Title: Lipidomics in autoimmune diseases with main focus on Systemic Lupus Erythematosus

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Highlights:

Systemic Lupus Erythematosus is an inflammatory chronic disease that lacks tools for prognostics;

Lipids and oxidized lipids have important role in inflammation and disease progression in SLE;

Lipidomics as tool to identify new lipid biomarkers of SLE relapsing episodes;

Lipidomics to identify lipid biomarkers to ensure better patient care and personalized medicine;

Lipidomics in autoimmune diseases with main focus on Systemic Lupus Erythematosus

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Abstract

Autoimmune diseases (AID) are a heterogeneous group of disorders that have in common a chronic inflammation and dysregulation of the immune system. Systemic lupus erythematosus (SLE) is one of the most frequent systemic autoimmune diseases characterized by autoimmune phenomena in multiple organs. The tests used for evolution and prognosis assessment are either non-specific or non-sensitive, impairing an adequate therapeutics. To face this drawback, lipidomics is being used to provide more knowledge and insights regarding autoimmune disorders. Through lipidomic approaches using MS, it is possible to identify and quantify the level of lipid molecular species in the biological system and this could be useful to identify biomarkers and to better understand the pathophysiology of autoimmune diseases. There are some evidence that lipids and oxidized lipids can play a key role in AID pathogenesis. Although this field has been scarcely explored, there are some studies that reported variations on the lipid profile at a molecular level using lipidomic approaches based on MS in SLE. The results gathered herein showed changes mainly in the level of phospholipids, with decrease of some plasmenyl lipids, fatty acids, with reduction of PUFA, and sphingolipids, with changes in fatty acyl chain composition. These changes may be the result of lipids` modifications due to oxidation and increase of ROS. Some alterations can be associated with changes in membrane of lymphocytes and with the deregulation of the immune system. Thus, exploring the knowledge from modern lipidomic approaches in the study of the role of lipids and oxidized lipids, in oxidative stress and in inflammatory diseases, could contribute for the identification of new lipid biomarkers. Lipid biomarkers are promising tools to prognosis and treatment monitoring, tailored for the best therapeutic response and highest safety to ensure better patient care and to be used for personalized medicine.

Keywords Autoimmune diseases; systemic lupus erythematosus; lipidomics; mass spectrometry; biomarkers; lipid peroxidation.

Abbreviations: AA, arachidonic acid; ANA, antinuclear antibodies; APCs, antigen-presenting cells; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acids; FFA, free fatty acids; LPC, lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; LPL, lyso-phospholipid; mtDNA, mitochondrial DNA; NET, neutrophil extracellular traps; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, phospholipid; pPC, plasmenylphosphatidylcholine; pPE, plasmenylphosphatidylethanolamine; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; SLE, systemic lupus erythematosus; SM, sphingomyelin; TG, triglyceride;.

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1. Autoimmune diseases

Autoimmune diseases are a highly heterogeneous group of disorders that cover a wide range of pathologies. Worldwide, they are known to have an estimated total prevalence of 7.6-9.4% [1]. This type of diseases is extremely debilitating in their acute phases and patients have their autonomy decreased. These diseases occur when the immune system begins to attack the body itself, recognizing some of the molecules from the own organism, as a foreign and undesirable pathogen [2]. The increased production of those antigens will induce the production of antibodies present in plasma [3]. The autoimmune diseases` pathogenesis is not a straightforward process, and it has the contribution of several factors (environmental, genetic and hormonal), yet they all work together to disrupt the normal tolerance to the system's own antigens [4]. However, there are certain mechanisms that are common to every autoimmune disease such as: the recognition of the autoantigen as a foreign body (disease initiation), enhanced production of antibodies, amplification of the disease by including multiple pathways of the immune response, chronic inflammation and tissue destruction [5].

The diagnosis of autoimmune diseases is based on clinical and laboratory data, including serologic tests, the symptoms reported by the patient and the signs observed by the clinician [6]. However, the diagnosis is not always easy to obtain due to insufficient clinical data and specific markers to ensure a positive diagnosis. Under these circumstances, only the unfolding of the clinical situation will confirm if the autoimmune disease is being responsible for the signs and symptoms of the patient. Moreover, autoimmune diseases have diverse forms of manifesting themselves, which also increases the difficulty of the diagnosis [4,6].

Autoimmune disorders can be classified in two different ways: organ-specific or

systemic (Table 1) [7]. In organ-specific autoimmune diseases, the autoimmune response is directed against antigens that are expressed only in a particular organ. Autoantibodies bind to autoantigens in the organ cells and can lead either to their destruction, overstimulation or suppression of the normal cellular function. On the other side, in systemic autoimmune diseases the autoimmune response is directed against autoantigens scattered throughout the organism, which ends into a widespread tissue damage [8].

Table 1. Classification of autoimmune diseases: organ-specific autoimmunity *vs* systemic autoimmunity, and some examples of the most common ones.

