noémie Gensous. Are lupus patients characterized by a phenomenon of accelerated aging? Analysis of literature and pilot study on epigenetic biomarkers of age.. Life Sciences [q-bio]. 2021. dumas-03351583

HAL Id: dumas-03351583

<https://dumas.ccsd.cnrs.fr/dumas-03351583v1>

Submitted on 22 Sep 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

U.F.R. DES SCIENCES MEDICALES

Année 2021

Thèse n° 3090

THESE POUR L'OBTENTION DU
DIPLOME D'ETAT de DOCTEUR EN MEDECINE

Présentée et soutenue publiquement

Par GENSOUS Noémie

Née le 07/10/1987 à Pau (64)

Le 14 septembre 2021

**Are lupus patients characterized by a phenomenon of
accelerated aging?**

Analysis of literature and pilot study on epigenetic biomarkers of age.

Sous la direction de Monsieur le Pr. Pierre DUFFAU

Rapporteur : Madame le Pr. Estibaliz LAZARO

Jury :

Monsieur le Pr. Patrick MERCIE
Monsieur le Pr. Patrick BLANCO
Monsieur le Pr. Pierre DUFFAU
Madame le Pr. Estibaliz LAZARO
Madame le Dr. Isabelle PELLEGRIN
Monsieur le Pr. Christophe RICHEZ

Président
Juge
Juge
Juge
Juge
Juge

REMERCIEMENTS

Au président du jury, Monsieur le Pr Patrick Mercié

Vous me faites l'honneur d'avoir accepté de présider mon jury de thèse. Merci pour votre pédagogie, votre bienveillance et pour la confiance que vous me portez en m'accueillant dans votre service.

Veillez recevoir ici l'expression de ma sincère reconnaissance et de tout mon respect.

Au rapporteur de thèse, Madame le Pr Estibaliz Lazaro

Merci d'avoir accepté de rapporter ce travail et de faire partie du jury. Ton sens clinique, ta bonne humeur, ta disponibilité et ton enthousiasme sont un modèle pour beaucoup. Je te remercie pour tout ce que tu m'as apporté au cours de mon semestre au G2 et par la suite.

Aux membres du jury

A Monsieur le Pr Patrick Blanco

Merci d'avoir accepté avec enthousiasme de faire partie de ce jury de thèse. Merci pour la confiance que tu me portes, pour l'année de Master 2, pour la qualité de ton encadrement et ton enseignement. Merci de m'avoir donné le goût de la recherche. Merci pour ta bonne humeur. J'espère pouvoir continuer longtemps à travailler avec toi.

A Madame le Dr Isabelle Pellegrin

Je te remercie d'avoir accepté de faire partie de mon jury de thèse. Ta volonté et ton enthousiasme, notamment vis-à-vis de la recherche sur le vieillissement sont précieux. Sois assurée de ma plus grande estime.

A Monsieur le Pr Christophe Richez

Merci d'avoir accepté de faire partie de ce jury. Merci pour ton encadrement et ta disponibilité au cours de l'année de Master 2 et lors du projet MARS. Merci pour tous tes précieux conseils, qu'ils soient professionnels ou personnels.

A mon directeur de thèse Monsieur le Pr Pierre DUFFAU

Merci d'avoir accepté de diriger ce travail. Merci pour tous tes précieux conseils, dans tous les domaines et ce depuis le début, et toujours dans la bonne humeur. Et enfin un immense merci pour la confiance que tu me portes. J'espère m'en montrer digne.

Aux Pr Jean-Luc Pellegrin et Pr Jean-François Viillard

Merci pour la qualité de vos enseignements, de l'externat à l'internat, à l'université ou au lit du malade. Le semestre passé dans votre service fut mémorable. Merci pour votre confiance et pour votre soutien au cours de ce long internat.

Au Pr Maïté Longy-Boursier

Merci de m'avoir donné le goût de la médecine interne pendant mon externat et de m'avoir accompagnée pendant mon premier semestre.

Aux Pr Marie-Sylvie Doutré, Pr Hilbert, Pr Neau, Pr Le Masson, Pr Salles

Aux PH et MCU-PH avec qui j'ai eu la chance de travailler

Dr Carine Greib, Dr Nam Bui, Dr Benjamin Clouzeau, Dr Hervé Dutronc, Dr Pierre Germain, Dr Maud Haaser, Dr Guilhem Solé, Dr Xavier Ferrer, Dr Geneviève Pinganaud, Dr Cécile Bordes, Dr Mamy Ralazamahaleo, Dr Julien Goret, Dr Anne Sarrat et Dr Emmanuel Ribeiro. Mention spéciale au plus béarnais d'entre tous, Charles Cazanave.

A mes chefs de clinique auprès desquels j'ai tant appris

Loïc, Émilie, Emmanuel, Marion, Caroline, Marc-Olivier, Fanny, Claire, Guillaume et pour finir en beauté Guillaume et Vivien (Guiiii).

A mes co-internes tout au long de l'internat

La fantastique team du G2, Hélène, Benjamin, Albane, Pauline, Jérémy, Pierre, Pascal, les biologistes, ... Et à l'équipe de l'unité 13, vous serez (enfin !) mes derniers co-internes !

Aux internes de médecine interne bordelais, à ma promo initiale, aux précédentes et aux suivantes.

Alexandre, Didier, Mathilde, Caroline, Nahéma, Irène, Etienne, Aurélie. A Gildas et Joëlle pour ces journées d'immunologie à St Louis, ces bo-buns au P'tit Cambodge et ces bières à Belleville.

Aux équipes infirmières et aides-soignantes avec qui j'ai eu le plaisir de travailler.

A la réanimation médicale. Merci pour ces 6 mois intenses et pour ces 3 ans de gardes qui ont suivi !

A ImmunoConCepT et tous ses membres. Merci pour cette année de Master 2 particulièrement enrichissante. A Isabelle Douchet, merci pour tout ! A Pao #contretouteattente.

A Monsieur le Professeur Jean-François Moreau

Merci pour votre transmission de l'amour de l'immunologie. Merci pour l'opportunité italienne.

A Bologna la rossa, la dotta, la grassa

Grazie per questi tre anni indimenticabili. A Maria Giulia, Paolo, Claudio, Stefano, Francesco, Elena, Chiara, Maddi, Cristina, Federica, Miriam, Maria, Gianluca, Sabrina ... Mi mancate tanto!

A Iva, Anna. Miss you girls! A mes co-thésards du projet européen PANINI.

A mes amis, palois, bordelais ou d'ailleurs

A Anne, des débuts en dermatologie en 2^{ème} semestre à aujourd'hui. Que de chemin parcouru (et de kilomètres de semi-marathons) ! Merci pour tout. Je n'oublie pas Quentin, Margot et Inès bien sûr.

A Pauline. Merci pour cette année de Master 2 pleine de rebondissements passée à tes côtés et pour ton amitié depuis. A Gaultier, pour son amour du labo, des dépôts glomérulaires et de l'orage cytokinique. A Pia et Anouk.

A Julien et Aurélie. Merci pour tout.

A Mathilde.

Aux basques repartis au pays : Tim, Marie, Jérémy, Océane, ...

A ma famille

A la famille Bise Danielle, Amandine, Pierre et Jeanne, Alain.

Merci pour votre soutien tout au long de ces années ! J'espère que les années à venir seront tout aussi riches que les précédentes.

A ma grand-mère pour son amour et son soutien. Merci pour les souvenirs d'enfance et merci pour ton accueil réconfortant tous les week-ends de 1^{ère} année.

A mes frères

Merci pour toutes ces années passées à vos côtés. Merci d'avoir toujours été de super grands frères.

A mes belles sœurs, mes neveux et nièce.

A mon père qui me manque terriblement.

A ma mère

Merci de m'avoir toujours aimée et soutenue, malgré toutes les épreuves et les doutes possibles. Merci d'être une super grand-mère également. Merci pour tout du fond du cœur.

A Sylvain

Merci pour ton amour et ton soutien sans faille. Love

A Augustin et Andrea

Vous êtes l'heureuse raison du décalage de la date de cette thèse. Vous voir grandir me remplit de joie. Je suis fière d'être votre maman, je vous aime.

Index

1. Introduction	7
2. SLE and the pillars of aging: analysis of literature	10
2.1 Aging and its pillars	10
2.2 SLE and the pillars of aging	11
2.2.1 Macromolecular damage.....	11
2.2.2 Metabolism	15
2.2.3 Stem cells	18
2.2.4 Proteostasis	19
2.2.5 Adaptation to stress.....	20
2.2.6 Inflammation and immunosenescence	21
2.2.7 Epigenetics	23
2.2.8 Impact of therapeutics	25
2.2.9 Conclusion	27
3. SLE and epigenetic biomarkers of age: a pilot study	28
3.1 Epigenetic biomarkers of aging: definition and concept	28
3.2 Methods	30
3.3 Results	32
3.3.1 Datasets	32
3.3.2 Lupus patients and controls.....	34
3.3.3 Epigenetic age.....	38
3.3.4 Epigenetic age acceleration	39
3.3.5 Influence of sex and lupus nephritis on the measure of epigenetic age acceleration	42
4. Discussion	44
5. References	49

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease characterized by a breakdown of tolerance against nuclear antigens, leading to autoantibody production, immune complex formation and organ damage (Tsokos 2011). Thanks to improvements during the last decades in diagnosis, therapeutics (immunosuppressive agents, biotherapies) and medical care (improved management of end stage renal and cardiovascular diseases especially), the lifespan of SLE patients has remarkably increased. While the five-year survival rates were about only 50% in the 1950's, they nowadays exceed 90% in SLE patients (95% from 2008 to 2016 in adults from high-income countries) (Tektonidou et al. 2017). However, despite the important increase in lifespan, standardized mortality ratio (SMR) (ratio of the number of deaths observed in a given cohort divided by the deaths reported in the general population for the same time period) are still high (Tselios et al. 2019; Lee et al. 2016; Yurkovich et al. 2014; Bernatsky et al. 2006; Scherlinger et al. 2020; Barber et al. 2021). Some clinical manifestations more commonly found during old age, such as cardiovascular (CV) events, renal disease, infections or cancers, appear frequently at a younger age in SLE patients, considered either as sequelae of the disease course or consequences of medications, and are responsible for increased mortality and morbidity in this population (Nossent et al. 2007; Thomas et al. 2014).

Thus, it is well established that CV events occur more frequently in patients with chronic inflammatory conditions, including SLE, as compared to the general population (Skaggs et al. 2012). The increased risk of CV diseases (CVD) in SLE patients is known since the 1970's, particularly with the initial publication of Urowitz *et al.* which described a bimodal mortality pattern in SLE, with important incidence of deaths associated with myocardial infarction in patients who die late in the course of their disease (Urowitz et al. 1976). Since this date, many studies have evaluated the incidence and epidemiology of CVD in patients with SLE, and have

confirmed that subclinical atherosclerosis and CVD occur more frequently and with an earlier onset in this population as compared to healthy individuals (Reviewed in (Skaggs et al. 2012; Schoenfeld et al. 2013; Liu & Kaplan 2018; Giannelou & Mavragani 2017; Lu et al. 2021)). The observed increased risk of CVD in SLE is the result of the combination of traditional CV risk factors and SLE-specific ones. Indeed, traditional CV risk factors (such as hypertension, smoking, diabetes or hypercholesterolemia) do not fully explain the high rates of CVD reported so far in SLE (Roman et al. 2003; Esdaile et al. 2001). Other several factors that are inherent to SLE itself have been described as contributors to the pathogenesis of premature and accelerated atherosclerosis in this disease. They include, amongst others, older age at diagnosis, longer disease duration of SLE, activity and chronicity, higher levels of SLE-associated damage, high cumulative glucocorticoid dosage, increased thrombosis in the setting of antiphospholipid antibodies. From a cellular and molecular point of view, they rely on specific inflammatory mediators and immune dysregulation, leading to chronic inflammation (Skaggs et al. 2012; Liu & Kaplan 2018; Giannelou & Mavragani 2017; Manzi et al. 1997; Roman et al. 2007; Roman et al. 2003; Doria et al. 2003).

Infections are also a leading cause of morbidity and mortality in the SLE population (Bernatsky et al. 2006; Yurkovich et al. 2014; Lee et al. 2016). Infections rates (especially regarding infections of the respiratory tract) are found at increased frequencies and with higher severity in the population of SLE patients (Danza & Ruiz-Irastorza 2013; Caza et al. 2014). Multiple factors contribute to the vulnerability to infections of this population, both related to the autoimmune disease itself and its imbalanced immune responses, but also regarding the use of certain treatments (especially high doses of glucocorticoids and immunosuppressive drugs). In addition to the increased susceptibility to infectious diseases, SLE population is also characterized by poorer vaccinations responses. Thus, according to recent systematic literature reviews and meta-analyses, while the safety profile of vaccines appeared to be good in SLE

patients, immunogenicity of some vaccines (against particular viral influenza strains for example) seems to be reduced in SLE patients as compared to the general population (Pugès et al. 2016; Rondaan et al. 2019; Huang et al. 2016; Izmirly et al. 2021).

Finally, different studies have indicated an increased susceptibility to certain types of cancer in individuals with SLE, especially for hematologic malignancies (predominantly non-Hodgkin's lymphoma), lung or cervical cancers (Reviewed in (Gayed et al. 2009; Goobie et al. 2015)). Several hypotheses have been suggested to explain the mechanisms responsible for the association between cancer and SLE, including genetic predispositions common to autoimmunity and hematologic malignancies, defects in the humoral and cellular immune systems responsible for tumor immunosurveillance, and increased influence of conventional cancer risk factors such as smoking. Except for cervical cancers, the impact of immunosuppressive therapies on the occurrence of malignancies appears controversial (Gayed et al. 2009; Bernatsky et al. 2005). Despite the increased incidence of cancer in the SLE population, it is to be noted that regarding cancer-related mortality, two recent meta-analyses reported that the risk of death related to malignancies was not significantly increased in SLE patients (Lee et al. 2016; Yurkovich et al. 2014).

As a consequence of the increased lifespan in SLE, more and more lupus patients are reaching an advanced age with longer disease duration. A large proportion of this population is getting to face early in life important co-morbidities, which are, for certain of them, more frequently found in older adults (*i.e.* age-related diseases), raising the question of the presence of an acceleration of the aging process in SLE patients.

2. SLE and the pillars of aging: analysis of literature

2.1 Aging and its pillars

Aging can be defined as the normal, time-dependent and progressive decline of function, occurring at the cellular, tissue and organismal levels over the lifespan, ultimately resulting in death. Aging is a complex and multifaceted phenomenon, resulting of a complex interplay between genetic, environmental, and stochastic variables. It is considered as the result of the progressive accumulation of damage coupled with a decline in maintenance (Goldsmith 2012; Blagosklonny 2013; Kowald & Kirkwood 2016). Aging is recognized as a major risk factor for a wide range of non-communicable and chronic diseases, including cancers, cardiovascular and neurodegenerative diseases or metabolic disorders. Scientific community agrees that aging is characterized by specific hallmarks, which rely on well-defined biological pathways. According to recent conceptualizations, an attempt has been made to clearly define a small set of these basic molecular and cellular mechanisms, highly interconnected, on which aging relies on (Kennedy et al. 2014; López-Otín et al. 2013) (**Figure 1**). It is considered that age-related diseases rely on the same mechanisms and are deviations from the normal trajectory of aging (Kennedy et al. 2014; Franceschi et al. 2018).

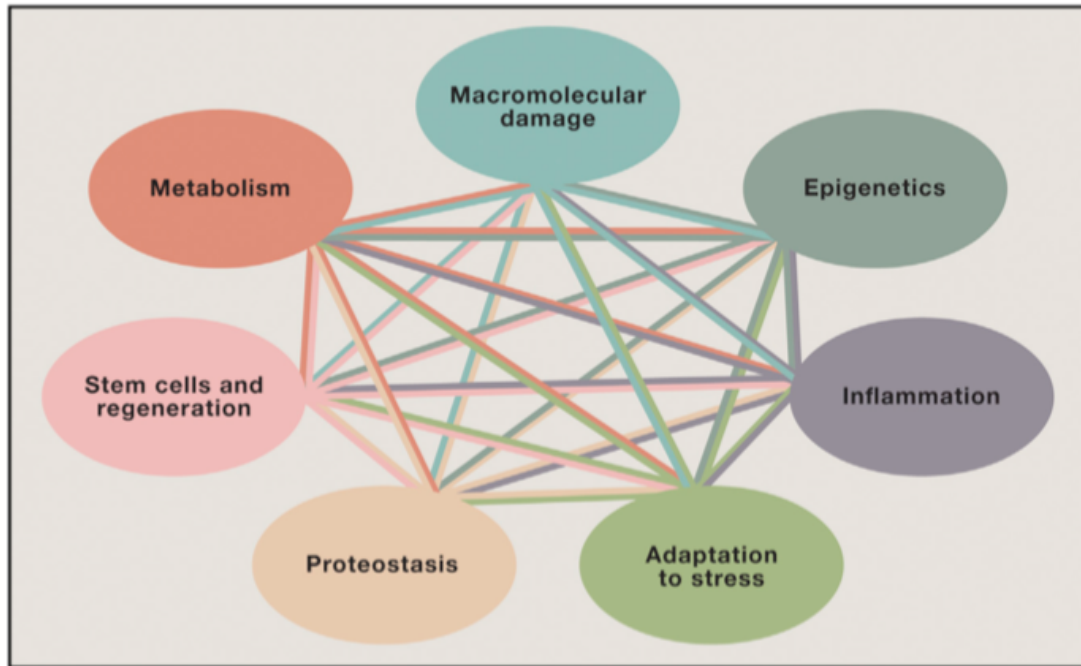


Figure 1: The seven pillars of aging, according to Kennedy *et al.* (2014).

2.2 SLE and the pillars of aging

Given the increased prevalence of some age-related diseases observed in SLE patients, we wanted to evaluate if lupus was associated with features of acceleration of the mechanisms which underly aging. To this aim, in this section, we reviewed and compared literature evidence on the pillars of aging evaluated in SLE patients and explored possible interconnections between these two frameworks.

2.2.1 Macromolecular damage

Accumulation of macromolecular damage throughout life, and especially DNA damage, is considered as a major contributor to aging (Moskalev *et al.* 2013; Soares *et al.* 2014). DNA is constantly exposed to endogenous and exogenous (physical, chemical and biological) agents that can stimulate a large variety of DNA lesions (point mutations, translocations, gene

disruption, double-stranded breaks, ...), both in nuclear and mitochondrial DNA. Different mechanisms are involved in the repair of these lesions (homologous recombination, nonhomologous end-joining, mismatch repairs, ...) and are collectively responsible for ensuring the integrity of DNA. However, while we age, both excessive macromolecular damage and declining efficiency of DNA repair systems favor the aging process. Notably, DNA damage accumulation can result in genomic instability and can promote malignant transformation, cellular senescence or cell death by apoptosis.

