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SYSTEMIC LUPUS ERYTHEMATOSUS THERAPY

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ABSTRACT

Systemic lupus erythematosus (SLE) is a prototypic, chronic, autoimmune disease, of variable severity with altering periods of flares and remissions and a multisystem character, predominately affecting women in a ratio 9:1 compared with men, especially in the reproductive years. Genetic predisposition is the main risk factor associated with the development and progression of the disease. SLE pathogenesis involves multiple hyperreactions of the immune system with overproduction of autoantibodies, directed against host-cells, tissues and organs. There present treatment of the disease is curative; it aims in the elimination of symptoms, improvement of the quality of life and delay of the disease progression.

The aim of this thesis is to provide information about SLE manifestation, demonstrate the risk factors and complications that influence the disease progression, with focus on its therapeutic approaches.

Keywords: Systemic lupus erythematosus, treatment, SLE, pharmacotherapy, autoimmune disease

ABSTRAKT

Systémový lupus erythematodes (SLE) je chronické autoimunitní onemocnění s různým stupněm závažnosti a střídajícími se periodami vzplanutí onemocnění a remisemi, které mají multisystémový charakter a postihují převážně ženy v poměru 9:1 oproti mužům, zejména produktivního věku. Za hlavní rizikový faktor spojený s rozvojem a progresí choroby je považována genetická predispozice. Patogeneze SLE zahrnuje mnohé patologické reakce imunitního systému, který ve zvýšené míře produkuje autoprotilátky, namířené proti buňkám, tkáním a orgánům. Současná léčba je zaměřena na zmírnění symptomů, zkvalitnění života a odklad progrese onemocnění.

Cílem této práce je poskytnout souhrn informací o SLE, manifestaci choroby, rizikových faktorech a komplikacích, které ovlivňují progresi onemocnění, se zaměřením na terapeutické přístupy.

Klíčová slova: systémový lupus erythematodes, léčba, SLE, farmakoterapie, autoimunitní choroba

DECLARATION

I declare that this thesis is my original copyrighted work. All literature and other resources I used while processing are listed in the bibliography and properly cited.

Author's signature

Mitsou Thomais

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I would like to dedicate this advanced master thesis to my parents, *Emmanouil Mitsou* and *Myranthi Mitsou* who have been my heroes. They have given me wings to fly and offered me the opportunity to reach my goals throughout life.

TABLE OF CONTENTS

1. INTRODUCTION

Systemic lupus erythematosus (SLE), also called the "disease with a thousand faces", is a prototypic, chronic, autoimmune disease, of variable severity with altering periods of flares and remissions and a multisystem character i.e. affecting various body parts including skin, musculoskeletal system, kidney, heart, blood, nervous system and reproductive system, mainly in women. The etymology of the disease originates from the Latin word for wolf *lupus* and erythro-, derived from the Greek word ερυθρός, meaning red. Both these terms are associated with the red, butterfly-shaped malar rash, typically demonstrated in the nose and cheeks of the patient with SLE, as it seemed to resemble a wolf bite. Systemic is used to emphasize on the multiple organ involvement of the disease. However, the name of the disease itself and its affiliation with such an aggressive and possible life-threatening wild animal has cause highly negative preoccupation on the disease and its possible outcomes. It is common that upon the sound of the diagnosis of lupus both patients and their social cycle can be misleaded in the belief that they are facing a disease as aggressive as a malignancy. Further education is significant for the society in order to clarify the actual dimension of the disease (American College of Rheumatology, 2018, Bertsias et al., 2012).

SLE predominately affects women in a ratio 9:1 compared with men, especially in the reproductive years with disease peak incidence rates between 16 and 55 years of age. By the end of the 20th century, the incidence of SLE diagnosis was increased; whereas its prevalence was not significantly altered, indicating the better and earlier diagnosis of the disease (Pons-Estel et al., 2010). Differences in the prevalence of SLE are distinguished among different geographical location and racial groups. SLE was less profound in Europe compared with USA and Australia, and in non-white Afro-Caribbean population (Rees et al., 2017). Mortality rates have shown encouraging data

referring in the life expectancy in SLE patients; approximately 93% survive 5 years, 85% survive for 10 years and 76% survive for 15 years (Cervena et al., 2009). Moreover, Lupus Foundation of America, stated that patients with effective and controlled therapy can lead a full life.

The pathogenesis of SLE includes multiple hyperreactions of the immune system with overproduction of autoantibodies, directed against host-cells, tissues and organs (Tsokos et al., 2007). The main risk factors for the development of the disease are female gender, especially during the reproductive years, genetic predisposition, epigenetic factors, environmental factors, certain infections and after specific medications that induce lupus-like symptoms (Dörner et al., 2011).

The most characteristic symptoms of SLE manifestation include fatigue, arthritis, malar rash, photosensitivity, leukopenia and in more severe occasions active lupus nephropathy, pericarditis, myocarditis, pleuritis, serositis, vasculitis, autoimmune hemolytic anemia, Raynaud's phenomenon etc. Moreover, SLE can coexist with relevant connective tissue diseases like rheumatoid arthritis, Sjögren's syndrome, scleroderma and dermomyositis (Bertsias et al., 2012).

SLE can occur only as discoid rash, that may or may not later have a systemic effect; also called Discoid Lupus Erythematosus. Furthermore, lupus passed from a childbearing woman to the fetus is known as Neonatal Lupus Erythematosus. Finally, besides the aforementioned drug-induced lupus, the arousal of neuropsychiatric manifestations is also called Neuropsychiatric Systemic Lupus Erythematosus (ACR, 2018).

Diagnostic methods used in the identification of SLE include the Antinuclear Antibody (ANA) test, anti-DNA antibodies (anti-dsDNA, anti-ssDNA), anti-ENA

antibodies (anti-Ro/SSA and anti-La/SSB, anti-Sm antibodies), complement levels. Furthermore, Antiphospholipid (aPL) antibodies (lupus anticoagulants, anticardiolipin antibodies etc) with possible existence of antiphospholipid syndrome (APS) that could lead in the formation of thromboses. Furthermore, a total blood count should be monitored revealing an organ-involvement (Gill et al., 2003, Bertsias et al., 2010).

The pharmaceutical management of SLE is adjusted in the amount of the systems involved and the severity of the disease. NSAIDs are used to treat mild-moderate general symptoms like arthralgias, myalgias, headache, fever etc. Corticosteroids are widely used orally alone or in combination in maintenance treatment and increased doses during disease flares, or in "pulse" high-dose in cases like LN. However, their long-term use should be avoided, due to potent dose-depended adverse effects (Kasturi et al., 2016). Antimalarials are also widely used due to their disease-modifying properties alone or in combination with corticosteroids and/or immunosuppressives in maintenance therapy, lupus flares and commonly in cutaneous lupus (Kalia et al., 2007). Immunosuppressive agents like cyclophosphamide, azathioprine, mycophenolate mofetil, cyclosporine, methotrexate and tacrolimus are administered during the onset of active lupus with major organ implications, although regular monitoring should take place due to some potent toxicities. Tacrolimus is also used in local preparations in lupus cutaneous disorders (Gurevitz et al., 2013). Interesting is the insertion of biologic agents, used in patients with active SLE receiving standard therapy. So far, belimumab is the only biologic agent approved in SLE therapy. Rituximab has been also suggested to be effective, although it has not been yet approved as a therapeutic in lupus due to some controversial results in various clinical trials. There are yet many potent and plausible drugs that are under research, with promising results in SLE pharmacotherapy (Lo et al., 2012).

2. THEORETICAL PART

2.1. THE IMMUNE SYSTEM

Immunity is defined as the ability of multicellular organisms to maintain adequate resistance to disease, infection, or unintended biological invasion, by harmful microorganisms. Moreover, immunity is also referred as the capability to refrain from allergic and autoimmune reactions (Abul et al., 2016). The *immune system* is constituted by numerous cells, tissues, as well as molecules, mediating resistance to any potential infection. Its function is to distinguish and diminish potentially threatening foreign molecules, also called *antigens*. In order to achieve this result, the immune system should be able to differentiate between antigens and self-molecules (Tao et al., 2016).

The immune system is divided into two major branches: the *innate (non-specific)* immunity and the *adaptive/acquired (specific)* immunity (Owen et al., 2013).

2.1.1. Innate (non-specific) immunity

Innate immunity is characterized as the first line defense against pathogens, providing immediate protection against microbial invasions (Janeway et al., 2005). Innate immunity is rapid, which means that it does not require a long start-up phase, and is broadly effective, but only to a certain degree.

Starting from the outside, the mucosal and epithelial linings of the respiratory and GI tracts, alongside with the skin, act as mechanical and chemical barriers against

germs (Hoebe et al., 2004). For instance, pathogens can be blocked from settling in the body due to the movements created by: hair-like structures in the nasal cavity, bronchi (cilia), and bowel muscles, as well as by the secretion of acids and enzymes (Hirayama et al., 2017).

In case microbes manage to enter the body, innate immunity is capable of destroying and excluding pathogens during the onset of the infection through an inflammatory response. Specifically, pathogen-associated molecular patterns (PAMPs) are obtained from pathogens or stresses, where pattern recognition receptors (PRRs) -found in the innate immune cells- can recognize them (Iwasaki et al., 2010). Furthermore, tissue damage, ischemia, or trauma, can trigger an immune response by the activation of damage-associated molecular patterns (DAMPs) whether there is a pathogenic infection involved, or not. Toll-like receptors (TLRs) are PRRs with a critical role in host cell recognition, responding to microbial pathogens (Schenten et al., 2011).

Phagocytes including macrophages, neutrophils and dendritic cells belong among the innate leukocytes, and are produced continuously by hematopoietic stem cells. They can form a bridge between specific bacterial surface antigens and cellular receptors, surround and engulf the bacteria, absorb them into the phagosome which is formed by the fusion of cell membranes (Iwasaki et al., 2010). **Macrophages** are able to recognize bacterial or viral components, like lipopolysaccharides (LPSs) or double-stranded RNA (dsRNA), secrete cytokines and start the process of phagocytosis, via the TLRs activation (Kawai et al., 2011). Both macrophages and dendritic cells serve as antigen-presenting cells (APCs), by presenting peptide antigens derived from digested pathogens, and thus stimulating acquired immunity through the activation of helper T cells. Macrophages present

antigens between the tissues, while **dendritic cells** present them in the lymph node and are the only ones that can activate naïve T cells into effector T cells (Hirayama et al., 2017). **Neutrophils** are the first cells reaching the site of infection and attack the pathogens by the activation of a respiratory burst, yielding in potent oxidizing agents. Moreover, **basophils** are histamine-releasing granulocytes, activated during an allergic reaction, while **eosinophils** function as strong anthelmintics by releasing toxic proteins against parasites, although they are implicated in tissue damage in allergic responses like asthma (Walsh et al., 2008, Wang et al., 2007). However, it has been revealed that eosinophils have a critical role in various facets of innate immunity, like manifestating tissue remodeling or connecting innate and adaptive immunity (Shamri et al., 2010). **Natural killer** (NK) cells can identify cells infected by a virus, or cells that became tumorous by searching for cell surface alterations. In case NK cells localize such cells, they release cytotoxins in order to destroy them (Bruns et al., 2014).

2.1.2. Adaptive (acquired) immunity

Adaptive immunity has a crucial role against pathogens that are able to resist innate immunity, and it is usually initiated four to seven days after the microbial invasion. It mainly consists of lymphocytes and their products, like B and T lymphocytes, antibodies as Y-shaped, soluble proteins in the bloodstream and cytokines. Substances identified by the adaptive immunity as hosts are called antigens (Abul et al., 2016). Lymphocytes of adaptive immunity have a high specificity, which refers to their ability to distinguish among millions of different antigens or antigen portions, compared to the innate immunity. This extraordinary

specificity of lymphocytes can be also called lymphocyte repertoire (Tao et al., 2016).

Adaptive immunity can be further divided into humoral immunity and cell-mediated immunity.

- **Humoral immunity** (antibody-mediated immunity) is created by circulating antibodies, generating specific immune responses to a specific foreign material. The name "humoral" refers to the medieval term of bodily fluids (Carroll M.C., 2012) Humoral immunity protects the extracellular spaces of the body, which is where the pathogens that entered the body multiply. Moreover, intracellular pathogens transfer from cell to cell via the extracellular space. The humoral immune response is initiated when a B cell recognizes antigens or pathogens in the lymph or bloodstream (Papenfuss et al., 2017) The antigens bind to B cells, and ILs or T_H cells costimulate B cells. In most cases, both a costimulatory and an antigen are required to activate a B cell and promote its proliferation. Plasma cells are produced, carrying antibodies with the identical antigen receptors of the activated B cells (Oracki et al., 2010). Afterwards, antibodies are released, in order to destroy pathogens. They can bind on the pathogen surface, neutralizing it, and thus preventing its entry to the cells (Abul et al., 2016). During *opsonization* the antibody-caught pathogens are exposed to phagocytosis by macrophages. When an antibody binds to a pathogen, the complement system is activated, where complement proteins bind to the antibody-bound pathogens and conscript phagocytic cells (Panawala, 2017).

- **Cell-mediated immunity** is mediated by antigen-specific T cells. Antigens should be exposed on the surface of APCs along with the major histocompatibility complexes (MHCs) markers, including cells invades by pathogens, transplanted cells and tumor cells (Kamperschroer et al., 2017). APCs and self-cells presenting foreign antigens bind to T cells and secrete ILs to further stimulate the T cell activation. Moreover, ILs can be also produced by helper T cells, in order to costimulate the activation of T cells (Playfair et al., 2017) If MHC class I and endogenous antigens are presented on the plasma membrane, T cells proliferate to produce cytotoxic T cells that destroy the infected cells by inducing apoptosis. Upon the event that MHC class II and exogenous antigens are presented on the plasma membrane, T cells proliferate to produce cytokines like ILs. These cytokines trigger B cells to produce antibodies that bind to the antigens by the B cell receptors and thus stimulate agents including NK cells and macrophages to attack and destroy the antigens (Piche et al., 2017).

In the table below are summarized the differences between humoral and cell-mediated immunity:

HUMORAL IMMUNITY	CELL-MEDIATED IMMUNITY
Refers to a component of the adaptive immunity where B cells secrete antibodies, which circulate in the blood as soluble proteins	Refers to the other component of adaptive immunity, which is mediated by the activated, antigen-specific T cells
Mediated by B cells	Mediated by T cells
Recruits T cells, B cells and macrophages	Recruits helper T cells, cytotoxic T cells, NK cells and macrophages
Acts on extracellular microbes and their toxins	Acts on intracellular microbes like bacteria, viruses, tumor cells and parasites
Involves BCR receptors	Involves TCR receptors
Igα, Igβ, CD40, CD21 and Fc receptors are the accessory receptors	CD2, CD3, CD4, CD8, CD28 and integrins are the accessory receptors
Recognizes unprocessed antigens	Antigens are processed and presented by MHC complexes
Plasma B cells secrete antibodies	T cells secrete cytokines
Rapid	A delayed hypersensitivity type
Does not act on tumor cells and transplants	Acts on tumor cells and transplants

Table 1 – Differences between humoral and cell-mediated immunity (retrieved from Panawala, 2017)

T cells are produced in the bone marrow as T progenitor cells and migrate to the thymus gland were they mature into T cells (Abul et al., 2016). In contrast to what was formerly known, McClory et al., at 2012, stated that T cells can also be developed in the tonsils. During their maturation process, T cells express T cell receptors (TCRs), and either $CD4^+$ or $CD8^+$ receptors. They are able to differentiate self from non-self-cells. TCRs, unlikely antibodies, can only recognize antigens bound to certain receptor molecules, named Major Histocompatibility Complex class 1 (MHCI) and class 2 (MHCII). MHC molecules are membrane-bound surface receptors on APCs. $CD4^+$ and $CD8^+$ receptors aid in T cell recognition and activation by binding to MHCI or MHCII. Specifically, MHCI presents to cytotoxic T cells, while MHCII presents to helper T cells (O'Leary et al., 2006).

The positive and the negative selection tests are the two selective processes that T cells have to undergo after they are released from the thymus, in order to confirm that they will perform properly. These tests ensure that T cells will protect the body cells and tissues against any autoimmune event.

- *Positive selection* is applied in order to ensure *MHC restriction* by testing if MHCI and MHCII are able to differentiate self from nonself proteins. Cells should be only binding to self-MHC. In case these cells bind to nonself molecules, they are eliminated by apoptosis.
- *Negative selection* is performed to test *self-tolerance*, testing $CD4^+$ and CD8 binding abilities. A T cell should only bind, either via $CD4^+$ or $CD8^+$, to self-MHC molecule that is presenting an antigen. In case that a T cell binds to a self-MHC molecule which is not presenting an antigen, or a self-

MHC presenting a self-antigen, will be eliminated by apoptosis (Starr et al., 2003).

After T cells undergo the aforementioned processes, they can be differentiated into *Helper T cells*, *Cytotoxic T cells*, and *T regulatory cells.*

- **T helper (T_H) cells**, also called ***$CD4^+$ T cells***, are activated when they recognize a peptide antigen with MHCII molecule on the surface of APCs. At that point, T_H cells release cytokines to aid in the activation of the immune response, including the activation of cytotoxic T cells and macrophages, as well as the maturation of B cells in memory B cells and plasma cells (O'Leary et al., 2006). Upon their activation, T_H cells can differentiate into T_H17, T_H9, T_H1, T_H2, T_H3, T_{FH}, each of which secrete different cytokines triggering different immune reactions (Gutcher et al., 2007).

  Interestingly, by the end of the twentieth century, it was observed that T_H17 has the ability to produce Interleukin-17 (IL-17), a proinflammatory cytokine, increasing the probability for an acute or chronic inflammation, and therefore the development of an autoimmune disease is also dramatically increased (Tabarkiewicz et al., 2015). A variety of publications based on animal and human models were able to verify the crucial influence of T_H17 in systemic and organ-specific autoimmune diseases. A variety of therapies are focused in the inhibition of T_H17-dependent pathways, providing clinical benefits, although they are frequently occurring adverse effects (Hu et al., 2011).
- **Cytotoxic T cells (CTLs)**, also called ***$CD8^+$ T cells***, are activated when they are presented with MHCI peptide antigens, found in every nucleated

cell. They play a critical role against bacteria, viruses and tumor surveillance, although they can also facilitate immune-mediated damage by an excessive immune response (Barry et al., 2002). When a CTL is exposed to its antigen, it rapidly activates, and attacks infected or malignant cells (Andersen et al., 2006). The cytotoxicity is accomplished directly by the Fas/FasL or perforin pathway, and indirectly by the cytokine release. Particularly, the cytokines primarily produced are tumor-necrosis factor α (*TNF-a*) and interferon γ (*IFNγ)* possessing antiviral and antitumor activities. Moreover, cytotoxic granules, that can be also found in NK cells, are secreted, containing the perforin and granzyme family of proteins (Zhang et al., 2011).