Organ-specific autoimmunity	**Systemic autoimmunity**
Type 1 diabetes	Systemic lupus erythematosus
Autoimmune hepatitis	Sjögren`s syndrome
Hashimoto`s thyroiditis	Rheumatoid arthritis
Goodpasture`s syndrome	Scleroderma
Psoriasis	Systemic Sclerosis
Addison`s disease	Antiphospholipid syndrome
Pernicious anemia	Dermatomyositis
Myasthenia gravis	Vasculitis
Graves` disease	Ankylosing spondylitis
Vitiligo	Polymyositis

Autoimmune disorders are polygenic and each type of autoimmune disease have its distinct clinical phenotype, with specific physiopathology and specific symptoms [5]. Each type of autoimmune disease has a different development and their prognostics are also dissimilar [5]. A characteristic feature of autoimmune diseases is that they have relapse

and remission periods [6]. A relapse, or flare, is an acute reactivation of the disease, manifested by the worsening of pre-existing symptoms or the development of new ones. Flare periods occur when the disease activity is enhanced and can manifest themselves in a variety of forms. Usually, treatment during flare episodes, requires specific therapy to attenuate symptoms. Meanwhile, remission is a period of inactivity of the disease. There are cases that the disease becomes permanently inactive, which means, in total remission, however this disease stage is rarely achieved. On the other side, partial remission is more frequent [9]. Relapse periods can occur for no apparent reason. In order to avoid flares, it is vital for the patient to adopt preventive measures or prediction tools. It is advisable to make routine visits to clinicians to reassess the symptoms and the signs, as well as the analysis and examinations that have been carried out. This way the treatment can be readjusted whenever it is necessary. The patient should also have other precautions such as: avoid stress, rest properly and avoid exaggerated physical activity [6]. However, there is a lack of specific diagnostic tools or biomarkers that allow the prediction of the relapse periods. To prevent the appearance of these highly debilitating periods of disease, new studies are needed to understand the mechanism of disease and to find new biomarkers. This should allow tailoring early diagnosis of flare episodes and promote most effective treatment to each patient with a chronic autoimmune disease.

Due to the high complexity of autoimmune diseases, this paper will only focus on one very common autoimmune disease, systemic lupus erythematosus (SLE) [4].

2. Systemic lupus erythematosus

SLE is a chronic, systemic autoimmune disease and it is characterized by systemic inflammation in multiple organs such as joints, vessels, skin, kidneys and central nervous

system [10]. A particular characteristic of SLE is that pathogenic autoantibodies are produced by dysfunctional immunocompetent cells, leading to multiple organ injuries [10,11]. It is most prevalent in women in childbearing age with a very strong 9:1 female to male ratio and the major clinical features of this disease are fatigue and musculoskeletal symptoms [12,13]. Clinical manifestations and the prognostic of the disease are influenced by age of onset and gender [14]. The male gender is often related with higher levels of disease activity, regardless their age or race, at the time of diagnosis. However, during disease course, gender does not seem to dramatically influence the clinical manifestations [15]. The death rate is also different according to the gender; female SLE patients have a lower death rate than men. Patients` age is also important for the cause of death; for patients with ages between 20 and 39 years, the most common cause of death is musculoskeletal and comorbidities that develop as a consequence of SLE, while patients with more than 40 years of age commonly die due to any type of cardiovascular diseases or malignancy, caused by the normal ageing process in combination with the added inflammation due to SLE [16].

Diagnosis of SLE is based on the criteria established by the American College of Rheumatology and besides the immuno-pathological features, there is also a clinical laboratory profile that suggests that the patient suffers from SLE (Table 2) [9,17]. It comprises mainly full blood cell count (with a decrease of red blood cells, usually associated with anaemia, and/or white blood cells and/or platelets), inflammation parameters (such as increase of C-reactive protein and erythrocyte sedimentation rate) and immunological changes (as the presence of some antibodies, mainly IgG antinuclear antibodies) [9]. The changes in the biochemical parameters are found in other autoimmune diseases, only the immunological parameters are specific for SLE. These changes in

laboratory parameters are not detected in the same patient at the same time, it is frequent that only a few alterations of these parameters are seen at a particular time of the disease. Also, the lab test results can vary from patient to patient [6,9]. In fact, the diagnosis of SLE is somehow difficult in many cases, therefore the interpretation of the laboratory results should be made carefully and only by the attending physician that is aware of the patient`s medical record.

Table 2. Principal alterations on laboratory parameters observed in SLE disease.

Blood count	Inflammation parameters	Immunology
		↓ Complement components C3, C4 and CH50
↓ Red blood cells (haemolytic anaemia)	↑ Erythrocyte sedimentation rate	**Presence of**:
↓ White blood cells (lymphopenia)	↑ C-reactive protein	Antinuclear antibodies
↓ Platelets (thrombocytopenia)		Anti-DNA and anti-Sm antibodies
		Anti-SS-A and anti-SS-B antibodies
		Anti-RNP antibodies
		Antiphospholipid antibodies

The development of SLE seems to have a strong genetic predisposition to a dysregulation of the immune system. Hyperexpression of B lymphocyte activity is therefore a consequence of SLE [4,18]. This autoimmune disorder is thus also associated with a polyclonal hypergammaglobulinemia and with elevated titters of antibodies against several auto-antigens, in particular, nuclear antigens. Nonetheless, there are also other factors that contribute to trigger this disease, namely ultraviolet light exposure, cross reactivity with infectious agents, drug hypersensitivities and several stress stimuli [19–21].

Exposure to ultraviolet light, specially UVB, can contribute to the pathogenesis and exacerbation of SLE, since it induces apoptosis in keratinocytes and alterations in DNA. Sunlight exposition also promotes the production of pro-inflammatory molecules that combined with the cell death mechanisms describe the cutaneous and flare reactions typical of SLE [22]. Infectious agents, such as Epstein-Barr virus, can also influence the development of SLE and induce flare periods, triggering the disease by molecular mimicry. It is true that EBV can prompt autoimmune processes, however, SLE patients have an abnormal viral latency period and a dysregulated anti-EBV response[23–25]. All these factors will enable T lymphocytes to recognize peptides presented by antigen-presenting cells (APCs) [26]. This process is known as antigen presentation and promotes the release of cytokines, inflammation process and B lymphocyte stimulation. However, T lymphocyte malfunction could be the result of defective APCs. In fact there are some studies that reflect the dysfunction of APCs in autoimmune diseases [27–29]. B lymphocyte stimulation leads to the hyperproduction of IgG autoantibodies that are responsible for the tissue damage, which is a characteristic of SLE [18,26,30]. These IgG antinuclear antibodies (ANA) that are elevated in SLE target cellular nuclear components. There are several types of ANA and their identification is of utterly importance in SLE diagnosis. In SLE, the most commonly found ANA are anti-native (double stranded) DNA antibodies, although they are not specific of this disease [31].