In SLE, multiple studies have demonstrated the presence of increased genomic DNA damage in patients as compared to healthy controls. Increased levels of damaged DNA have been observed in neutrophils (McConnell et al. 2002), low density granulocytes (Singh et al. 2014), cell samples from buccal mucosa (Al-Rawi et al. 2014) or circulating mononuclear cells (Souliotis & Sfikakis 2015) of SLE patients. Likewise, damage to mitochondrial DNA is more common in peripheral blood mononuclear cells (PBMC) of SLE patients as compared to healthy individuals, with a positive correlation between mitochondrial DNA lesions and disease duration (López-López et al. 2014). Repair mechanisms of DNA damage appear to be also impaired and less efficient with delayed kinetics in SLE patients, both in pediatric and adult subpopulations (Davies et al. 2012; McCurdy et al. 1997; Souliotis et al. 2016). In PBMC from adults SLE patients, DNA repair deficiencies were observed regardless of disease activity level and were found correlated with apoptosis rates (Souliotis et al. 2016).

Several polymorphisms in genes coding for mediators of crucial pathways for DNA repair have been associated with SLE (Lin et al. 2009; Jahantigh et al. 2015; Lin et al. 2010; Bassi et al. 2008; De Azevêdo Silva et al. 2014). Moreover, it has been demonstrated that accumulation of damaged DNA is also related to impaired function of some critical enzymes involved in DNA degradation, such as three prime repair exonuclease 1 (TREX1) (Yan 2017), RNaseH2 (Günther et al. 2015) or Dnase1 (Napirei et al. 2000).

Thereby, there is extensive evidence that increased DNA damage formation, defective repair and defective DNA degradation are important factors implicated in the pathogenesis of SLE. They play a crucial role in the early initiation and the progression of the disease, by the induction of increased rates of cell death, accumulation of cytosolic DNA and generation of neoantigens, that could trigger innate immune system activation and auto-antibodies production (Souliotis et al. 2019). Also, as it has been observed, for example, that damage of mitochondrial DNA correlate with disease duration (López-López et al. 2014), indicating that damage accumulate over the years, it could be hypothesized that these phenomena also participate to an acceleration of the aging process in SLE patients.

Some critical chromosomal regions are particularly susceptible to age-related accumulation of DNA damage: telomeres. Telomeres are repetitive DNA sequences, consisting of multiple TTAGGG repeats, located at the ends of the chromosomes, responsible for their stability and protection (Sfeir 2012). At each cell division, a certain number of nucleotides at these telomeric ends is lost, and telomers become slightly shorter at every DNA replication, ultimately leading to cell senescence. To compensate telomeric loss, some cells express the enzyme telomerase which is able to elongate telomeres by adding telomere repeats. However, telomerase activity declines with age, and telomere shortening still occurs progressively. Telomeric length is widely recognized as a powerful index of cellular aging and is probably one of the most studied biomarker of age (Jylhävä et al. 2017). Correlations between short telomeres and mortality risk, cancer, CVD risk or cognitive status, particularly at younger ages, have been well demonstrated (Jylhävä et al. 2017; Rode et al. 2015).

Several researchers have investigated telomere shortening in SLE. Collectively, these studies show that granulocytes, mononuclear leucocytes or lymphocytes of SLE patients have shorter telomeres when compared to age-matched healthy subjects, indicating a clear pattern of

accelerated senescence (Honda et al. 2001; Kurosaka et al. 2003; Kurosaka et al. 2006; Fasching et al. 2017; Haque et al. 2013; Hoffecker et al. 2013; Wu et al. 2007; Skamra et al. 2013). Interestingly, this difference is even more pronounced in younger age groups (Honda et al. 2001; Kurosaka et al. 2003; Fasching et al. 2017; Wu et al. 2007). No association between telomere length and disease activity or disease duration was identified (Haque et al. 2013). Wu *et al.* quantified the telomere shortening observed in SLE patients and tried to convert it in terms of chronological time. According to their study, the average reduction in telomere length in SLE patients was equivalent to a premature senescence of 16.5 years in mononuclear cells and of 13.4 years in polymorphonuclear neutrophils (Wu et al. 2007). Different attempts to use telomere length as a marker of CV risk in SLE patients were performed. Shorter telomere lengths were observed in young SLE women with carotid plaque (Skamra et al. 2013). On the contrary and counterintuitively, Haque *et al.* observed that carotid plaque was particularly prevalent in patients with the higher tertile of telomere length (Haque et al. 2013). The explanation for this observation is unclear. The cross-sectional design of the study could have led to a censorship of data, as the authors discuss in the publication. Patients with atherosclerotic disease and shortest telomeres may have been too ill to participate, and this could have led to a specific survival effect in the studied population. Moreover, it is possible that more of these patients are receiving CVD-protective drugs (such as statins or aspirin) which could have an indirect impact on telomere length.

Regarding telomerase activity, it generally seems increased in SLE patients as compared to controls (Kurosaka et al. 2003; Kurosaka et al. 2006; Klapper et al. 2004; Kurosaka et al. 2001; Katayama & Kohriyama 2001). Unfortunately, this up-regulated telomerase activity is not capable of counteracting accelerated telomere attrition, resulting in shorter telomeres in SLE patients, thus possibly contributing to an accelerated senescence phenomenon.

2.2.2 Metabolism

Aging is characterized by a progressive deterioration in the maintenance of metabolic homeostatic processes (Barzilai et al. 2012). Particularly, a decline in the nutrient-sensing pathways is a key feature of aging and is the focus of intense investigation. The insulin-like growth factor 1 (IGF-1) and insulin signaling, and the mechanistic target of rapamycin (mTOR) pathways are major contributors to the aging process, highly conserved through evolution. mTOR is a serine/threonine kinase belonging to the highly conserved phosphoinositide 3-kinase (PI3K)-related kinase family, composed of two complexes, mTOR complex 1 (mTORC1) and mTORC2. The mTOR signaling pathway senses and integrates a variety of environmental cues in order to regulate the growth and the metabolism of cells. This pathway has been implicated in an increasing number of age-related pathological conditions, including cancer, obesity, type 2 diabetes or neurodegeneration (Laplante & Sabatini 2012). It is, as well, a critical mediator of mammalian longevity, as intense trophic and anabolic activities are important accelerators of aging whereas many of the mutations that extend lifespan decrease the activity of nutrient-signaling pathways (Fontana et al. 2010).

Human aging is also associated with a progressive mitochondrial dysfunction, which plays a complex role in regulating longevity (Wang & Hekimi 2015). Defective mitochondrial function is associated with defects in mitochondrial integrity, reduced mitochondrial biogenesis, and with a decrease in the efficacy of the respiratory chain and an increased reactive oxygen species (ROS) production. Imbalance between ROS production and antioxidant defenses can participate to increased oxidative stress, highly detrimental (Liguori et al. 2018).

In SLE, a lot of research has been carried out on the metabolic determinants of the disease pathogenesis. Studies in murine models as well as in lupus patients have clearly established that immune cells and especially T lymphocytes have profound rearrangements of

several metabolic pathways. They have an hypermetabolic state characterized by high glucose flux, oxidation, mitochondrial abnormalities and increase in mTOR kinase activities (Reviewed in (Morel 2017; Teng et al. 2020)). mTOR pathway participates in the proliferation of immune cells and has a fundamental role in T cell lineage commitment. Thus, mTOR signaling pathway promotes the differentiation of the pro-inflammatory Th1 and Th17 cells subsets and is central in the differentiation of T follicular helper (Tfh) cells, which are all critical to the pathogenesis of the disease (Zeng et al. 2016; Delgoffe et al. 2009). mTOR signaling pathway also affects the secretion of inflammatory cytokines (Lai et al. 2013) and the increased mTORC1 activity observed in SLE patients has been associated with a contraction of the T regulatory (Treg) cells compartment (Kato & Perl 2014; Kato & Perl 2018).

SLE T cells also depict mitochondrial dysfunction and increased oxidative stress. T cells mitochondria are characterized by a large size, membrane hyperpolarization, enhanced activity with increased production of ROS and ATP depletion (Doherty et al. 2014; Yin et al. 2015; Gergely et al. 2002). In one study, increased oxidative stress appeared to be independent of disease activity levels (Avalos et al. 2007).

Targeting metabolic pathways with specific interventions have shown promising effects in slowing down the aging process and is an area of intense research (Longo et al. 2015). One intervention that has proven to be by far the most effective in delaying or preventing an important range of age-related processes and extending the maximum lifespan in a wide range of organisms is caloric restriction (CR), *i.e.* the reduction of caloric intake (by 10% to 40%) without causing malnutrition (Liang et al. 2018). CR is an intervention likely to be difficult to implement in the long run in humans and pharmacological interventions have been proposed. Thus, inhibition of the mTOR signaling pathway with rapamycin significantly extends lifespan in model organisms (such as invertebrates and mice) and confers protection against several age-

related diseases (Kapahi et al. 2010; Harrison et al. 2009; Johnson et al. 2013). Additionally, metformin has emerged as a powerful antiaging drug (Barzilai et al. 2016): it increases lifespan of nematodes and mice (Anisimov et al. 2011; Cabreiro et al. 2013; Onken & Driscoll 2010) and is associated with reduction of age-associated pathologies such as dementia (Campbell et al. 2018).

Targeting these pathways have also shown promising effects in SLE. CR appeared to be protective in murine models of SLE. It decreases serum levels of anti-dsDNA antibodies, attenuates lupus nephritis, decreases pro-inflammatory cytokines expression and increases mice survival (Sun et al. 2004; Troyer et al. 1997; Muthukumar et al. 2000; Fernandes et al. 1976; Fernandes et al. 1978). In mouse models of the disease, antioxidants and rapamycin treatment seem to ameliorate lupus nephritis and to markedly prolong the animals survival (Warner et al. 1994; Gu et al. 2016; Suwannaroj et al. 2001). In humans, reducing mTOR activation with rapamycin or treatments with antioxidant effects (such as N-acetylcysteine) appeared to reduce disease severity in small groups of selected patients (Lai et al. 2012; Lai et al. 2018; Fernandez et al. 2006). Finally, oral administration of metformin in sanroque mice (Roquin^{san/san}) normalizes T cell responses and suppresses the development of autoantibody-producing plasma cells (Lee et al. 2017). Metformin, delivered as an add-on treatment to standard of care, appears to improve disease parameters and reduce disease flares in patients with SLE (Wang et al. 2015; Sun et al. 2020).

Activation of mTOR pathway and oxidative stress appear to be substantial contributors to disease pathogenesis and to organ damage in SLE patients (Perl 2013). Targeting specifically these pathways seems to have a therapeutic benefit in murine models and in lupus patients. To our knowledge, the impact of these treatments was mainly focused on disease activity and

features of autoimmunity. No data seems available regarding the eventual impact of these interventions on co-morbidities and age-related diseases in this specific population.

2.2.3 Stem cells

Aging is associated with a decline in stem cell function leading to a loss of regenerative potential of tissues (Schultz & Sinclair 2016; Ahmed et al. 2017). This declining function affects all adult stem cell compartments: neural (NSCs), mesenchymal, adult skeletal muscle (also known as satellite cells) and hematopoietic stem cells (HSCs). Stem cell exhaustion is believed to be the result of the combination of surrounding environmental changes, and other hallmarks of aging described in the other sections, including increase in DNA damage, telomere shortening, mitochondrial dysfunction, metabolic changes and epigenetic patterns modifications.

In SLE, research has been mainly focused on bone marrow mesenchymal stem cells (BM-MSCs). These cells are bone-marrow-derived non-hematopoietic cells of mesodermal origin, characterized by self-renewal and pluripotent differentiation capability. They provide a supportive microenvironment for the maintenance of HSCs and for hematopoiesis. They also possess the ability to differentiate into various cell types, such as bone, fat or cartilage, under appropriate environmental conditions. Multiple papers have demonstrated that BM-MSCs from SLE patients are clearly defective. As compared to matched controls, lupus BM-MSCs exhibit an accelerated senescent phenotype, with lower proliferation capacity, flattened morphology and cytoskeleton abnormalities (Sun et al. 2007; Gu et al. 2014; Gao et al. 2017; Li et al. 2012; Nie et al. 2010; Gu et al. 2012; Gu et al. 2014). This senescent phenotype was observed in both untreated and treated SLE patients (Gu et al. 2012). BM-MSCs are more prone to undergo apoptosis, they exhibit increased DNA damage, overactivation of the mTOR pathway, increased intracellular ROS levels and increased production of proinflammatory cytokines,

similar to a senescent-associated secreted phenotype (Gao et al. 2017; Li et al. 2012; Gu et al. 2013; Gu et al. 2016).

To our knowledge, very limited data is available on the other stem cell compartments in SLE. Regarding HSCs, their function was investigated in one study on a lupus mouse model (Niu et al. 2013). Animals with active disease have an expanded HSCs population in the bone marrow and are characterized by a biased hematopoietic output toward the myeloid rather than the lymphoid lineage, driven by both genetic factors and chronic inflammation (Niu et al. 2013). Here, there was no investigation on the presence of an eventual accelerated senescence in HSCs.

Conclusively, there is evidence that SLE BM-MSCs display an enhanced aging process. Unfortunately, to our knowledge, the exact significance of this accelerated cellular senescence, from a clinical point of view on SLE disease activity and on the development of other comorbidities, was not clearly investigated.

2.2.4 Proteostasis

Protein homeostasis, or proteostasis, corresponds to the collection of cellular mechanisms involved in maintaining the homeostasis of the proteome. Proteostasis is maintained through the coordinated action of several quality control mechanisms. The main components of the proteostasis maintenance system are chaperones, and two proteolytic systems, the ubiquitin-proteasome system and the lysosomal pathways (autophagy). All together, they coordinate protein synthesis with correct folding, conservation of protein conformation and protein degradation. Many studies have demonstrated that proteostasis is altered with age (Hipp et al. 2019; Kaushik & Cuervo 2015). Accumulation of external and endogenous stresses lead to the decline of the proteostasis network capacity and to the accumulation of misfolded and aggregated proteins. More stable proteomes have been observed in the longest-living species (Treaster et al. 2014; Pérez et al. 2009), whereas accumulation and

aggregation of misfolded proteins are found in common age-associated diseases, especially in neurodegenerative ones (*i.e.*, Alzheimer's and Parkinson's diseases) (Hipp et al. 2019; Kaushik & Cuervo 2015).

To our knowledge, few studies have investigated protein homeostasis during SLE. Some reports have been made on the implication of Heat Shock proteins (HSPs) in autoimmune diseases. HSPs are small molecular chaperones which interact and assist in the folding and assembly of the native polypeptide chains into its final structure. HSPs are expressed constitutively and in response to stressful conditions. Elevated expression of the 90 kDa HSP (HSP90) has been found in serum and kidneys of SLE patients (Kenderov et al. 2002; Shimp et al. 2012; Ripley et al. 2001; Hu et al. 2006). The SLE patients with active disease exhibit higher HSP90 levels than patients with inactive lupus (Hu et al. 2006). Positive correlations were found between HSP90 levels and IL-6 levels in SLE patients (Hu et al. 2006; Ripley et al. 2001). However, to our knowledge, no study has reported the presence of misfolded proteins or the evidence of a deficiency in the proteostasis network capacity in SLE under resting or stressful conditions.

2.2.5 Adaptation to stress

All along their lifetime, organisms have to face multiple stressors, which can be external perturbations, such as exposure to radiations, changes in oxygen levels, in temperature or in nutrient availability, as well as internal perturbations, such as protein or DNA damage. In response, cellular and organismal homeostasis are maintained through the coordinated action of multiples stress responses and repair pathways. During aging, there is progressive decline in all these critical response systems, as we have seen in the previous sections (IGF signaling, mTOR pathway, mitochondrial function, DNA damage response and proteostasis especially), leading to a deterioration of stress resistance (Haigis & Yankner 2010). In higher levels

organisms such as humans, stressors are not just physical, but are also social and psychological, adding another layer of complexity to the concept of stress and stress response. The impact of these specific stressors and their associated mechanisms on the aging process and aging rate is far to be completely understood currently (Epel & Lithgow 2014).

If we consider stress in the context of SLE, research has been mainly focused on the impact of psychological stressors on lupus occurrence and activity. For example, exposure to trauma and post-traumatic stress disorder have been associated with increased risk of developing SLE (O'Donovan et al. 2015; Feldman et al. 2019; Roberts et al. 2017). Furthermore, stress can have an impact on patients' functionality, as it is an important contributor to fatigue and as it can worsen the perceived clinical symptomatology (Williams et al. 2014; Azizoddin et al. 2019; Peralta-Ramírez et al. 2004). However, to our knowledge, there has been little specific investigation on the systems biology of stress response and its possible implication in the occurrence of age-related comorbidities in SLE.

2.2.6 Inflammation and immunosenescence

Aging is characterized by a chronic, sterile, low-grade inflammation, called inflammaging (Franceschi et al. 2000), which results from an imbalance between inflammatory and anti-inflammatory networks (Franceschi et al. 2007). It is characterized by increased basal levels of circulating pro-inflammatory factors, such as C-reactive protein (CRP), IL-6, IL-1 or TNF- α , as well as chronic immune activation. Inflammaging contributes greatly to the pathogenesis of multiple chronic diseases and has been associated with higher risk of mortality (Franceschi & Campisi 2014). It results from multiple causes, such as danger signals which can be exogenous (persistent viral infections such as cytomegalovirus, failure to effectively clear pathogens) or endogenous (accumulation of damaged and senescent cells, cell debris, extracellular mitochondrial DNA).

Inflammaging is tightly linked to immunosenescence. Interestingly, it is known that a chronic immune activation, such as seen in autoimmune diseases, can accelerate the immunosenescence process. Thus, different autoimmune diseases, such as rheumatoid arthritis, have been associated with phenotypic changes that can be interpreted as an accelerated immunosenescence (Bauer 2020). This appears to be also the case in SLE patients who present elevated markers of immunosenescence. For example, as compared to age-matched healthy controls, they have expanded effector T cells subpopulations that have lost the expression of CD28, a marker that has been associated with immune aging (Kalim et al. 2020; Thewissen et al. 2007; Ulfah et al. 2019). SLE patients present an accumulation of terminally differentiated memory T cells, which harbor a decreased proliferative capacity and an increased tendency to undergo apoptosis (Fritsch et al. 2006). Interestingly, the immune-senescent profile observed in SLE patients has been associated with cognitive impairment (Kalim et al. 2020).

One believed key parameter of immunosenescence is the thymus, as it undergoes major changes during aging with a process of involution and shrinking, and a constant decline in the output of newly generated T lymphocytes (Thomas et al. 2020). Thymus functional capacity can be estimated by the frequency of peripheral cells expressing T-cell receptor excision circles (TRECs). In SLE patients as compared to age-matched healthy controls, it was observed a significant decrease in the number of TRECs in peripheral blood (Thewissen et al. 2007; Kayser et al. 2004; Fasching et al. 2017). Interestingly, the decrease in naïve CD4⁺ T cells assessed with TRECs was found in older SLE patients, whereas no difference was observed for young patients and their respective controls, suggesting a disproportional decline in thymic output (Fasching et al. 2017).