- **T regulatory cells** (former **suppressor T cells**), also named T_{reg} cells, are able to express both $CD4^+$ and $CD25^+$ (a component of IL-2 receptor), and are activated upon the expression of the nuclear transcription factor *Forkhead box P3* (*FoxP3*). FoxP3 is fundamental for maintaining suppression of the immune system. Moreover, they suppress activation, proliferation and cytokine production by $CD4^+$ and $CD8^+$ T cells (Fontenot et al., 2003, Baecher-Allan et al., 2001). T_{reg} cells control the immune response to self-molecules and antigens and are able to aid in the prevention of an autoimmune disease (Khattri et al., 2003).
- **T follicular helper (T_{fh}) cells** are essential for the maturation, affinity and maintance of the humoral memory. They stimulate B cells to initiate extrafollicular and germinal center (GC) antibody responses, where GC B cells with higher affinity for the pathogen are generated and selected (Deenick et al., 2011, Schwickert et al., 2011), while they restrict low-affinity B cells from entering the GC (Victora and Nussenzweig, 2012).

Due to the fact that the majority of GC B cells cannot facilitate BCR signaling (Khalil et al., 2012), they depend on help signals of T_{fh} cells to distinguish which GC B cells proliferate (Gitlin et al., 2014).

- **Memory T cells** are generated from the progeny of antigen-stimulated lymphocytes and remain inactive in the body, unless they are stimulated by the antigen that induced their development. Upon that event, they rapidly respond by orchestrate antigen-specific recall responses and triggering secondary immune responses (Rahimi et al., 2018, Baecher et al., 2001).

B cells are produced and matured in the bone marrow. They construct a fundamental pillar of the humoral adaptive defense, as they are responsible for the release antibodies. Markedly, B cells produce IL-10, which is the regulatory cytokine that suppresses harmful immune responses by regulating the Th1/Th2 balance and directly attenuating innate cell-mediated inflammatory responses (Mizoguchi et al., 2006). Unlike the other two classes of lymphocytes, they express B cell receptors (BCRs) on their cell membrane. B cells are activated in the secondary lymphoid organs (SLOs), i.e. the spleen and the lymph nodes, were they circulate after maturation (LeBien et al., 2008).

Germinal centers (GCs) are sites of the SLOs where mature B cells undergo cycles of proliferation, somatic hypermutation and affinity discrimination. GC formation is relying on the activation of antigen-specific B-cells by T-cells able to recognize epitopes of the same antigenic complex (Hamel KM et al., 2012, Gatto D et al., 2010).

Naïve B cells express antibodies on their cell surface, also called *membrane-bound antibodies*. Upon the event in which a naïve B cell encounters an antigen that matches its membrane-bound antibody, the antigen is directly bound with the B cell membrane-

bound antibody. Afterwards, the B cell rapidly divides and begins differentiating at the GCs into a memory B cell or an effector B cell, also called plasma cell, or a regulatory B cell (Khalil et al., 2012).

- **Memory B cells** provide protective immunity against recurring infectious agents. They are generated only in response to T-dependent antigens in GC or extrafollicular reactions (Kurosaki et al., 2015). Memory B cells persist in the body when their specific antigen is not present, however they do not produce antibodies. They have an increased lifespan as well as rapid and strong response to stimulation and expression during the presence of an antigen (Seifert M et al., 2016).
- **Effector B cells** (Plasma cells) are specialized, long-lived, terminally differentiated B cells that arise in SLOs, from antigen-activated B cells. They produce and release antibodies, maintaining the humoral immunity (Allman D. et al., 2008). However, plasma cells can produce autoantibodies, and thus contribute in the development of autoimmune disorders, allergies and transplant rejection (Nutt et al., 2015).
- **Regulatory B cells (Bregs)** are able to suppress inflammation by stabilizing the T_{H1}/T_{H2} balance (Mauri et al., 2003, Fillatreau et al., 2002) exacerbations of inflammations by directly diminishing proinflammatory networks like IL-1 and TNF-α production by macrophages (Mizoguchi et al., 2002). Furthermore, Knoecher et al., at 2005 have stated that Bregs that possess secondary APC activity are possibly involved in the induction of respiratory and systemic immune tolerances. Due to the fact that B cells respond directly to naïve antigen because of the BCR existence, Bregs are immediately involved in an immune response, although its effect and lifespan is much shorter compared to that of a T lymphocyte. Bregs have also the ability to trigger alterations in the

T-cell behavior via interaction with T-helper cells. Although Bregs represent a very low proportion of the B cell population, they could be used as novel therapeutic options in autoimmune diseases, as they can interact with a variety of other immune subsets and inhibit pro-inflammatory signals (Ray et al., 2017).

Adaptive immunity can be further subdivided as either natural or artificial. Both natural and artificial immunity have active and passive components. Active immunity is a result of an infection or immunization, whereas passive immunity is arised from naturally or artificially antibody receiving (Tao et al., 2016, Abul et al., 2016). Specifically:

- **Active immunity** can be either natural or artificial.
  - **I.** **Natural** active immunity occurs when the organism is exposed to a pathogen, develops the disease, and finally acquires immunity against this pathogen resulting from the primary immune response (Kroger et al., 2012, Baxter, 2007) Natural active immunization develops when an antigen is obtained by an organism that meets for the first time the pathogen, and therefore does not posses antibodies against this specific pathogen (Strikas et al., 2018).
  - **II.** **Artificial** active immunity occurs when the pathogen, or part of it, are inserted in the organism before the body it is naturally infected. A characteristic example of this category is the **vaccination** where the antigens are attenuated so the primary immune response against the antigen occurs, without causing symptoms of the disease (Kumari et al., 2014). A vaccine can also obtain particles of the pathogen, weakened or dead pathogens, toxins from the pathogen, or one of its

surface proteins, depending on the type of the disease (Baxter, 2007). Nowadays, twenty-six available vaccines exist, including the ones against influenza, Human Papilloma virus (HPV), chickenpox, smallpox, measles, mumps, Hepatitis A/B/E, pneumococcus and tetanus. Furthermore, the existence of twenty-four more candidate vaccines in development has been stated (WHO, 2018). Recently the World Health Organization, announced that the first Ebola vaccine is established. Specifically, in a WHO-led Guinea ring vaccination trial in December 2016, 11000 people were voluntarily vaccinated with the newly prepared Ebola vaccine and no cases of Ebola virus disease occurred.

- **Passive immunity** can be also categorized in natural or artificial.

  I. **Natural** passive immunity occurs during the pregnancy, and breastfeeding. Systemic immunity (IgG) is transferred from the mother to the offspring via the placenta or yolk sac, and after childbirth via the colostrum and breast-milk (Slifka et al., 2018). Although, this immunization has a short duration, it already decreases during the first months of the infant life, and completely fade in approximately 6 months after birth (Raab, 2011)

  II. **Artificial** passive immunity refers to an immediate, but short-term immunization, administered by the injection of antibodies (e.g. gamma globulin), that are not provided by the recipient's cells (Baxter, 2007). The administered antibodies have an animal or a foreign human origin. When they are obtained from the blood of animals, severe anaphylactic reactions can occur for the

recipient, due to immune response against animal serum (Stiehm et al., 2012). Their route of administration varies, e.g. they can be given in the form of e.g. blood plasma, blood serum, intravenous human Ig, intramuscularly, monoclonal antibodies (Slifka et al., 2018). Artificial passive immunization is used in the treatment of a variety of severe acute infections, like tetanus, as well as prophylaxis in immunodeficiency diseases. Rapid action is provided; however their effect typically ranges from a few weeks to four months (Goddard et al., 2018).

2.2. AUTOIMMUNE DISEASES

In the previous chapter, the function of the immune system as a prophylactic and disease-fighting system, was thoroughly discussed.

Autoimmunity refers to the immune responses where the body produces autoantibodies that attack its own healthy cells, tissues and organs. A disease derived from such responses is called **autoimmune disease** (**AID**) (Ganguly, 2018). During the early days of immunology, the first description of an autoimmune disorder was pointed by Ehrlich, who put the dogma of *"horror autotoxicus"*, referring to the inability of the immune system to recognize self-constitutes (Avrameas et al., 2018)

AIDs are chronic reactions defined by inflammatory reactions against self-antigens. These reactions are not necessarily organ specific, they can also be systemic. Body compartments that can be damaged include the heart, brain, joints, muscles, skin, glands (e.g. thyroid), lungs, GIT, nerves, eyes, kidneys, blood vessels, alone or in some combinations. For example, SLE can interfere with the joints, kidneys, nerves, skin, heart, blood vessels

etc. In Diabetes Mellitus type 1, the immune system attacks the insulin producing β-cells of the pancreas, where the elevated blood sugar levels can injure the heart, kidneys, nervous system, eyes, as well as blood vessels (Shuayb et al., 2018, Al-Khalidi, 2017).

The American Autoimmune Related Diseases Association has provided a list containing the currently existing autoimmune diseases. Some of them include Addison's disease, Alopecia areata, Behcet's disease, Crohn's disease, celiac disease, dermatitis herpetoformis, systemic lupus erythematosus, discoid lupus, endometriosis, fibromyalgia, Grave's disease, Hashimoto's thyroiditis, glomerulonephritis, Diabetes mellitus type 1 (juvenile diabetes), myasthenia gravis, multiple sclerosis, psoriasis, pernicious anemia, rheumatoid arthritis, Sjögren's syndrome, ulcerative colitis, vasculitis and vitiligo.

The cause of an autoimmune disease is not apparent and understood, although the triggering event may be T and/or B cell mediated. T and B cells share a common feature, well preserved during evolution, *auto-polyreactivity*. Both T- and B- cell receptors distinguish environmental components, acquired from proteins employed in their embryonic development. In order to have the ability to recognize environmental components, they lost part of their competence to recognize self (Zhou et al., 2007, Avrameas, 2016). During the years, these auto-polyreactive immune receptors could recognize more avidly the external components of the organism, compared to the internal. Meanwhile, further capacity for the production of specific receptors in order to recognize environmental antigens was added. Due to their ability to bind to self and non-self-molecules, they can bind to e.g., cytokines, masking them from the adaptive immune system, and thus inhibiting pathogenic autoimmune reactions (Watanabe et al., 2010). Under normal conditions, the active site of auto-polyreactive immune receptors are blocked by the elevated quantity of self-antigens. Additionally, these receptors can monitor vital homeostatic biological activities like enzymatic catalysis and cellular repairing (Wright et al., 2009).

A complex interplay of various factors contributes in the development of an AID, including genetic factors, epigenetic factors, external factors and internal compartments influenced by environmental factors. Although none of these factors alone can sufficiently induce AIDs, their (temporal) combinations can give the lead signal to autoimmunity. Environmental cues can contain vitamin D deficiency, sexual hormones i.e. gender, smoking, pollution and some drugs that can trigger autoimmune reactions (Anaya et al., 2016).

In the figure below, a brief summary of the factors contributing in the development of an AID are illustrated.

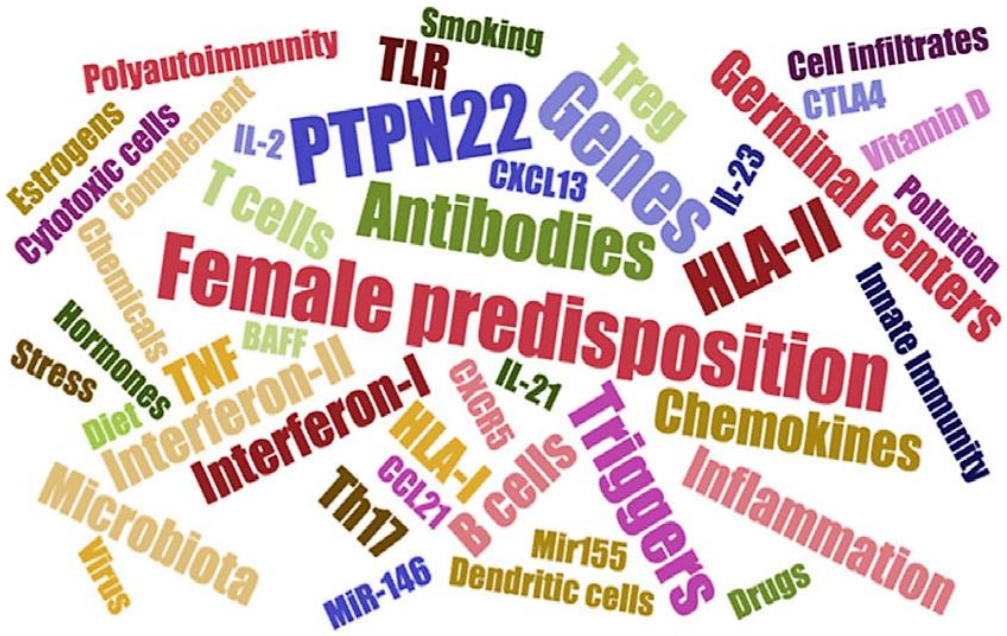


Figure 1 – Common features triggering the development of autoimmune diseases (retrieved from Sudres et al., 2018).

Chronic stimulation of the immune system is able to lead to escalation of naturally present auto-polyreactive clones and, especially in individuals with a genetic predisposition, it can result in the development of an AID (Avrameas et al., 2018). Furthermore, age-associated B cells, constantly accumulated with age, have seem to be a newly discovered factor that has been linked with the onset of SLE in murine models (Rubtsova et al., 2017).

Autoimmune diseases can be described using the **Witebsky's postulates**. In order to confirm the autoimmune nature of a disease, it must comply with the following statements:

- The autoimmune reaction should be an autoantibody or a cell-mediated immune reaction.
- The equivalent antigen is known.
- An akin response in experimental should cause an analogous disease (Witebsky et al., 1957, Rose et al., 1993, van Gaalen et al., 2005).

2.3. EPIDEMIOLOGY

Systemic lupus erythematosus is a chronic autoimmune disease, of variable severity with altering periods of flares and remissions, with multisystem character i.e. affecting various body parts including skin, musculoskeletal system, heart, kidneys, blood, nervous system and reproductive system in women. The etiology of the disease is not yet fully understood, although it is believed to be multifactorial (Jakes et al., 2012, Lupus Foundation of America, 2018).

Prevalence data concerning SLE demonstrate that an exceptional high percentage of people affected by this disease are women -especially in the reproductive years- in a ratio 9:1, compared to men (Bretsias et al., 2012, Lupus Research Alliance, 2018). One study, performed by Ramsey-Goldman et al., at 2000, indicated that this ratio in women's childbearing years was 12:1, suggesting the key role of hormonal factors in the pathogenesis of the disease. Men tend to have an older age diagnosis of SLE, less photosensitivity and more serositis compared to women (Bretsias et al., 2012). Elderly diagnosed with SLE, tend to have milder disease symptoms and progression, i.e. decreased incidence of malar rash,

purpura, alopecia, Raynaud's phenomenon, photosensitivity and renal and central nervous system (CNS) disfunctions. However, elderly have increased prevalence of musculoskeletal manifestations, serositis, pulmonary complications and sicca symptoms (Deng et al., 2017, Lupus UK, 2018).

Age distribution is broad, which means that it can range from children in the first two years of age, to older adults 80 years of age and older. It is estimated that twenty percent of the patients with SLE have disease onset before the age of 16 years, sixty-five percent between the ages of 16 and 55 and fifteen percent after the age of 55. These data reinforce the statement of higher prevalence of the disease in childbearing years (Pons-Estel et al., 2010, Danchenko et al., 2006). The organization Lupus UK has recorded that the peak age of the diagnosis of the disease onset is in women from fifteen years of age to forty. However, the actual age of diagnosis is thirty-seven to fifty years, indicating the delayed diagnosis of SLE. On the contrast, by the end of the twentieth century the number of patients diagnosed with lupus was tripled. Specifically, the incidence of SLE was increased, while the prevalence was not significantly altered. Regarding these data, we can assume the better diagnosis of mild disease the past years (Danchenko et al., 2006).

The prevalence of SLE cases fluctuates in a high rate, depending on the geographical location and races, indicating the influence of genetic and environmental factors, although further research is still required. Throughout the history, the SLE cases among Europe were lower compared to USA and Australia (Pons-Estel et al., 2010). Notably, higher disease prevalence has been observed in non-white racial groups. Afro-Caribbean population followed by Aborigines have demonstrated the highest SLE prevalence in a worldwide scale. However, the transitory nature of certain groups (immigration/emigration) has caused an unstable racial composition. Well suited countries for epidemiological studies are the ones

with racially and homogenic stable population, e.g. Iceland (Rees et al., 2017, Danchenko et al.,2006).

Describing SLE in the continents -i.e. Europe, USA (North America, South America), Asia, Africa and Australia-, it is observed that the incidence and prevalence rate is two to three times greater in people of Asian or African background compared to white populations. The disease is also most frequently met in Aboriginal than Caucasian Australians, as well as in African Americans compared with whites (Pons-Estel et al., 2010, Schur et al., 2014). Referring to UK, SLE was more frequent in individuals with Afro-Caribbean origin, followed by Asians and last were the Caucasians. (Rees et al., 2016). In a summarized worldwide incidence report, that local secondary care hospitals or National Health Insurance databases were used for the gathering of the information, the highest incidence was observed in North America (i.e. 23.2/100000 person-years). Furthermore, the lowest incidences were observed in Africa (0.3/100000 person-years) and Ukraine (0.2/100000 person-years). Once more, Europe had the lowest SLE incidence rate, when Asia, Australia and USA had a higher incidence rate (Rees et al., 2017).

Morbidity characteristics and information of European countries are provided and updated since the last decade of the twentieth century by the "Euro-Lupus Project, where a variety of clinical and immunological prognostic factors are also described. Moreover, comparisons of the statistics in Europe, Asia and America also take place. Arthritis is the most prominent SLE manifestation in Europe, occurring in 48.1% of the patients, and seems to be greatly increased throughout the years. In the corresponding study in America 88.1% of the SLE patients developed *arthritis* and the 50.5% of patients in Asia. The second leading manifestation was the *malar rash*, prominent in 31.1% of Europeans (58% in America, 76.1% in Asia). *Active nephropathy* was reported in 27.9% of SLE cases in Europe (40.2% in America, 74% in Asia), followed by the *photosensitivity*, diagnosed in 22.9% of patients

(60.2% in America, 41.2% in Asia). The aforementioned lower frequencies of SLE clinical manifestations, could be reasoned by the genetic and/or environmental differences among Europeans, Americans and Asians. On the contrast, they could be the result of the medical care during the study (Cervera et al., 2009). In a smaller ratio, Stefanidou et al., performed a 5-year study, comparing the morbidities between men and women. This study was composed of 594 patients, where 535 were women and 59 men, i.e. female: male ratio was 9:1, aged from fourteen to fifty years of age. Male SLE patients had a lower incidence of *arthralgia*, *photosensitivity*, *hair loss* and *Raynaud's phenomenon*, compared to women. However, they would more often develop *antiphospholipid syndrome* (APS) that could possibly lead to *thromboses*, playing a key role in the prevalence of *strokes* (Soto et al., 2004, Andrade et al., 2007). Moreover, a variety of studies have come in the conclusion that *renal disease* (nephropathy), *arthritis* and *neurological disorders* (chorea, strokes, neuropathy and absence seizures) are the main manifestations, after a 5-year follow up. Referring to the developed *infections*, predominately affecting the respiratory tract, was present in both men and women, although this incidence was notably more common in the male population. These observations would lead us in the conclusion that despite the significantly higher prevalence of the disease among women, the disease appears to be more severe in men (Stefanidou et al., 2011).