Genetic factors are one of the major effectors of SLE susceptibility. The development of specific autoantibodies and SLE`s clinical features are genetically encoded, thus the determination of the specific genes that directly contribute to this disease would be a scientific breakthrough that would widely improve the knowledge of this autoimmune disorder. This topic is being intensely investigated and will not be discussed

in this paper (to further clarify this matter please check review article [14]). The development of SLE is also aggravated by epigenetic changes, namely the DNA methylation that is implicated in the pathogenesis of this disorder, although there is not much information regarding hydroxymethylation in this process [14].

Metabolic alterations are also found in patients with SLE; there is evidence that plasma, sera and urine of SLE patients have higher levels of reactive oxygen species (ROS) as well as 8-hydroxy-2`-deoxyguanosine, an oxidative DNA damage biomarker [32,33]. Also, first and second line of defences against mitochondrial ROS, or from other intracellular sources, are found to be decreased in SLE patients [33,34]. First line of cellular antioxidant system (manganese superoxide dismutase and copper/zinc superoxide dismutase), as well as second line of antioxidant enzymes (catalase, glutathione peroxidase 1 and glutathione peroxidase 4), are significantly lower in plasma/serum and in neutrophils/lymphocytes/leukocyte in SLE [33,34]. The disease activity and extent of organ damage are related with glutathione and the ratio of glutathione/oxidized glutathione, which are found to be downregulated in patients with SLE [34,35]. The glucose metabolism is also altered in lupus, in particular, it was found that leukocytes have lower levels of pyruvate dehydrogenase mRNA and of transcripts of mtDNA-encoded peptides and mitochondrial transcription factor A, which are fundamental for electron-chemical transport, oxidative phosphorylation and mtDNA replication [33]. It was likewise shown that the Krebs cycle in SLE patients is reduced, as well as the levels of the enzymes hexose kinase, glucose phosphate isomerase, phosphofructokinase and glyceraldehyde 3-phosphate dehydrogenase [33,36]. The alteration of DNA and glucose metabolism contributes to an inefficient immune system leading to the development of SLE.

In spite of the difficulties in diagnostics and prediction of relapsing periods, the survival rate of SLE patients has significantly improved over the last decades owing to some progress in early diagnosis. The detection of specific antibodies and the development of more efficient treatment strategies for both disease and its comorbidities revealed to be important for the improvement of SLE patients` quality of life. The detection of anti-double stranded DNA (anti-dsDNA) antibodies, ANA and complement activation are used to support either the diagnosis or the evaluation of disease activity of SLE [37]. Disease activity has a direct impact on the complications and associated comorbidities, depending whether it is more or less active. Side effects of immunosuppressant drugs are also a key factor for the development of complications in these patients. In fact, infections are one of the most frequent comorbidities in SLE patients and are responsible for a major contribution to the morbidity and mortality rate of this disorder [14]. People suffering from SLE showed higher probability to have infections of the nervous system and inflammatory bowel disease. Moreover, they have a substantial higher risk of developing cardiovascular complications (such as coronary heart disease and accelerated atherosclerosis) that may lead to the patient's death. Usually, the peak of occurrence of cardiovascular disease in SLE is observed 7 to 10 years after the diagnosis of the autoimmune disorder [38].

2.1 Diagnosis

As reported above, nowadays, SLE diagnostic is based on the assessment of the clinical symptoms and signals evaluated by the physician, and by laboratory tests. The laboratory tests more specific of SLE relies on the detection of specific antibodies, namely the detection of anti-dsDNA , ANA, anti-Sm and anti-RNP antibodies [7,39] The antibodies anti-Sm are detectable in SLE patients comprised in a percentage of 5 and 30% while anti-RNP are detectable in 25-47% of SLE patients. These antibodies are detected by counter

279 immunoelectrophoresis (CIE), immunoblot and ELISA, and are quite important in clinics.
280 Expression of anti-Sm is always associated with anti-RNP in patients with SLE. Although
281 its mechanism remains uncertain, they seem to have some modulatory effect on monocytes
282 [39,40].

283 Complement components activation are used to support either the diagnosis or the
284 evaluation of disease activity in SLE [37]. These laboratory tests are made to help
285 clinicians to better understand the patients' health status and to provide the patient an
286 accurate diagnosis, prognosis and treatment. It is of utmost importance to highlight the fact
287 that inadequate use of such tests can lead to misdiagnosis, inappropriate therapy and
288 needless health-care expenses [41]. The presence of specific autoantibodies is a key
289 parameter for the diagnosis of autoimmune diseases like SLE [42]. Throughout the years,
290 several diagnostic techniques have been developed for the detection of these specific
291 antibodies, always bearing in mind the improvement of the specificity and sensitivity of
292 the analysis. The main techniques used are immunofluorescence (IIF), enzyme-linked
293 immunosorbent assay (ELISA) and recombinant autoantigen technology.
294
295 These laboratory techniques have their own advantages and disadvantages; IIF is being
296 replaced by enzymatic immunoassays, ELISA`s requires autoantigens to have extremely
297 high purity and the technology of recombinant autoantigens is highly demanding. On the
298 other side, recombinant autoantigens are widely used for the diagnosis of autoimmune
299 diseases since it brought major developments onto diagnostic techniques. The same
300 progress is needed to be made with autoantibodies used as controls in diagnostic tests.
301 However, considering that autoimmune diseases are characterized by remission
302 (asymptomatic periods) and relapsing periods (with acute symptoms) that occur with

different degrees of severity, more reliable biomarkers are needed to predict, or early diagnose, these periods and to evaluate their degree of severity. They are also important to avoid disease related damage of affected organs, to decrease associated morbidity and to help evaluating treatment efficiency.