2.2.7 Epigenetics

Epigenetics refers to the range of heritable reversible changes in chromatin structure and gene function that do not involve alterations of the DNA sequence itself. The major epigenetic mechanisms, *i.e.* DNA methylation, post-translational histone modifications and small non-coding RNAs, affect cells and tissues throughout life and play crucial roles in cellular differentiation, growth and development. Moreover, these three main categories of epigenetics marks are considered as central regulators of the aging process (Pal & Tyler 2016; Sen et al. 2016). Firstly, important changes in DNA methylation patterns have been observed during aging and have been associated with several age-related conditions. Different types of DNA methylation changes coexist during aging: the global genomic DNA methylation level decreases with age, mainly reflecting a decrease in DNA methylation of repetitive genomic regions and interspersed elements (Bollati et al. 2009; Luo et al. 2014); differential methylation of specific genomic loci occurs, especially with an age-related hypermethylation in gene promoters (Bell et al. 2012; Bell et al. 2016; Rakyan et al. 2010; Christensen et al. 2009); and there is an increase in the inter-individual divergence between patterns of DNA methylation (named DNA methylation drift) (Fraga et al. 2005; Maegawa et al. 2017; Tan et al. 2016; Mendelsohn & Larrick 2017), and an increase in the rate of epimutations (Gentilini et al. 2015). Secondly, histones, which are proteins that form an octamer core particle around which DNA is wrapped, undergo different types of posttranslational modifications (methylation, acetylation, ubiquitination, phosphorylation) that can induce changes in chromatin structure. Changes in the abundance and distribution of certain of these specific histone marks have been observed during aging in diverse species (Wang et al. 2018). Finally, noncoding RNAs, and especially microRNAs (miRNAs) which are a broad class of small noncoding RNAs that exert key roles in the post-transcriptional regulation of gene expression by targeting specific mRNAs,

are differentially expressed during aging (Huan et al. 2018; Noren Hooten et al. 2013; Smith-Vikos & Slack 2012; Inukai et al. 2012).

Contribution of epigenetic mechanisms to SLE pathogenesis has been extensively investigated (Reviewed in (Hedrich 2017; Long et al. 2016; Surace & Hedrich 2019)), especially regarding DNA methylation and identification of specific miRNAs signatures (Stagakis et al. 2011; Te et al. 2010; Dai et al. 2007; Tang et al. 2009; Chauhan et al. 2014). SLE is characterized by wide-spread DNA methylation changes. A lower overall DNA methylation level is observed in SLE subjects as compared to non-SLE individuals (Javierre et al. 2010). This global hypomethylation appears to be particularly important in repetitive regions and interspersed elements, derived from transposable elements, which form the majority of genomic DNA (long interspersed nuclear elements (LINEs), short interspersed nuclear elements (SINEs, such as Alu sequences), long-terminal repeat (LTR) transposons, or DNA transposons). Methylation levels of LINE-1 sequences and of Human Endogenous Retrovirus (HERV) sequences are decreased in SLE patients as compared to controls (Nakkuntod et al. 2011; Fali et al. 2014; Huang et al. 2014; Nakkuntod et al. 2013). Hypomethylation of these regions can increase the expression of their respective transcripts and can increase DNA immunogenicity, providing potent stimuli for an innate immune response (Crow 2010). Epigenetic mechanisms are also crucial in the dysregulation of the expression of genes pertinent to SLE pathogenesis. This influence of the expression of SLE-related genes is particularly true in T cells (Reviewed in (Zhang et al. 2013)), leading to imbalanced expression of crucial factors, such as CD40L, FoxP3, perforin, or several cytokines (IL-10, IL-17, IL-4, IL-6) (Hedrich et al. 2014; Zhao et al. 2010; Apostolidis et al. 2013; Lal et al. 2009, p.3; Kaplan et al. 2004). Epigenetic alterations are finally important contributors to the interferon signature in SLE. Thus, several studies on DNA methylation have reported striking patterns of hypomethylation

at interferon-signature genes, such as *IFI44L* (Interferon Induced Protein 44 Like), *MX1* (Interferon-Induced GTP-Binding Protein Mx1), *IFITM1* (Interferon Induced Transmembrane Protein 1), or *IRF7* (Interferon Regulatory Factor 7) (Imgenberg-Kreuz et al. 2018; Coit, Yalavarthi, et al. 2015; Yeung et al. 2017; Absher et al. 2013; Chen et al. 2019; Zhao et al. 2016; Coit, Renauer, et al. 2015; Imgenberg-Kreuz et al. 2019; Zhu et al. 2016; Coit et al. 2013).

Epigenetic modifications are highly complex and have appeared to be crucial contributors to SLE pathogenesis. To our knowledge, the contribution of these changes to SLE co-morbidities has not been investigated so far. Aside from lower DNA methylation levels in repetitive sequences which are also observed during the aging process, it does not seem that there is a significant overlap between SLE epigenetic signatures and age-related ones.

2.2.8 Impact of therapeutics

SLE is a chronic disease characterized by a fluctuating disease course. Organ-threatening/life-threatening manifestations of the disease require an intensive treatment with an initial period of high-intensity immunosuppressive therapy to control disease activity (Fanouriakis et al. 2020). Subsequently, therapeutic consolidation and prevention of relapses/flares are based on long-term administration of specific therapeutics, such as hydroxychloroquine or glucocorticoids (GCs), which can be prescribed for many years (Mathian et al. 2020).

Some therapeutics used in SLE are known to have severe side effects, and they could have a negative impact on the aging process by favoring its acceleration. For example, high cumulative GCs dosage has been associated with atherosclerosis in patients with SLE (Skaggs et al. 2012), as GCs can have deleterious effects on insulin resistance, blood pressure and lipid profile. Additionally, it has been well demonstrated that cancer therapeutics can cause accelerated aging (Hill et al. 2020; Cupit-Link et al. 2017). In SLE, it is thus possible that exposure to intense

immunosuppressive therapy, with cyclophosphamide for example, may accelerate mechanisms associated with aging, such as precipitation of genomic instability.

Nevertheless, it should also be considered the fact that these therapeutics may have a positive impact of the aging process and its associated diseases. As mentioned earlier, it is clearly established that chronic inflammation plays a pathogenic role in the development of accelerated atherosclerosis, and therapeutic suppression of inflammation can have a favorable impact on CVD. Thus, anti-inflammatory strategies have been proposed for secondary prevention of atherosclerotic disease in the general population (therapeutic inhibition of interleukin-1 β with canakinumab or treatment with methotrexate) (Ridker et al. 2017; Ridker et al. 2019). In the context of autoimmunity, it has been particularly investigated in rheumatoid arthritis. Lowering disease activity with the use of disease-modifying antirheumatic drugs (DMARDs) (methotrexate in particular) and/or biological agents (such as anti-TNF α agents) decreases CVD risk (Jagpal & Navarro-Millán 2018). In mice, B cell depletion therapy reduces the development of atherosclerosis (Ait-Oufella et al. 2010; Kyaw et al. 2010). In patients with SLE, hydroxychloroquine has been associated with decreased aortic stiffness (Selzer et al. 2001) and reduced carotid plaque burden (Roman et al. 2003). The anti-inflammatory effects of GCs, when used in a specific therapeutic window with low dose, in addition of preventing flares (Mathian et al. 2020), may be atheroprotective and may have beneficial impact on CVD risk.

While the clinical impact of the SLE therapeutics has been examined, especially regarding CVD risk, their exact influence on the basic cellular and molecular mechanisms of aging should be better investigated. Influence of medications on DNA damage and telomere length has been explored in some reports. In three different studies, no correlations have been found between genomic instability and SLE treatment modalities (Singh et al. 2014; Souliotis & Sfikakis 2015;

López-López et al. 2014). Influence of GCs treatment on telomere length and on telomerase activity is disputed (Wu et al. 2007; Haque et al. 2013; Kurosaka et al. 2001; Kurosaka et al. 2003). Regarding stem cells, the senescent phenotype of BM-MSCs was observed in both untreated and treated SLE patients (Gu et al. 2012). To our knowledge, the exact impact of SLE medications on the other pillars of aging has been poorly investigated. Their beneficial or detrimental effects, difficult to capture owing to several confounding factors such as disease activity and disease duration, require longitudinal studies in SLE patients cohorts in order to clarify this issue.

2.2.9 Conclusion

SLE pathogenesis is extremely complex, involving genetic, environmental, hormonal and immunological factors (Tsokos et al. 2016). Multiple pathways involved in this pathogenesis appear to have a complex interplay with the ones which underpin the aging process (Kennedy et al. 2014; López-Otín et al. 2013). Some of the biological hallmarks that have been proposed for aging can be identified chronologically earlier in subjects with SLE, and typical age-associated mechanisms, such as DNA damage or telomere attrition, are frequently found in SLE patients at young age. Of particular importance is the evidence of the presence of an acceleration of aging in the immune system during SLE. The exact relevance and implications of these observations on SLE disease course on one hand, but mostly on the presence of a clinically significant acceleration of the aging process at the systemic level on the other hand is not clear and several questions remain to be answered. Longitudinal studies with frequent serial measurements of aging biological hallmarks, evaluation of the occurrence of age-related diseases and evaluation of the impact of therapeutics are greatly needed.

3. SLE and epigenetic biomarkers of age: a pilot study

3.1 Epigenetic biomarkers of aging: definition and concept

In order to evaluate if a condition is associated or not with an acceleration of the aging process, we need to find a way to properly measure biological aging. To this aim, identification of biomarkers of aging has been a critical and intense area of research since decades. Ideally, these markers could be used to predict the occurrence of adverse outcomes during aging and to identify populations with accelerated aging which could benefit from early interventions. Biomarkers of age could also be used as surrogate endpoints for clinical trials and to evaluate the effectiveness of an intervention.

There is currently no consensus on how to best measure one individual's biological age and until now a valid and unique aging biomarker has not yet been identified. However, in the last decade, there has been tremendous interest in epigenetic biomarkers of aging (also known as epigenetic clocks or DNA methylation-based biomarkers). Epigenetic clocks are algorithms based on the combination of DNA methylation levels at some specific CpG sites, that can predict the age of a DNA source. They are nowadays considered as among the most robust predictors of chronological age in humans (Horvath & Raj 2018; Field et al. 2018; Jylhävä et al. 2017; BLUEPRINT consortium 2016). Two of these clocks, Horvath's (based on 353 CpG sites) and Hannum's (based on 71 CpG sites) calculators, developed in large samples cohorts covering the entire adult lifespan, show high correlations with age and small deviations from chronological age (Horvath 2013; Hannum et al. 2013). Besides these two models, researchers have also developed several other age-prediction models with different characteristics and performances (Levine et al. 2018; Lu et al. 2019; Horvath et al. 2018; Bocklandt et al. 2011; Weidner et al. 2014). These clocks are mostly derived by linear regression algorithms that train against the chronological age of the sample and ultimately select a set of CpG sites. The weights associated with each CpG site are determined and the product of the methylation level at each

site with the learned coefficient is summed, leading to an estimated epigenetic age, also called DNAm age (**Figure 2**).

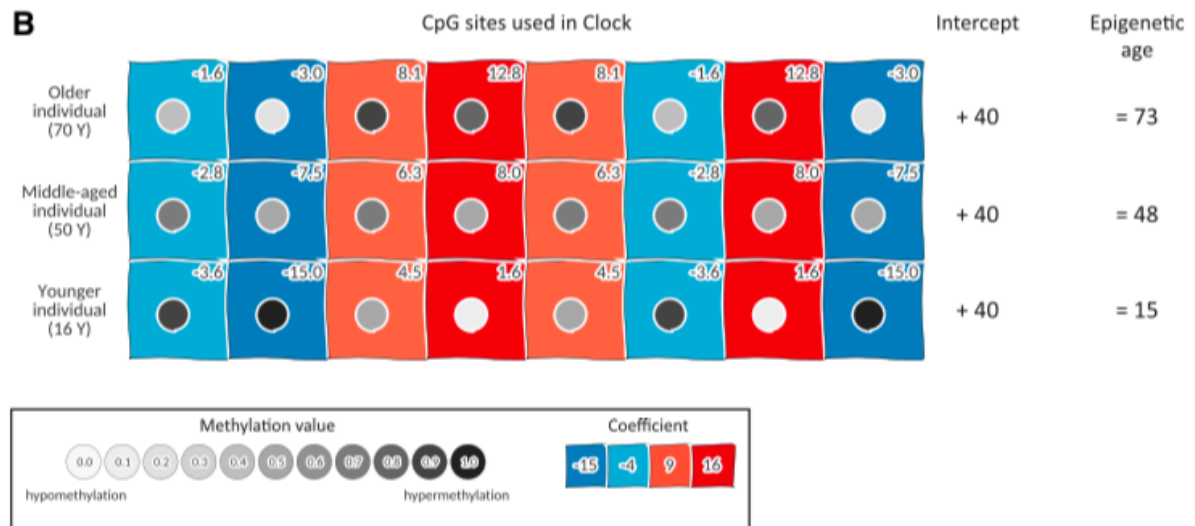


Figure 2: DNA methylation clock: how it works. Adapted from Field *et al.* 2018.

Epigenetic clocks are not only able to predict chronological age with high accuracy, but evidence also suggests that they can be informative of the biological age of an individual. Thus, a positive or negative deviation of the biological age to chronological age according to these models reflects the presence of an accelerated or decelerated aging phenomenon, respectively. Horvath's and Hannum's clocks are able to predict all-cause mortality independently of classic risk factors (Marioni *et al.* 2015; Chen *et al.* 2016; Dugué *et al.* 2018; Zheng *et al.* 2016; Christiansen *et al.* 2016; Perna *et al.* 2016; Fransquet *et al.* 2019; Marioni *et al.* 2016) and they also can be informative on other age-related outcomes such as frailty, cognitive and physical functioning, neurodegenerative or cardiovascular diseases (Horvath & Raj 2018). Finally, it was also demonstrated that Horvath's clock is able to capture the age acceleration phenomenon associated with progeroid syndromes, such as Down or Werner syndromes (Maierhofer *et al.* 2017; Horvath, Garagnani, *et al.* 2015) and to detect age-deceleration effects in models of

healthy aging and longevity, such as centenarians and their offspring (Horvath, Pirazzini, et al. 2015). Interestingly, epigenetic clocks have also been applied to cohorts of patients with chronic HIV infection, which is associated with early onset of diseases linked to aging (Wing 2016). According to two studies, HIV-infected treated patients are characterized by a 5 year increase in biological age in blood samples as compared to healthy controls, reflecting an acceleration of the aging process from a biological point of view (Gross et al. 2016; Horvath & Levine 2015).

In the current study, we sought to test the hypothesis that SLE would be associated with an accelerated biological aging. The objective of this work was to assess the association between lupus status and epigenetic age acceleration, by comparing DNA methylation profiles of SLE patients *versus* healthy controls.

3.2 Methods

Publicly available DNA methylation datasets involving SLE patients were searched on NCBI's Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/gds). The database was searched for DNA methylation data generated with the genome-wide Illumina[®] Infinium platforms (Illumina Inc., San Diego, CA, USA). All generations of platforms were considered: 27K (GEO Accession: GPL8490), 450K (GEO Accession: GPL13534) and EPIC (GEO Accession: GPL21145). The Illumina[®] Infinium HumanMethylation27 BeadChip array interrogates 27 578 methylation sites quantitatively across the genome at single-nucleotide resolution, while the HumanMethylation450 BeadChip array interrogates over 450 000 methylation sites and the EPIC one over 850 000.

With these arrays, DNA methylation levels at individual CpG sites are evaluated following the same protocol. Firstly, genomic DNA is extracted from the tissue/cell type of

interest. Subsequently, 500 ng or 1 mg of DNA are bisulfite converted, using EZ DNA Methylation Kits from Zymo Research Corporation (Zymo, Orange, CA, USA). After bisulfite conversion, DNA is whole genome amplified, enzymatically fragmented and hybridized to the Illumina® Infinium BeadChips (Illumina Inc., CA, USA), according to manufacturer's protocols. Finally, arrays are scanned using specific instruments and DNA methylation data are extracted for processing and normalization (**Figure 3**).

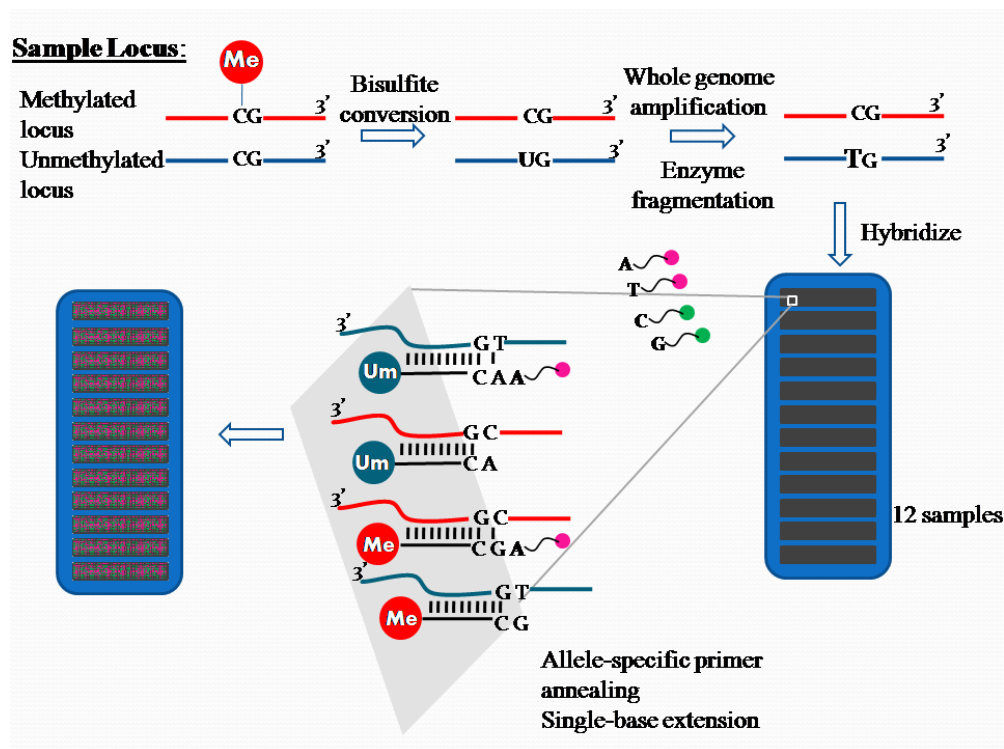


Figure 3: Illumina® Infinium HumanMethylation 27K workflow (source: Wikimedia commons).

For this study, pre-processed DNA methylation data were directly downloaded from GEO database. Information regarding studied samples, when available, was also retrieved: tissue/cell type used, socio-demographic factors of the participants and status (lupus patient / control).

DNA methylation data were then uploaded into the DNA methylation age calculator, developed by Steve Horvath and freely available at the website: <https://dnamage.genetics.ucla.edu>, in order to calculate the epigenetic age of the individuals,

named DNAm age. DNAm age is calculated using the weighted average of DNA methylation levels at 353 CpG sites, as described in the seminal paper (Horvath 2013). We also considered a measure of epigenetic age acceleration, denoted AgeAccel, which corresponds to the residual that results from regressing DNAm age on chronological age and denoted AgeAccelerationResidual in the online calculator. A positive (or negative) value indicates that the biological age of the sample is higher (or lower) than expected based on chronological age.

All values are expressed as mean \pm standard deviation or median \pm interquartile range for continuous variables, and as counts and percentages for categorical variables. Pearson correlation coefficient was calculated to describe the linear correlation between measures of epigenetic age and chronological age. Wilcoxon tests were used to compare measures of epigenetic age acceleration between the different groups. Statistical significance was defined as p-value < 0.05 . All statistical analyses and figures were made using R v3.3.2.