Mortality rates had a striking improvement over the past 50 years. Studies performed in the 1950's, were claiming a survival rate les than 50% in the first five years after the disease was firstly diagnosed. Recently, it has been stated that over 93% of SLE patients survive 5 years and 85% survive for 10 years. Slightly longer survival rates are observed in Europeans when compared with the Americans (Bernatsky et al., 2006, Cervera et al., 2009). Historically, SLE was referred as a quickly mortal disease, and the term "early death" was defined as the death within the first year of the disease diagnosis and only 50% of the patients would make it to the 5-year survival (Stefanidou et al., 2011). Nowadays, Ippolito et al.,

claimed that the term “early death” is redefined within the first five years of the disease and the 5-, 10- and 15-year survival are 96%, 93% and 76% respectively. It is believed that the higher survival rate is due to the better understanding of the SLE pathogenesis, earlier diagnosis in milder forms of the disease and the progression of the therapeutic approaches (i.e. more intensive pharmacotherapy including cytotoxics, immunosuppressives, high-dose prednisolone, antihypertensives, antibiotics, possible renal dialysis even transplantation) (Cervera et al., 2009, Pons-Estel et al., 2010, Stefanidou et al., 2011).

Great interested was attracted towards the socioeconomic status of the populations, as it appears to be affecting the SLE mortality rates. It has been observed that minority populations the death-rate associated with the disease is increased, due to difficult access in healthcare, lack in therapy adherence and health coverage, although further research is still required (Durán et al., 2007). Moreover, higher mortality rate has been estimated in males with SLE, and in late-onset (>50 years of ages) lupus (Ippolito et al., 2008).

Causes of death in SLE patients in a 10-year period are 26.5% by active disease, 26.5% by thromboses and 25% by infections. Nevertheless, the main cause of death within the 5-year survival were active disease and infections, both by 28.9%, followed by thromboses in 26.1% of patients (Cervera et al., 2009). Furthermore, renal complications are also very common causes of SLE mortality and especially in black patients compared with whites, by the high probability for the doubling of creatinine, leading to end-stage renal disease (Korbet et al., 2007).

The prevalence, morbidity and mortality of SLE, its diversity across countries, as well as across different age, sex, racial and socioeconomical groups within the countries, reveal the multiple factors contributing in its manifestation and development. Further researches are needed in order to obtain a clear view.

2.4. PATHOPHYSIOLOGY OF SLE

The manifestation of SLE requires multiple events and abnormalities among the innate and the adaptive immunity, resulting in the induction and perpetuation of auto-reactivity. However, all pathways end up in endogenous nucleic acids-mediated production of **interferon α** (**IFNα**). IFNα is a pluripotent cytokine, mainly produced by pDCs, T-cells, B-cells, neuronal cells, endothelial cells and renal cells. A variety of lupus-related genes encode proteins regulating or mediating TLR signals, related to elevated IFNα plasma levels in patients with specific autoantibodies that may transfer stimulatory nucleic acids in the intracellular compartments of TLR7 or TLR9. Upon the activation of the IFN pathway, autoantibodies specific for RNA-associated proteins are present. In addition, the production of IFN and various other proinflammatory cytokines is strongly influenced by the RNA-mediated activation of TLR.

One major ongoing event in SLE is the *loss of tolerance* to nuclear auto-antigens and to specific other self-antigens.

Toll-like Receptors (TLR)

TLRs are a protein family that belongs to the innate immunity and are expressed in a wide range of immune cells. They serve as a first line defense, rapidly and extraordinary effectively clearing the majority of the invading pathogens, by the recognition of PAMPs by TLRs-3, -7, -8 and -9. Certain nuclear autoantigens, through TLR7 and TLR9, directly activate B cells. TLR ligation stimulates upregulation of IFNα by pDCs, which is a characteristic event in SLE. Autoreactive T and B cells coexist with the respective antigens without any immunological response, although they can become pathogenic after the involvement of TLRs. B cells in the active lupus form, have elevated TLR-9 expression.

Neutrophils

Neutrophils have recently attracted attention in SLE pathogenesis, especially in the organ damage (Mantovani et al., 2011). Due to their short lifespan (≥90 hours) (Tak et al., 2013), the role of neutrophils in the SLE pathophysiology was never examined thoroughly, until recently. Because of the TLR ligation, neutrophils release inflammatory cytokines, activating immune cells and form the immune response (Jog et al., 2013). Defensins and lactoferrin are some oh the neutrophil granule bactericidal proteins, are observed to be increased in SLE, corresponding to the disease activity. Moreover, anti-lactoferrin antibodies can be detected in the serum of SLE patients as candidate biomarkers. Furthermore, neutrophil granule proteins (NGAL) could possibly indicate lupus nephritis (Deng et al., 2017a).

Following activation, neutrophils that have emerged in the site of infection, undergo apoptosis and are phagocytosed by local macrophages or monocyte-derived macrophages. This procedure serves as an anti-inflammatory stimulus that curbs inflammation (Mantovani et al., 2011). In SLE, neutropenia (low number of circulating neutrophils) is often observed. Lupus neutrophils undergo increased apoptosis, although lupus macrophages are shown to have defective phagocytic ability, which is inversely related to the SLE disease activity score (SLEDAI). Elevated apoptotic neutrophils have been observed in SLE blood serum, also corresponding to the disease severity (Jog et al., 2013).

Neutrophils can regulate the recruitment and function of immune system cells including cells of the adaptive immunity by the secretion of a variety of cytokines. They release high amounts of the chemokine IL-8, a potent neutrophil chemoattractant. Thus, we can conclude that the increased secretion of IL-8 is utilized as a feedback mechanism to recruit further neutrophils (Lande et al., 2011). In addition, the higher the IL-8 concentration in the site of infection, the higher the possibility to also immobilize the cells that had migrated to the

tissues by receptor desensitization. Finally, neutrophils produce biologically active C-C motif ligand (CCL) 20 and CCL19, two strong chemotactics for DCs, as well as lactoferrin, cathepsin G and LL-37, serving as immature DC chemoattractants (Deng et al., 2017a).

A characteristic trait of neutrophils is the formation of Neutrophil Extracellular Traps (NETs). They are filamentous structures, made of nuclear material, i.e. DNA coated with either histones or chromatin proteins or granule proteins including elastase and myeloperoxidase (Knight et al., 2012). They are formed to regulate bacterial infection, while filaments act as webs to trap bacteria. NETs are formed in the last stage of neutrophil life, suggesting that their formation is a specialized form of cell death defined as NETosis (Deng et al., 2017a). Some SLE patients do not have the ability to normally clear NETs, further allowing neutrophils to exacerbate pathological immune responses, a phenotype related with the disease activity. Moreover, dying neutrophils secrete self DNA/LL-37 complexes by the NET formation, activating pDCs to release IFNα (Lande et al., 2011).

The figure below briefly illustrates the influence of neutrophils in SLE pathophysiology.

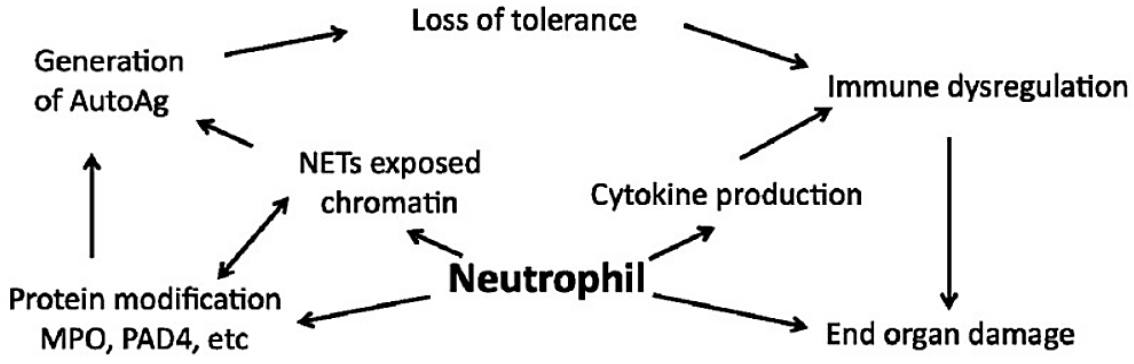


Figure 2 – **Role of neutrophils in SLE pathogenesis. (**MPO – myeloperoxidase, PAD4 – Protein Arginine Deiminases 4) (retrieved from Jog et al., 2013)

B lymphocytes

B cells regulate various aspects of the immune reactivity and additionally differentiate to antibody-producing cells. It has been proposed that in SLE, the intrinsic tendency of B cell excessive response to immune stimulation or *hyperactivity* is a crucial pathogenic event. Another characteristic of this "B cell hypothesis" is the relation of SLE with the production of certain patterns of autoantibodies like *anticardiolipin* in thrombosis, *anti-DNA* in glomerulonephritis and *anti-Ro* in congenital heart block, which are definitely involved in tissue damage (Lipsky, 2001, Iikuni et al., 2009). B cells can differentiate in cytokine-producing effector cells and can potentially influence the DC function. They also have a key role in the lymphoid organogenesis and in the initiation and regulation T- and B- cell responses. Therefore, an excessive or insufficient B cell activity can reinforce immune activity and increase the possibility to initiate autoimmunity (Dörner et al., 2011).

In SLE, genetic factors can enhance B cell reactivity, leading to autoantibody production, as well as end organ damage. The capacity of B cells to reinforce the function of cells that contribute to B cell responses is also increased (Zhang et al., 2001). Enhanced B cell responsiveness, resulting in autoimmunity, can be also caused by the genetic factors-influenced alteration of T cell and APCs function, the cytokine production and the availability of endogenous antigens (Iikuni et al., 2009).

B cell abnormalities observed in SLE include alterations in pre-immune B cell maturation, negative selection at certain maturation checkpoints and receptor editing, antigen responsiveness issues like somatic hypermutation and effector B cell generation (Dörner et al., 2011). Pre-immune B cells are also able to release IL-10, and thus suppress Th1 and Th2 functions, resulting in defective control of T-cell and monocyte responses. Regarding the early defects in the selection against autoreactive B cells, it has been estimated that during both active and inactive lupus it is impossible to remove B cells expressing self-

reactive BCRs expressed by naïve B cells (Iikuni et al., 2009). Increased amounts of memory B cells contributes to the development of autoimmunity as they can be rapidly activated in a non-antigen-specific way by TLR agonists, combined with BAFF and APRIL (which are later discussed) (Dörner et al., 2001, Morel et al., 2009). Moreover, a B cell subset called $IgD^{-}CD27^{+}$ is related with the presence of certain autoantibodies – anti-sdDNA, anti-Smith and anti-ribonucleoprotein (Wei et al., 2007).

Autoantibodies in SLE can either arise in the periphery by somatic hypermutation from non-autoantigen reactive B cells or be selected by increased apoptosis or decreased clearance of apoptotic cells in GCs (Dörner et al., 2011). Moreover, autoreactive B cells lose tolerance to nuclear autoantigens, producing autoantibodies which bind to the autoantigens, forming immune complexes (ICs) (Iikuni et al., 2009).

Furthermore, a novel B cell subset named age-associated B cells (ABCs), has been involved in the pathogenesis of SLE. In contrast to other B cells, ABCs are able to express increased levels of CD11c, as well as the transcription factor T-bet. T-bet is needed for the appearance of this subset and stimulates BCR, IFN-γ receptor, as well as TLR7 on B cells, and thus elevated T-bet expression levels occur. ABCs are strong APCs and can trigger autoimmunity by presenting self-antigens to autoreactive T cells. Since increased T-bet expression in B cells can be observed in SLE blood mononuclear cells, we can assume the critical role of this event in the development of the disease (Rustova et al., 2015, Rubtsova et al., 2017)

In the figure below, the role of B cells in a) normal immunity and b) SLE are briefly summarized.

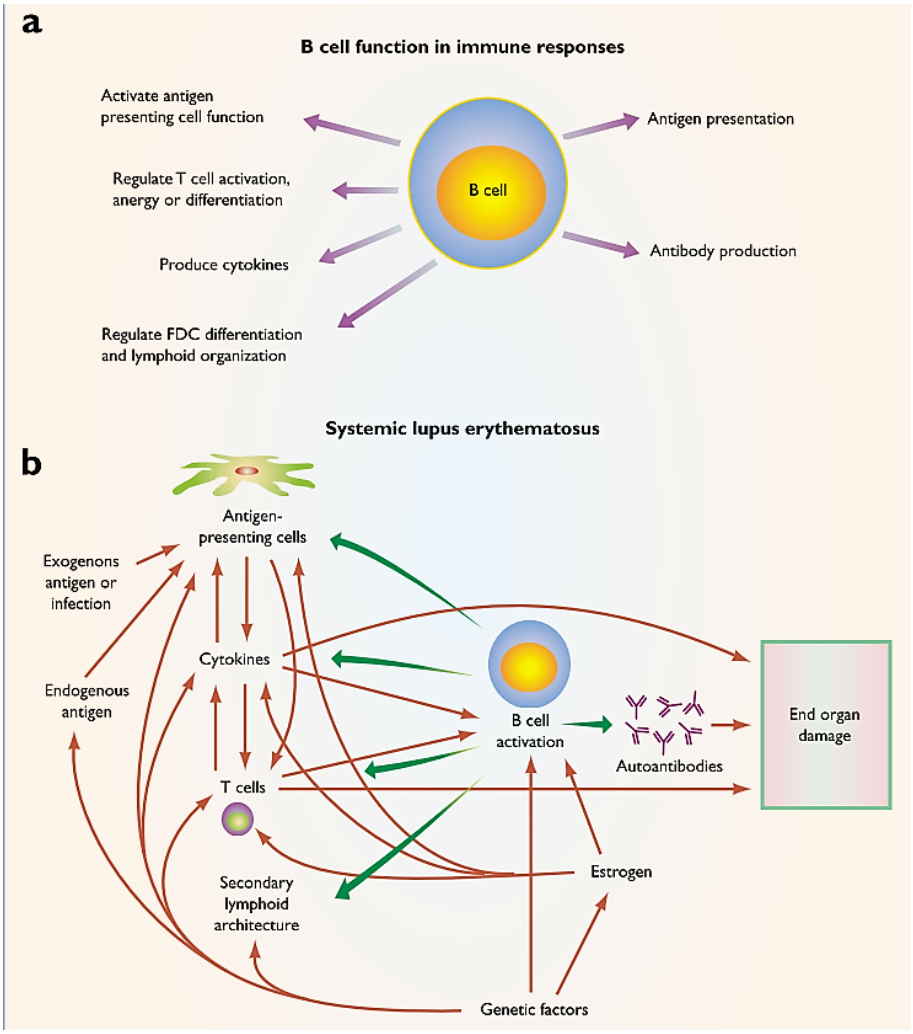


Figure 3 – B cell regulation of normal immunity and SLE (retrieved by Lipsky, 2001).

The BAFF/APRIL system

A major breakthrough discovery, in 1999, was related to an essential *B cell survival factor, TNF ligand superfamily member 13B* (also called B cell-activating factor of the TNF family [**BAFF**], B-lymphocyte stimulator [BLyS], zTNF$_4$, THANK, TALL-1), possessing a crucial role in SLE pathogenesis (Mackay et al., 1999). BAFF is proven to be essential for B-cell maturation and survival, as BCRs were not able to perform them alone. Its expression is increased by IFNα signaling and B cell deficiency (Vincert et al., 2014, Samy et al., 2017). Moreover, a related cytokine, *TNF ligand superfamily member 13* (also called as a

proliferation-inducing ligand [**APRIL**]), was later also observed in specific subsets of SLE patients. APRIL affects B1 cell activity, humoral responses, and is critical for antibody class-switching and plasma cell survival (Treamtrakanpon et al., 2012).

BAFF and APRIL are produced as type II transmembrane proteins by myeloid and lymphoid cells, although they can be both expressed by non-haemotopoietic cells (BAFF by osteoclasts and synovial fibroblasts, APRIL by osteoclasts, epithelial and tumor cells). They share two receptors; transmembrane activator and cyclophilin ligand interactor (TACI) and B cell maturation antigen (BCMA). Both receptors are expressed on B cells, where TACI was additionally detected on a subset of activated T cells (Vincert et al., 2013). APRIL has strong affinity for BCMA and fairly for TACI and can also bind heparin sulfate proteoglycans (HSPG). APRIL serum levels were related with the renal histology severity in lupus nephritis (LN), i.e. higher APRIL concentration indicates higher severity of LN. In addition, APRIL serum levels could possibly predict the patients' responses to immunosuppressive therapy (Treamtrakanpon et al., 2012). BAFF has strong affinity for TACI and weak affinity to BCMA. It is also strongly bound to the BAFF specific receptor (BAFF-R) (also called BAFF receptor 3 – BR3), which is observed on B cells, mediating most BAFF-elicited B cell survival signals. BR3 expression is obtained on immature B cells upon obtaining a functional BCR inducing their survival and maturation (Morel et al., 2009). Notably, it has been proven that BAFF expression is upregulated by estrogens, potentially revealing the strong tendency of females to develop lupus mainly in their reproductive years and especially during pregnancy when the estrogen peak reaches its zenith (Deng et al., 2017b).

It has been repetitively and undoubtfully demonstrated that elevated serum concentration of functional, soluble BAFF is observed in SLE patients. It should be related to predict specific SLE manifestations like LN, rather than the overall disease activity (Vincent et al.,

2013). Furthermore, increased expression of BAFF mRNA has also been reported in peripheral blood leukocytes, and increased expression of the membrane-bound form was observed in peripheral blood mononuclear cells of SLE patients. BAFF and IFNs function together in the disease pathogenesis in both a TLR-dependent and independent manner (Samy et al., 2017). APRIL's role has not well broadly and undoubtfully been established, although elevated APRIL serum levels, tend to correlate with the modulation of the disease activity, particularly during immunosuppressive therapy as the cells regulating the production of APRIL are not affected by immunosuppressives (Rédei 2008, Morel et al., 2009).