2.2 The role of lipids in SLE pathogenesis

Autoimmune diseases are strongly influenced by gene mutations and the immune system will be affected as a consequence. SLE pathogenesis is not only associated with genetic dysregulations but it has also been associated with alteration in lipid metabolism including lipid oxidation. In fact, lupus has been strongly associated with oxidative damage in different levels due to the increase of oxidative stress conditions, which can be correlated with mitochondrial dysfunction and cell death. Enhanced neutrophil death called NETosis is a characteristic event of lupus pathogenesis . Also, oxidative stress and lipid alteration and oxidation have been related with T lymphocyte dysfunctions, as well as to the systemic inflammation. In all of these events, lipids seem to play a fundamental role in their regulation.

2.2.1 Oxidative stress and lipids in SLE

Since autoimmune diseases have an effect on the innate immunity, response neutrophils, as they are the first line of defence of the immune system, will therefore be affected as well [43]. To eliminate pathogens, neutrophils produce superoxide anion, which is a precursor of several types of ROS. It has been reported that neutrophils in SLE are malfunctioning, which is associated with the increase of ROS production that is implicated in the pathogenesis of this disease [43–45]. In this way, uncontrolled or chronic production of ROS by neutrophils may lead to severe oxidative damage in several

biomolecules, in particular in lipids of the membranes [46,47]. Also, a very important finding was the fact that ROS production by neutrophils decreased in patients in relapsing phases when compared to patients in remission [43]. This reduction has also been seen in other autoimmune diseases such as multiple sclerosis, Behcet`s disease and Guillain-Barre syndrome [48–50], supporting the importance of neutrophils` dysfunction in autoimmune diseases. A possible explanation for this reduction could be the exhausted status of neutrophils due to overproduction of ROS during active phase of the disease [45]. However, Elloumi *et al* suggested that in neutrophils, after the oxidative burst and the production of ROS, there is a second stage with lower levels of ROS production in these immune cells, inferred by the observed decrease of malondialdehyde levels, a lipid peroxidation product, in neutrophils of patients with lupus [43]. They also observed that, within patients, malondialdehyde levels were higher in patients in relapsing than in patients in remission stages, which is indicative of higher levels of oxidative stress during aggressive periods of this disease. [43].

SLE is a multifactorial disorder, lipids and lipid metabolism have been correlated with this autoimmune disease in several ways. The overproduction and increase of ROS and oxidative stress is usually associated with lipid peroxidation and alteration in lipid metabolism [51]. Reaction of ROS with lipids in membranes can lead to the formation of lipid hydroperoxides that can be further degraded into small reactive carbonyl species, such as 4-hydroxynonenal (4-HNE), a toxic aldehyde that can react with proteins and modify their structure and function. The 4-HNE is proven to be significantly elevated in SLE [52]. People with SLE suffer from inflammation, oxidative stress and alteration of energy production pathways, alongside with a prothrombotic state and a disturbance in the lipid profile. Besides malondialdehyde and lipid peroxidation products, elevated levels

were also observed for gamma-glutamyl peptides, gamma-glutamyltransferase, leukotriene B4 and 5-hydroxyeicosatetraenoic acid [36]. Lipids` oxidation can also lead to the formation of other products such as F2 isoprostanes, formed by oxidation of arachidonic acid, and found in plasma and urine of SLE patients [53]. Isoprostanes are biomarkers of oxidative stress and are usually elevated in acute phases of the disease [51]. Overall, it is well accepted that oxidized lipids have a significant role in the pathogenesis of lupus, however their action is far from being completely elucidated.

2.2.2 NETosis and lipids in SLE

Neutrophils` dysfunction is also associated with their degradation through a specific activation-induced cell death process called NETosis, which is similar to apoptosis. The result of this process is the formation of a transient web-like organelle known as neutrophil extracellular trap (NET) [54]. The development of NET has been recognized as a significant mechanism in SLE`s pathogenesis, that is associated with NETosis, characteristic of this AID (Figure 1) [55]. NET is responsible for the release of active oxidative enzymes that will induce a proatherogenic mechanism. Those enzymes can modify HDL to such an extent that they lose their anti-inflammatory and vasoprotective properties and start failing in mediating reverse cholesterol transport [56]. This data is in agreement with the altered lipoprotein profile that is characteristic of SLE` patients and is going to be addressed further ahead.. Oxidized lipids, for example oxidized cardiolipin and oxidized phosphatidylethanolamine (PE), have been reported as important players in apoptosis and ferroptosis, it would be interesting to develop more research in order to understand the role of lipids and oxidized lipids in NETosis.

In addition to NETosis, apoptosis of SLE lymphocytes, mitochondrial dysfunctions, the enhancement of the inflammation cascade and the oxidative stress conditions that occur

in these patients also contribute for the multifactorial aspect of SLE. Dendritic cells (DC) are considered the professional APCs and their main function is to prime naïve T cells activation [57]. DC have been correlated with SLE pathogenesis, essentially in the induction and progression of this disease [58]. However, their action is not completely clear so far.