3.3 Results

3.3.1 Datasets

Ten publicly available datasets involving SLE patients were found on GEO database. Six of them were excluded as they did not contain the chronological age of the included subjects, making the analysis impossible (GSE110607, GSE76056, GSE59250, GSE57869, GSE27895, GSE118144). Additionally, two datasets did not include control subjects (GSE80553 and GSE161476), making subsequent comparison impossible. In the end, a total of two datasets, GSE65097 and GSE82218, were selected for further analysis (**Figure 4** and **Table 1**).

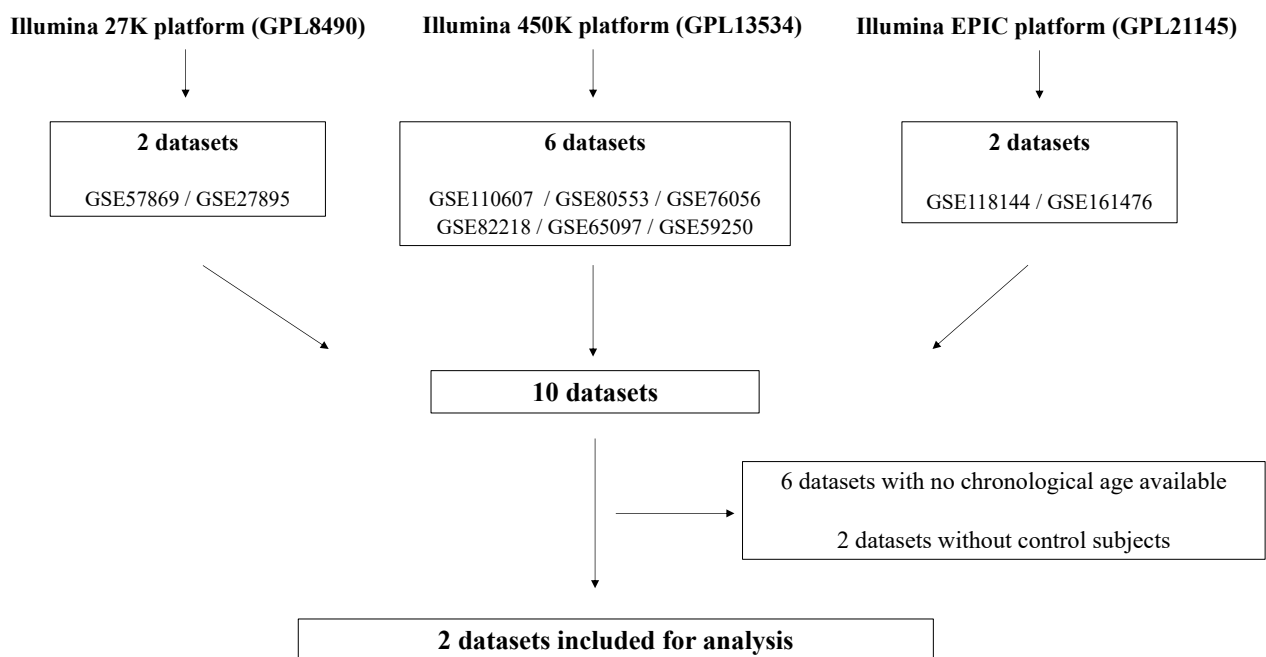


Figure 4: GEO datasets selection process.

GEO ID	Citation	Tissue source	Platform	N (total)	N SLE subjects	Female (%)	Mean age (years)
GSE65097	(Coit, Yalavarthi, et al. 2015)	Neutrophils	450K	30	15	100	37.8
GSE82218	(Zhu et al. 2016)	PBMC	450K	55	30	84	30.5

Table 1: Overview of the DNA methylation datasets included in the analysis.

PBMC: Peripheral Blood Mononuclear Cells.

3.3.2 Lupus patients and controls

GSE65097

The GSE65097 dataset was generated by Coit *et al.* (Coit, Yalavarthi, et al. 2015). It consisted of 15 lupus patients and 15 age, sex and ethnicity matched healthy controls. Participants were recruited from the University of Michigan rheumatology clinics or from the Lupus Natural History Protocol at the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) of the National Institutes of Health (NIH). Mean age of patients and controls was 36.9 years (± 10.2) and 38.7 years (± 9.6), respectively. All study participants were female. Main characteristics of the lupus patients are presented in **Table 2**.

Age (mean ± SD)	36.9 years (± 10.2)
Ethnicity (n, %)	European-American: 6 (40%) African American: 5 (33%) Hispanic/White: 2 (13%) Asian: 2 (13%)
SLEDAI score (mean ± SD)	1.67 (± 1.84)
Clinical manifestations (n, %)	Cutaneous: 10 (67%) Articular: 10 (67%) Renal: 6 (40%) Hematologic: 8 (53%) Serositis: 7 (47%) Neurologic: 2 (13%)
Treatments (n, %)	Hydroxychloroquine: 10 (67%) Steroids: 11 (73%) Mycophenolate: 6 (40%) Azathioprine: 2 (13%) Rituximab: 1 (7%)

Table 2: Demographic and clinical information of the lupus patients included in the GSE65097 dataset.

SD: Standard Deviation. SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.

In this study, neutrophils were extracted from the granulocyte layer after Ficoll density gradient centrifugation of fresh peripheral blood samples (25 mL). DNA methylation profiling was performed using the Infinium HumanMethylation450 BeadChip Kit.

In the published paper, authors identified 293 differentially methylated CpG sites in neutrophils between SLE patients and controls, the majority of them (68%) being hypomethylated. A robust and consistent hypomethylation of interferon signature genes in neutrophils of SLE patients was observed (Coit, Yalavarthi, et al. 2015).

GSE82218

The GSE82218 dataset was generated by Zhu *et al.* (Zhu et al. 2016). Thirty SLE patients (15 SLE with lupus nephritis (LN+) and 15 SLE without lupus nephritis (LN-)) and 25 controls were included in this study. Patients and controls were matched for age, sex, and ethnicity. They were all recruited from Xiangya Hospital, Central South University of China (Changsha, Hunan, China). Mean age of SLE LN+ patients, SLE LN- patients and controls was 30.13 (\pm 7.68), 28.2 (\pm 6.7) and 32.2 years (\pm 9.04) respectively. Main characteristics of the lupus patients are presented in **Table 3**.

	SLE LN-	SLE LN+
Age (mean ± SEM)	28.2 years (± 6.7)	30.13 years (±7.68)
Sex (M/F)	2/13	2/13
Disease duration (months) (mean ± SEM)	4.90 ± 4.18	3.33 ± 4.21
SLEDAI score (mean ± SEM)	9.06 (± 4.06)	14.93 (± 4.65)
Clinical manifestations (n, %)	Malar rash: 8 (53%) Discoid rash: 1 (7%) Photosensitivity: 4 (27%) Oral ulcers: 2 (13%) Arthritis: 8 (53%) Serositis: 1 (7%) Renal: 0 (0%) Neurologic: 1 (7%) Hematologic: 2 (13%)	Malar rash: 11 (73%) Discoid rash: 3 (20%) Photosensitivity: 3 (20%) Oral ulcers: 1 (7%) Arthritis: 6 (40%) Serositis: 3 (20%) Renal: 15 (100%) Neurologic: 0 (0%) Hematologic: 7 (47%)

Table 3: Demographic and clinical information of the lupus patients included in the GSE82218 dataset.

SEM: Standard Error of the Mean. SLEDAI: Systemic Lupus Erythematosus Disease Activity Index. SLE LN-: Systemic Lupus Erythematosus without lupus nephritis. SLE LN+: Systemic Lupus Erythematosus with lupus nephritis.

SLE LN+ was defined as clinical and laboratory manifestations that meet American College of Rheumatology (ACR) criteria (persistent proteinuria > 0.5 g/day or greater than 3+ by dipstick, and/or urine cellular casts including red blood cells, hemoglobin, granular, tubular, or mixed). SLE LN+ patients exhibited higher disease activity (SLEDAI score) and lower level of

complement C3 (293.2 ± 196.2 versus 534.5 ± 280.1 mg/dL) than SLE LN- patients. There was no other statistically significant difference between the two groups.

PBMC were isolated from heparinized blood by Ficoll density gradient centrifugation. DNA methylation profiling was performed using the Infinium HumanMethylation450 BeadChip Kit.

Here, the authors identified 1813 hypermethylated CpG sites and 3785 hypomethylated ones in SLE patients as compared to controls. Correlating the DNA methylation data with transcriptomic analysis, they demonstrated that DNA hypomethylation was related with overexpression of IFN-related genes (Zhu et al. 2016).

In both datasets, all patients studied fulfilled the ACR classification criteria for lupus (Tan et al. 1982). All patients and controls signed an informed consent prior to participation in the respective study, and protocols were approved by local ethics committees.

3.3.3 Epigenetic age

For each dataset individually, epigenetic age (also referred to as DNA methylation age (DNAm age)) was calculated using the online age calculator. As expected, DNAm age was significantly associated with chronological age ($p < 0.0001$) in both cohorts (**Figure 5**).

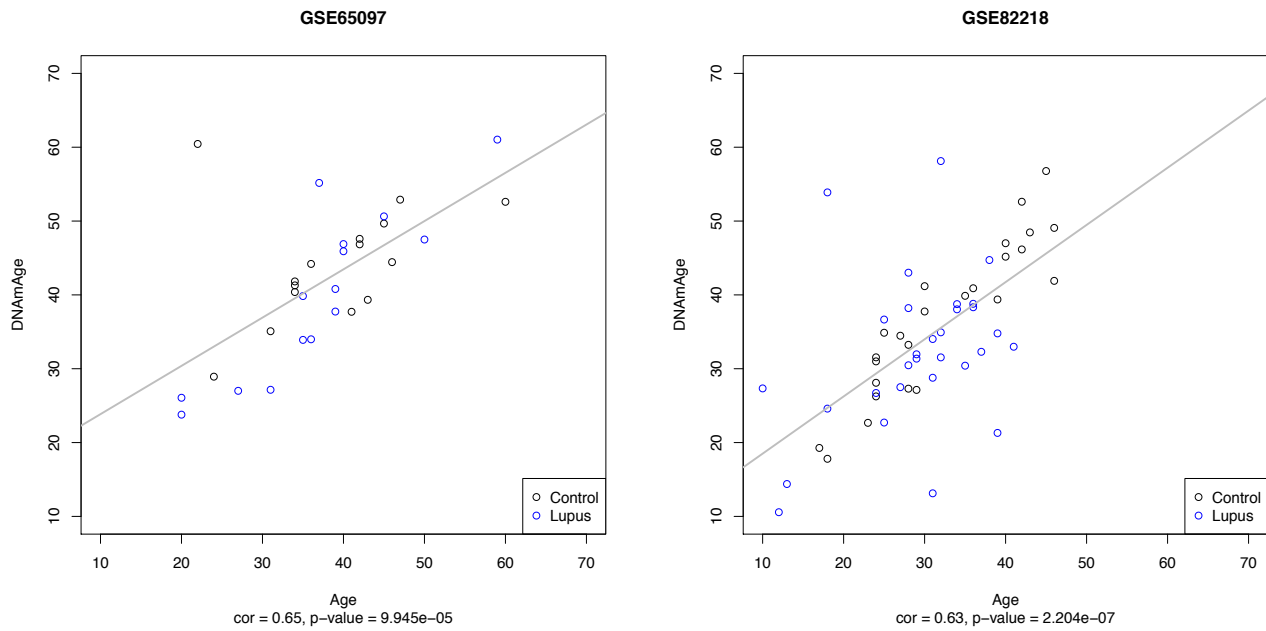


Figure 5: Significant association between DNAm age and chronological age in both cohorts.

Scatter plots of chronological age (x-axis) versus DNAm age (y-axis) in the different groups (Control subjects = black; Lupus patients = blue). Grey lines represent fitted linear regressions.

3.3.4 Epigenetic age acceleration

For each subject, we also considered the measure of epigenetic age acceleration, named AgeAccel, defined as the residual from regressing epigenetic age on chronological age. Positive values of epigenetic age acceleration indicate an epigenetic age older than expected, while negative values indicate an epigenetic age younger than expected on the basis of chronological age (**Table 4**).

GEO ID	Status	Number of subjects	Chronological age (years) mean \pm SD	DNAm age (years) mean \pm SD	AgeAccel (years) mean \pm SD
GSE65097	Controls	15	38.7 \pm 9.6	44.2 \pm 7.9	2.0 \pm 8.8
	SLE	15	36.9 \pm 10.2	39.8 \pm 11.3	- 1.0 \pm 6.0
GSE82218	Controls	25	32.2 \pm 9.0	36.8 \pm 10.4	1.1 \pm 4.9
	SLE	30	29.1 \pm 8.1	32.3 \pm 10.4	- 0.9 \pm 10.2

Table 4: DNAm age and measure of epigenetic age acceleration in the two cohorts.

SD: standard deviation. DNAm age: DNA methylation age. AgeAccel: Epigenetic age acceleration.

Subsequently, in both cohorts, we compared the average epigenetic age acceleration measure in controls and in lupus patients, in order to evaluate if lupus patients are biologically older than their age-matched controls. We did not observe any statistically significant difference between the groups (GSE65097: p-value = 0.30; GSE82218: p-value = 0.08) (**Figure 6**). In both datasets, lupus patients appeared to be slightly biologically younger as compared to the control subjects, but the trend was not statistically significant.

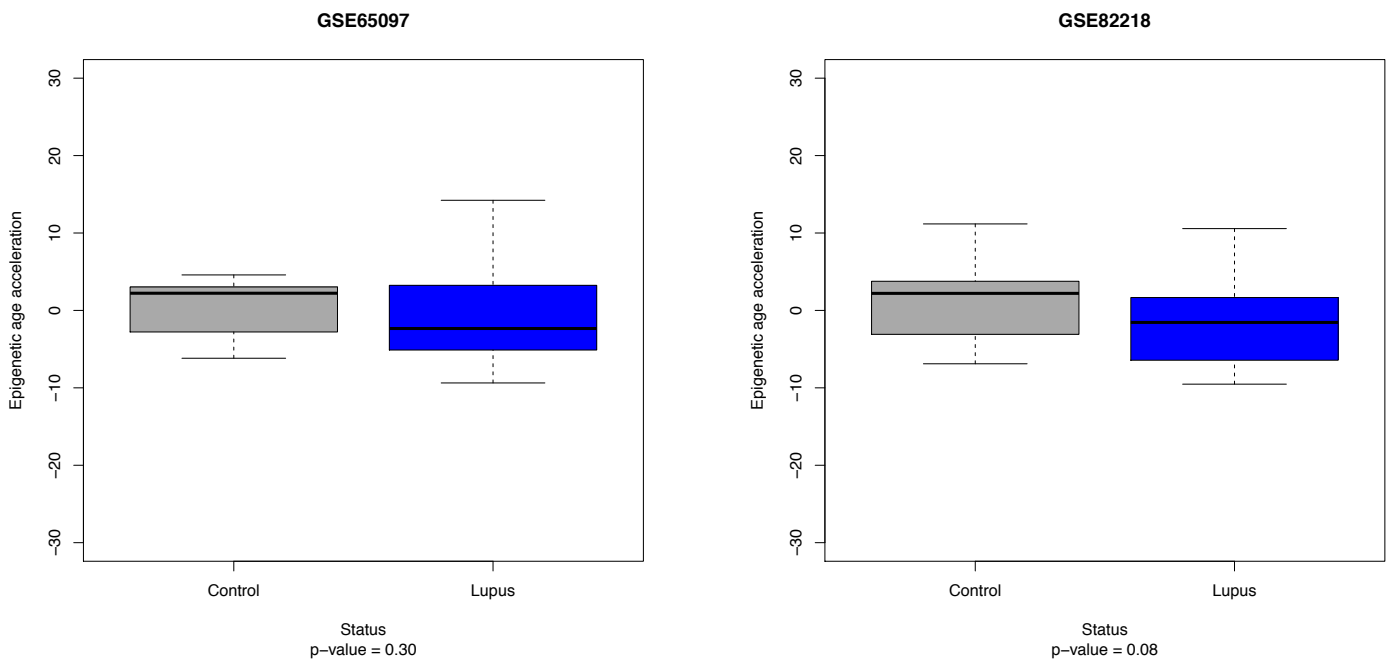


Figure 6: Boxplots of predicted epigenetic age acceleration in control subjects (grey) and in lupus patients (blue).

3.3.5 Influence of sex and lupus nephritis on the measure of epigenetic age acceleration

In GSE82218 dataset, males and females were included. We divided the cohort of lupus patients according to sex in order to evaluate the potential relationship between sex and the measure of epigenetic age acceleration in this specific population. There was no difference between the two groups (p-value = 0.62) (**Figure 7**). It should be noted that the number of male subjects is extremely low (n=4).

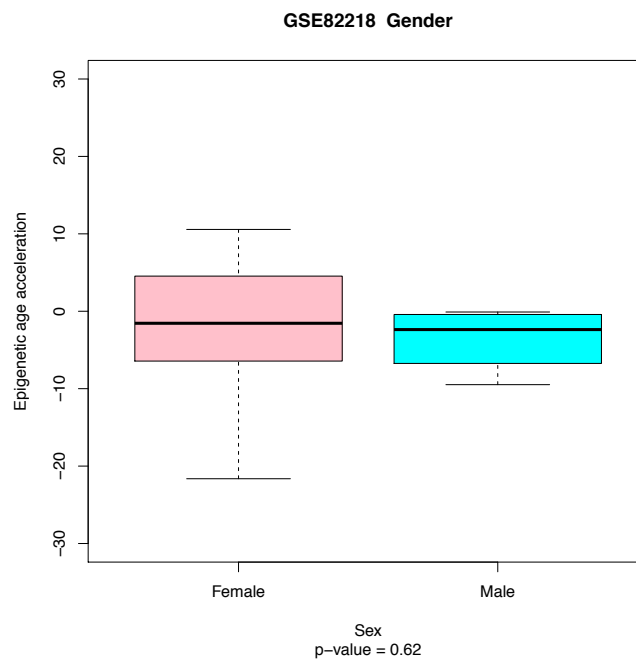


Figure 7: Boxplot of predicted epigenetic age acceleration in SLE females (pink) and SLE males (blue).

Finally, in this dataset, we also divided SLE patients according to the presence or the absence of lupus nephritis. Despite a trend toward a higher epigenetic age acceleration measure in patients with lupus nephritis (Mean AgeAccel = -0.5 years) as compared to subjects without nephritis (Mean AgeAccel = -1.3 years), the difference between the two groups was not statistically significant (p-value = 0.62) (**Figure 8**).