Despite the fact that both BAFF and APRIL are elevated in SLE serum (as well as in primary Sjögren's syndrome and rheumatoid arthritis [RA]), a linkage between co-expression of these molecules is not yet clearly defined. Serum APRIL levels are inversely related with serum BAFF levels and weakly with anti-dsDNA titres (Morel et al., 2009). It is indicated that alterations in the BAFF/APRIL system can affect the capacity of the innate immunity to regulate B cell activation. They are related in splenic neutrophil activation of the marginal zone B cells, increase Ig production and promote plasma cells differentiation. BAFF production is also performed by neutrophils triggered by anti-neutrophil Abs in the cytoplasm, as well as by human NK cells when triggered by IL-2. (Vincert et al., 2014).

T cells

Irregular T cell function in SLE can be attributed to their failure to correctly regulate the immune response, provide support to the auto-reactive B cells and to infiltrate target-organs like the kidneys. Increased concentrations of cytokines like IFNα, IL-6, IL-10 and IL-12, affect T cell function by chronic stimulation. These cytokines bind in the surface of T cells, activating **STAT3** (signal transducer and activator of transcription 3) by phosphorylation and dimerization (Bonelli et al., 2008, Harada et al., 2007). STAT3 then moves into the

nucleus to bind to a target sequence (SBS – STAT-binding site) in the promoter of genes like myeloid cell leukemia-1 (Mcl-1), IFN regulated factor-1, c-Myc, or even STAT3 itself. STAT3-dependent genes have an important role in cellular functions like cell proliferation, motility and invasiveness, resulting in increased cell migration in inflammatory (and neoplastic) diseases (Miyara et al., 2005). It has been concluded that in SLE, T cells show increased STAT3 levels, in its activated form p-STAT3. Moreover, STAT3 mRNA and protein are also increased, but only after constant and chronic exposure of SLE T cells to inflammatory cytokines (Hendrich et al., 2014).

The underlying cause for the increased cytokine concentration could be either genetic (e.g. IL-10) or due to a response of the innate immunity to the apoptotic material and ICs (IFNα). Additionally, autoantigens secreted by apoptotic cells are presented to T cells by dendritic cells, resulting in T cell activation. Activated T cells by releasing cytokines like IL10, IL23, and by cell surface molecules like CD40L and CTLA-4, assist B cells to produce antibodies to the self-constituents (Dörner et al., 2011).

T helper cell subpopulation 17 (**T_{H17} cells**) have been characterized as potent stimulators of acute and chronic inflammations, playing a key role in the development and progression of autoimmune disorders like SLE due to their ability to produce the proinflammatory cytokines IL-17 (IL-17A and IL-17B). Th17 cells need certain transcription factors and cytokines in order to be activated and proliferated (Henriques et al., 2010). They can be stimulated by IL-6/transforming growth factor (TGF)-β or IL-23p40 pathway, whereas IFN-γ (produced by Th1) and IL-4 (produced by Th2) act as its inhibitors. It has been observed that IL-17 was elevated during a new onset of SLE and during the disease flares, as IL-17 is related with the severity of the disease (Crispín et al., 2008). Additionally, IL-17^+ T cells can also release cytokines like TNF, IL-2 and IFN-γ, that are correlated with the disease severity. IL-17 by itself or in combination with BAFF, can regulate B cell survival, proliferation and

differentiation into Ig-secreting cells, aiding in SLE development (Chen et al., 2010b). For instance, decreased IL-21R expression on B lymphocytes during SLE is strongly related with nephritis. Furthermore, IL-23 is a major cytokine in the development, expansion and proliferation of Th17 cells, existing in high levels in the SLE serum and is also associated with lupus renal disorders (Tabarkiewicz et al., 2015).

Dendritic cells (DCs)

Immature DCs permit *tolerance*. Specifically, tissue DCs sample their environment, capture antigens in normal tissue turnover and migrate at low numbers to the draining lymph nodes. Since there is no inflammation, DCs are immature and antigens that are presented have no analogous costimulation, resulting in either T cell deletion or production of Tregs (Obermoser et al., 2011).

Mature DCs permit *immunity*. Tissue inflammation leads to large amounts of DCs to maturate and migrate to draining lymph nodes. Translocation of MHC peptide complexes, activation of NK cells, NK T-cells as well as influence on B cell proliferation, differentiation and isotype switching, leas to the initiation of an adaptive immune response (Gilliet et al., 2008).

Dendritic cells can be differentiated in two main subcategories: **myeloid DCs** (**mDCs**) (Langerhans cells; skin epidermis and stratified epithelia, and interstitial DCs; dermis and various tissues), and **plasmacytoid DCs** (**pDCs**). *DCs precursors* include monocytes (the most abundant), lineage-negative CD_{11c+} myeloid and CD_{11c}- IL-3R-α^{+} pDCs which are the main type I IFN-producing cells (Pascual et al., 2003).

In SLE, autoantigens or exogenous factors/antigens (UV, viruses etc.), detected by the innate immunity receptors activate DC activity and differentiation, and thus IFNα production

by the CD_{123+} pDCs. Moreover, factors like IFNα , ICs and TLRs, induce mDC maturation, leading to autoreactivity (Lande et al., 2011).

The excretion of self-DNA in the extracellular environment is a characteristic of both necrotic and apoptotic cell death. In order for the immune system to discriminate between pathogen DNA and self-DNA, three district actions take place. First, the subcellular localization of TLR9 allows pathogen DNA immune responses to invade the cells by endocytosis, while self-DNA cannot randomly enter at this compartment (Collona et al., 2004). Furthermore, the rapid degradation of self-DNA released by dying cells, but not the DNA of viruses or pathogens, is ensured by the elevated concentration of DNases in the extracellular environment. This is a crucial action, as it has been noticed that some SLE patients have mutations in DNase 1 (Obermoser et al., 2011). Finally, bacterial or viral DNA consists of various unmethylated CpG motifs, binding and activating TLR9, while human self-DNA contains less respective motifs which are mainly masked by methylation. Nevertheless, hypomethylated CpG islands showing reactivity to TLR9 have been also observed in human DNA. They are preferentially enriched in DNA fragments, secreted by apoptotic or necrotic cells and are common in the DNA of SLE ICs (Vollmer et al., 2004, Gilliet et al., 2008).

In humans, mDCs can express TLR1, TLR2, TLR3 and TLR8, while pDCs can only express TLR7 and TLR9 (Guiducci et al., 2010). In lupus the barriers that prevent pDCs from sensing self-DNA are not flawless and responses to self-DNA can occur as an autoimmune event. Specifically, pDCs are consecutively activated by circulating ICs compromising self-DNA and antibodies to DNA or nucleoproteins. The continuing production of type I IFNs by pDCs causes an irreducible activation and maturation of mDCs, stimulating autoreactive T cells. Moreover, pDC-derived type I IFNs, as well as IL-6, trigger the differentiation of autoreactive B cells into antibody-secreting plasma cells (Colonna et

al., 2004). In SLE, the collapse of the innate tolerance to self-DNA has been related to the formation of complexes between self-DNA and DNA-specific Abs, which can stimulate the production of type I IFN by pDCs via the binding of the DNA-specific autoantibody to low affinity Fc receptor for IgG (Obermoser et al., 2011).

ICs from SLE patients contained LL37, an essential gene for the ability of DNA-specific antibodies to uptake self-DNA to pDCs to stimulate an early TLR9 response. Moreover, due to the high abundancy of LL37 in SLE blood serum, elevated expression levels of type I IFNs and IFN-induced genes ("IFN signature"), as well as disease activity is observed (Lande et al., 2011, Obermoser et al., 2011).

Finally, a nuclear DNA-binding protein called high-mobility group box 1 protein (HMGB1) is released by dying cells. The DNA-HMGB1 complex binds to the RAGE (receptor for advanced glycation end-products) of pDCs, and thus increases the production of IFNα by facilitating correlation of DNA with TLR9 (Colonna et al., 2004).

Respectively, self-RNA released by dying cells, normally cannot activate pDCs due to its rapid extracellular degradation by RNases, limiting its ability to approach TLR7-containing endosomes. Self-RNA low immunogenicity is attributed to vertebrate-specific RNA modifications, including nucleotide methylation and polyA tails (Zhang et al., 2010). Nevertheless, self-RNA molecules, especially the ones abundant in uridine and guanosine (U/UG-RNA), as well as the ones in small nuclear ribonucleoprotein (snRNP), can stimulate pDCs to produce IFNα through TLR7 when handed to endosomes by liposomes or autoantibodies. Autoantibody and self-RNA complexes are also able to activate autoreactive B cells through the BCRs and endosomal TLR7 (Ganguli et al., 2009). SLE is characterized by the appearance of autoantibodies of both self-DNA/chromatin and snRNPs containing U/UG-RNA (Ganguly et al., 2009, Obemoser et al., 2011, Lande et al., 2011).

2.5. RISK FACTORS

One of the main issues in SLE pathology is the breakdown of B cell tolerance, leading to tissue-specific and non-specific autoantibodies development, responsible for numerous pathogenic outcomes. The most common manifestations are arthritis, glomerulonephritis, pericarditis, serositis, vasculitis, cytopenia and cerebritis. These autoantibodies target ubiquitous nuclear antigens including chromatin, ssDNA (single-stranded DNA), dsDNA (double-stranded DNA) and nuclear proteins like Ro/SS-A or U1RNP. (Dörner et al., 2011, Bertsias et al., 2012).

The majority of the patients are genetically predisposed to develop SLE, although the risk alleles by themselves are not potent enough to provoke a "full-blown" disease. Additional factors provide further their pathophysiological impact that aids in the expression of the disease. These factors include female gender, hormonal impact, environmental factors like infections, certain medications, toxins and chemicals, epigenetic events and immune regulatory factors (Tsokos et al., 2007).

2.5.1. Gender

It has been widely and undoubtedly been proved that the gender of an individual has a crucial role in the development of SLE. Precisely, it has been estimated that the ratio of women and men developing the disease is 9:1 (Jakes et al., 2012, Rees et al., 2017, Lupus Research Alliance, ACR, LFA). One study, performed by Ramsey-Goldman et al., at 2000, suggested that this ratio in women's childbearing years was 12:1, suggesting the key role of hormonal factors in the pathogenesis of the disease.

Sex hormones **estrogen** and **prolactin** have been found to have a key role in this genetic gender-specific predisposition, as -among all their functions- they also influence B cell

maturation and selection, allowing B cells maturation to immunocompetence. Their effect in SLE is the more frequent occurrence of flares as well as affecting target organ sensitivity to autoantibodies (Cohen-Solal et al., 2006). It has been evidenced that endogenous sex hormones can control the disease, i.e. estrogen is able to exacerbate the disease, whereas androgen can lower the disease susceptibility. Accordingly, women with SLE have decreased plasma androgen and abnormal estradiol metabolism patterns, resulting in elevated estrogenic activity (Deng et al., 2017). An interesting concern has been also taken in consideration, referring to the safety of exogenous estrogen therapies, oral contraception (OCP) and hormone replacement therapy (HRT). A trial conducted in 2003 by Buyon et al., demonstrated that HRT can increase the occurrence of mild and moderate flares, whereas OCP does not.

Estrogens are steroid hormones, mainly produced in in the ovary in response to follicle-stimulating hormone (FSH), with 17β-estradiol being the most abundant estrogenic compound in the circulation. Progesterone, dehydroepiandrosterone and testosterone include the metabolic precursors of estrogen, and 16-hydroxyestrone, displaying increased estrogenic activity, is one of the main 17β-estradiol catabolites (Cohen-Solal et al., 2006). The two types of estrogen receptors, estrogen receptor α (ERα) and β (ERβ) are not only expressed in the reproductive system, they can also be detected in various systems including cells of the immune system like B and T lymphocytes, monocytes and macrophages, as well as NK cells. The breakdown of tolerance by estrogen is related with the upregulation of molecules from both B cell apoptotic pathways and the BCR signalosome, resulting in the survival of autoreactive cells and maturation of a marginal zone (MZ) phenotype (Cohen-Solal et al., 2006). Membrane-associated ERs are involved in nongenomic rapid responses to estrogen and are expressed by macrophages and T cells but not B cells. 17β-estradiol inhibits B cell development in the bone marrow and decreases B cell lymphopoiesis during pregnancy. Moreover, it increases TNFα production while it decreases IL-10 synthesis.

Estrogen affects monocyte differentiation and can also induce, in a ERβ-dependent matter, monocyte apoptosis (Deng et al., 2017, Buyon et al., 2005).

Prolactin (**PRL**) is a peptide hormone secreted by the anterior pituitary gland and by cells of the immune system. It binds to surface receptors of the cytokine superfamily (PRLR), which are expressed in the breast and uterus in females and in the prostate in males, but they are also seen on lymphohematopoietic cells. Prolactin is the lactogenic master hormone, but is also an immunomodulator affecting apoptosis, activation and proliferation of immune cells (Cohen-Solal et al., 2006). It promotes lymphocyte development, enhancing pro-B cells generation and CD4, CD8 double-negative thymocyte maturation in double-positive cells. Prolactin influences both Th1 and Th2 cytokine production by prolactin-mediated upregulation of IL-6 and IFNγ and downregulation of IL-2 (Tanev et al., 2016). It has been reported that approximately 15%-20% of SLE patients of either gender have hyperprolactinemia, whereas the etiology in not yet fully understood (Fojtikova et al., 2010).

2.5.2. Age

SLE has a characteristic age of onset after menarche and before menopause. Outside the period of female reproductive activity, the onset of disease is uncommon and without sex preference. It has been shown that 65% of the SLE patients have been diagnosed with the disease between the ages of 16 and 55. These data reinforce the statement of higher prevalence of the disease in childbearing years (Pons-Estel et al., 2010, Danchenko et al., 2006). The organization Lupus UK has recorded that the peak age of the diagnosis of the disease onset in women is 15-40 years., enhancing the key role of hormones in the pathogenesis of lupus.

2.5.3. Genetic factors

It is now apparent that the predisposition to produce autoantibodies is genetically determined, and that many genes and genetic loci can further stimulate this predisposition. Many studies have concluded that both genetic and allelic heterogeneity have an impact in the initiation of SLE (Tsokos et al., 2007). The production of autoantibodies can precede clinical disease by several years. Recently, it has become clear that target organ vulnerability to autoimmune attack is also genetically determined. Thus, some individuals will experience more tissue destruction than others, despite harboring the same autoreactivity (Ramos et al., 2010). Javierre et al. demonstrated an increased concordance rate between monozygotic twin (24%-69%), almost 10 times higher compared to dizygotic twins (2%-9%), further supporting the genetic influence in SLE. However, the lack of 100% in monozygotic twins, indicates the impact of environmental and other factors in the development of the disease.

MHC was the first region that was found to be associated with SLE. It comprises the human leukocyte antigen (HLA) class I and II regions encoding the genes participating in antigen presentation. Moreover, it comprises the class III region containing various immune genes such as cytokines. The **class II alleles HLA-DR2** has been the most persistent SLE genetic risk factor (mainly in Caucasians), indicating an overall 2-to-3-fold increased risk for the development of the disease. Additionally, TNFα in class III as well as **TAP1** (transporter 1, ATP binding cassette subfamily B member) and **TAP2** (transporter 2, ATP binding cassette subfamily B member) genes in class II have been also associated with SLE (Eroglu et al., 2002, Ramos et al., 2010).

Complement components include plasma proteins, key components of the innate immunity, can be activated by three pathways: classical, alternative and lectin pathway. They are important in host resistance to bacterial infection and in the clearance of ICs, therefore they have a protective role against autoimmunity (Yang et al., 2015). They also participate

in the lymph node organization, B-cell maturation, differentiation and tolerance, as well as IgG isotype switching. Complements including C_2, C_4A, C_4B and factor B are located in the MHC class III region. Complement deficiency is mainly inherited as recessive trait except C_1 inhibitor, CR_1 and properdin deficiency, leading in SLE susceptibility (Vaughn et al., 2013).

FCγ receptors (**FCγR**), are divided in three distinct but closely related classes, named **FCγRI** (**CD64**), **FCγRII** (**CD32**) and **FCγRIII** (**CD16**) have demonstrated a strong association with both susceptibility and severity of lupus. Their function is to bind and clear IgG antibodies and IgG-containing ICs from the circulation. They can either be *stimulatory* (FCγRIIA, FCγRIIIA, FCγRIIIB, FCγRIIC) or *inhibitory* (FCγRIIB) to immune responses. FCγIIB is the only gene of this family possessing an immunoreceptor tyrosine-based inhibitory motif (ITIM) and can transmit inhibitory signals in B-cells and myelomonocytic cells. Furthermore, the FCRIIIA-F176 allele polymorphism can increase the risk of lupus nephritis (LN) (Alvarez-Errico et al., 2016).

The TNF superfamily member OX40L (**TNFSF4**) gene has also been related with the development of SLE, expressed upon activation of APCs and vascular endothelial cells. It interacts with the single OX40 receptor expressed on activated T cells, sustaining their survival. Increased TNFSF4 expression can either quantitatively augment T cell – APC interaction or influence the functional consequences of T cell activation via TNFRSF4 (Vaughn et al., 2012). Additionally, **TNFAIP3** (TNFα-induced protein 3) catalyzes the ubiquitin modification of adaptor proteins downstream of TLR, IL1R, TNFR and acts as a negative regulator of the NF-κB pathway (Tsokos et al., 2007). **TREX1** (transcription export), the main DNA repair exonuclease, causes ssDNA damage in caspase-independent apoptosis activated by granzyme A and is a necessary negative regulator of the IFN-stimulatory DNA response. Defective TREX1 can lead in the disability to degrade ssDNA

or dsDNA resulting to immune activation and development of autoantibodies. (Yang et al., 2015). **TNF$_2$** is a common susceptibility allele for SLE, as well as other autoimmune rheumatic diseases, whereas it has a protective role against tuberculosis (TB), leading in the proposal that autoimmune diseases could result of natural selection for enhanced TB resistance (Cui et al., 2013).

Furthermore, **BANK1** (B-cell scaffold protein with ankyrin repeats 1) can contribute to preserved B-cell receptor signaling, breakdown of B-cell tolerance, production of autoantibodies and B-cell hyperactivity (Vaughn et al., 2012). **BLK** (B-lymphocyte tyrosine kinase) is also associated with SLE. The risk allele at BLK is related with decreased expression of BLK in B-cell lines, as it normally interacts with the B-cell receptor and mimics pre-B cell receptor signaling. Altered BLK protein levels can induce B-cell tolerance mechanisms (Eroglu et al., 2002).