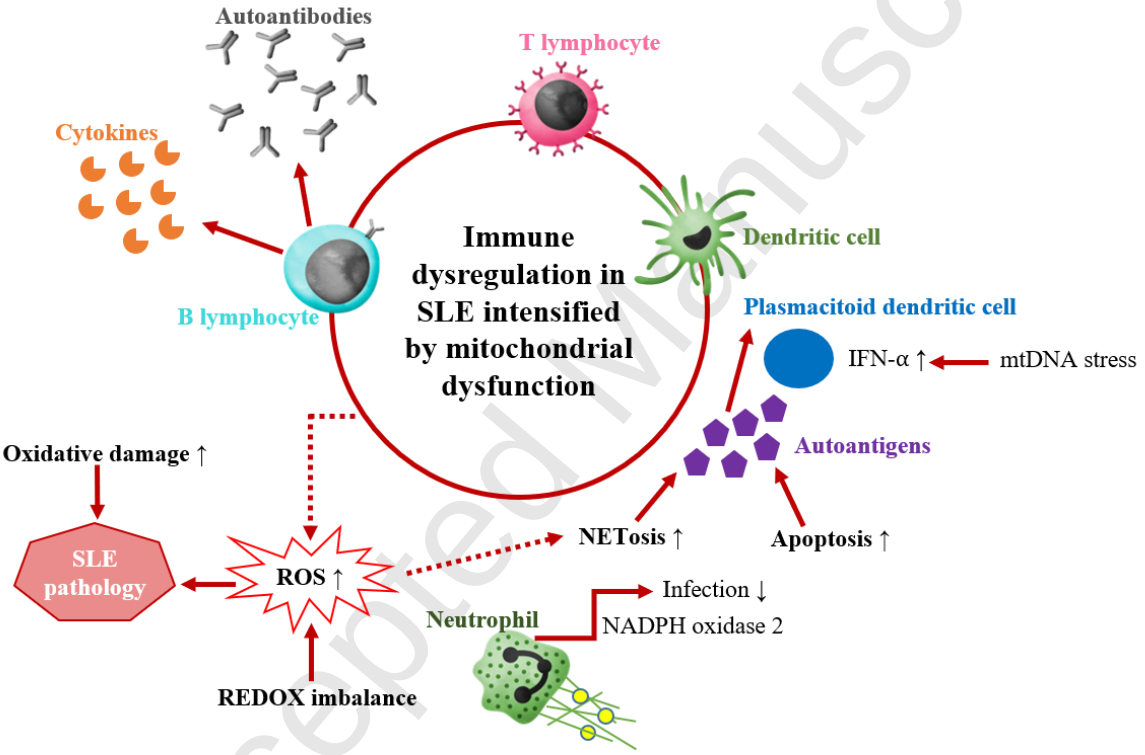

Figure 1. Schematic representation of NETosis` role in SLE pathogenesis. Under sufficient concentrations of ROS, a neutrophil undergoes NETosis to form NET, which is a web-like structure that contains nucleic acid and nucleic protein as well as cell remnants. NET is the source of several autoantigens. Those autoantigens will activate plasmacytoid dendritic cells to release IFN-α and consequently prompt autoimmune destruction. Adapted from H.T. Lee *et al* [11]. Cellular and autoantibodies pictures withdrawn from "BigPicture" [59].

2.2.3 T lymphocytes, mitochondrial dysfunction and lipids in SLE

The incorrect functioning of mitochondria in lupus T lymphocytes leads to an elevated mitochondrial transmembrane potential or persistent mitochondrial hyperpolarization [60]. This hyperpolarized state of mitochondria is found to be markedly higher in SLE patients than in healthy controls which may lead to cell death pathways [32,60,61].

Chronic inflammation is one key characteristic of SLE and it is exacerbated by oxidative stress and mitochondria dysfunction. Leishangthem *et* al found significantly increased levels of superoxide anion free radicals in mitochondria of lupus` patients which is suggestive of mitochondria dysfunction [61]. Lipids in mitochondria play an important role in controlling mitochondria functions; they are part of the membrane transport events; therefore, the lipid specific regulation of channel transport mechanisms is a decisive aspect related to membrane functions. Malfunctioning of lipid specific mitochondrial membrane transport may have a dangerous effects on cellular health status and raise cell based disease states [62]. T cell dysfunction in SLE have also been correlated with altered lipid metabolism [63]. It was determined that T lymphocytes of SLE patients have an altered glycosphingolipid profile in their membranes, specifically, lactosylceramide, globotriaosylceramide and monosialotetrahexosylganglioside levels were significantly increased when compared with healthy controls [64]. Although glycosphingolipids are not so abundant in cell membranes, they are very important in inflammation/immune response and cell-cell signalling.

There are some evidences that alterations in lipid profile and in lipid metabolism in these patients have an important role in SLE pathogenesis. Therefore, it is of utmost importance to improve the screening methodologies to evaluate the alterations in lipid

profile of SLE and other AID patients by using robust and accurate approaches. Omics platforms and lipidomics, in particular, are suitable tools to contribute to understand the role of dysregulation in lipid metabolism in SLE and also in other AID. Besides, lipidomics is also important to comprehend how these alterations are reflected in the complexity of the biologic processes and for fingerprinting lipid profile alterations for new potential biomarkers.

3. Lipidomics in SLE and in other autoimmune diseases

The identification of the lipid profile at a molecular level in biological systems is nowadays performed using lipidomic approaches which could be used to identify biomarkers and to understand the contribution of lipids in disease pathogenesis, useful for new therapeutic strategies. The main analytical technique in lipidomics is MS combined with LC or GC. MS is an extremely sensitive technique and it requires a very small quantity of sample. Lipidomics starts with sample collection followed by lipid extraction The lipid extract is analysed in a mass spectrometer to identify the individual lipid species, usually using a LC-MS approaches [65]. The acquired data is analysed by using bioinformatics tools and statistical analysis.

In spite of the lack of knowledge regarding the variation of the lipid profile at a molecular level in SLE, there is evidence that lipid metabolism suffers alterations namely in lipoprotein metabolism during the course of SLE disease [52,66–73].