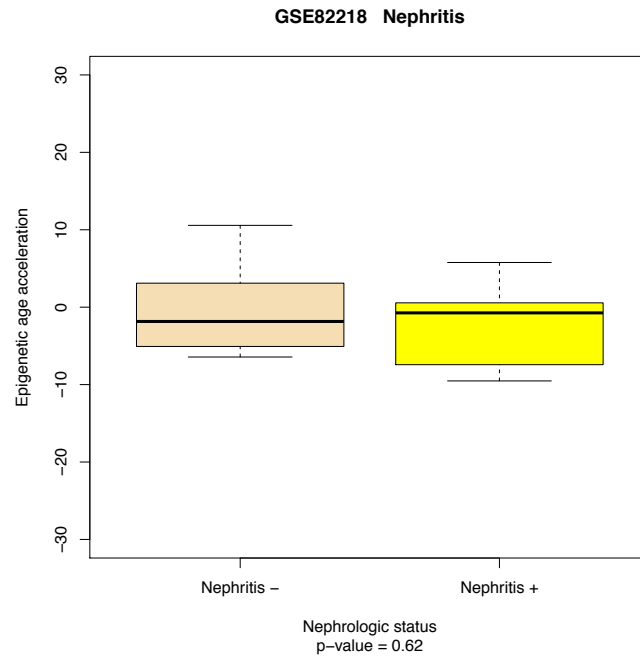


Figure 8: Boxplot of predicted epigenetic age acceleration in SLE patients with lupus nephritis (yellow) and without lupus nephritis (beige).

4. Discussion

SLE is a complex systemic autoimmune disease, with diverse clinical manifestations and an unpredictable clinical course. Despite improvements in diagnosis and treatments, many SLE patients can suffer from the accumulation of organ damage and mortality risks remain high for this population. Especially, clinical progression is associated with the development of comorbidities, such as infections or cardiovascular events, which are classically considered as age-related in the general population. Thus, the high prevalence of these comorbidities has raised the question of the presence of an acceleration of the aging process in the SLE population.

Interestingly, from a clinical point of view, two recent papers have evoked the importance of frailty in SLE (Katz et al. 2017; Legge et al. 2020). Frailty has been conceptualized as a state of increased vulnerability which results in a diminished ability to respond to physiologic stressors and in poorer outcomes. While the concept was so far mainly developed and applied in geriatrics communities, it has also been extended to other populations. In 2017, Katz *et al.* applied the classic Fried frailty criteria (Fried et al. 2001) in a cohort of 152 women with SLE (mean age = 48 (\pm 12) years) (Katz et al. 2017). Prevalence levels of frailty were found really high, as compared to the ones that can be observed in populations of older adults. Thus, 20% of the sample was classified as frail and 50.7% as prefrail. In this study, presence of frailty was associated with an increased risk of functional decline and mortality. More recently, using data from the Systemic Lupus International Collaborating Clinics (SLICC) inception cohort, Legge *et al.* developed a frailty index specially tailored for lupus patients, named SLICC-FI, based on the combination of 48 health deficits (Legge et al. 2020). Here also, high prevalence of frailty (27.1%) was found among SLE patients (mean age = 35.7 (\pm 13.4) years), higher than expected for similarly aged individuals of the general population.

From a biological point of view, as we have seen in the first part of this work, multiple pathways involved in the pathogenesis of SLE have a complex interplay with the ones which

underpin the aging process. Some of the typical biological hallmarks of aging, such as telomere attrition, DNA damage or stem cells decline, can be identified prematurely in SLE. Particularly, patients with SLE demonstrate several signatures of accelerated aging in the immune system.

In order to test the accelerated aging hypothesis in the SLE population, in the second part of the present work, we investigated the cross-sectional association between lupus status and epigenetic age acceleration in two cohorts of lupus patients. We did not find any evidence of an accelerated biological aging according to this measure of biological age. The results we obtained were consistent between the two independent datasets which used different cell types for the measure of DNA methylation. In the opposite direction from what would be expected, SLE patients appeared to be slightly biologically younger than controls, but the difference was not statistically significant. On the contrary, in one dataset, measures of epigenetic age acceleration in patients with SLE nephritis were higher than the ones measured in patients without nephritis, suggesting a discrete acceleration of the aging process in the first subgroup, but again the difference was not significant. While SLE patients appear to have an acceleration of the aging process according to other indices of aging such as telomere attrition, we did not find such an association with epigenetic biomarkers of age. Different hypothesis can be put forward to explain these null associations. It is possible that SLE patients do not have an acceleration of the aging process, or it is possible that it exists but is not captured by the model used in this work, and that DNAm age is not a suitable biomarker for age in SLE patients.

Our study has important limitations. Firstly, it relies on data of public repositories and highlights the problematic of the accessibility of data. While dozens of studies have been made on SLE and DNA methylation, and several of them used the Illumina® platform (more than 20 studies according to our research in PubMed), very few data are made publicly available. In the

end, we were able to include only two datasets. Moreover, these datasets are characterized by a cross-sectional design, small sample sizes and narrow age range. Data on disease activity, disease duration and therapeutics were presented in the original publication, but the information was not available individually in the downloaded data. We tried to reach corresponding authors of both publications (A.H Sawalha; X. Zuo and Q-Z. Li) by e-mail in order to obtain raw data, but we did not get a reply so far. As this individual information was not available to us, we were not able to assess the effect of these factors on DNAm age.

The two datasets included relatively young lupus patients, with relatively inactive disease and short disease duration. It would be interesting to perform analysis of biomarkers of age in cohorts of SLE patients with wider ranges of age, of disease activity indexes and of disease durations in order to evaluate the impact of these parameters on the biological age of the individuals. Moreover, it should be noted that inclusion of young patients, especially in the second dataset (GSE82218), with age between 10 and 20 years old, raises the problem of the performance of the mathematical model to properly predict the biological age of the younger individuals, as it is known that there is non-linear (logarithmic) pattern of the clock during teenage years (Horvath 2013).

As mentioned earlier, in the two datasets, DNA methylation analysis were performed using different cell types: isolated neutrophils (GSE65097) or PBMC (GSE82218). Considering that DNA methylation is cell type specific, it is known that differences in cell counts can impact the prediction of the model. Using Horvath's model, measures of epigenetic age acceleration can be adjusted for cellular heterogeneity (Horvath & Ritz 2015), but this correction is only possible for whole blood samples and thus this normalization could not be applied here. Furthermore, we could not apply the other widely used model of epigenetic clock, *i.e.* the Hannum's model (Hannum et al. 2013), as it only applies to whole blood samples.

We did not identify any significant overlap between the CpG sites found differentially methylated into the two original publications and the CpG sites included in Horvath's clock. Thus, in the study by Coit *et al.* (GSE65097), there was no common CpG sites between the 293 differentially methylated positions and the CpG sites included in the Horvath's clock. In the other study (GSE82218), 5 CpG sites were common. Four of them (cg21096399 associated with *MCAM* gene; cg17285325 associated with *ECGF1* gene; cg04121983 associated with *CASKIN2* gene; cg06810647) were found hypomethylated in SLE patients as compared to controls, whereas the other one (cg26297688 associated with *C12orf23* gene) was found hypermethylated. It seems unlikely that these five positions have a strong influence on the outcome of the biological age prediction. Indeed, DNAm age is calculated through averaging the information of 353 CpG sites scattered across the genome and in the final model, each CpG site has a limited weight in the algorithm. Therefore, changes in DNA methylation levels at these 5 CpG sites have possibly only a marginal impact on the final outcome.

The ability of properly measuring biological age can provide interesting opportunities to identify individuals with premature aging who are at increased risk for adverse outcomes. Early identification of these subjects can lead to preventative measures for co-morbidities and precocious interventions to try to slow down aging. These tools can also be used to better predict long-term outcomes in SLE patients. To date, such instruments are limited in SLE. While interesting results have been recently obtained with the development of frailty index (Legge *et al.* 2020), more work is required for biological markers. We need more data on the complex interplay between SLE pathogenesis and the aging process, as well as on the possible influence of environmental factors and SLE medications especially. There is a need of longitudinal, prospective studies following individuals over the course of their disease, in order to better

characterize features of aging in SLE and assess the dynamics of potential biomarkers of age in this population.

5. References

- Absher DM, Li X, Waite LL, Gibson A, Roberts K, Edberg J, Chatham WW & Kimberly RP (2013) Genome-wide DNA methylation analysis of systemic lupus erythematosus reveals persistent hypomethylation of interferon genes and compositional changes to CD4⁺ T-cell populations. *PLoS Genet.* 9, e1003678.
- Ahmed ASI, Sheng MH, Wasnik S, Baylink DJ & Lau K-HW (2017) Effect of aging on stem cells. *World J. Exp. Med.* 7, 1–10.
- Ait-Oufella H, Herbin O, Bouaziz J-D, Binder CJ, Uyttenhove C, Laurans L, Taleb S, Van Vré E, Esposito B, Vilar J, Sirvent J, Van Snick J, Tedgui A, Tedder TF & Mallat Z (2010) B cell depletion reduces the development of atherosclerosis in mice. *J. Exp. Med.* 207, 1579–1587.
- Al-Rawi ZS, Gorial FI, Tawfiq RF, Mohammed AK, Al-Naaimi AS, Al'aadhmi MA & Hayyawi AA (2014) Brief report: a novel application of buccal micronucleus cytome assay in systemic lupus erythematosus: a case-control study. *Arthritis Rheumatol. Hoboken NJ* 66, 2837–2841.
- Anisimov VN, Berstein LM, Popovich IG, Zabezhinski MA, Egorin PA, Piskunova TS, Semchenko AV, Tyndyk ML, Yurova MN, Kovalenko IG & Poroshina TE (2011) If started early in life, metformin treatment increases life span and postpones tumors in female SHR mice. *Aging* 3, 148–157.
- Apostolidis SA, Rauen T, Hedrich CM, Tsokos GC & Crispin JC (2013) Protein phosphatase 2A enables expression of interleukin 17 (IL-17) through chromatin remodeling. *J. Biol. Chem.* 288, 26775–26784.
- Avalos I, Chung CP, Oeser A, Milne GL, Morrow JD, Gebretsadik T, Shintani A, Yu C & Stein CM (2007) Oxidative stress in systemic lupus erythematosus: relationship to disease activity and symptoms. *Lupus* 16, 195–200.
- Azizoddin DR, Gandhi N, Weinberg S, Sengupta M, Nicassio PM & Jolly M (2019) Fatigue in systemic lupus: the role of disease activity and its correlates. *Lupus* 28, 163–173.
- Barber MRW, Drenkard C, Falasinnu T, Hoi A, Mak A, Kow NY, Svenungsson E, Peterson J, Clarke AE & Ramsey-Goldman R (2021) Global epidemiology of systemic lupus erythematosus. *Nat. Rev. Rheumatol.*
- Barzilai N, Crandall JP, Kritchevsky SB & Espeland MA (2016) Metformin as a Tool to Target Aging. *Cell Metab.* 23, 1060–1065.
- Barzilai N, Huffman DM, Muzumdar RH & Bartke A (2012) The critical role of metabolic pathways in aging. *Diabetes* 61, 1315–1322.
- Bassi C, Xavier D, Palomino G, Nicolucci P, Soares C, Sakamoto-Hojo E & Donadi E (2008) Efficiency of the DNA repair and polymorphisms of the XRCC1, XRCC3 and XRCC4 DNA repair genes in systemic lupus erythematosus. *Lupus* 17, 988–995.
- Bauer ME (2020) Accelerated immunosenescence in rheumatoid arthritis: impact on clinical progression. *Immun. Ageing A* 17, 6.
- Bell CG, Xia Y, Yuan W, Gao F, Ward K, Roos L, Mangino M, Hysi PG, Bell J, Wang J & Spector TD (2016) Novel regional age-associated DNA methylation changes within human common disease-associated loci. *Genome Biol.* 17, 193.
- Bell JT, Tsai P-C, Yang T-P, Pidsley R, Nisbet J, Glass D, Mangino M, Zhai G, Zhang F, Valdes A, Shin S-Y, Dempster EL, Murray RM, Grundberg E, Hedman AK, Nica A, Small KS, MuTHER Consortium, Dermitzakis ET, McCarthy MI, Mill J, Spector TD & Deloukas P (2012) Epigenome-wide scans identify differentially methylated regions for age and age-related phenotypes in a healthy ageing population. *PLoS Genet.* 8, e1002629.
- Bernatsky S, Boivin J-F, Joseph L, Manzi S, Ginzler E, Gladman DD, Urowitz M, Fortin PR, Petri M, Barr S, Gordon C, Bae S-C, Isenberg D, Zoma A, Aranow C, Dooley M-A, Nived O, Sturfelt G, Steinsson K, Alarcón G, Senécal J-L, Zummer M, Hanly J, Ensworth S, Pope J, Edworthy S, Rahman A, Sibley J, El-Gabalawy H, McCarthy T, St Pierre Y, Clarke A & Ramsey-Goldman R (2006) Mortality in systemic lupus erythematosus. *Arthritis Rheum.* 54, 2550–2557.
- Bernatsky S, Boivin JF, Joseph L, Rajan R, Zoma A, Manzi S, Ginzler E, Urowitz M, Gladman D, Fortin PR, Petri M, Edworthy S, Barr S, Gordon C, Bae SC, Sibley J, Isenberg D, Rahman A, Aranow C, Dooley MA, Steinsson K, Nived O, Sturfelt G, Alarcón G, Senécal JL, Zummer M, Hanly J, Ensworth S, Pope J, El-Gabalawy H, McCarthy T, St Pierre Y, Ramsey-Goldman R & Clarke A (2005) An international cohort study of cancer in systemic lupus erythematosus. *Arthritis Rheum.* 52, 1481–1490.
- Blagosklonny MV (2013) Aging is not programmed. *Cell Cycle* 12, 3736–3742.
- BLUEPRINT consortium (2016) Quantitative comparison of DNA methylation assays for biomarker development and clinical applications. *Nat. Biotechnol.* 34, 726–737.
- Bocklandt S, Lin W, Sehl ME, Sánchez FJ, Sinsheimer JS, Horvath S & Vilain E (2011) Epigenetic predictor of age. *PLoS One* 6, e14821.
- Bollati V, Schwartz J, Wright R, Litonjua A, Tarantini L, Suh H, Sparrow D, Vokonas P & Baccarelli A (2009) Decline in genomic DNA methylation through aging in a cohort of elderly subjects. *Mech. Ageing Dev.* 130, 234–239.

Cabreiro F, Au C, Leung K-Y, Vergara-Irigaray N, Cochemé HM, Noori T, Weinkove D, Schuster E, Greene NDE & Gems D (2013) Metformin retards aging in *C. elegans* by altering microbial folate and methionine metabolism. *Cell* 153, 228–239.

Campbell JM, Stephenson MD, de Courten B, Chapman I, Bellman SM & Aromataris E (2018) Metformin Use Associated with Reduced Risk of Dementia in Patients with Diabetes: A Systematic Review and Meta-Analysis. *J. Alzheimers Dis. JAD* 65, 1225–1236.

Caza T, Oaks Z & Perl A (2014) Interplay of infections, autoimmunity, and immunosuppression in systemic lupus erythematosus. *Int. Rev. Immunol.* 33, 330–363.

Chauhan SK, Singh VV, Rai R, Rai M & Rai G (2014) Differential microRNA profile and post-transcriptional regulation exist in systemic lupus erythematosus patients with distinct autoantibody specificities. *J. Clin. Immunol.* 34, 491–503.

Chen BH, Marioni RE, Colicino E, Peters MJ, Ward-Caviness CK, Tsai P-C, Roetker NS, Just AC, Demerath EW, Guan W, Bressler J, Fornage M, Studenski S, Vandiver AR, Moore AZ, Tanaka T, Kiel DP, Liang L, Vokonas P, Schwartz J, Lunetta KL, Murabito JM, Bandinelli S, Hernandez DG, Melzer D, Nalls M, Pilling LC, Price TR, Singleton AB, Gieger C, Holle R, Kretschmer A, Kronenberg F, Kunze S, Linseisen J, Meisinger C, Rathmann W, Waldenberger M, Visscher PM, Shah S, Wray NR, McRae AF, Franco OH, Hofman A, Uitterlinden AG, Absher D, Assimes T, Levine ME, Lu AT, Tsao PS, Hou L, Manson JE, Carty CL, LaCroix AZ, Reiner AP, Spector TD, Feinberg AP, Levy D, Baccarelli A, van Meurs J, Bell JT, Peters A, Deary IJ, Pankow JS, Ferrucci L & Horvath S (2016) DNA methylation-based measures of biological age: meta-analysis predicting time to death. *Aging* 8, 1844–1865.

Chen S, Pu W, Guo S, Jin L, He D & Wang J (2019) Genome-Wide DNA Methylation Profiles Reveal Common Epigenetic Patterns of Interferon-Related Genes in Multiple Autoimmune Diseases. *Front. Genet.* 10, 223.

Christensen BC, Houseman EA, Marsit CJ, Zheng S, Wrensch MR, Wiemels JL, Nelson HH, Karagas MR, Padbury JF, Bueno R, Sugarbaker DJ, Yeh R-F, Wiencke JK & Kelsey KT (2009) Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. *PLoS Genet.* 5, e1000602.

Christiansen L, Lenart A, Tan Q, Vaupel JW, Aviv A, McGue M & Christensen K (2016) DNA methylation age is associated with mortality in a longitudinal Danish twin study. *Aging Cell* 15, 149–154.

Coit P, Jeffries M, Altorok N, Dozmorov MG, Koelsch KA, Wren JD, Merrill JT, McCune WJ & Sawalha AH (2013) Genome-wide DNA methylation study suggests epigenetic accessibility and transcriptional poisoning of interferon-regulated genes in naïve CD4⁺ T cells from lupus patients. *J. Autoimmun.* 43, 78–84.

Coit P, Renauer P, Jeffries MA, Merrill JT, McCune WJ, Maksimowicz-McKinnon K & Sawalha AH (2015) Renal involvement in lupus is characterized by unique DNA methylation changes in naïve CD4⁺ T cells. *J. Autoimmun.* 61, 29–35.

Coit P, Yalavarthi S, Ognenovski M, Zhao W, Hasni S, Wren JD, Kaplan MJ & Sawalha AH (2015) Epigenome profiling reveals significant DNA demethylation of interferon signature genes in lupus neutrophils. *J. Autoimmun.* 58, 59–66.

Crow MK (2010) Long interspersed nuclear elements (LINE-1): potential triggers of systemic autoimmune disease. *Autoimmunity* 43, 7–16.

Cupit-Link MC, Kirkland JL, Ness KK, Armstrong GT, Tchkonja T, LeBrasseur NK, Armenian SH, Ruddy KJ & Hashmi SK (2017) Biology of premature ageing in survivors of cancer. *ESMO Open* 2, e000250.

Dai Y, Huang Y-S, Tang M, Lv T-Y, Hu C-X, Tan Y-H, Xu Z-M & Yin Y-B (2007) Microarray analysis of microRNA expression in peripheral blood cells of systemic lupus erythematosus patients. *Lupus* 16, 939–946.

Danza A & Ruiz-Irastorza G (2013) Infection risk in systemic lupus erythematosus patients: susceptibility factors and preventive strategies. *Lupus* 22, 1286–1294.

Davies RC, Pettijohn K, Fike F, Wang J, Nahas SA, Tunuguntla R, Hu H, Gatti RA & McCurdy D (2012) Defective DNA double-strand break repair in pediatric systemic lupus erythematosus. *Arthritis Rheum.* 64, 568–578.

De Azevêdo Silva J, Pancotto JAT, Donadi EA, Crovella S & Sandrin-Garcia P (2014) LIG4 and RAD52 DNA repair genes polymorphisms and systemic lupus erythematosus. *Mol. Biol. Rep.* 41, 2249–2256.

Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, Xiao B, Worley PF, Kozma SC & Powell JD (2009) The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* 30, 832–844.