Numerous genes have been associated with the onset, development and severity of SLE as an autoimmune disease, or of an organ-specific lupus-associated damage, although a variety of trials and researches are still conducted. More genes that are known to participate in the susceptibility of the disease include **IRF5** (related with type I IFN production), immunoreceptor tyrosine-based inhibitory motif (**ITIM**), pituitary tumor transforming protein 1 (**PTTG1**), autophagy protein 5 (**ATG5**), interleukin-1 receptor-associated kinase 2 (**IRAK2**), methyl CpG binding protein 2 (**MECP2**), integrin-a_M (**ITGAM**), programmed cell death 1 gene (**PDCD1**) etc (Ramos et al., 2010).

2.5.4. Epigenetic factors

Epigenetic mechanisms are reversible and heritable patterns that can control gene expression *without* changing the underlying DNA sequence. They govern the accessibility of DNA to the transcriptional complex (RNA polymerases, transcriptional factors) and the

gene expression in a tissue- and signal-specific way (Tsokos et al., 2007). Various molecular mechanisms can incorporate to what is called "epigenome", including **DNA methylation, histone modification**s and **non-coding transcripts**. Alterations of the epigenome are related to the dysregulation of signaling molecules and receptors, common in SLE, although they are also interesting potential targets in the search for SLE pathological mechanism and in future therapeutic approaches (Long et al., 2016).

During **DNA methylation** a methyl group is added to the 5' carbon position of cytosine-phosphate-guanosine (CpG) dinucleotides. DNA methylation is the most well-understood and well-studied epigenetic event. This procedure is carried out by enzymes called DNA methyltransferase (DNMT) that can be divided in maintenance DNMTs (DNMT1), responsible for the re-methylation in cell division, and de novo DNMTs (DNMT3a, DNMT3b) that confer DNA methylation, independent of pre-existing patterns. Later, it was also confirmed that DNMT1s can also confer de novo DNA methylation (Hedrich et al., 2017). Methylated-CpG-binding proteins are able to recruit histone deacetylases (HDACs) and further remodeling factors. Interrupted DNA methylation is one central contributor to SLE and various autoimmune/inflammatory disorders (Hedrich et al., 2011). Mechanisms implicated in altered DNA methylation in immune cells during SLE include *altered DNMT expression and activity*, directed by the action of *Mitogen Activated Protein Kinases (MAPK)* whose uncontrolled activation can result in altered methylation of genomic DNA. Moreover, *growth-arrest* and *DNA damage inducible protein45a* (*GADD45a*) cause DNA demethylation in T cells (Alvarez-Errico et al., 2017) *TET proteins* (hydroxytransferase ten eleven translocation) by the oxidation of methylated cytosines in the CpG dinucleotides cause *DNA hydroxymethylation* which is involvedin the pathophysiology of autoimmune diseases like SLE. Finally, *dysregulated transcription factor network* contributes in the generation of effector T cells in lupus, in contrast with healthy organisms, where

transcription factors instruct epigenetic remodeling, and thus defining the phenotype of cells and tissues (Hedrich et al., 2014).

2.5.5. Environmental factors

There has been a tremendous progress in examining the potential SLE environmental risk factors yet so much more are needed. Gene-environment interactions are able to explain why individuals respond differently to the same environmental trigger, why some of them may develop the disease under the exposure of an environmental factor, whereas others do not (Miller et al., 2012).

Occupational exposure to **silica** was established as a contributor in the development of SLE. Exposure to particulate silica (crystalline silica or quartz) is usually typical in mining and "dusty trades" including granite cutting, cement work, brick and tile laying, sandblasting and construction work. It is a dose-dependent response with increased risk for those with increased exposure (Tsokos et al., 2007, Kamen, 2014)

Tobacco smoking has been included in the factors that can *possibly* contribute in SLE development with a modestly elevated risk of the disease in current smokers compared to those who have never smoked. However, smoking appears to mostly affect the course of the disease and especially the skin manifestations with current smoking related with active SLE rashes and ex-smoking related with discoid rash and photosensitivity (Costenbader et al., 2004). Despite the fact that smokers are slightly more prone to develop SLE, the risk is further increased in those with certain polymorphisms in genes for metabolic enzymes participating in reactive oxygen species (ROS) production, e.g. polymorphism in CYP1A1 (Harel-Meir et al., 2007),

Epstein-Barr Virus (**EBV**) **Infection** is a human herpesvirus 4 (HHV4). SLE and EBV-induced infectious mononucleosis (IM) have similar symptoms and clinical manifestations,

demonstrating a correlation. SLE patients have been shown to have a minimum 10-fold elevated frequency of EBV-infected peripheral B cells and increased viral load in the peripheral blood mononuclear cells compared to a healthy organism (Miller et al., 2012, Draborg et al., 2012). The lack of control of EBV infection can result in a widespread latent infection and reoccurring reactivation, indicating higher amounts of EBV-infected B cells and epithelial cells, possibly leading to increased cell apoptosis and load of amplified cellular waste. Defective EBV-specific T cells, expression of viral genes and increased EBV IgA antibodies in SLE have demonstrated genetic and/or acquired difficulties in suppressing the infection and keeping the virus in latent state. It has been suggested that the infection and reactivation of EBV has a pathologic impact in the development of SLE, mainly in genetically predisposed patients (Lossius et al., 2012), as SLE susceptibility genes influence EBV replication and immune evasion (Kamen, 2014).

Vitamin D

Vitamin D is an essential steroid hormone with crucial role in mineral metabolism, as well as skeletal cardiovascular and immune system. It demonstrates regulatory effects on growth, proliferation, function and apoptosis of immune cells related with the pathophysiology of SLE (Pakpoor et al., 2013). Vitamin D regulates the immune system by its involvement in the lymphocyte proliferation, IL-2 inhibition and antibody production. 1,25-dihydroxy vitamin D_3 (1,25(OH)D_3) inhibits IFN-γ secretion and inversely controls IL-12 production by downregulating NF-κB. Some of the vitamin D deficiency results include fatigue and decreased muscle strength as vitamin D receptors are also located on muscle cells and SLE patient have a lower quality of life and exercise capacity. Serum levels of 25(OH)D in lupus are directly associated with serum albumin, glomerulonephritis and the magnitude of proteinuria (Mihaylov et al., 2016, Hassannalilou et al., 2017). Moreover, a connection between 25(OH)D_3 level and cardiovascular diseases like stroke, increased artery

calcification, myocardial infraction (MI) and hypertension are also been observed (Tsokos et al., 2007). The risk of fractures and osteoporosis is increased in SLE patients with lack of Vitamin D. 1,25(OH)2D normally aids in the improvement of intestinal absorption and renal resorption of calcium by mediating the interactions between Vitamin D and Vitamin D receptors (VDRs) (Ruiz-Irastorza et al., 2010).

Vitamin D deficiency is usually reported in SLE patients, as a bidirectional connection between them is revealed; SLE can result in decreased Vitamin D levels, whereas vitamin D deficiency may have a causative role in the disease etiology and aggravation. SLE can lead to Vitamin D deficiency due to the avoidance of sunshine, photoprotection, renal insufficiency, as well as the use of pharmacotherapies like glucocorticoids, antimalarials, anticonvulsants, calcineurin inhibitors that alter Vitamin D metabolism of downregulate the function of Vitamin D receptor (Hassanalilou et al., 2017). Notably lower serum 25-hydroxyvitamin D (25OHD) levels have been observed in newly diagnosed SLE patients (Kamel, 2014).

Furthermore, various other potential environmental candidates related with SLE development and/or aggression like UV radiation, dietary alfalfa sprouts are examined, although numerous studies are still needed. These factors include metals like mercury (related with antinuclear antibody and inflammatory cytokine production), persistent organic pollutants (POPs), asbestos, industrial chemicals and solvents etc. (Kamen 2014).

Drug-induced lupus erythematosus (DIL) refers to the development of a lupus-like syndrome after the exposure to certain drug. Typically, the resolution of the clinical features is rapid after the discontinuation of the offending agent, however autoantibodies might persist for a prolonged period. Moreover, evidence suggest that genetic predisposition is also related to the determination of which patients will develop lupus-like syndrome. DIL should be suspected in patients without any previous diagnosis or history of SLE, and yet they

developed a positive ANA (antinuclear antibodies) test and at least one of the main clinical symptoms of lupus including arthritis, myalgia, rash, fever and serositis. In more than 95% of these cases antihistone antibodies are present, while anti-DNA antibodies and hypocomplementemia are rare, except if the disease is induced by IFNα and anti-TNF medications (ACR, Dalle Vedove et al., 2009)

In the table below are listed the drugs that have been reported to induce lupus-like disease and associated autoantibodies.

Agent	Risk	Agent	Risk	Agent	Risk
Antiarrhythmics		Perphenazine	Very low	Mesalamine	Low
Procainamide	High	Phenelzine	Very low	*Diuretics*	
Quinidine	Moderate	Lithium carbonate	Very low	Chlorthalidone	Very low
Disopyramide	Very low	*Anticonvulsants*		Hydrochlorothiazide	Very low
Propafenone	Very low	Carbamazepine	Low	*Hypolipidemics*	
Antihypertensives		Phenytoin	Very low	Lovastatin	Very low
Hydralazine	High	Trimethadione	Very low	Simvastatin	Very low
Methyldopa	Low	Primidone	Very low	*Miscellaneous*	
Captopril	Low	Ethosuximide	Very low	Propylthiouracil	Low
Enalapril	Low	*Antibiotics*		Levodopa	Very low
Acebutolol	Low	Isoniazid	Low	Aminoglutethimide	Very low
Labetalol	Very low	Minocycline	Very low	Timolol eye drops	Very low
Pindolol	Very low	Nitrofurantoin	Very low	*Biologic agents*	
Clonidine	Very low	*Anti-inflammatory*		TNAα blockers	High
Minoxidil	Very low	D-Penicillamine	Low	Interferon α	Low
Prazosin	Very low	Sulfasalazine	Low		
Antipsychotics		Phenylbutazone	Very low		
Chlorpromazine	Low	Zafirlukast	Very low		

Table 2 – Drugs reported to induce lupus-like disease and associated autoantibodies (retrieved from Bertsias et al., 2012).

Hydralazine is an antihypertensive agent and it is the first medication *undoubtedly associated with DIL*. It has been estimated that approximately 24%-54% of patients receiving hydralazine therapy will develop anti-nuclear antibodies, although only 2%-21% of them will develop lupus-like symptoms. The main symptoms include fever, arthralgias, arthritis, malaise and less commonly serositis. Renal impairment has been also observed during this therapy, however it is still unclear if the renal manifestations are actually

associated with hydralazine or if they are related to the underlying hypertension (Tsokos et al., 2007). The development of DIL is related linked to the HLA class II and HLA class III genes, as a noteworthy increased frequency of HKA-DR4 antigen is observed. This issue is dose-dependent, meaning that patients receiving a dose greater than 200mg hydralazine per day have a significant increase risk of development DIL compared to those receiving lower doses (Chamsi-Pasha et al., 2014). The risk is also linked to the acetylator phenotype which is under genetic control, i.e. slow acetylators are more prone to develop DIL. This is due to the slower rate of inactivation of the parent compound through acetylation of its hydrazine group by the hepatic acetyl transferase enzymes (Finks et al., 2006). Anti-nuclear and anti-histone antibodies have been observed in patients receiving hydrazine, as well as anti-phospholipid, lymphotoxic, anti-neutrophilic cytoplasmic antibodies to poly A and Z-DNA (Chamsi-Pascha et al., 2014, Kamen 2014).

Procainamide is a class I antiarrhythmic agents that is also highly related with the development of DIL. 90% of the patients will have a positive ANA test, whereas approximately 30% of them will develop lupus-like symptoms. Common clinical features include musculoskeletal and constitutional symptoms where about half of the patients will develop pleuropulmonary involvement and/or pericarditis (Dalle Vedove et al., 2009, Tsokos et al., 2007) These symptoms can be manifested 1month to 12 months after the initiation of the procainamide therapy, with an average of 1 year. Similar to hydralazine, patients taking procainamide have anti-phospholipid, lymphotoxic antibodies, antibodies to poly A and Z-DNA, as well as lupus anticoagulants. Additionally, antibodies to procainamide have been observed (Chang et al., 2011).

Quinidine is another class I antiarrhythmic agent also linked with DIL, although in a lower extend compared to procainamide. Quinidine-related lupus is primary seen in older (>63 years of age) white patients, with no gender preference (Kamen, 2014). Symptoms are

usually developed after 1-12 months of therapy and can be extinguished within 1-4 weeks after its discontinuation. Frequently met symptoms include arthralgia and arthritis symmetrical involved in hands and wrists mainly and thus, DIL could be mistaken for rheumatoid arthritis (Borchers et al., 2007). Other, less frequently observed symptoms are rash, fever, serositis and peripheral neuropathy. Additionally, thrombocytopenia has been observed in approximately 47% of patients and leukopenia in 24% of patients (Chang et al., 2011).

Anti-TNFα drugs have been also highly associated with DIL, most commonly in patients on **infliximab**, followed by those on **etanercept** and **adalimumab**. The exact mechanism is not yet fully understood although three district pathways exist, all resulting in autoantibody production (Almoallim et al., 2012). A positive ANA test, anticardiotropin antibodies in approximately 25% of the patients and anti-dsDNA antibodies of IgG, IgM and IgA subtypes can be observed (Zhu et al., 2010). Despite the fact that antihistone antibodies can been also detected, the number of patients who will express them is not yet clear (Costa et al., 2008). Main symptoms of anti-TNFα-induced lupus most commonly consist of malar rash, purpura, discoid rash and photosensitive rash, with an onset ranging from a month to 4 years and usually resolve after the discontinuation of the offending agent. Moreover, fever, malaise and arthralgia can be present (Shakoor et al., 2011).

Numerous drugs are associated with DIL, as listed in Table 2, with a notably observation indicating an increased frequency of a positive ANA test in children on anticonvulsant therapy compared to the adult patients on the same treatment. Further research is needed in order to establish the role of certain drugs in DIL, as well as their correlation with genetic factors. In the feature below, a summary of the correlation between environmental, genetic and epigenetic influences on SLE is demonstrated:

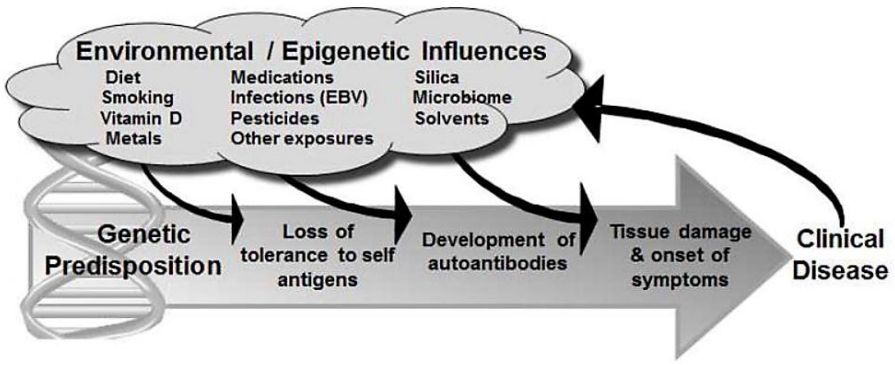


Figure 4 - Interplay between environmental factors, genetics and epigenetics in SLE development (retrieved from Kamen, 2014)

2.6. CLINICAL MANIFESTATIONS

Even though various possible SLE manifestations exist, lupus is a disease with many facets meaning that rarely do two people have the exact same symptoms. However, a list of generally most common symptoms exists including the following:

2.6.1. General symptoms

Fatigue is one of the most common and often the most debilitating symptom of lupus patients, interfering with their ability to perform daily tasks, during both periods of flares and remissions. To distinguish between SLE-related fatigue or fatigue due to another connective tissue disease, in SLE this symptom decreases in the morning and is increased in the evening (Heinlen et al., 2007, Tsokos et al., 2007).

Arthritis is also a major presenting symptom of SLE, affecting 80-95% of patients. It is usually a non-erosive, non-deforming and symmetric arthropathy, with tender, swollen and effusive joints. Arthritis mainly accompany SLE onset or flare. The small joints most frequently involved, especially proximal interphalangeal, metacarpal phalangeal, wrists and knees (Tsokos et al., 2016).

Fever in SLE is usually low grade and rarely exceeds 38°C. Although it is a very common symptom during the onset of SLE, fever can indicate numerous disorders (Bertsias et al., 2012). Unexplained **weight loss** of approximately 5% of body weight can be also observed in lupus patients. Unexplained weight gain is also observed in some cases (Lupus UK).

2.6.2. Musculoskeletal manifestations

As discusses above, arthritis is the main musculoskeletal manifestation of SLE is arthritis. Furthermore, inflammation of the synovial membrane lining the joints, also called *synovitis* can also occur. It is reversible and even resolve within a few days in some patients, migratory and is accompanied with pain and stiffness. The term "rhupus" is used in some rare, extreme cases, where synovitis can be so intense that it cannot be distinguished from rheumatoid arthritis. *Costochondritis* (inflammation of the rib cage cartilage) is accompanied with chest pain, discomfort and tenderness, although conditions like angina pectoris or pericarditis should be first excluded. Relapsing *polychondritis* (multi-system episodes of inflammation and deterioration of cartilage) can also occur and is usually treated with low-dose corticosteroids.

Myositis (muscle tissue inflammation) is characterized by generalized myalgia, muscle tenderness and swelling, involving mainly the proximal muscles can occur any time during SLE, especially in exacerbations. *Avascular bone necrosis* is a major cause of morbidity and disability among SLE patients, affecting 5%-12% of them. It can be indicated by acute joint pain inn shoulders, hips and knees and can be induced by Raynaud's phenomenon, corticosteroids, antiphospholipid syndrome, vasculitis and fat emboli. Moreover, osteonecrosis can occur shortly after the onset of high-dose corticosteroids.

2.6.3. Renal manifestations

Clinical evidence of kidney involvement and particularly lupus nephritis (LN), is the most common organ-threatening SLE manifestation, occurring in 40%-70% of patients, especially within the first 5 years after the SLE diagnosis (Ayoub et al., 2018). More susceptible to renal pathology are male patients, young-ages (<33 years) and non-Caucasians. IFN-α, even when administered therapeutically, is able to induce SLE/LN. Interestingly, it has been estimated that children have a high incidence of LN, reaching 80% (Ilori et al., 2016). Intrinsic antigens like extracellular matrix components or cell surface glycoproteins may be utilized as targets for autoantibody binding. Furthermore, renal injury can occur either due to autoantibodies binding either to the circulating antigens, resulting in circulating performed ICs, or to antigens deposited in glomerular and vessel walls from the circulation, leading in the formation of in situ ICs. Following, the binding of Fc receptor and complement initiate an inflammatory and cytotoxic reaction that, if not treated early and successfully may result in end-stage kidney disease (ESKD) (Hahn et al., 2012, Tsokos et al., 2007).