435

436 Disturbance of lipoproteins levels was reported in patients with SLE. Dyslipidemia,
437 an imbalance of lipids in the blood, is known to have a significant influence in
438 atherogenesis [66]. Dyslipoproteinemia deregulation was observed in SLE, namely by low
439 levels of HDL cholesterol and elevated levels of both VLDL cholesterol and triglycerides
440 (TG) [67]. Lupus patients also show increased concentrations of oxidized LDL and an
441 anomalous chylomicron metabolism, which is consistent with the higher levels of 4-HNE
442 [52,68,69]. Levels of oxidized LDL detected by the monoclonal antibody E06 are
443 significantly higher in SLE patients and are associated with cardiovascular disease that
444 develop in SLE [74,75]. SLE`s dyslipoproteinemia has a multifactorial origin and it is yet
445 unclear which factors are definitely involved in the pathophysiology of this disorder [70].
446 Treatment using some drugs, in particular steroids, contribute to this dyslipoproteinemia
447 especially when they are administrated at a high dosage. Dislypidemia can also occur as a
448 consequence of renal failure, one of the most common comorbidities of SLE. Thus, renal
449 involvement and disease activity are some other factors that seem to have the most
450 influence on this characteristic lipoprotein pattern of SLE patients [71–73]. HDL is a cell
451 cholesterol efflux promoter through the reverse cholesterol transport system and prevents
452 LDL oxidation as well, thereby it has an atheroprotective role in the organism. NETosis, as
453 described above, may be a fundamental intervenient in the mechanism implicated in this
454 HDL dysregulation [76]. SLE patients have a much higher risk of developing
455 cardiovascular diseases and this high susceptibility may be partially due to impaired HDL
456 metabolism. In this way, the development of strategies to improve HDL metabolism could
457 have promising effects on the lipid metabolism, ameliorating patients` lipoprotein values
458 and disease management [77,78].

Disease activity is one factor that may have a leading role on the changes of the patients` lipid profile. Differences in the lipid profile between flare and remission need to be considered to better understand lupus lipid metabolism. So far, and to the best of our knowledge, there is only one study that evaluates the changes in lipoprotein values between flare and remission of adult patients with SLE, but no significant differences were observed [70]. The study only detected a tendency of lipoproteins` values during flare to be worse when compared with the same parameters during remission. Patients in flare had higher values of total cholesterol, LDL and TG and lower values of HDL comparing to the same lipoproteins' values in patients in remission[70]. The tendency to higher levels of LDL, main targets of ROS, would promote the enhance ox-LDL levels, with contribution to inflammation and atherogenesis risk factors for SLE relapsing and comorbidities.Lipoprotein dysregulation in SLE patients has been associated with high active phases of the disease. It is also considered a risk factor for the development of cardiovascular diseases, once they have an atherogenic lipoprotein profile [66]. Lipidomics is a high throughput method that allows to evaluate the variation of lipids at a molecular level and has been used for the study of several chronic diseases. It is now well known that changes in lipids at molecular level can occur as a consequence of metabolism adaptation in a disease environment therefore can be used as important biomarkers of the disease. Lipidomics at molecular level is important to disease pathology explanation, identification of changes in lipid metabolism, discovery of new biomarkers and definition of new treatment strategies [79]. The study of the variations on the lipid profile at a molecular level using lipidomics has been applied in some autoimmune diseases, for instance rheumatoid arthritis and multiple sclerosis, however little is known about this issue in SLE (Table 3). Nonetheless, there are already a few studies that provide some evidence

considering a possible adaptation of the lipid profile regarding FA, free FA (FFA), PL, lysoPL (LPL), sphingolipids, TG and 4-HNE species profiles in SLE patients.

Table 3. Lipidomics studies on AID. Research made on PubMed data base under the terms lipid profile, lipidomic(s), phospholipid(s) and fatty acid (all studies published until 2019 were analysed). All the studies that did not use MS techniques were not taken into consideration.

Autoimmune disease	Lipidomic approach	Class of lipids	Reference
Systemic Lupus Erythematosus	GC-MS; MDMS-SL	FA; FFA; PL; LPL; Sphingolipids; TG; 4-hydroxyalkenal species	[38,52,80–83]
Rheumatoid Arthritis	GC-MS; LC-MS/MS	FA; PL	[84–91]
Multiple Sclerosis	MALDI-TOF/TOF; GC-MS; ESI-MS; LC-MS	PL; FA; Sphingolipids; Very long chain FA	[92–97]
Systemic Sclerosis	LC-MS/MS	PL; LPL	[98]
Psoriasis	GC-MS; HPLC-MS	FA; Sphingolipids; PL	[99–103]
Polymyositis/ Dermatomyositis	GC-MS; LC-MS/MS	FA; TG; PL; LPL; Sphingolipids	[104]
Celiac disease	HPLC-MS; GC-MS	PL; FA	[105–107]

Lipidomics revealed a marked increase in TG, individual lyso--phosphatidylethanolamine (LPE) molecular species and total LPE levels, and a significant decrease in some species of PE and phosphatidylcholine (PC), as it is detailed (Table 4)[52,83]. Serum total concentration of TG were increased, with markedly differences in its FA composition. It was determined a raise in TG either with 16:1, 20:3, 20:2, 20:1 or