Doherty E, Oaks Z & Perl A (2014) Increased mitochondrial electron transport chain activity at complex I is regulated by N-acetylcysteine in lymphocytes of patients with systemic lupus erythematosus. *Antioxid. Redox Signal.* 21, 56–65.

Doria A, Shoenfeld Y, Wu R, Gambari PF, Puato M, Ghirardello A, Gilburd B, Corbanese S, Patnaik M, Zampieri S, Peter JB, Favaretto E, Iaccarino L, Sherer Y, Todesco S & Pautetto P (2003) Risk factors for subclinical atherosclerosis in a prospective cohort of patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* 62, 1071–1077.

Dugué P-A, Bassett JK, Joo JE, Baglietto L, Jung C-H, Wong EM, Fiorito G, Schmidt D, Makalic E, Li S,

Moreno-Betancur M, Buchanan DD, Vineis P, English DR, Hopper JL, Severi G, Southey MC, Giles GG & Milne RL (2018) Association of DNA Methylation-Based Biological Age With Health Risk Factors and Overall and Cause-Specific Mortality. *Am. J. Epidemiol.* 187, 529–538.

Epel ES & Lithgow GJ (2014) Stress biology and aging mechanisms: toward understanding the deep connection between adaptation to stress and longevity. *J. Gerontol. A. Biol. Sci. Med. Sci.* 69 Suppl 1, S10-16.

Esdaile JM, Abrahamowicz M, Grodzicky T, Li Y, Panaritis C, du Berger R, Côte R, Grover SA, Fortin PR, Clarke AE & Senécal JL (2001) Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. *Arthritis Rheum.* 44, 2331–2337.

Fali T, Le Dantec C, Thabet Y, Jousse S, Hanrotel C, Youinou P, Brooks WH, Perl A & Renaudineau Y (2014) DNA methylation modulates HRES1/p28 expression in B cells from patients with Lupus. *Autoimmunity* 47, 265–271.

Fanouriakis A, Kostopoulou M, Cheema K, Anders H-J, Aringer M, Bajema I, Boletis J, Frangou E, Houssiau FA, Hollis J, Karras A, Marchiori F, Marks SD, Moroni G, Mosca M, Parodis I, Praga M, Schneider M, Smolen JS, Tesar V, Trachana M, van Vollenhoven RF, Voskuyl AE, Teng YKO, van Leew B, Bertsias G, Jayne D & Boumpas DT (2020) 2019 Update of the Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of lupus nephritis. *Ann. Rheum. Dis.*

Fasching, J, Fessler, A, Raicht, A, Lackner, R, Husic, W, Graninger, W, Schwinger, M, Stradner, & C. Dejaco (2017) Premature Senescence of Naive CD4+ T-Cells in Systemic Lupus Erythematosus [abstract].

Feldman CH, Malspeis S, Leatherwood C, Kubzansky L, Costenbader KH & Roberts AL (2019) Association of Childhood Abuse with Incident Systemic Lupus Erythematosus in Adulthood in a Longitudinal Cohort of Women. *J. Rheumatol.* 46, 1589–1596.

Fernandes G, Friend P, Yunis EJ & Good RA (1978) Influence of dietary restriction on immunologic function and renal disease in (NZB x NZW) F1 mice. *Proc. Natl. Acad. Sci. U. S. A.* 75, 1500–1504.

Fernandes G, Yunis EJ & Good RA (1976) Influence of diet on survival of mice. *Proc. Natl. Acad. Sci. U. S. A.* 73, 1279–1283.

Fernandez D, Bonilla E, Mirza N, Niland B & Perl A (2006) Rapamycin reduces disease activity and normalizes T cell activation-induced calcium fluxing in patients with systemic lupus erythematosus. *Arthritis Rheum.* 54, 2983–2988.

Field AE, Robertson NA, Wang T, Havas A, Ideker T & Adams PD (2018) DNA Methylation Clocks in Aging: Categories, Causes, and Consequences. *Mol. Cell* 71, 882–895.

Fontana L, Partridge L & Longo VD (2010) Extending healthy life span--from yeast to humans. *Science* 328, 321–326.

Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suñer D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu Y-Z, Plass C & Esteller M (2005) Epigenetic differences arise during the lifetime of monozygotic twins. *Proc. Natl. Acad. Sci. U. S. A.* 102, 10604–10609.

Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E & De Benedictis G (2000) Inflammaging. An evolutionary perspective on immunosenescence. *Ann. N. Y. Acad. Sci.* 908, 244–254.

Franceschi C & Campisi J (2014) Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J. Gerontol. A. Biol. Sci. Med. Sci.* 69 Suppl 1, S4-9.

Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panourgia MP, Invidia L, Celani L, Scurti M, Cevenini E, Castellani GC & Salvioli S (2007) Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech. Ageing Dev.* 128, 92–105.

Franceschi C, Garagnani P, Morsiani C, Conte M, Santoro A, Grignolio A, Monti D, Capri M & Salvioli S (2018) The Continuum of Aging and Age-Related Diseases: Common Mechanisms but Different Rates. *Front. Med.* 5, 61.

Fransquet PD, Wrigglesworth J, Woods RL, Ernst ME & Ryan J (2019) The epigenetic clock as a predictor of disease and mortality risk: a systematic review and meta-analysis. *Clin. Epigenetics* 11, 62.

Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, McBurnie MA, & Cardiovascular Health Study Collaborative Research Group (2001) Frailty in older adults: evidence for a phenotype. *J. Gerontol. A. Biol. Sci. Med. Sci.* 56, M146-156.

Fritsch RD, Shen X, Illei GG, Yarboro CH, Prussin C, Hathcock KS, Hodes RJ & Lipsky PE (2006) Abnormal differentiation of memory T cells in systemic lupus erythematosus. *Arthritis Rheum.* 54, 2184–2197.

Gao L, Bird AK, Meednu N, Dauenhauer K, Liesveld J, Anolik J & Looney RJ (2017) Bone Marrow-Derived Mesenchymal Stem Cells From Patients With Systemic Lupus Erythematosus Have a Senescence-Associated Secretory Phenotype Mediated by a Mitochondrial Antiviral Signaling Protein-Interferon-β Feedback Loop. *Arthritis Rheumatol. Hoboken NJ* 69, 1623–1635.

Gayed M, Bernatsky S, Ramsey-Goldman R, Clarke A & Gordon C (2009) Lupus and cancer. *Lupus* 18, 479–485.

Gentilini D, Garagnani P, Pisoni S, Bacalini MG, Calzari L, Mari D, Vitale G, Franceschi C & Di Blasio AM (2015) Stochastic epigenetic mutations (DNA methylation) increase exponentially in human aging and correlate with X chromosome inactivation skewing in females. *Aging* 7, 568–578.

Gergely P, Grossman C, Niland B, Puskas F, Neupane H, Allam F, Banki K, Phillips PE & Perl A (2002) Mitochondrial hyperpolarization and ATP depletion in patients with systemic lupus erythematosus. *Arthritis Rheum.* 46, 175–190.

Giannelou M & Mavragani CP (2017) Cardiovascular disease in systemic lupus erythematosus: A comprehensive update. *J. Autoimmun.* 82, 1–12.

Goldsmith TC (2012) On the programmed/non-programmed aging controversy. *Biochem. Biokhimiia* 77, 729–732.

Goobie GC, Bernatsky S, Ramsey-Goldman R & Clarke AE (2015) Malignancies in systemic lupus erythematosus: a 2015 update. *Curr. Opin. Rheumatol.* 27, 454–460.

Gross AM, Jaeger PA, Kreisberg JF, Licon K, Jepsen KL, Khosroheidari M, Morsey BM, Swindells S, Shen H, Ng CT, Flagg K, Chen D, Zhang K, Fox HS & Ideker T (2016) Methylome-wide Analysis of Chronic HIV Infection Reveals Five-Year Increase in Biological Age and Epigenetic Targeting of HLA. *Mol. Cell* 62, 157–168.

Gu Z, Cao X, Jiang J, Li L, Da Z, Liu H & Cheng C (2012) Upregulation of p16INK4A promotes cellular senescence of bone marrow-derived mesenchymal stem cells from systemic lupus erythematosus patients. *Cell. Signal.* 24, 2307–2314.

Gu Z, Jiang J, Tan W, Xia Y, Cao H, Meng Y, Da Z, Liu H & Cheng C (2013) p53/p21 Pathway involved in mediating cellular senescence of bone marrow-derived mesenchymal stem cells from systemic lupus erythematosus patients. *Clin. Dev. Immunol.* 2013, 134243.

Gu Z, Tan W, Feng G, Meng Y, Shen B, Liu H & Cheng C (2014) Wnt/ β -catenin signaling mediates the senescence of bone marrow-mesenchymal stem cells from systemic lupus erythematosus patients through the p53/p21 pathway. *Mol. Cell. Biochem.* 387, 27–37.

Gu Z, Tan W, Ji J, Feng G, Meng Y, Da Z, Guo G, Xia Y, Zhu X, Shi G & Cheng C (2016) Rapamycin reverses the senescent phenotype and improves immunoregulation of mesenchymal stem cells from MRL/lpr mice and systemic lupus erythematosus patients through inhibition of the mTOR signaling pathway. *Aging* 8, 1102–1114.

Günther C, Kind B, Reijns MAM, Berndt N, Martinez-Bueno M, Wolf C, Tüngler V, Chara O, Lee YA, Hübner N, Bicknell L, Blum S, Krug C, Schmidt F, Kretschmer S, Koss S, Astell KR, Ramantani G, Bauerfeind A, Morris DL, Cunninghame Graham DS, Bubeck D, Leitch A, Ralston SH, Blackburn EA, Gahr M, Witte T, Vyse TJ, Melchers I, Mangold E, Nöthen MM, Aringer M, Kuhn A, Lütke K, Unger L, Bley A, Lorenzi A, Isaacs JD, Alexopoulou D, Conrad K, Dahl A, Roers A, Alarcon-Riquelme ME, Jackson AP & Lee-Kirsch MA (2015) Defective removal of ribonucleotides from DNA promotes systemic autoimmunity. *J. Clin. Invest.* 125, 413–424.

Haigis MC & Yankner BA (2010) The aging stress response. *Mol. Cell* 40, 333–344.

Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sada S, Klotzle B, Bibikova M, Fan J-B, Gao Y, Deconde R, Chen M, Rajapakse I, Friend S, Ideker T & Zhang K (2013) Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol. Cell* 49, 359–367.

Haque S, Rakieh C, Marriage F, Ho P, Gorodkin R, Teh LS, Snowden N, Day PJR & Bruce IN (2013) Shortened telomere length in patients with systemic lupus erythematosus. *Arthritis Rheum.* 65, 1319–1323.

Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS, Pahor M, Javors MA, Fernandez E & Miller RA (2009) Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 460, 392–395.

Hedrich CM (2017) Epigenetics in SLE. *Curr. Rheumatol. Rep.* 19, 58.

Hedrich CM, Rauen T, Apostolidis SA, Grammatikos AP, Rodriguez Rodriguez N, Ioannidis C, Kyttaris VC, Crispin JC & Tsokos GC (2014) Stat3 promotes IL-10 expression in lupus T cells through trans-activation and chromatin remodeling. *Proc. Natl. Acad. Sci. U. S. A.* 111, 13457–13462.

Hill A, Sada J, LaBarge MA & Hurria A (2020) How cancer therapeutics cause accelerated aging: Insights from the hallmarks of aging. *J. Geriatr. Oncol.* 11, 191–193.

Hipp MS, Kasturi P & Hartl FU (2019) The proteostasis network and its decline in ageing. *Nat. Rev. Mol. Cell Biol.* 20, 421–435.

Hoffecker BM, Raffield LM, Kamen DL & Nowling TK (2013) Systemic lupus erythematosus and vitamin D deficiency are associated with shorter telomere length among African Americans: a case-control study. *PloS One* 8, e63725.

Honda M, Mengesha E, Albano S, Nichols WS, Wallace DJ, Metzger A, Klinenberg JR & Linker-Israeli M (2001) Telomere shortening and decreased replicative potential, contrasted by continued proliferation of telomerase-positive CD8⁺CD28(lo) T cells in patients with systemic lupus erythematosus. *Clin. Immunol. Orlando Fla* 99, 211–221.

Horvath S (2013) DNA methylation age of human tissues and cell types. *Genome Biol.* 14, R115.

Horvath S, Garagnani P, Bacalini MG, Pirazzini C, Salvioli S, Gentilini D, Di Blasio AM, Giuliani C, Tung S, Vinters HV & Franceschi C (2015) Accelerated epigenetic aging in Down syndrome. *Aging Cell* 14, 491–495.

Horvath S & Levine AJ (2015) HIV-1 Infection Accelerates Age According to the Epigenetic Clock. *J. Infect. Dis.* 212, 1563–1573.

Horvath S, Oshima J, Martin GM, Lu AT, Quach A, Cohen H, Felton S, Matsuyama M, Lowe D, Kabacik S, Wilson JG, Reiner AP, Maierhofer A, Flunkert J, Aviv A, Hou L, Baccarelli AA, Li Y, Stewart JD, Whitsel EA, Ferrucci L, Matsuyama S & Raj K (2018) Epigenetic clock for skin and blood cells applied to Hutchinson Gilford Progeria Syndrome and ex vivo studies. *Aging* 10, 1758–1775.

Horvath S, Pirazzini C, Bacalini MG, Gentilini D, Di Blasio AM, Delledonne M, Mari D, Arosio B, Monti D, Passarino G, De Rango F, D'Aquila P, Giuliani C, Marasco E, Collino S, Descombes P, Garagnani P & Franceschi C (2015) Decreased epigenetic age of PBMCs from Italian semi-supercentenarians and their offspring. *Aging* 7, 1159–1170.

Horvath S & Raj K (2018) DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat. Rev. Genet.*

Horvath S & Ritz BR (2015) Increased epigenetic age and granulocyte counts in the blood of Parkinson's disease patients. *Aging* 7, 1130–1142.

Hu S, Xu Q, Xiao W & Huang M (2006) The expression of molecular chaperone HSP90 and IL-6 in patients with systemic lupus erythematosus. *J. Huazhong Univ. Sci. Technol. Med. Sci. Hua Zhong Ke Ji Xue Xue Bao Yi Xue Ying Wen Ban Huazhong Keji Daxue Xuebao Yixue Yingdewen Ban* 26, 664–666.

Huan T, Chen G, Liu C, Bhattacharya A, Rong J, Chen BH, Seshadri S, Tanriverdi K, Freedman JE, Larson MG, Murabito JM & Levy D (2018) Age-associated microRNA expression in human peripheral blood is associated with all-cause mortality and age-related traits. *Aging Cell* 17.

Huang X, Su G, Wang Z, Shangguan S, Cui X, Zhu J, Kang M, Li S, Zhang T, Wu F & Wang L (2014) Hypomethylation of long interspersed nucleotide element-1 in peripheral mononuclear cells of juvenile systemic lupus erythematosus patients in China. *Int. J. Rheum. Dis.* 17, 280–290.

Huang Y, Wang H, Wan L, Lu X & Tam WWS (2016) Is Systemic Lupus Erythematosus Associated With a Declined Immunogenicity and Poor Safety of Influenza Vaccination?: A Systematic Review and Meta-Analysis. *Medicine (Baltimore)* 95, e3637.

Imgenberg-Kreuz J, Almlöf JC, Leonard D, Sjöwall C, Syvänen A-C, Rönnblom L, Sandling JK & Nordmark G (2019) Shared and Unique Patterns of DNA Methylation in Systemic Lupus Erythematosus and Primary Sjögren's Syndrome. *Front. Immunol.* 10, 1686.

Imgenberg-Kreuz J, Carlsson Almlöf J, Leonard D, Alexsson A, Nordmark G, Eloranta M-L, Rantapää-Dahlqvist S, Bengtsson AA, Jönsen A, Padyukov L, Gunnarsson I, Svenungsson E, Sjöwall C, Rönnblom L, Syvänen A-C & Sandling JK (2018) DNA methylation mapping identifies gene regulatory effects in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* 77, 736–743.

Inukai S, de Lencastre A, Turner M & Slack F (2012) Novel microRNAs differentially expressed during aging in the mouse brain. *PLoS One* 7, e40028.

Izmirly PM, Kim MY, Samanovic M, Fernandez-Ruiz R, Ohana S, Deonaraine KK, Engel AJ, Masson M, Xie X, Cornelius AR, Herati RS, Haberman RH, Scher JU, Guttmann A, Blank RB, Plotz B, Haj-Ali M, Banbury B, Stream S, Hasan G, Ho G, Rackoff P, Blazer AD, Tseng C-E, Belmont HM, Saxena A, Mulligan MJ, Clancy RM & Buyon JP (2021) Evaluation of Immune Response and Disease Status in SLE Patients Following SARS-CoV-2 Vaccination. *Arthritis Rheumatol. Hoboken NJ.*

Jagpal A & Navarro-Millán I (2018) Cardiovascular co-morbidity in patients with rheumatoid arthritis: a narrative review of risk factors, cardiovascular risk assessment and treatment. *BMC Rheumatol.* 2, 10.

Jahantigh D, Salimi S, Mousavi M, Moossavi M, Mohammadoo-Khorasani M, Naroocai-nejad M & Sandoughi M (2015) Association Between Functional Polymorphisms of DNA Double-Strand Breaks in Repair Genes XRCC5, XRCC6 and XRCC7 with the Risk of Systemic Lupus Erythematosus in South East Iran. *DNA Cell Biol.* 34, 360–366.

Javierre BM, Fernandez AF, Richter J, Al-Shahrouf F, Martin-Subero JI, Rodriguez-Ubreva J, Berdasco M, Fraga MF, O'Hanlon TP, Rider LG, Jacinto FV, Lopez-Longo FJ, Dopazo J, Forn M, Peinado MA, Carreño L, Sawalha AH, Harley JB, Siebert R, Esteller M, Miller FW & Ballestar E (2010) Changes in the pattern of DNA methylation associate with twin discordance in systemic lupus erythematosus. *Genome Res.* 20, 170–179.

Johnson SC, Rabinovitch PS & Kaeberlein M (2013) mTOR is a key modulator of ageing and age-related disease. *Nature* 493, 338–345.

Jylhävä J, Pedersen NL & Hägg S (2017) Biological Age Predictors. *EBioMedicine* 21, 29–36.

Kalim H, Pratama MZ, Mahardini E, Winoto ES, Krisna PA & Handono K (2020) Accelerated immune aging was correlated with lupus-associated brain fog in reproductive-age systemic lupus erythematosus patients. *Int. J. Rheum. Dis.*

Kapahi P, Chen D, Rogers AN, Katewa SD, Li PW-L, Thomas EL & Kockel L (2010) With TOR, less is more: a key role for the conserved nutrient-sensing TOR pathway in aging. *Cell Metab.* 11, 453–465.

Kaplan MJ, Lu Q, Wu A, Attwood J & Richardson B (2004) Demethylation of promoter regulatory elements contributes to perforin overexpression in CD4+ lupus T cells. *J. Immunol. Baltim. Md 1950* 172, 3652–3661.

Katayama Y & Kohriyama K (2001) Telomerase activity in peripheral blood mononuclear cells of systemic connective tissue diseases. *J. Rheumatol.* 28, 288–291.