The glomerular patterns of immune complex-mediated injury are associated with the site of Ig accumulation, their antigen specificity, their binding and activation complement as well as various other serine proteases and the initiation of a cellular inflammatory response. These patterns are divided in three groups. The **mesangial pattern** resulting in IgA nephropathy or mesangial proliferative LN, leading in *hematuria*, subnephrotic *proteinuria* with well-preserved or minimally decreased glomerular filtration rate (GFR). The **endothelial pattern** is characterized by leukocyte accumulation, endothelial cell injury and endocapillary proliferation, resulting in capillary wall destruction. This pattern is associated with renal diseases like severe postinfectious glomerulonephritis and systemic vasculitis. Moreover, it is also strongly related with acute GFR reduction, *hematuria* and mild-to-moderate *proteinuria*. Finally, the **epithelial pattern** results in a non-exudative, non-proliferative

capillary wall lesion seen in both SLE-related and idiopathic membranous glomerulopathy. It is implicated with significant *proteinuria*, nephrotic syndrome and preservation or gradual reduction of GFR (Nimri et al., 2012, Ayoub et al., 2018).

2.6.4. Dermal manifestations

It has been estimated that 90% of SLE patients will have **skin** involvement during the course of the disease. Most skin disorders in SLE are *acute cutaneous lupus* (30%-50%) and *subacute cutaneous lupus* (10-15%). A typical dermal effect of lupus is the **malar rash** (also called **butterfly rash**), characterized by erythema and edema of cheeks, sparing in the nasolabial folds, with rapid onset (ACR, Lupus UK, Tsokos et al., 2016). **Photosensitivity** is strongly related with disease manifestation, proposing an abnormal reactivity to UV light resulting in skin lesion or (in more severe cases) sunburn after a considerable sun exposure (Kuhn et al., 2010).

Lupus can be manifested and develop only in the skin area, with no systemic effects, although it can later develop also systemic effects. Chronic cutaneous lupus erythematosus occurs in 80% of cutaneous LE patients, whereas 25% of these patients also have SLE (BAD, 2018). The main cutaneous forms of lupus include:

- **Acute cutaneous lupus erythematosus** is observed in at least 50% of SLE cases, characterized by a malar rash remaining for hours or days, erythematous popular rash on arms or even large plaques, photosensitivity, mouth ulcers, erosions and blisters (bullous LE) (Grönhagen et al., 2014).
- **Subacute cutaneous LE** is observed in 15% of cases, whereas one third of them are causes due to prior phototoxic drug exposure and can either precipitate or aggravate

by sun exposure. Non-itchy psoriasis-like rashes on the upper back, chest and arms can occur with no scarring after resolution (Kuhn et al., 2010, BAD 2018).

- **Intermittent cutaneous LE** affects sun-exposed dermal area like cheeks, neck and anterior chest. It occurs in erythematosus, urticaria-like plaques and patches with annular or round shape, clearing during the winter without scarring (Moura et al., 2014).
- **Drug-induced subacute cutaneous LE**
- **Neonatal cutaneous LE** can occur within 2 months of birth in infants whose mothers have known or subclinical cutaneous or systemic LE. It is characterized by a periorbital annular erythematosus rash and photosensitivity. Moreover leukopenia, haemolytic anemia and thrombocytopenia can occur, as well as hepatobiliary disease and persistent congenital heart block that can lead by 20% in mortality (Tsokos et al., 2016, Grönhagen et al., 2014).
- **Discoid lupus erythematosus** is the most common form of chronic cutaneous LE (BAD, 2018) and is mainly located on the cheeks, nose ears and scalp, although it can spread to the upper back and chest, forearms and hands. The scalp can reveal atrophic alopecic patches. The affected area could have edema initially, related with follicular plugging and erythema, keratotic plugs, red dots and enlarged branching vessels (Moura et al., 2014). Photosensitivity is prominent. Slow healing results in post-inflammatory pigmentation and white scars (Oboite et al., 2016) Recurrent oral ulcerative lesions with difficult healing should be also taken into consideration (BAD, 2018).
- **Mucosal LE** includes plaques, ulcers and scaling found on the lips, inside the mouth, lower eyelids and can predispose to squamous cell carcinoma (Grönhagen et al., 2014)

- **Lupus profundus** (also called **lupus panniculitis** and **subcutaneous LE**) are firm, deep and tender nodules which can develop at any age (BAD, 2018).

2.6.5. Neuropsychiatric Systemic Lupus Erythematosus (NPSLE)

Neuropsychiatric manifestations are highly recognized in SLE patients including a variety of neurologic and psychiatric issues, ranging from mild to severe. It is estimated that the cumulative incidence of NPSLE is 30%-40% and almost half of them occur either during the onset of SLE or 1-2 years after the diagnosis, whereas the most severe symptoms are associated with older age (Bertsias et al., 2010). The cause attribution of the neuropsychiatric symptoms is quite challenging as approximately 60% of them represent complications of the disease or its therapy (secondary NPSLE), or causes irrelevant to SLE like metabolic reactions, infections and adverse drug reactions. Common *symptoms* include headache, mild to moderate cognitive dysfunction, anxiety, mood disorders and seizure disorders. Moreover, recurrent seizures can develop in epilepsy and they are also related with concurrent cerebrovascular disease and psychosis (ACR, 2018). The presence of antiphospholipid antibodies is responsible for cerebrovascular disorders, seizures, myelopathy, chorea and moderate-to-severe cognitive dysfunction (Hanly et al., 2009).

2.6.6. Hematologic manifestations

Hematologic abnormalities are common and presenting SLE features. The main clinical manifestations are anemia, neutropenia, autoimmune hemolytic anemia (AIHA), lymphopenia, thrombocytopenia, hemophagocytic syndrome, thrombotic thrombocytopenic purpura (TTP) and the antiphospholipid syndrome. Nevertheless, none of these symptoms are SLE specific, therefore it is essential to differentiate between SLE-related hematologic

disorders from the results of a coexisting hematologic disease or of immunosuppressive agents (Domiciano et al., 2010).

Anemia is a common SLE feature, related with the disease activity. It can be induced by chronic disease, renal insufficiency, hemolysis (autoimmune or microangiopathic), blood loss (e.g. secondary to medications), hypersplenism, infections, medications, myelofibrosis or as aplastic anemia. Autoantibodies can result in cell and tissue damage by Fc receptor-mediated inflammation and by direct complement-dependent cytotoxicity resulting in antibody-mediated hemolytic anemia. Overt autoimmune hemolytic anemia has been observed in SLE patients, who may have a positive Coombs test without overt hemoptysis. A microangiopathic hemolytic anemia with or without symptoms including fever, kidney involvement, thrombocytopenia and neurologic manifestations of thrombotic thrombocytopenic purpura (TTP) can been observed in SLE. Hallmarks of this disorder are schistocytes in the peripheral blood smear and elevated lactate dehydrogenase (LDH) levels. When this happens under generalized SLE activity the term TTP-like syndrome is used. Rarely, red cell aplasia due to antibodies against erythrocyte progenitors.

Leukopenia is a common presenting symptom in SLE and is related with disease activity. A white blood cell count <4500/mm^3 has been observed in up to 40% of SLE patients, especially during the active disease. *Lymphopenia* (lymphocyte count < 1500/mm^3) is the most frequent white blood cell abnormality in SLE, persist throughout the disease and can fluctuate during flares. Severe lymphopenia (<5000/mm^3) is very rare. Moreover, immunosuppressives and glucocorticoids can also contribute to SLE lymphopenia (Yu et al., 2016). *Neutropenia* (neutrophil count < 1000/mm^3) is a common feature in SLE, and it is possibly caused by the antibodies directed against neutrophil cell surface antigens. Mild neutropenia can be found in up to 40% of SLE patients, whereas severe neutropenia is rare.

Thrombocytopenia (platelet counts 100000-150000/mm^3) in SLE can be a result of immune-mediated platelet destruction or even elevated platelet consumption in case of microangiopathic hemolytic anemia, hypersplenism, secondary to medications, or as a result of a bone marrow involvement like the hemophagocytic syndrome or myelofibrosis (Shah et al., 2007). *Idiopathic thrombocytopenic purpura* (*ITP*) can also be the first SLE sign and especially when it is accompanied with high ANAs or *exactable nuclear antigens* (*ENAs*). Mild thrombocytopenia is a common finding in patients with antiphospholipid syndrome. Thrombocytopenia that appears at the early course of SLE is related with a more severe and active disease (Jung et al., 2016).

2.6.7. Cardiac manifestations

Cardiac involvement is one of the most common incidences of SLE where any part of the heart can be affected, like the pericardium, myocardium, valves, conduction system and coronary arteries. SLE patients have significantly increased morbidity and mortality from cardiovascular diseases (CVD) (Appenzeller et al., 2011).

Pericarditis is the most frequent cardiac manifestation in SLE, related with active disease in the organs. In acute pericarditis, patients experience substernal or precordial chest pain, positional in nature. Symptoms worsen with supine position, coughing, inspiration, swallowing and are relieved with sitting up or bending forward. Furthermore, dyspnea, tachycardia and fever can occur (Gustafsson et al., 2012). On auscultation, diminished heart sounds and a pericardial friction rub are heard, although in order for these sounds to be better heard, the patient should be leaning forward at end-expiration (Yu et al., 2014).

Acute *myocarditis* is involved in up to 10% of SLE patients, with similar symptoms of myocarditis. Patients possibly recall a recent GI illness (nausea, diarrhea) or seek medical attention for mild-nonspecific viral flu-like symptoms (Bertsias et al., 2012). Moreover, they

may experience severe stabbing chest pain, palpitations, lethargy and dyspnea, resulting in complications like arrhythmias and cognitive heart failure. In all the above-mentioned cases any responsible infections should be identified, such as Mycobacterium tuberculosis (MTB), Staphylococcus aureus etc. (Yu et al., 2014).

Valvular abnormalities like valve vegetations, thickening of the mitral and aortic valves or dysfunction and stenosis are associated with antiphospholipid syndrome in SLE patients. Stroke, heart failure, peripheral embolism, infective myocarditis and need for valve replacement is approximately 3 times increased in SLE patients with valvular disease. Systolic cardiac murmurs that can be heard in up to 70% of patients is also related with the antiphospholipid syndrome. Cardiac tamponade, where there is a pericardial fluid buildup around the heart, is a rare and life-threatening situation in SLE (Bertsias et al., 2012, Tsokos et al., 2007).

2.6.8. Pulmonary manifestations

Over 50% of SLE patients will develop a form of pleural disease like pleuritis, pulmonary arterial hypertension, lupus pneumonitis, diffuse alveolar hemorrhage, pulmonary embolism associated with antiphospholipid syndrome, or even chronic interstitial lung disease in their lifetime (Bouros et al., 2008, Condliffe et al., 2009).

Pleuritis is the most common pleuropulmonary SLE manifestation, affecting approximately 40%-60% of patients, and correlates with disease activity in other organs. It can be unilateral (affect one lung), but in the majority of cases it is bilateral, and of moderate intensity in SLE (Toworakul et al., 2011). Increased risk for the development of pleuritis among SLE patients has been observed in those with concomitant positive anti-Sm and anti-RNP serology, higher cumulative damage, prolonged disease duration and younger age of SLE onset. Patients

report sharp chest pain when coughing or breathing, shortness of breath, dyspnea, cough and possibly low-grade fever (Mittoo et al., 2010).

Pulmonary hemorrhage is a life-threatening SLE complication, seen in patients with severe, multi-organ and increased disease activity. It can result from vasculitis of the pulmonary vessels and it is usually marked by hemoptysis and confirmed by bronchoscopy (Gulhane et al., 2012). The symptoms are non-specific; however, its characteristic manifestations include dyspnea, hypoxemia, diffuse alveolar infiltrates and anemia. This condition mostly occurs in patients with known SLE history, active extrapulmonary disease and increased titres of anti-DNA antibodies (Mittoo et al., 2014).

Acute Lupus pneumonitis presents abruptly with cough, dyspnea, fever, chest pain, hypoxia and occasionally hemoptysis, and is characterized by 50% mortality if not treated immediately (Gulhane et al., 2012). These symptoms are similar with bacterial infections and alveolar hemorrhage, so further examinations should be performed in order to exclude them (Mittoo et al., 2010). **Pulmonary hypertension** (**PH**) is a rare but potentially life-threatening SLE complication that may be secondary to chronic pulmonary emboli or arise from the disease itself (Leslie et al., 2007).

2.6.9. Raynaud's Phenomenon (RP)

Raynaud's phenomenon is characterized by vessel hyperactivity causing a reduction of the blood flow, especially in the extremities, as a response to low temperatures and sudden emotional stress leading in skin color changes typically in the fingers and less commonly in the toes. Rarely, the lips, nose and ears can be involved. RP implicates excessive peripheral vasoconstriction where the fingers undergo distinctive color changes, reflecting the lack of oxygenation and tissue perfusion. Ischemia causes pain and hypoesthesia, resulting in discomfort and loss of functionality. In fact, RD can be manifested in any body area

containing thermoregulatory vessels. A vasospasm episode is presented as a triphasic or diphasic color change of the fingers. Ischemia is manifested as paleness that may be followed by cyanosis with a blue coloration, due to the blood desaturation. Finally, reflex vasodilation occurs, where the blood flow is restored, presented as erythema due to the sudden blood flow, accompanied with a burning sensation (Mpaltagiannis et al., 2017). RP affects one-third of SLE patients. Although the exact mechanism by which RP can be triggered in lupus, it has been suggested that it results from the nervous system involvement and the presence of pulmonary hypertension (Heimovski et al., 2015).

2.6.10. Gastrointestinal (GI) manifestations

GI involvement is usually mild. The most common SLE GI manifestations are painless ulcers in the mouth and nose, something almost all patients will develop during the course of their disease, whereas esophageal ulcerations and dysphagia are rarely seen. The most common causes of acute pain are pancreatitis, mesenteric vasculitis, gastroenteritis, hepatobiliary disease and appendicitis that may lead to peritonitis (Ellen et al., 2011).

Pancreatitis is a rare condition, manifested with abdominal pain, followed by nausea and/or vomiting, fever, abdominal distension, absent bowel sounds and an increasement in serum amylase or lipase (Nesher et al., 2006). It has been estimated that more than half of the cases develop acute pancreatitis within 2 years of the SLE diagnosis or during active disease (Tian et al., 2010). SLE patients may have acute pancreatitis secondary to non-SLE causes including cholelithiasis, alcohol consumption, certain medications, hypocalcemia, hypertriglycemia, viral infections. Elevated serum amylase or lipase are the main pancreatitis markers (Makol et al., 2010).

Peritonitis can result from small vessel involvement in the bowel serosa or retro peritoneum, or from perforation of the bowel. Rebound nausea, vomiting, diarrhea,

tenderness and fever occur. Clinical signs can be masked by the use of corticosteroids and immunosuppressives Bacterial peritonitis is frequent in patients with nephrotic syndrome (Ebert et al., 2011). The exact pathogenesis of SLE-related peritonitis remains obscure. Inflammatory infiltrates, immunoglobulin and complement deposits can be observed in peritoneal tissues and peritoneal vessels (Bertsias et al., 2012). **Ascites,** the pathologic fluid buildup in the abdominal cavity, may or may not be present in this case. Furthermore, chronic ascites is also related to SLE can be due to nephrotic syndrome, protein-losing enteropathy, heart failure, constrictive pericarditis or irrelevant infection like TB.

Hepatic involvement

Hepatomegaly (abnormally enlarged liver) can be identified in approximately 40% and splenomegaly (abnormally enlarged spleen) can be seen in 6% of SLE patients, both endangered for rupture (Tian et al., 2010). *Autoimmune hepatitis* (*AIH)* is a rare SLE clinical feature and is presented with a non-specific onset like fatigue, malaise and anorexia. In more severe cases, specific symptoms like jaundice, hepatomegaly and ascites may occur (Ebert et al., 2011).

2.6.11. Ocular manifestations

Ocular manifestations are fairly common in SLE, and almost any part of the eyes visual pathways like eyelid, ocular adnexa, sclera, cornea, uvea, retina as well as the optic nerves can be affected. The most common ocular disease in SLE is **keratoconjunctivitis sicca**, also associated with secondary Sjögrens's syndrome (Bertsias et al., 2010).

In the table below, some of the main clinical features compatible with the SLE diagnosis are listed:

Main clinical features compatible with the diagnosis of SLE		
Abdominal pain	Nausea or vomiting	Psychosis
Alopecia	Nasopharyngeal ulcerations	Pulmonary hemorrhage
Arthralgia	Oral ulceration	Pulmonary hypertension
Arthritis	Organic brain syndrome	Purpura
Butterfly rash	Optic neuropathy	Raynaud's phenomenon
Cranial neuropathies	Panniculitis	Ring-shaped cutaneous lesions
Discoid rash	Pericarditis	Seizures
Fatigue	Photosensitivity	Splenomegaly
Fever (in the absence of infection)	Peripheral neuropathies	Transverse myelitis
Hepatomegaly	Pleuritis	Urticaria
Lymphadenopathy	Pneumonitis	Vasculitis
Myocarditis	Proteinuria	Weight loss
Chilblain-like lesions	Myositis	

Table 3 – Main clinical features compatible with the diagnosis of SLE (retrieved from Arnaud et al., 2018).

2.7. SLE DIAGNOSIS

The American College of Rheumatology (ACR) established 11 criteria for the diagnosis of SLE. The patient is classified with SLE if 4 or more of the manifestations are present, either serially or simultaneously, during any interval of observations (ACR, 2018).

The table below lists the 1997 update of the 1982 ACR revised criteria for SLE classification:

Criterion	Definition
1. Malar rash	Fixed, flat or raised erythema over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by physician
5. Arthritis	Nonerosive, involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Serositis	I. *Pleuritis* – convincing history of pleuritic pain or rubbing heard by a physician or evidence of pleural effusion OR II. *Pericarditis* – documented by electrocardiogram or rub or evidence of pericardial effusion

7. Renal disorder	I. Persistent proteinuria > 0.5 grams per day or > than 3+ if quantitation not performed OR II. Cellular casts – may be red cell, hemoglobin, granular, tubular, or mixed
8. Neurologic disorder	I. *Seizures* – in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance OR II. *Psychosis* – in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance
9. Hematologic disorder	I. *Hemolytic anemia* – with reticulocytosis OR II. *Leukopenia* - < 4000/mm^3 on ≥ 2 occasions OR III. *Lymphopenia* - < 1500/mm^3 on ≥ 2 occasions OR IV. *Thrombocytopenia* - < 100000/mm^3 in the absence of offending drugs
10. Immunologic disorder	I. *Anti-DNA*: antibody to native DNA in abnormal titer OR II. *Anti-Sm*: presence of antibody to Sm nuclear antigen OR III. Positive finding on antiphospholipid antibodies on: a. An abnormal serum level of IgG or IgM anticardiolipin antibodies b. A positive test result for lupus anticoagulant using a standard method, or c. A false-positive test result for at least 6 months confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test
11. Antinuclear antibody (ANA)	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs

Table 4 – ACR 1997 Update of 1982 Revised Criteria for Classification of SLE (retrieved from ACR, www.rheumatology.org)

2.7.1. Antinuclear Antibody (ANA) Test

Anti-nuclear antibodies (**ANA**) are autoantibodies binding to the contents of the cell nucleus and can be detected in the blood several years prior SLE diagnosis. It has been estimated that 98% of SLE patients will have a positive ANA test, indicating it as the most sensitive diagnostic test for lupus. The test for ANA is named as the immunofluorescent antinuclear antibody test (Yu et al., 2014).