22:6 FA [52]. The most significantly increased LPE species included 20:4 and 22:6 FA [52]. All plasmenylphosphatidylethanolamine (pPE) and plasmenylphosphatidylcholine (pPC) species, which are a subclass of PE and PC respectively, were substantially reduced in SLE patients` serum [52]. The species that suffered the most statistically significant reduction were pPE species of 16:0-20:4 and 18:0-20:4 and pPC species of 18:0-18:0 and 16:0-18:2. The authors attributed the reduction of these species to three major metabolic pathways: decrease of phospholipase A_2 activation, peroxisomal dysfunction and peroxidation-mediated degradation. Oxidation of pPE is considered the major mechanism responsible for the reduction of pPE levels, which is corroborated by the increase of 4-HNE. It is important to consider that under oxidative stress conditions pPE species have an anti-oxidant role, which also supports the favourable oxidation and decrease of the content of its non-oxidized precursors. Another possible hypothesis proposed by the authors was that the activation of phospholipase A_2 was decreased, however, this hypothesis was discharged because there was not an accumulation of alkenyl LPE. The peroxisomal dysfunction hypothesis was also rejected since it appears to be normal in SLE patients, once plasmanylphosphatidylethanolamine and plasmanylphosphatidylcholine levels were unchanged [52]. pPE may become a biomarker for diagnosis/prognosis of SLE whereas its reduction is strongly associated with disease activity (SLEDAI), oxidative stress (ROS) and pro-inflammatory cytokines. It has also been reported an increase in plasma levels of PE species (16:0-18:2), (18:0-18:2), (18:1-18:2), (16:0-22:6) and (18:0-22:6), and also an increase of phosphatidylinositol (PI) species with (18:0-18:2) [83]. It was determined a reduction as well in other PE species such as PE (16:0-18:1), (18:1-20:4), (20:0-20:0) and (20:0-20:4), in PI species with (18:1-20:4) and a reduction in lyso-phosphatidylcholine (LPC), namely, LPC 18:2 [83].

The total content of sphingomyelin (SM) and ceramide species in SLE patients is not different from that found in healthy people. On the other side, it was described that the FA composition of both SM and ceramide species was modified in patients with SLE[83]. In SLE patients it was identified a significant increase in SM with N18:0, N18:1 and N22:0 comparing to the healthy control group ("N" stands for the amide linage of the acyl chain). In ceramide species, it was determined a significant decrease in ceramides with N22:0, N23:0 and N24:0 with a hydroxyl group on the second position of the acyl chain and a significant increase in N24:1[83].

FA composition of plasma and red blood cells can be changed by diet and steroids use, consequently, those alterations also affect circulating lipid profile and thus PL`s composition [38]. The variation of FA in PL have a direct impact on the fluidity of cell membrane of immune cells [108]. Moreover, changes in FA composition in PL of membranes of immune cells can affect the quantity and quality of essential FA, which are released by phospholipases and to be available for the production of inflammatory mediators that are released by these cells. Essential FA that are used in eicosanoid synthesis derive from PL pools. For instance, arachidonic acid (AA, 20:4*n*-6) that promotes production of inflammatory molecules, while eicosapentaenoic acid (EPA, 20:5*n*-3) is a precursor of anti-inflammatory mediators [109]. Plasma levels of FA depict recent dietary fat intake while red blood cells levels reflect longer dietary patterns. There are a few reports that suggest a relationship between FFA metabolism and SLE [37,110,111]. FFA can change their levels according with disease activity [112–114].

High levels of saturated FA and low concentrations of PUFA have been detected in plasma of SLE patients, which were correlated with autoimmunity and inflammatory processes [110]. It was also found that oleic acid and AA are decreased in patients with

lupus and that higher disease activity are usually associated with a lower level of linoleic acid [37]. Using LC-MS and GC-MS technologies, Wu *et al* found that medium-chain FAs and serum FFA were upregulated, while long-chain FAs (including ω-3 and ω-6 essential FA) were markedly downregulated, which is suggestive of oxidative conditions [115]. Most probably, the decrease of PUFA could be correlated with oxidation of the unsaturated FA due to the increase of ROS products associated with enhanced oxidative stress conditions. However, with GC-MS platforms, Shin and co-workers detected significantly higher levels of palmitoleic and oleic acids (known as anti-inflammatory FA) once they regulate the activation of immune cells (as well as myristic and eicosenoic acids). On the other side, they found markedly decreased concentrations of caproic, caprylic, linoleic, stearic, AA, eicosanoic, behenic, lignoceric and hexacosanoic acids [82]. In general, having in consideration the total content of FFA, saturated FFA levels were reduced and PUFA concentrations were increased [82]. On the contrary, it was also discovered that these patients have lower levels of EPA (which is an ω-3 PUFA) and a reduction of ω-3 index along with a significantly higher ratio of the inflammatory mediator AA to EPA, which is clearly favouring an inflammatory environment [38]. These findings are not in agreement with those reported before presenting the need for more studies in this field. Hereupon, alterations in FA profile in SLE may result from low dietary intake (however it cannot be generalized to every SLE patient due to body mass index differences), lipid peroxidation and/or defects in essential FA desaturation and elongation enzymatic reactions. However, there are controversial findings regarding the changes of FA profiles and its correlation with disease activity, which suggests that more studies are needed in this field of lipidomics [82,111]. Some of these MS studies present contradictory results which

may be due to the different methods used for lipid extraction, the type of analysed sample, the number of patients under study or even the chosen lipidomic approach (Table 4).

It seems that lipid metabolism in SLE can be improved with dietary fish oil supplementation, which have been shown to have lipid lowering effects. Fish oil has in its composition omega-3 PUFAs, specially EPA and DHA. EPA will promote the formation of anti-inflammatory mediators and thus having an anti-atherogenic and anti-inflammatory effect [116]. Therefore, fish oil supplementation is prone to balance immune, atherosclerotic and inflammatory events in patients suffering from lupus [117,118]. It was noticed an increase in EPA and DHA incorporation into cell membranes as well as a decrease in AA after fish oils supplementation, as well as the level of lipoproteins [80]. In fact, TG, VLDL concentrations and the ratio of total to HDL decreased, and it was detected a significant elevation of HDL levels in the group of SLE patients that were receiving fish oil supplementation [80]. However, the effects of fish oil supplementation are dependent of the dose administrated. Also, the administration of highly purified EPA alone has proved to have beneficial effects on the lipid profile of patients with SLE by decreasing the oxidative stress. After treatment, patients showed significantly increased levels of EPA, comparing with the pre-treatment period, as well as markedly decreased concentrations of AA [81].