Kato H & Perl A (2018) Blockade of Treg Cell Differentiation and Function by the Interleukin-21-Mechanistic Target of Rapamycin Axis Via Suppression of Autophagy in Patients With Systemic Lupus Erythematosus. *Arthritis Rheumatol. Hoboken NJ* 70, 427–438.

Kato H & Perl A (2014) Mechanistic target of rapamycin complex 1 expands Th17 and IL-4+ CD4-CD8- double-negative T cells and contracts regulatory T cells in systemic lupus erythematosus. *J. Immunol. Baltim. Md 1950* 192, 4134–4144.

Katz PP, Andrews J, Yazdany J, Schmajuk G, Trupin L & Yelin E (2017) Is frailty a relevant concept in SLE? *Lupus Sci. Med.* 4, e000186.

Kaushik S & Cuervo AM (2015) Proteostasis and aging. *Nat. Med.* 21, 1406–1415.

Kayser C, Alberto FL, da Silva NP & Andrade LEC (2004) Decreased number of T cells bearing TCR rearrangement excision circles (TREC) in active recent onset systemic lupus erythematosus. *Lupus* 13, 906–911.

Kenderov A, Minkova V, Mihailova D, Giltiay N, Kyurkchiev S, Kehayov I, Kazatchkine M, Kaveri S & Pashov A (2002) Lupus-specific kidney deposits of HSP90 are associated with altered IgG idiotypic interactions of anti-HSP90 autoantibodies. *Clin. Exp. Immunol.* 129, 169–176.

Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, Franceschi C, Lithgow GJ, Morimoto RI, Pessin JE, Rando TA, Richardson A, Schadt EE, Wyss-Coray T & Sierra F (2014) Geroscience: linking aging to chronic disease. *Cell* 159, 709–713.

Klapper W, Moosig F, Sotnikova A, Qian W, Schröder JO & Parwaresch R (2004) Telomerase activity in B and T lymphocytes of patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* 63, 1681–1683.

Kowald A & Kirkwood TBL (2016) Can aging be programmed? A critical literature review. *Aging Cell* 15, 986–998.

Kurosaka D, Ozawa Y, Yasuda J, Yamada A, Akiyama M, Saito S & Tajima N (2001) Telomerase activity in peripheral blood mononuclear cells from patients with SLE. *Ann. Rheum. Dis.* 60, 1158–1159.

Kurosaka D, Yasuda J, Yoshida K, Yokoyama T, Ozawa Y, Obayashi Y, Kingetsu I, Saito S & Yamada A (2003) Telomerase activity and telomere length of peripheral blood mononuclear cells in SLE patients. *Lupus* 12, 591–599.

Kurosaka D, Yasuda J, Yoshida K, Yoneda A, Yasuda C, Kingetsu I, Toyokawa Y, Yokoyama T, Saito S & Yamada A (2006) Abnormal telomerase activity and telomere length in T and B cells from patients with systemic lupus erythematosus. *J. Rheumatol.* 33, 1102–1107.

Kyaw T, Tay C, Khan A, Dumouchel V, Cao A, To K, Kehry M, Dunn R, Agrotis A, Tipping P, Bobik A & Toh B-H (2010) Conventional B2 B cell depletion ameliorates whereas its adoptive transfer aggravates atherosclerosis. *J. Immunol. Baltim. Md 1950* 185, 4410–4419.

Lai Z-W, Borsuk R, Shadakshari A, Yu J, Dawood M, Garcia R, Francis L, Tily H, Bartos A, Faraone SV, Phillips P & Perl A (2013) Mechanistic target of rapamycin activation triggers IL-4 production and necrotic death of double-negative T cells in patients with systemic lupus erythematosus. *J. Immunol. Baltim. Md 1950* 191, 2236–2246.

Lai Z-W, Hanczko R, Bonilla E, Caza TN, Clair B, Bartos A, Miklossy G, Jimah J, Doherty E, Tily H, Francis L, Garcia R, Dawood M, Yu J, Ramos I, Coman I, Faraone SV, Phillips PE & Perl A (2012) N-acetylcysteine reduces disease activity by blocking mammalian target of rapamycin in T cells from systemic lupus erythematosus patients: a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum.* 64, 2937–2946.

Lai Z-W, Kelly R, Winans T, Marchena I, Shadakshari A, Yu J, Dawood M, Garcia R, Tily H, Francis L, Faraone SV, Phillips PE & Perl A (2018) Sirolimus in patients with clinically active systemic lupus erythematosus resistant to, or intolerant of, conventional medications: a single-arm, open-label, phase 1/2 trial. *Lancet Lond. Engl.* 391, 1186–1196.

Lal G, Zhang N, van der Touw W, Ding Y, Ju W, Bottinger EP, Reid SP, Levy DE & Bromberg JS (2009) Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. *J. Immunol. Baltim. Md 1950* 182, 259–273.

Laplane M & Sabatini DM (2012) mTOR signaling in growth control and disease. *Cell* 149, 274–293.

Lee S-Y, Moon S-J, Kim E-K, Seo H-B, Yang E-J, Son H-J, Kim J-K, Min J-K, Park S-H & Cho M-L (2017) Metformin Suppresses Systemic Autoimmunity in Roquinsan/san Mice through Inhibiting B Cell Differentiation into Plasma Cells via Regulation of AMPK/mTOR/STAT3. *J. Immunol. Baltim. Md 1950* 198, 2661–2670.

Lee YH, Choi SJ, Ji JD & Song GG (2016) Overall and cause-specific mortality in systemic lupus erythematosus: an updated meta-analysis. *Lupus* 25, 727–734.

Legge A, Kirkland S, Rockwood K, Andreou P, Bae S-C, Gordon C, Romero-Diaz J, Sanchez-Guerrero J, Wallace DJ, Bernatsky S, Clarke AE, Merrill JT, Ginzler EM, Fortin P, Gladman DD, Urowitz MB, Bruce IN, Isenberg DA, Rahman A, Alarcón GS, Petri M, Khamashta MA, Dooley MA, Ramsey-Goldman R, Manzi S,

Zoma AA, Aranow C, Mackay M, Ruiz-Irastorza G, Lim SS, Inanc M, van Vollenhoven RF, Jonsen A, Nived O, Ramos-Casals M, Kamen DL, Kalunian KC, Jacobsen S, Peschken CA, Askanase A & Hanly JG (2020) Construction of a Frailty Index as a Novel Health Measure in Systemic Lupus Erythematosus. *J. Rheumatol.* 47, 72–81.

Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, Hou L, Baccarelli AA, Stewart JD, Li Y, Whitsel EA, Wilson JG, Reiner AP, Aviv A, Lohman K, Liu Y, Ferrucci L & Horvath S (2018) An epigenetic biomarker of aging for lifespan and healthspan. *Aging* 10, 573–591.

Li X, Liu L, Meng D, Wang D, Zhang J, Shi D, Liu H, Xu H, Lu L & Sun L (2012) Enhanced apoptosis and senescence of bone-marrow-derived mesenchymal stem cells in patients with systemic lupus erythematosus. *Stem Cells Dev.* 21, 2387–2394.

Liang Y, Liu C, Lu M, Dong Q, Wang Z, Wang Z, Xiong W, Zhang N, Zhou J, Liu Q, Wang X & Wang Z (2018) Calorie restriction is the most reasonable anti-ageing intervention: a meta-analysis of survival curves. *Sci. Rep.* 8, 5779.

Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D & Abete P (2018) Oxidative stress, aging, and diseases. *Clin. Interv. Aging* 13, 757–772.

Lin Y-J, Lan Y-C, Wan L, Huang C-M, Lin C-W, Hsueh K-C, Chen D-Y, Lin T-H & Tsai F-J (2010) The NBS1 genetic polymorphisms and the risk of the systemic lupus erythematosus in Taiwanese patients. *J. Clin. Immunol.* 30, 643–648.

Lin Y-J, Wan L, Huang C-M, Chen S-Y, Huang Y-C, Lai C-H, Lin W-Y, Liu H-P, Wu Y-S, Chen C-M, Tsai Y-H, Tsai C-H, Sheu J-J & Tsai F-J (2009) Polymorphisms in the DNA repair gene XRCC1 and associations with systemic lupus erythematosus risk in the Taiwanese Han Chinese population. *Lupus* 18, 1246–1251.

Liu Y & Kaplan MJ (2018) Cardiovascular disease in systemic lupus erythematosus: an update. *Curr. Opin. Rheumatol.* 30, 441–448.

Long H, Yin H, Wang L, Gershwin ME & Lu Q (2016) The critical role of epigenetics in systemic lupus erythematosus and autoimmunity. *J. Autoimmun.* 74, 118–138.

Longo VD, Antebi A, Bartke A, Barzilai N, Brown-Borg HM, Caruso C, Curiel TJ, de Cabo R, Franceschi C, Gems D, Ingram DK, Johnson TE, Kennedy BK, Kenyon C, Klein S, Kopchick JJ, Lepperdinger G, Madeo F, Mirisola MG, Mitchell JR, Passarino G, Rudolph KL, Sedivy JM, Shadel GS, Sinclair DA, Spindler SR, Suh Y, Vijg J, Vinciguerra M & Fontana L (2015) Interventions to Slow Aging in Humans: Are We Ready? *Aging Cell* 14, 497–510.

López-López L, Nieves-Plaza M, Castro M del R, Font YM, Torres-Ramos CA, Vilá LM & Ayala-Peña S (2014) Mitochondrial DNA damage is associated with damage accrual and disease duration in patients with systemic lupus erythematosus. *Lupus* 23, 1133–1141.

López-Otín C, Blasco MA, Partridge L, Serrano M & Kroemer G (2013) The hallmarks of aging. *Cell* 153, 1194–1217.

Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, Hou L, Baccarelli AA, Li Y, Stewart JD, Whitsel EA, Assimes TL, Ferrucci L & Horvath S (2019) DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging* 11, 303–327.

Lu X, Wang Y, Zhang J, Pu D, Hu N, Luo J, An Q & He L (2021) Patients with systemic lupus erythematosus face a high risk of cardiovascular disease: A systematic review and Meta-analysis. *Int. Immunopharmacol.* 94, 107466.

Luo Y, Lu X & Xie H (2014) Dynamic Alu methylation during normal development, aging, and tumorigenesis. *BioMed Res. Int.* 2014, 784706.

Maegawa S, Lu Y, Tahara T, Lee JT, Madzo J, Liang S, Jelinek J, Colman RJ & Issa J-PJ (2017) Caloric restriction delays age-related methylation drift. *Nat. Commun.* 8, 539.

Maierhofer A, Flunkert J, Oshima J, Martin GM, Haaf T & Horvath S (2017) Accelerated epigenetic aging in Werner syndrome. *Aging* 9, 1143–1152.

Manzi S, Meilahn EN, Rairie JE, Conte CG, Medsger TA, Jansen-McWilliams L, D’Agostino RB & Kuller LH (1997) Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. *Am. J. Epidemiol.* 145, 408–415.

Marioni RE, Harris SE, Shah S, McRae AF, von Zglinicki T, Martin-Ruiz C, Wray NR, Visscher PM & Deary IJ (2016) The epigenetic clock and telomere length are independently associated with chronological age and mortality. *Int. J. Epidemiol.*

Marioni RE, Shah S, McRae AF, Chen BH, Colicino E, Harris SE, Gibson J, Henders AK, Redmond P, Cox SR, Pattie A, Corley J, Murphy L, Martin NG, Montgomery GW, Feinberg AP, Fallin MD, Multhaup ML, Jaffe AE, Joehanes R, Schwartz J, Just AC, Lunetta KL, Murabito JM, Starr JM, Horvath S, Baccarelli AA, Levy D, Visscher PM, Wray NR & Deary IJ (2015) DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biol.* 16, 25.

Mathian A, Pha M, Haroche J, Cohen-Aubart F, Hié M, Pineton de Chambrun M, Boutin THD, Miyara M, Gorochov G, Yssel H, Cherin P, Devilliers H & Amoura Z (2020) Withdrawal of low-dose prednisone in SLE

patients with a clinically quiescent disease for more than 1 year: a randomised clinical trial. *Ann. Rheum. Dis.* 79, 339–346.

McConnell JR, Crockard AD, Cairns AP & Bell AL (2002) Neutrophils from systemic lupus erythematosus patients demonstrate increased nuclear DNA damage. *Clin. Exp. Rheumatol.* 20, 653–660.

McCurdy D, Tai LQ, Frias S & Wang Z (1997) Delayed repair of DNA damage by ionizing radiation in cells from patients with juvenile systemic lupus erythematosus and rheumatoid arthritis. *Radiat. Res.* 147, 48–54.

Mendelsohn AR & Larrick JW (2017) Epigenetic Drift Is a Determinant of Mammalian Lifespan. *Rejuvenation Res.* 20, 430–436.

Morel L (2017) Immunometabolism in systemic lupus erythematosus. *Nat. Rev. Rheumatol.* 13, 280–290.

Moskalev AA, Shaposhnikov MV, Plyusnina EN, Zhavoronkov A, Budovsky A, Yanai H & Fraifeld VE (2013) The role of DNA damage and repair in aging through the prism of Koch-like criteria. *Ageing Res. Rev.* 12, 661–684.

Muthukumar AR, Jolly CA, Zaman K & Fernandes G (2000) Calorie restriction decreases proinflammatory cytokines and polymeric Ig receptor expression in the submandibular glands of autoimmune prone (NZB x NZW)F1 mice. *J. Clin. Immunol.* 20, 354–361.

Nakkuntod J, Avihingsanon Y, Mutirangura A & Hirankarn N (2011) Hypomethylation of LINE-1 but not Alu in lymphocyte subsets of systemic lupus erythematosus patients. *Clin. Chim. Acta Int. J. Clin. Chem.* 412, 1457–1461.

Nakkuntod J, Sukkapan P, Avihingsanon Y, Mutirangura A & Hirankarn N (2013) DNA methylation of human endogenous retrovirus in systemic lupus erythematosus. *J. Hum. Genet.* 58, 241–249.

Napirei M, Karsunky H, Zevnik B, Stephan H, Mannherz HG & Möröy T (2000) Features of systemic lupus erythematosus in Dnase1-deficient mice. *Nat. Genet.* 25, 177–181.

Nie Y, Lau C, Lie A, Chan G & Mok M (2010) Defective phenotype of mesenchymal stem cells in patients with systemic lupus erythematosus. *Lupus* 19, 850–859.

Niu H, Fang G, Tang Y, Xie L, Yang H, Morel L, Diamond B & Zou Y-R (2013) The function of hematopoietic stem cells is altered by both genetic and inflammatory factors in lupus mice. *Blood* 121, 1986–1994.

Noren Hooten N, Fitzpatrick M, Wood WH, De S, Ejiogu N, Zhang Y, Mattison JA, Becker KG, Zonderman AB & Evans MK (2013) Age-related changes in microRNA levels in serum. *Ageing* 5, 725–740.

Nossent J, Cikes N, Kiss E, Marchesoni A, Nasonova V, Mosca M, Olesinska M, Pokorny G, Rozman B, Schneider M, Vlachoyiannopoulos PG & Swaak A (2007) Current causes of death in systemic lupus erythematosus in Europe, 2000–2004: relation to disease activity and damage accrual. *Lupus* 16, 309–317.

O'Donovan A, Cohen BE, Seal KH, Bertenthal D, Margaretten M, Nishimi K & Neylan TC (2015) Elevated risk for autoimmune disorders in Iraq and Afghanistan veterans with posttraumatic stress disorder. *Biol. Psychiatry* 77, 365–374.

Onken B & Driscoll M (2010) Metformin induces a dietary restriction-like state and the oxidative stress response to extend *C. elegans* lifespan via AMPK, LKB1, and SKN-1. *PLoS One* 5, e8758.

Pal S & Tyler JK (2016) Epigenetics and aging. *Sci. Adv.* 2, e1600584.

Peralta-Ramírez MI, Jiménez-Alonso J, Godoy-García JF, Pérez-García M, & Group Lupus Virgen de las Nieves (2004) The effects of daily stress and stressful life events on the clinical symptomatology of patients with lupus erythematosus. *Psychosom. Med.* 66, 788–794.

Pérez VI, Buffenstein R, Masamsetti V, Leonard S, Salmon AB, Mele J, Andziak B, Yang T, Edrey Y, Friguet B, Ward W, Richardson A & Chaudhuri A (2009) Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole-rat. *Proc. Natl. Acad. Sci. U. S. A.* 106, 3059–3064.

Perl A (2013) Oxidative stress in the pathology and treatment of systemic lupus erythematosus. *Nat. Rev. Rheumatol.* 9, 674–686.

Perna L, Zhang Y, Mons U, Holleczer B, Saum K-U & Brenner H (2016) Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. *Clin. Epigenetics* 8, 64.

Pugès M, Biscay P, Barnette T, Truchetet M-É, Richez C, Seneschal J, Gensous N, Lazaro E & Duffau P (2016) Immunogenicity and impact on disease activity of influenza and pneumococcal vaccines in systemic lupus erythematosus: a systematic literature review and meta-analysis. *Rheumatol. Oxf. Engl.* 55, 1664–1672.

Rakyan VK, Down TA, Maslau S, Andrew T, Yang T-P, Beyan H, Whittaker P, McCann OT, Finer S, Valdes AM, Leslie RD, Deloukas P & Spector TD (2010) Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. *Genome Res.* 20, 434–439.

Ridker PM, Everett BM, Pradhan A, MacFadyen JG, Solomon DH, Zaharris E, Mam V, Hasan A, Rosenberg Y, Iturriaga E, Gupta M, Tsigoulis M, Verma S, Clearfield M, Libby P, Goldhaber SZ, Seagle R, Ofori C, Saklayen M, Butman S, Singh N, Le May M, Bertrand O, Johnston J, Paynter NP, Glynn RJ, & CIRT Investigators (2019) Low-Dose Methotrexate for the Prevention of Atherosclerotic Events. *N. Engl. J. Med.* 380, 752–762.

Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, Kastelein JJP, Cornel JH, Pais P, Pella D, Genest J, Cifkova R, Lorenzatti A, Forster T, Kobalava Z,

Vida-Simiti L, Flather M, Shimokawa H, Ogawa H, Dellborg M, Rossi PRF, Troquay RPT, Libby P, Glynn RJ, & CANTOS Trial Group (2017) Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N. Engl. J. Med.* 377, 1119–1131.

Ripley BJ, Isenberg DA & Latchman DS (2001) Elevated levels of the 90 kDa heat shock protein (hsp90) in SLE correlate with levels of IL-6 and autoantibodies to hsp90. *J. Autoimmun.* 17, 341–346.

Roberts AL, Malspeis S, Kubzansky LD, Feldman CH, Chang S-C, Koenen KC & Costenbader KH (2017) Association of Trauma and Posttraumatic Stress Disorder With Incident Systemic Lupus Erythematosus in a Longitudinal Cohort of Women. *Arthritis Rheumatol. Hoboken NJ* 69, 2162–2169.

Rode L, Nordestgaard BG & Bojesen SE (2015) Peripheral blood leukocyte telomere length and mortality among 64,637 individuals from the general population. *J. Natl. Cancer Inst.* 107, djv074.

Roman MJ, Crow MK, Lockshin MD, Devereux RB, Paget SA, Sammaritano L, Levine DM, Davis A & Salmon JE (2007) Rate and determinants of progression of atherosclerosis in systemic lupus erythematosus. *Arthritis Rheum.* 56, 3412–3419.