Procedure: The serum from the patient's blood sample is added to a microscopic slide prepared with sections of rodent liver/kidney or human tissue culture cell lines on the slide surface. In the presence of ANAs in the sample, their serum binds to the cells on the slide. Afterwards, a second antibody tagged with a fluorescent dye is added in order to attach to the serum ANA-cell complex previously formed and observed under a fluorescence microscope. The intensity of staining and pattern of binding are scored at numerous dilutions, with a test marked as positive if fluorescent cells are detected. ANA test results are measured in titers and patterns. The titer corresponds to the number of times the blood plasma was dilutes in order to obtain a sample of ANAs and each titer involves doubling the amount of test fluid (i.e. 1:100 and 1:200 difference is one dilution) (Chernecky et al., 2007).

The existence of ANA is commonly at a titer ≥1:80. In extremely rare cases, an ANA-negative lupus exists, although further examinations should be done to confirm the existence of lupus. The ANA test is not SLE-specific, and thus positive results can occur in healthy individuals (titer < 1:80), especially during infections, with the consumption of certain drugs (as discusses in chapter 2.5.5.) and in other connective tissue diseases (Abeles et al., 2013). The most frequent screening method for ANA is **immunofluorescence on human epithelial** (**HEp2**) **tissue**, while an **enzyme-linked immunosorbent assay** (**ELISA**) **test**, **bead-based**

tests and **solid phase assays** are also available (Chernecky et al., 2007). Low titers (1:40 – 1:80) can normally occur in healthy individuals, especially in women older than 40 years of age or elderly and consequently, a titer > 1:80 is considered significant for the diagnosis of connective tissue diseases (Satoh et al., 2009). A highly positive ANA test can be sensitive to various diseases like DIL, mixed connective tissue disease, scleroderma, pauciarticular juvenile chronic arthritis, Sjögren's disease, antiphospholipid syndrome, polymyositis, rheumatoid arthritis (RA), rheumatoid vasculitis, Raynaud's phenomenon and discoid lupus, as well as in some organ-specific autoimmune diseases like primary autoimmune cholangitis, autoimmune hepatitis, Grave's disease or Hashimoto's thyroiditis (Bertsias et al., 2010).

2.7.2. Anti-DNA Antibodies

A homogenous/peripheral pattern usually indicates antibodies to histone/dsDNA/chromatin, whereas SLE-specific molecules (anti-SSA, SSB RNP, Sm) demonstrate speckled patterns of various sizes and densities (Yu et al., 2014).

Anti-dsDNA antibodies can be identified in 60%-80% of SLE patients. They are highly SLE-specific (≈95%-98%) and are included among the disease classification criteria. Their pathogenic involvement is due to the fact that DNA/anti-dsDNA complexes activate complement and are nephritogenic (Bertsias et al., 2012). The most prevalent tests to detect anti-dsDNA, mentioned in decreasing sensitivity and increasing specificity, are the ELISA, CLIFT and Farr. Increased anti-dsDNA levels are associated with SLE clinical activity, proliferative lupus nephritis and hypocomplementemia (close monitoring is needed) (Gill et al., 2003).

Anti-ssDNA antibodies have a very limited diagnostic value because of their low specificity and are not used in routine clinical practice. **Anti-histone** antibodies are identified

in approximately 50%-80% of SLE patients, however they are seldom used anymore due to their non-specificity for SLE and their inability to properly distinguish DIL (Pavlovic et al., 2010). **Anti-nucleosome** antibodies have a good sensitivity (approximately 60%) and high specificity (approximately 90%) for SLE and are also associated with lupus nephritis. The majority of autoantigens recognized by anti-nucleosome antibodies are conformational epitopes and do not react with histone or DNA alone. They can successfully be utilized as markers for the diagnosis and the activity assessment of anti-dsDNA negative SLE (Riboldi et al., 2005).

2.7.3. Anti-ENA antibodies

Extractable nuclear antigen (ENA) antibodies are good SLE markers after when anti-dsDNA is absent, whereas they are also able to indicate specific lupus manifestations.

Anti-Ro/SSA and **Anti-La/SSB antibodies** are frequently detected in patients with SLE and/or Sjögren's syndrome. Immunodiffusion, ELISA, Western blot and RNA immunoprecipitation are performed to detect them, although ELISA has very highly sensitivity and also provide a quantitative result (Gill et al., 2003). **Anti-Ro/SSA** antibodies are related with photosensitivity, subacute cutaneous lupus, cutaneous vasculitis (palpable purpura), neonatal lupus, interstitial lung disease and congenital heart block. These antibodies can be observed in up to 70% of patients with Sjögren's syndrome and can also be occasionally detected in patients with RA, juvenile RA, cutaneous vasculitis, progressive systemic sclerosis, connective tissue disease, chronic active hepatitis, primary biliary cirrhosis and homozygous C_2 or C_4 deficiency (Defendenti et al., 2011). It is very usual to encounter SLE patients' sera with Anti-Ro/SSA antibodies also have **anti-La/SSB** antibodies. Anti-La/SSB are also frequently detected in Sjögren's syndrome and can be further be associated with scleroderma,

dermato/polymyositis, RA, primary biliary cirrhosis and autoimmune hepatitis (Franceschini et al., 2005).

Anti-Sm antibodies bind to the Smith antigen. The Smith antigen is a nuclear nonhistone protein consisting of a series of proteins complexed with small nuclear RNA. These complexes are named small nuclear ribonucleoprotein particles (snRNPs); important in the splicing of precursor messenger RNA, an integral step in the processing of DNA-transcribed RNA. Anti-Sm antibodies are measured either by ELISA or by hemagglutination; however, ELISA is easily quantitated. They are insensitive (up to 30%) but strongly SLE specific, and thus they remain positive when anti-DNA titers have fallen to the normal range (Alba et al., 2003). **Anti-RNP** antibodies react with proteins containing only U1-RNA and form U1snRNP, although the are not SLE specific; they can typically be observed in connective tissue diseases. Interestingly, Tápanes et al., suggested an association of anti-Sm and anti-RNP with the less severe form or with the absence of glomerulonephritis. **Anti-C1q** antibodies can be found in up to 60% of SLE patients without being SLE-specific; they are related with global and renal disease activity (Bertsias et al., 2010).

2.7.4. Other specificities

Other, less frequent auto-antibodies include **anti-ribosomal P** (**anti-Ribo P**) antibody which provides a finely granular cytoplasmic pattern in immunofluorescence. It has a low sensitivity (up to 10%) but high SLE-specificity. It has been thought that anti-Ribo P antibodies are related with certain SLE features like NP, renal or hepatic manifestations, although this role is not yet established (Tsokos et al., 2007). **Anti-dense fine speckled 70** (**DFS70**) antibodies are observed in some SLE patients, however it has

been reported that they are negatively-associated with the existence of autoimmune diseases (Bertsias et al., 2012).

2.7.5. Complement levels

Homozygous and/or heterozygous deficiencies of the classical complement pathway (C_{1q}, C_{1r}, C_{1s}, C_{4B} and C_2) are related with elevated SLE susceptibility. Moreover, decreased C_3, C_4 and CH50 (total complement hemolytic activity) levels, indicate the activation of the classical pathway by immune complexes in SLE, resulting from the consumption of complement factors. Hypocomplementemia is not SLE-specific, it can occur is any disease with circulating ICs (Parra-Medina et al., 2013). However, C_3 and C_4 consumption is mostly observed in patients with active LN and hematological manifestations (Ishizaki et al., 2014).

2.7.6. Antiphospholipid (aPL) antibodies (APA)

Antiphospholipid antibodies are recognized causes of thromboembolic complications, thrombocytopenia and several obstetrical adverse effects, although they can sometimes be found in normal asymptomatic individuals. They occur with increased frequency in women with more than three spontaneous recurrent abortions. Four types of APA have been described: Antibodies that give a false-positive serologic test for syphilis (STS), lupus anticoagulants (LA), anticardiolipin antibodies (aCL) and anti-β-glycoprotein 1 antibodies (Meroni et al., 2011).

False-Positive serologic Test for Syphilis

Approximately 20% of SLE have a false-positive STS; when the serum from a patient with SLE contains cardiolipin antibodies it is bound with the antigen used in the test. Consequently, a reaction against this molecule will be incorrectly interpreted as being

directed against the treponemal antigen. STS is not recommended to screen aPL as it has low sensitivity and specificity (Ruiz et al., 2010).

Lupus *Anticoagulants* (LA)

LA are antibodies directed against plasma proteins (e.g. prothrombin or β-2-glycoprein I) bound to anionic phospholipids. LA blocks *in vitro* assembly of the prothrombinase complex leading in the prolongation of *in vitro* clotting assays like the activated partial thromboplastin time (aPTT), the dilute Russell viper venom time (dRVVT), the kaolin clotting time and seldomly the prothrombin time (Meroni et al., 2011). When LA were firstly discovered, they were named based on the misunderstanding that due to their ability to prolong a clotting assay, they would also induce an elevated tendency for bleeding, whereas they actually increase the ability of the blood to clot. Moreover, LA can also increase the frequency of arterial and venous thrombotic events. Finally, the term "lupus" in their name is also misleading, as they can extensively exist in various autoimmune and rheumatic diseases (Petri, 2010).

Anticardiolipin antibodies (aCLs)

Anticardiolipin antibodies react with proteins (β_2-glycoprotein 1, annexin V etc.) bound to anionic phospholipids like cardiolipin and phosphatidylserine. An 85% concurrence between aCL and LA has been estimated, although they can form two separate populations; testing therefore should be performed for each one. LA-positivity implies a greater thrombosis risk than aCL (Ruiz-Irastorza et al., 2010). Moreover, aCL is associated with different Ig isotypes and subclasses, including IgA, IgG and IgG subclasses 1-4, as well as IgM. IgG aCL, and especially IgG2, possesses a higher thrombosis risk compared to the other Ig isotypes (Petri, 2010).

Anti-β$_2$ Glycoprotein I Antibodies (Anti-β2GP1)

Anti-β2GP1 antibodies bind directly to β2GP1 (in contrast with aCL) and are phospholipid-binding inhibitors of coagulation. Patients with primary or secondary antiphospholipid syndrome (APS) have notably high percentage of anti-β2GP1 antibodies (Galli et al., 2003).

Antiprothrombin antibodies are closely related with both clotting and pulmonary hemorrhage (Miyakis et al., 2006).

Both LA and aCL have been detected in patients with multiple autoimmune and rheumatic diseases including hemolytic anemia, rheumatoid arthritis, juvenile arthritis, psoriatic arthritis, idiopathic thrombocytopenic purpura, scleroderma, Sjögren's syndrome, mixed connective tissue disease, polymyositis, dermomyositis, polymyalgia rheumatica, osteoarthritis, chronic discoid LE and occasionally in multiple sclerosis and gout (Petri, 2010, Meroni et al., 2011)

Antiphospholipid antibody Syndrome (APS) is diagnosed in patients who experience complications from antiphospholipid antibodies and is a condition that can occur in both SLE and non-SLE patients. It is marked by the existence of one or more clinical manifestations of thrombotic etiology, pregnancy complications (miscarriage, premature birth) in combination with significantly elevated level of aCL antibodies, LA and/or anti-β2GP1 antibodies. In order to confirm the APS diagnosis, a second blood examination should take place approximately 12 weeks after the initial diagnosis (Ruiz-Irastorza et al., 2010, Bertsias et al., 2012)

2.7.7. NPSLE diagnosis

The first step in the NPSLE is to determine whether the NP events are primarily induced by SLE (disease complication, therapy side effect), or if a coincidental disease exists. Primarily, the **cerebrospinal fluid** (**CSF**) should be examined in order to exclude infections. Afterwards, an assessment of brain structure and brain function should be performed (Bertsias et al., 2012). **Computer tomography** (**CT**) scanning is executed for the diagnosis of acute intracranial hemorrhage, however **magnetic resonance** (**MRI**) is nowadays more popular due to its increased sensitivity and ability to detect further abnormalities. T_2-weighted MRI detects pathological processes resulting in edema and is more sensitive than T_1-weighted imaging for NPSLE abnormalities. In order to further enhance the T_2-weighted images utility, the technique of *fluid-attenuating inversion recovery* (*FLAIR*) is applied to dampen the CSF signal and highlight the locations with edema (Bertsias et al., 2010). Diffuse NP clinical manifestations are related with transient subcortical white matter and patchy hyperintensities in gray matter. Additional pathologic conditions identified on MRI in SLE patients are venous sinus thrombosis, cerebral infraction and elevated signal in the spinal cord accompanying the clinical presentation of myelopathy (Utset et al., 2006).

Single-photon-emission computer tomography (SPECT) scanning provides semiquantitative analysis of regional cerebral blood flow and metabolism. It is extremely sensitive and is able to detect both diffuse and focal deficits, which may be reversible or fixed (Yu et al., 2014). **Magnetic resonance spectroscopy** aids in the identification and quantification of brain metabolites, and thus provide indirect evidence of cellular changes (Utset et al., 2006). Furthermore, it has been observed that the amount of N-acetyl (NA) compounds, reflecting the quantity and integrity of neuronal cells, is decreased in the brains of SLE patients. An association between brains with decreased NA levels and neurocognitive dysfunction, independently with high IgG antiphospholipid antibodies. Inflammation and

ischemia may be detected by increased brain lactate levels, whereas Damaged cell membranes and myelin destruction can be indicated by increased choline compounds (Bertsias et al., 2012).

2.7.8. Musculoskeletal system

Regarding to the **musculoskeletal system** disorders, increased serum **creatinine phosphokinase** (**CPK**) can be observed in patients with connective tissue disease like SLE. CPK is an enzyme found in heart, brain and skeletal muscles and it is leaked in the bloodstream after muscle injury**.** Therefore, CPK can be used as a marker for musculoskeletal, cardiac and brain abnormalities (Bertsias et al., 2010). **MRI** is the more sensitive diagnostic procedure used in the detection of avascular necrosis, myositis and various musculoskeletal complications. Furthermore, bone scan [Tc990] and radiograph can be also used (Yu et al., 2014)

2.7.9. Renal assessment

In order to assess the renal function, the following diagnostic methods are established:

- **Serologic analysis:** C_3, C_4 and C_{H50} complements and anti-DNA antibodies are mainly used as markers to indicate renal function, although rising titers of the latter are more important than their absolute values. Clinically, C_3 is more useful than C_4 in this occasion as C_4 deficiency is common in SLE, whereas C_3 levels are related with the renal histology on repeated renal biopsies (Hahn et al., 2012). Upon the activation of the complement system, breakdown of precursor molecules occurs, so these products could be potent markers of the disease activity, although further research is still needed. Furthermore, creatinine levels are also measured in order to establish total kidney function (Weening et al., 2004).

- **Urinalysis** is the most important and effective way to detect and monitor disease renal activity. To assure its quality an expeditious examination of an early morning, fresh, non-refrigerated, clean, midstream urine specimen (Li et al., 2006). The sample is tested for *hematuria* (microscopic, in severe cases macroscopic) with fragmented or dysmorphic erythrocytes, indicating inflammatory glomerular or tubulointerstitial disease. *Proteinuria* can be reflected by granular and fatty casts (Ayoub et al., 2018). Nephritic states are indicated by red blood cells, white blood cells and mixed cellular casts, while broad and waxy casts reflect chronic renal failure. Urine sediment with a full range of cells and casts (telescopic urine sediment) is an indication of severe glomerular and tubular ongoing disease obtained by chronic renal damage. (Ilori et al., 2016).

- **Renal Biopsy**

  In the presence of objective urinary and/or serologic abnormalities (e.g. rapidly elevated creatinine), kidney biopsy is important for the assessment of the renal pathology and for better disease prognosis (Li et al., 2006). Early biopsy (i.e. before the initiation of therapy) is performed in patients with nephritic urine sediment, glomerular hematuria with proteinuria 0.5-1.0 g/day, decreased C_3 and/or positive anti-dsDNA, or in patients with proteinuria 1.0-2.0 g/day and especially if C_3 is decreased and/or positive anti-dsDNA. Patients with unexplained worsening of proteinuria and/or renal function, persistent glomerular hematuria with proteinuria higher than 2g/day and /or significant C_3 decrease are indicated for an urgent renal biopsy (Ayoub et al., 2018).

  Renal biopsies should always include two components: immunofluorescence, light and electron microscopy (Nimri et al., 2012). The most serious complications of this procedure are pericapsular hemorrhage or clot obstruction in patients with lupus procoagulants (Ilori et al., 2016). According to the findings of the aforementioned

diagnostic methods, International Society of Nephrology/Renal Pathology Society (ISN/RPS), formed a classification of LN, listed below:

THE ISN/RPS CLASSIFICATION OF LUPUS NEPHRITIS

CLASS I	**Minimal Mesangial Lupus Nephritis** Normal glomeruli by light microscopy, but mesangial immune deposits by immunofluorescence microscopy
CLASS II	**Mesangial Proliferative Lupus Nephritis** Purely mesangial hypercellularity of any degree and/or mesangial matrix expansion by light microscopy, with mesangial immune deposits. A rare isolated subepithelial of subendothelial deposit may be visible by immunofluorescence microscopy or electron microscopy
CLASS III	**Focal Lupus Nephritis** Active or inactive focal, segmental or global endo- or extra-capillary glomerulonephritis involving <50% of glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations. **III (A)** Active lesions focal proliferative lupus nephritis **III (A/C)** Active and chronic lesions (focal proliferative sclerosing lupus nephritis) **III (C)** Chronic inactive lesions with scars (focal lupus nephritis) *indicate the proportion of glomeruli with active and with sclerotic lesions *indicate the proportion of glomeruli with fibrinoid necrosis and with cellular crescents *indicate and grade (mild, moderate, severe) tubular atrophy, interstitial inflammation and fibrosis, arteriosclerosis or other vascular disease
CLASS IV	**Diffuse Lupus Nephritis** Active or inactive diffuse, segmental, or global endo- and/or extra-capillary glomerulonephritis involving ≥50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is divided into diffuse segmental (IV-S) when ≥50% if the involved glomeruli have segmental lesions, and diffuse global (IV-G) when ≥50% of the involved glomeruli have global lesions. Segmental is defined as a glomerular lesion that involves less

	than half of the glomerular tuft. This class includes cases with diffuse wire-loop deposits, but with little or no global proliferation. **IV-S (A) or IV-G (A)** Active lesions (diffuse segmental or global proliferative lupus nephritis) **IV-S (A/C) or IV-G (A/C)** Active and chronic lesions (diffuse segmental or global proliferative and sclerosing lupus nephritis) **IV-S (C) or IV-G (C)** Chronic inactive lesions with scars (diffuse segmental or global sclerosing lupus nephritis) *indicate the proportion of glomeruli with active and with sclerotic lesions and/or with fibrinoid necrosis and with cellular crescents *indicate and grade (mild, moderate, severe) tubular atrophy, interstitial inflammation and fibrosis, arteriosclerosis or other vascular disease	
CLASS V	**Membranous Lupus Nephritis** Global or segmental subepithelial immune deposits or their morphologic sequelae by light microscopy and by immunofluorescence or electron microscopy, with or without mesangial alterations *May occur in combination with III or IV, in which case both will be diagnosed *May show advanced sclerosis	
CLASS VI	**Advanced Sclerosing Lupus Nephritis -** of glomeruli globally sclerosed without residual activity	≥90%

Table 5 – ISN/RPS classification of lupus nephritis (retrieved from Nimri et al., 2012).