Through multi-dimensional mass spectrometry-based shotgun lipidomics it was found an increase of the levels of 4-hydroxyalkenal species [52]. The elevation of 4-hydroxyalkenals levels alongside with the upregulation of LPE and the increased levels of oxidized LDL mention above, are undoubtedly indicative of lipid peroxidation [52,68]. Isoprostanes are biomarkers of lipid peroxidation formed through the non-enzymatic

peroxidation of AA. The urinary 8-isoprostane levels were determined to be significantly decreased in SLE patients` after the administration of the EPA treatment [81].

All of these studies clearly demonstrate the role of lipids in inflammatory processes, in immunity, and in the onset and development of AID. Also, lipidomics has potential as a tool to aid both in AID diagnosis and therapeutics by allowing a detailed lipidome profiling of SLE and AID patients, which is a first step for the identification of new lipid biomarkers of disease.

Table 4. Main lipid species that showed variation in SLE reported in published lipidomics studies, in PubMed data base, using MS approaches.

4. Concluding remarks and future perspectives

Lipids have a plethora of important biological functions and play key roles in many intra and intercellular signalling pathways that are related with the maintenance of cell and tissue homeostasis. Alterations in lipid profile and individual lipid molecular species are important players of several pathologies, including SLE and other AID. There are evidences that there are alterations in lipids and in lipids metabolism in SLE patients, showing the importance that lipid regulation has in SLE pathogenesis. SLE is usually associated with dyslipidemia. Some studies also reported alterations of some molecular lipid species, from particular classes of lipids, such as variation of plasmalogens and FA. Oxidized PL were also reported in few studies. Alteration in lipids seems to be a common issue in SLE, that has been scarcely addressed and are far from being completely elucidated. Therefore, it is of outmost importance to improve the screening methodologies

to evaluate the alterations in lipid profile of SLE, also useful in other AID. Lipidomics is nowadays the best methodological approach to understand the modulation of lipid metabolism in SLE, and advance knowledge on SLE`s pathology. Lipidomics is a promising tool to better understand autoimmune diseases` lipidome, enabling the identification of potential disease biomarkers. Thus, it would contribute to a more precise and early diagnosis, evaluation of disease progression, to predict relapse episodes, and evaluate therapy outcomes. Utmost, lipidomics approaches could be a promising tool to personalise medicine, allowing the reduction of the morbidity and mortality of SLE and other AID.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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945 **List of Tables**

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957 Figure 1. Schematic representation of NETosis` role in SLE pathogenesis. Under sufficient
958 concentrations of ROS, a neutrophil undergoes NETosis to form NET, which is a
959 web-like structure that contains nucleic acid and nucleic protein as well as cell
960 remnants. NET is the source of several autoantigens. Those autoantigens will activate
961 plasmacytoid dendritic cells to release IFN-α and consequently prompt autoimmune
962 destruction. Adapted from H.T. Lee *et al* [11]. Cellular and autoantibodies pictures
963 withdrawn from "BigPicture" [59]. 18
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970 *Table 4.* ***Main lipid species that showed variation in SLE reported in published lipidomics***
971 ***studies, in PubMed data base, using MS approaches.***

Reference	Analytical method	Lipid extraction method	Type of sample	Sample size	Results	
					↓ Reduction	↑ Increase
Aghdassi *et al* [38]	GC-MS	Chloroform/methanol (2:1, v/v)	Red blood cell total lipids (%) Plasma total lipids (%)	33 F	EPA, ω-3 index, total PUFA, total ω-6 Linoleic acid	AA/EPA plasma total trans-FA
Hu *et al* [52]	MDMS-SL	Modified Bligh and Dyer	Serum (nmol/mL serum)	30 F	PE species (16:0-18:1), (16:0-20:4), (18:0-20:4), (18:0-22:4), (18:0-22:5) PC species (18:0-18:0) and (16:0-18:2) TG with 18:2	LPE with 20:4 and 22:6 Total LPE content TG with 16:1, 20:3, 20:2, 20:1, 22:6 4-HNE
Clark *et al* [80]	GC-MS	Modified Bligh and Dyer	Platelet membrane phospholipids	8 F + 4 M	AA	EPA, DHA
Nakamura *et al* [81]	GC-MS	Folch *et al*	Plasma phospholipid fraction (mol%)	5 F + 1 M	Linoleic acid, AA, DHA	EPA/AA, EPA, DPA
Shin *et al* [82]	GC-MS	Paik *et al*	Plasma (FFA%)	41 F	Caproic, caprylic, linoleic, stearic, AA, eicosanoic, behenic, lignoceric and hexacosanoic acids	Myristic, palmitoleic, oleic, and eicosenoic acids
Lu *et al* [83]	MDMS-SL	Modified Bligh and Dyer	Serum (mol%)	30 F	PE species (16:0-18:1), (18:0-20:4), (18:1-20:4), (20:0-20:0), (20:0-20:4) PI species with (18:1-20:4) LPC with 18:2 Ceramides with N22:0, N23:0 and N24:0 with hydroxyl group on the 2nd position of the acyl chain	PE species (16:0-18:2), (18:0-18:2), (18:1-18:2), (16:0-22:6) and (18:0-22:6) PI species with (18:0-18:2) SM with N18:1 and N18:0 Ceramides with N24:1

F- Female; M-Male