Roman MJ, Shanker B-A, Davis A, Lockshin MD, Sammaritano L, Simantov R, Crow MK, Schwartz JE, Paget SA, Devereux RB & Salmon JE (2003) Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. *N. Engl. J. Med.* 349, 2399–2406.

Rondaan C, Furer V, Heijstek MW, Agmon-Levin N, Bijl M, Breedveld FC, D’Amelio R, Dougados M, Kapetanovic MC, van Laar JM, Ledefoged de Thurah A, Landewé R, Molto A, Müller-Ladner U, Schreiber K, Smolar L, Walker J, Warnatz K, Wulffraat NM, van Assen S & Elkayam O (2019) Efficacy, immunogenicity and safety of vaccination in adult patients with autoimmune inflammatory rheumatic diseases: a systematic literature review for the 2019 update of EULAR recommendations. *RMD Open* 5, e001035.

Scherlinger M, Mertz P, Sagez F, Meyer A, Felten R, Chatelus E, Javier R-M, Sordet C, Martin T, Korganow A-S, Guffroy A, Poindron V, Richez C, Truchetet M-E, Blanco P, Schaefferbeke T, Sibilia J, Devillers H & Arnaud L (2020) Worldwide trends in all-cause mortality of auto-immune systemic diseases between 2001 and 2014. *Autoimmun. Rev.*, 102531.

Schoenfeld SR, Kasturi S & Costenbader KH (2013) The epidemiology of atherosclerotic cardiovascular disease among patients with SLE: a systematic review. *Semin. Arthritis Rheum.* 43, 77–95.

Schultz MB & Sinclair DA (2016) When stem cells grow old: phenotypes and mechanisms of stem cell aging. *Dev. Camb. Engl.* 143, 3–14.

Selzer F, Sutton-Tyrrell K, Fitzgerald S, Tracy R, Kuller L & Manzi S (2001) Vascular stiffness in women with systemic lupus erythematosus. *Hypertens. Dallas Tex* 1979 37, 1075–1082.

Sen P, Shah PP, Nativio R & Berger SL (2016) Epigenetic Mechanisms of Longevity and Aging. *Cell* 166, 822–839.

Sfeir A (2012) Telomeres at a glance. *J. Cell Sci.* 125, 4173–4178.

Shimp SK, Chafin CB, Regna NL, Hammond SE, Read MA, Caudell DL, Rylander M & Reilly CM (2012) Heat shock protein 90 inhibition by 17-DMAG lessens disease in the MRL/lpr mouse model of systemic lupus erythematosus. *Cell. Mol. Immunol.* 9, 255–266.

Singh N, Traisak P, Martin KA, Kaplan MJ, Cohen PL & Denny MF (2014) Genomic alterations in abnormal neutrophils isolated from adult patients with systemic lupus erythematosus. *Arthritis Res. Ther.* 16, R165.

Skaggs BJ, Hahn BH & McMahon M (2012) Accelerated atherosclerosis in patients with SLE—mechanisms and management. *Nat. Rev. Rheumatol.* 8, 214–223.

Skamra C, Romero-Diaz J, Sandhu A, Huang Q, Lee J, Pearce W, McPherson DD, Sutton-Tyrrell K, Pope R & Ramsey-Goldman R (2013) Telomere length in patients with systemic lupus erythematosus and its associations with carotid plaque. *Rheumatol. Oxf. Engl.* 52, 1101–1108.

Smith-Vikos T & Slack FJ (2012) MicroRNAs and their roles in aging. *J. Cell Sci.* 125, 7–17.

Soares JP, Cortinhas A, Bento T, Leitão JC, Collins AR, Gaivã I & Mota MP (2014) Aging and DNA damage in humans: a meta-analysis study. *Aging* 6, 432–439.

Souliotis VL & Sfikakis PP (2015) Increased DNA double-strand breaks and enhanced apoptosis in patients with lupus nephritis. *Lupus* 24, 804–815.

Souliotis VL, Vlachogiannis NI, Pappa M, Argyriou A, Ntoulos PA & Sfikakis PP (2019) DNA Damage Response and Oxidative Stress in Systemic Autoimmunity. *Int. J. Mol. Sci.* 21.

Souliotis VL, Vougas K, Gorgoulis VG & Sfikakis PP (2016) Defective DNA repair and chromatin organization in patients with quiescent systemic lupus erythematosus. *Arthritis Res. Ther.* 18, 182.

Stagakis E, Bertias G, Verginis P, Nakou M, HatziaPOSTOLOU M, Kritikos H, Iliopoulos D & Boumpas DT (2011) Identification of novel microRNA signatures linked to human lupus disease activity and pathogenesis: miR-21 regulates aberrant T cell responses through regulation of PDCD4 expression. *Ann. Rheum. Dis.* 70, 1496–1506.

Sun D, Krishnan A, Su J, Lawrence R, Zaman K & Fernandes G (2004) Regulation of immune function by calorie restriction and cyclophosphamide treatment in lupus-prone NZB/NZW F1 mice. *Cell. Immunol.* 228, 54–65.

Sun F, Geng S, Wang H, Wang H, Liu Z, Wang X, Li T, Wan W, Lu L, Teng X, Morel L & Ye S (2020) Effects of metformin on disease flares in patients with systemic lupus erythematosus: post hoc analyses from two randomised trials. *Lupus Sci. Med.* 7, e000429.

Sun LY, Zhang HY, Feng XB, Hou YY, Lu LW & Fan LM (2007) Abnormality of bone marrow-derived mesenchymal stem cells in patients with systemic lupus erythematosus. *Lupus* 16, 121–128.

Surace AEA & Hedrich CM (2019) The Role of Epigenetics in Autoimmune/Inflammatory Disease. *Front. Immunol.* 10, 1525.

Suwannaroj S, Lagoo A, Keisler D & McMurray RW (2001) Antioxidants suppress mortality in the female NZB x NZW F1 mouse model of systemic lupus erythematosus (SLE). *Lupus* 10, 258–265.

Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N & Winchester RJ (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 25, 1271–1277.

Tan Q, Heijmans BT, Hjelmborg JVB, Soerensen M, Christensen K & Christiansen L (2016) Epigenetic drift in the aging genome: a ten-year follow-up in an elderly twin cohort. *Int. J. Epidemiol.* 45, 1146–1158.

Tang Y, Luo X, Cui H, Ni X, Yuan M, Guo Y, Huang X, Zhou H, de Vries N, Tak PP, Chen S & Shen N (2009) MicroRNA-146A contributes to abnormal activation of the type I interferon pathway in human lupus by targeting the key signaling proteins. *Arthritis Rheum.* 60, 1065–1075.

Te JL, Dozmorov IM, Guthridge JM, Nguyen KL, Cavett JW, Kelly JA, Bruner GR, Harley JB & Ojwang JO (2010) Identification of unique microRNA signature associated with lupus nephritis. *PLoS One* 5, e10344.

Tektonidou MG, Lewandowski LB, Hu J, Dasgupta A & Ward MM (2017) Survival in adults and children with systemic lupus erythematosus: a systematic review and Bayesian meta-analysis of studies from 1950 to 2016. *Ann. Rheum. Dis.* 76, 2009–2016.

Teng X, Brown J, Choi S-C, Li W & Morel L (2020) Metabolic determinants of lupus pathogenesis. *Immunol. Rev.*

Thewissen M, Somers V, Venken K, Linsen L, van Paassen P, Geusens P, Damoiseaux J & Stinissen P (2007) Analyses of immunosenescent markers in patients with autoimmune disease. *Clin. Immunol. Orlando Fla* 123, 209–218.

Thomas G, Mancini J, Jourde-Chiche N, Sarlon G, Amoura Z, Harlé J-R, Jouglu E & Chiche L (2014) Mortality associated with systemic lupus erythematosus in France assessed by multiple-cause-of-death analysis. *Arthritis Rheumatol. Hoboken NJ* 66, 2503–2511.

Thomas R, Wang W & Su D-M (2020) Contributions of Age-Related Thymic Involution to Immunosenescence and Inflammaging. *Immun. Ageing A* 17, 2.

Treaster SB, Ridgway ID, Richardson CA, Gaspar MB, Chaudhuri AR & Austad SN (2014) Superior proteome stability in the longest lived animal. *Age Dordr. Neth.* 36, 9597.

Troyer DA, Chandrasekar B, Barnes JL & Fernandes G (1997) Calorie restriction decreases platelet-derived growth factor (PDGF)-A and thrombin receptor mRNA expression in autoimmune murine lupus nephritis. *Clin. Exp. Immunol.* 108, 58–62.

Tselios K, Gladman DD, Sheane BJ, Su J & Urowitz M (2019) All-cause, cause-specific and age-specific standardised mortality ratios of patients with systemic lupus erythematosus in Ontario, Canada over 43 years (1971-2013). *Ann. Rheum. Dis.* 78, 802–806.

Tsokos GC (2011) Systemic lupus erythematosus. *N. Engl. J. Med.* 365, 2110–2121.

Tsokos GC, Lo MS, Costa Reis P & Sullivan KE (2016) New insights into the immunopathogenesis of systemic lupus erythematosus. *Nat. Rev. Rheumatol.* 12, 716–730.

Ulfah F, Octaviani H, Handono K & Kalim H (2019) T cell senescence and its correlation to inflammaging process of patients with systemic lupus erythematosus. *AIP Conf. Proc.* 2108, 020031.

Urowitz MB, Bookman AA, Koehler BE, Gordon DA, Smythe HA & Ogryzlo MA (1976) The bimodal mortality pattern of systemic lupus erythematosus. *Am. J. Med.* 60, 221–225.

Wang H, Li T, Chen S, Gu Y & Ye S (2015) Neutrophil Extracellular Trap Mitochondrial DNA and Its Autoantibody in Systemic Lupus Erythematosus and a Proof-of-Concept Trial of Metformin. *Arthritis Rheumatol. Hoboken NJ* 67, 3190–3200.

Wang Y & Hekimi S (2015) Mitochondrial dysfunction and longevity in animals: Untangling the knot. *Science* 350, 1204–1207.

Wang Y, Yuan Q & Xie L (2018) Histone Modifications in Aging: The Underlying Mechanisms and Implications. *Curr. Stem Cell Res. Ther.* 13, 125–135.

Warner LM, Adams LM & Sehgal SN (1994) Rapamycin prolongs survival and arrests pathophysiologic changes in murine systemic lupus erythematosus. *Arthritis Rheum.* 37, 289–297.

Weidner CI, Lin Q, Koch CM, Eisele L, Beier F, Ziegler P, Bauerschlag DO, Jöckel K-H, Erbel R, Mühleisen TW, Zenke M, Brummendorf TH & Wagner W (2014) Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biol.* 15, R24.

Williams EM, Bruner L, Penfield M, Kamen D & Oates JC (2014) Stress and Depression in Relation to

Functional Health Behaviors in African American Patients with Systemic Lupus Erythematosus. *Rheumatol. Sunnyvale Calif* 2014. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4662575/> [Accessed April 30, 2020].

Wing EJ (2016) HIV and aging. *Int. J. Infect. Dis. IJID Off. Publ. Int. Soc. Infect. Dis.* 53, 61–68.

Wu C-H, Hsieh S-C, Li K-J, Lu M-C & Yu C-L (2007) Premature telomere shortening in polymorphonuclear neutrophils from patients with systemic lupus erythematosus is related to the lupus disease activity. *Lupus* 16, 265–272.

Yan N (2017) Immune Diseases Associated with TREX1 and STING Dysfunction. *J. Interferon Cytokine Res. Off. J. Int. Soc. Interferon Cytokine Res.* 37, 198–206.

Yeung KS, Chung BH-Y, Choufani S, Mok MY, Wong WL, Mak CCY, Yang W, Lee PPW, Wong WHS, Chen Y-A, Grafodatskaya D, Wong RWS, Lau CS, Chan DTM, Weksberg R & Lau Y-L (2017) Genome-Wide DNA Methylation Analysis of Chinese Patients with Systemic Lupus Erythematosus Identified Hypomethylation in Genes Related to the Type I Interferon Pathway. *PLoS One* 12, e0169553.

Yin Y, Choi S-C, Xu Z, Perry DJ, Seay H, Croker BP, Sobel ES, Brusko TM & Morel L (2015) Normalization of CD4+ T cell metabolism reverses lupus. *Sci. Transl. Med.* 7, 274ra18.

Yurkovich M, Vostretsova K, Chen W & Aviña-Zubieta JA (2014) Overall and cause-specific mortality in patients with systemic lupus erythematosus: a meta-analysis of observational studies. *Arthritis Care Res.* 66, 608–616.

Zeng H, Cohen S, Guy C, Shrestha S, Neale G, Brown SA, Cloer C, Kishton RJ, Gao X, Youngblood B, Do M, Li MO, Locasale JW, Rathmell JC & Chi H (2016) mTORC1 and mTORC2 Kinase Signaling and Glucose Metabolism Drive Follicular Helper T Cell Differentiation. *Immunity* 45, 540–554.

Zhang Y, Zhao M, Sawalha AH, Richardson B & Lu Q (2013) Impaired DNA methylation and its mechanisms in CD4(+)T cells of systemic lupus erythematosus. *J. Autoimmun.* 41, 92–99.

Zhao M, Tang J, Gao F, Wu X, Liang Y, Yin H & Lu Q (2010) Hypomethylation of IL10 and IL13 promoters in CD4+ T cells of patients with systemic lupus erythematosus. *J. Biomed. Biotechnol.* 2010, 931018.

Zhao M, Zhou Y, Zhu B, Wan M, Jiang T, Tan Q, Liu Y, Jiang J, Luo S, Tan Y, Wu H, Renauer P, Del Mar Ayala Gutiérrez M, Castillo Palma MJ, Ortega Castro R, Fernández-Roldán C, Raya E, Faria R, Carvalho C, Alarcón-Riquelme ME, Xiang Z, Chen J, Li F, Ling G, Zhao H, Liao X, Lin Y, Sawalha AH & Lu Q (2016) IFI44L promoter methylation as a blood biomarker for systemic lupus erythematosus. *Ann. Rheum. Dis.* 75, 1998–2006.

Zheng Y, Joyce BT, Colicino E, Liu L, Zhang W, Dai Q, Shrubsole MJ, Kibbe WA, Gao T, Zhang Z, Jafari N, Vokonas P, Schwartz J, Baccarelli AA & Hou L (2016) Blood Epigenetic Age may Predict Cancer Incidence and Mortality. *EBioMedicine* 5, 68–73.

Zhu H, Mi W, Luo H, Chen T, Liu S, Raman I, Zuo X & Li Q-Z (2016) Whole-genome transcription and DNA methylation analysis of peripheral blood mononuclear cells identified aberrant gene regulation pathways in systemic lupus erythematosus. *Arthritis Res. Ther.* 18, 162.

Serment d'Hippocrate

Au moment d'être admise à exercer la médecine, je promets et je jure d'être fidèle aux lois de l'honneur et de la probité.

Mon premier souci sera de rétablir, de préserver ou de promouvoir la santé dans tous ses éléments, physiques et mentaux, individuels et sociaux.

Je respecterai toutes les personnes, leur autonomie et leur volonté, sans aucune discrimination selon leur état ou leurs convictions. J'interviendrai pour les protéger si elles sont affaiblies, vulnérables ou menacées dans leur intégrité ou leur dignité. Même sous la contrainte, je ne ferai pas usage de mes connaissances contre les lois de l'humanité.

J'informerai les patients des décisions envisagées, de leurs raisons et de leurs conséquences.

Je ne tromperai jamais leur confiance et n'exploiterai pas le pouvoir hérité des circonstances pour forcer les consciences.

Je donnerai mes soins à l'indigent et à quiconque me les demandera.

Je ne me laisserai pas influencer par la soif du gain ou la recherche de la gloire.

Admise dans l'intimité des personnes, je tairai les secrets qui me seront confiés. Reçue à l'intérieur des maisons, je respecterai les secrets des foyers et ma conduite ne servira pas à corrompre les mœurs.

Je ferai tout pour soulager les souffrances. Je ne prolongerai pas abusivement les agonies. Je ne provoquerai jamais la mort délibérément.

Je préserverai l'indépendance nécessaire à l'accomplissement de ma mission. Je n'entreprendrai rien qui dépasse mes compétences.

Je les entretiendrai et les perfectionnerai pour assurer au mieux les services qui me seront demandés.

J'apporterai mon aide à mes confrères ainsi qu'à leurs familles dans l'adversité.

Que les hommes et mes confrères m'accordent leur estime si je suis fidèle à mes promesses ; que je sois déshonorée et méprisée si j'y manque.

Résumé

Du fait de l'amélioration du diagnostic et de la prise en charge thérapeutique, l'espérance de vie des patients avec lupus érythémateux systémique (LES) a augmenté au cours des dernières décennies. Cependant, cette population est toujours caractérisée par une morbi-mortalité élevée. Certaines manifestations cliniques, plus fréquemment observées à un âge avancé en population générale telles que événements cardio-vasculaires, cancers ou infections, apparaissent fréquemment à un âge plus précoce, soulevant l'hypothèse d'une accélération du processus de vieillissement chez les patients avec LES. Dans la première partie de ce travail, nous avons analysé les données de la littérature concernant les sept piliers du vieillissement (*i.e.* dommages macromoléculaires, métabolisme, cellules souches, proteostasis, adaptation au stress, inflammation et épigénétique) chez les patients avec LES, et exploré les interconnexions possibles entre ces deux cadres. Dans la seconde partie de ce travail, nous avons appliqué des biomarqueurs de méthylation de l'ADN, qui sont les biomarqueurs du vieillissement les plus robustes actuellement, à des données de patients avec LES. Nous avons inclus dans notre analyse deux bases de données distinctes comprenant 30 et 55 individus respectivement. Pour chaque patient LES et chaque contrôle, nous avons calculé son âge épigénétique et une mesure d'accélération de l'âge biologique, selon le modèle d'horloge épigénétique d'Horvath. Nous n'avons pas observé d'argument en faveur d'une accélération du vieillissement biologique selon cette mesure chez les patients avec LES, puisqu'il n'y avait pas de différence statistiquement significative entre les mesures d'accélération de l'âge épigénétique des patients et des contrôles.

Abstract

Thanks to improvements in diagnosis, therapeutics and medical care, lifespan of systemic lupus erythematosus (SLE) patients has remarkably increased over the last decades. However, mortality ratio are still high in this population. Some clinical manifestations, more commonly found during old age in the general population such as cardiovascular events, cancers or infections, appear frequently at a younger age, raising the question of the presence of an acceleration of the aging process in SLE patients. In the first part of this work, we reviewed and compared literature evidence on the seven pillars of aging (*i.e.* macromolecular damage, metabolism, stem cells, proteostasis, adaptation to stress, inflammation and epigenetics) evaluated in SLE patients, and explored possible interconnections between these two frameworks. In the second part of this work, we applied DNA methylation biomarkers, which are the most robust biomarkers of age developed so far, in publicly available datasets of SLE patients. We included in our analysis two distinct DNA methylation datasets of 30 and 55 subjects respectively. For every SLE patient and control, we calculated their epigenetic age and a measure of epigenetic age acceleration, according to Horvath's epigenetic clock model. We did not observe any evidence of an accelerated biological aging according to this measure in SLE patients, as there was no statistically significant difference in the measure of epigenetic age acceleration between SLE patients and controls.