2.7.10. Cardiological assessment

Referring to the **cardiological assessment** in SLE patients the following procedures are used:

- **Electrocardiography** (**ECG**) is the electrical activity is recorded over a period of time by the attachment of electrons over specific skin areas. It can indicate finding such as PR interval depression with diffuse concave ST segment elevation,

tachyarrhythmia (atrial fibrillation and flutter) less commonly, and rarely bradyarrhythmia (Yu et al., 2014). **Echocardiography** refers to a heart sonogram and is the diagnostic method of choice for the evaluation of pericardial tamponade (Tsokos et al., 2007).

- **CT** and **MRI** have been performed to detect both clinical and subclinical cardiac involvement. They are more effective than echocardiography in the identification of loculated effusions and will also demonstrate the pericardial thickening seen on echography in chronic disease and constrictive pericarditis (Bertsias et al., 2012, Appenzeller et al., 2011).

Furthermore, the pericardial fluid in SLE-related pericarditis is straw-colored, although it can also be serosanguinous or hemorrhagic. It is exudative with increased protein levels, normal to decreased glucose levels and increased white blood cell count with a predominance of polymorphonuclear cells (Bertsias et al., 2012). Autoantibodies like antinuclear (ANA) and ds-DNA, can be observed in the pericardial fluid. Despite that ANA-positive pericardial fluid can be an indication of SLE, it is not specific, as it can be also observed in various autoimmune diseases, infections like TB and malignancies (Gustafsson et al., 2012).

2.7.11. Pulmonary assessment

In **pulmonary SLE manifestations**, most frequently **chest radiography** is performed, in order to confirm or distinguish among the possible pulmonary diseases (Mittoo et al., 2010). For further investigation, **CT** is performed as it reveals patchy consolidations surrounded by ground glass appearance. **Thoracentesis** can also take place when an infection is suspected, where the pleural fluid is usually exudative (Gulhane et al., 2012). Furthermore, **fiberoptic bronchoscopy, bronchoalveolar lavage** or even **transbronchial lung biopsies** are important in order to substantiate the diagnosis (Bouros et al., 2008).

2.7.12. Hepatic assessment

Hepatic diseases during SLE can be assessed by blood tests indicating the presence of hypergammaglobulinemia, autoantibodies against hepatic antigens or liver-kidney microsomal proteins like ANA, anti-smooth muscle antibodies (anti-SMA) and anti-LKM antibodies. Moreover, the liver functionality and state are revealed by the levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), albumin, direct and indirect bilirubin, L-lactate dehydrogenase (LD), as well as prothrombin time. If persistent and severe abnormalities in liver function are noticed, further examination is needed, including ultrasonography and liver biopsy to determine the underlying causes of hepatic damage (Tian et al., 2010, Yu et al., 2014).

2.7.13. BILAG/SLEDAI scores

The **British Isles Lupus Assessment Group *(*BILAG*)*** refers to a computerized index in order to measure the clinical disease activity in SLE, developed according to the principle of the physician's "intention to treat". This system includes distinct alphabetic scores to each of the eight organ-based systems (mucocutaneous, neuropsychiatric, musculoskeletal, cardiorespiratory, gastrointestinal, ophthalmic, renal, hematological) and constitutional manifestations, although the is no calculation of a total score. It requires the assessment of (1) improved, (2) the same, (3) worse or (4) new, over the past month (Castrejón et al., 2014). In the table below the scoring BILAG index system is summarized.

Scoring system for the BILAG index

Category A	Denotes disease thought to be sufficiently active to require disease-modifying treatment (prednisolone > 20mg daily or immunosuppressants)
Category B	Denotes disease which is less active than in "A"; mild reversible problems requiring only symptomatic therapy such as antimalarials, non-steroidal anti-inflammatory drugs or prednisolone < 20mg/day
Category C	Indicated stable mild disease
Category D	System previously affected but currently inactive
Category E	Indicates system never involved

Table 6 – Scoring system for the BILAG index (retrieved from Hay et al., 1993).

Systemic lupus erythematosus disease activity index *(*SLEDAI*)* provides an overall measurement of disease activity and individual organ/system assessment scales, describing the disease activity in single organs. It consists of list of 24 components; 16 of them are clinical features like seizure, psychosis, organic brain syndrome, visual disturbance, other neurological problems, hair loss, new rash, muscle weakness, arthritis, blood vessel inflammation, mouth sores, chest pain worse with deep breathing and manifestations of pleurisy and/or pericarditis and fever. Eight of the 24 features are laboratory results (e.g. urinalysis), blood complement levels, elevated anti-DNA antibody levels, low platelets, and low white blood cell count. These items are scored based on whether these manifestations are present or absent in the previous 10 days. The existing scores are 0-none, 1-mild, 2-moderate and 3-severe disease activity (Romero-Diaz et al., 2011). SLEDAI score determines the global improvement, whereas the BILAG domain scores to ensure no significant worsening in heretofore unaffected organ systems (Tsokos et al., 2007).

2.8. COMORBIDITIES

Connective tissue diseases (CTDs) are systemic autoimmune disorders with a wide spectrum of clinical manifestations. CTDs include rheumatoid arthritis (RA), SLE, Sjögren's syndrome (SS), Scleroderma/systemic sclerosis, dermatomyositis (DM) and polymyositis (PM). The clustering of multiple autoimmune diseases, also called overlap syndrome (OS) suggests some degree of common genetic susceptibility and common serologic markers involved in the pathogenesis. The main coexisting CTDs with SLE are listed below.

2.8.1. Sjögren's syndrome (SS)

SLE and Sjögren's syndrome are two significant conditions, both characterized by chronicity and autoimmunity. SS develops from the accumulation of lymphocytes on exocrine glands, and can be seen in other autoimmune rheumatic diseases, like rheumatoid arthritis (RA) (ACR, 2018). It is an inflammatory disease affecting various parts of the body, however the lacrimal and salivary glands are most often affected. The main clinical features of SS include xerostomia, accompanied with difficulty in mastication, dental symptoms, oral fissures and ulcers, xeropthalmia, swelling of the lymph nodes or in the parotid gland. Additionally, dryness in nasal passages, throat, vulvo-vaginal and skin dryness, nonthrombocytopenic purpura, vasculitis as well as symptoms of acid reflux can occur. Furthermore, arthralgias and myalgias can occur; arthritis and inflammatory myopathy are rare except in secondary SS. Later, serious complications can occur including lymphoma (especially non-Hodgkin), as well as lung, liver and kidney involvements (Soliotis et al., 2004, Baer et al., 2010). It has been estimated that up to 90% of SLE patients have been reported with secondary SS. Anti-La/SSB antibodies are one of the main serological markers of this overlap, incredibly increased compared to the existence of SLE alone, whereas SLE-related antibodies are less frequent in patients with SLE/SS (González et al., 2017). HLA-DR2 and HLA-DR3 bearing haplotypes are related to both SLE and SS. SSA/Ro antibodies,

found in SLE and SS are linked with the subacute cutaneous lupus. Patients with SLE/SS seem to have milder SLE features and a predominance of SS-related features and especially the sicca symptoms (Richard-Miceli et al., 2012). Baer et al., in a large prospective series suggested that skin manifestations like malar rash and photosensitivity, oral ulcers, arthritis, psychosis and Raynaud's phenomenon are more common in SLE/SS patients. Furthermore, the patients with SLE/SS are older and have a decreased risk to develop glomerulonephritis, and increased frequency of fatigue and thrombocytopenia compared to SLE patients.

2.8.2. Rheumatoid Arthritis (RA)

RA is the most frequent form of autoimmune arthritis, affecting women approximately 3 times more often than men. The main clinical symptom of the disease is morning joint stiffness, differentiating RA from any other rheumatic disease (ACR, 2018). Other common disease manifestations include joint pain, swelling, redness, warmth and tenderness, mainly affecting the small joints of hands and feet in a symmetrical pattern (i.e. both wrists). Furthermore, the knees, shoulders, elbows, neck and hips can be affected with the progression of the disease. Intense hand deformations are observed in more severe cases. Other symptoms include fatigue, muscle pain, malaise, poor appetite, rheumatic nodules, vasculitis and in more severe cases carpal tunnel syndrome, anemia, lung fibrosis, atherosclerosis, stroke, pericarditis and endocarditis (Ramos et al., 2011, González et al., 2017).

The coexistence of SLE and RA if often also called "rhupus", although it is still debated (Fernández et al., 2004). It has been also claimed that rhupus syndrome should be considered an erosive subset of lupus arthropathy (Fernández et al., 2006).

Anti-citrullinated peptides (ACPA) are well-known, strongly specific RA serological markers, that can also be observed (in lower quantities) in other inflammatory rheumatic conditions like SLE. In rhupus ACPA are found in the majority of patients, especially in

those with erosive arthritis. However, SLE-related antibodies including ANA, anti-dsDNA, anti-U_1RNP and anti-Sm were similar whether erosive arthritis is present, or not (Amezcua-Guerra et al., 2006). Additionally, Simons et al., in a cohort study among 22 rhupus patients, supported by genetic HLA-DR phenotyping, found that none of the cases presented thrombosis or morbidity during pregnancy even though they presented a high frequency of anticardiolipin antibodies. Confirmed loci associated with the coexistence of SLE and RA are HLA-DRB_1, PTPN22 (Protein tyrosine phosphatase, non-receptor type 22), $PRDM_1$, $STAT_4$, $FCGR_2A$, IRF_5, $TNFAIP_3$ and PXK. In 2011, Orozco et al., confirmed the implication of BLK and UBE_2L_3. BLK encodes a tyrosine kinase implicated in B-cell activation. UBE_2L_3 encodes a ubiquitin-conjugated enzyme implicated in the IFN production and the signaling pathways of TLR_7 and TLR_9 (Iaccarino et al., 2013).

2.8.3. Scleroderma

Scleroderma (also called systemic sclerosis) is an autoimmune connective tissue disease resulting in fibrosis and vascular abnormalities. It originates from the Greek words sclero- (σκληρός) meaning hard and derma (δέρμα) meaning skin, which is the hallmark of the disease; forming a thick, hard, buildup of scar tissue (Bertsias et al., 2012). Two main types of scleroderma exist: localized and systemic scleroderma. In **localized scleroderma**, only the skin is usually affected, manifested with discolored patches on the skin (morphea) or streaks or bands of thick, hard skin on the arms and legs (linear scleroderma) (Furtado et al., 2002). **Systemic scleroderma** is the most severe form of the disease affecting the skin, joints, muscles, heart, blood vessels, kidneys, lungs etc. *Limited cutaneous systemic sclerosis* is also called CREST syndrome, naming its common features: calcinosis, Raynaud phenomenon, esophageal motility dysfunction, sclerodactyly and telangiectasia. This form is also related with pulmonary hypertension (Richard-Miceli et al., 2012). Centromere antibodies are potent markers of this condition. In *diffuse cutaneous systemic scleroderma*, the skin thickening spreads above the wrists, whereas internal organs like lungs, kidney or

GIT are more commonly implicated. Internal organ involvement occurs earlier than the in other types. Various antibodies can indicate this form, although the most common is Scl-70. *Systemic sclerosis sine scleroderma* refers to the fibrosis that affects one or more internal organs but does not demonstrate any skin manifestations (Ramos et al., 2011)

SLE and systemic sclerosis coexistence is a rare condition. Regardless the incidence of both anti-dsDNA and anti-Scl70 antibodies in these two conditions, SLE patients have low anti-Scl70 reactivity levels, and thus the antibody titer can aid in the differentiation between SLE and systemic sclerosis (Richard-Miceli et al., 2012). Patients of both conditions have polyserositis, avascular bone necrosis, pancreatitis, skin rashes, arthritis, pulmonary hypertension and glomerulonephritis. However, in patients with hypertension and renal disease it is crucial to distinguish the origin of these complications as different management will be required (Iaccarino et al., 2013).

2.8.4. Dermomyositis (DM)

Dermomyositis (derma-skin, myo-muscle, sitis-inflammation) is an idiopathic, progressive, chronic, inflammatory, autoimmune myopathy with specific cutaneous features. It is presented 2-3 times more frequently in women compared to men, however the adult-onset of DM that is marked as a paraneoplastic syndrome, may act as a trigger for the development of cancer including ovarian, pancreatic, lung, colorectal, stomach cancer and non-Hodgkin lymphoma. It has been estimated that up to 50% of DM patients will develop pulmonary disease in the form of interstitial lung disease. (Tartar et al., 2018). Patients present with a skin rash accompanied by symmetric, proximal muscle inflammation and weakness, although the skin-related symptoms can be present more than a year before the muscle symptoms develop. This statement is not absolute, as approximately 20% of the patients can be amyopathic. The differentiations between polymyositis (PM) and DM are

mainly the cutaneous manifestations which are present only in DM, as well as the increased relation of DM patients with underlying malignancies; the main cause of DM-associated morbidity and mortality (Iaccarino et al., 2013). The six hallmark skin manifestations of DM include the following:

- *Gottron papules* described as raised, smooth, indurated, red-violet lesions affecting mainly bony prominences like the knuckles, elbows or knees. They are observed in up to 80% of DM patients and are a pathognomonic DM symptom; cutaneous knuckle lesions are extremely rare in SLE.
- *Heliotrope rash* is a macular, red-purple rash on the eyelid, presented with dilated eyelid veins to violet, edematous rash, that can be accompanied with periorbital edema and scaling. This is also a pathognomonic DM symptom (Marvi et al., 2012).
- *Violacious/erythematous pruritic macular rash* which can be presented over any portion of the body but tends to concentrate in sun-exposed areas. Areas involved are the bony prominences of knuckles, elbows, knees, as well as face, V-sign of neck, Shawl sign of back, holster sign of hips and extensor arms. The macules can be associated with severe, or even debilitating pruritus. They occur in a diffuse, confluent or patchy symmetrical distribution and may be scaling. Secondary to the initial assault, hyperkeratosis, ulcerations and pigment changes can occur. However, SLE does not present with pruritus, which is an aid for the differentiation of the diagnosis.
- *Periungual telangiectasias* are visible blood vessels that appear like irregular splinters on the proximal nail folds that can be accompanied by ragged, dystrophic cuticles.
- *Alopecia,* accompanied with scaly red-violet lesions in the scalp

- *Poikiloderma* is characterized with finely variegated skin, with areas of hyper- and hypopigmentation, telangiectasia and atrophy, represented in the aforementioned sun-exposed areas (Ruhlman et al., 2014).

Although SLE and DM/PM share some common clinical and laboratory features, district differentiations exist, aiding in the proper diagnosis. Anti-Ro/SSA and Anti-U_1RNP autoantibodies are commonly found in SLE, as well as in DM/PM; they are myositis-associated autoantibodies alongside with anti-Ku and anti-PM-Sc. SLE-specific autoantibodies do not share any known similarity with the myositis specific autoantibodies. Myositis specific autoantibodies include anti-MDA_5 (associated with the relative decrease in CK levels), anti-SAE (associated with HLA-DRB_1 halotype), anti-TIF_1, anti-NXP_2 (also used for the diagnosis of the disease), anti-Jo-1, anti-SRP, anti-Mi-2 and less commonly anti-OJ, anti-EJ etc (Richard-Micel et al., 2012).

2.9. SYSTEMIC LUPUS ERYTHEMATOSUS PHARMACOTHERAPY

Systemic lupus erythematosus is a systemic, chronic autoimmune disease; its pharmacologic treatment should be a careful consideration of a multi-system and patient-specific aspect. SLE is related with symptomatology which should be alleviated and can potentially result in severe and irreversible damage in the affected organs and tissues; thus, the prevention or elimination of this process is a critical issue. Specifically, it is important to prevent the accumulation of disease-induced and treatment-induced damage.

Fries had successfully described the five dimensions of treating chronic illnesses as the "**five Ds**":

- Death: preventing mortality
- Discomfort: relieving symptoms
- Disability: preventing functional decline
- Drug side effects: minimizing toxicities due to the treatment
- Dollar cost: finding an appropriate health-economic balance

Regarding SLE, mortality is rarely attributed to the disease itself, although it can cause severe and fatal complications, i.e. renal, cardiovascular, pulmonary. Moreover, the medications used for SLE can contribute in short-term mortality (e.g. immunosuppressants can lead to fatal infections) and long-term morbidity (e.g. medications accelerating arteriosclerosis). Corticosteroids are effectively and widely used to alleviate discomfort and disability; however, they are related with serious adverse effects. Corticosteroids, as most of the current medications used in SLE are inexpensive; more recently approved and experimented biologic agents are quite expensive. SLE pharmacotherapy can be divided according to the intended result which can either be the immediate control of the disease process (during the onset or a flare), or the maintenance therapy aiming to keep the disease under control and eliminate/prevent flares.

2.9.1. Nonsteroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are widely used in SLE; up to 80% of patients will use them throughout the course of the disease for headache, fever, musculoskeletal symptoms (arthralgia, myalgia) or synovitis and serositis. Their therapeutic function is attributed to the inhibition cyclooxygenase (COX) -1 and/or COX-2, leading in the inhibition of prostaglandin (PG) production (Bertsias et al., 2012). PGs and thromboxanes are produced in the cellular membrane bilayer. In this process arachidonic acid (AA) is metabolized by two distinct cyclooxygenases (COXs). COX-1 is the main constitutive form regulating prostanoid-dependent functions in tissues and cells, with stable production; although it can be upregulated in a 2-3-fold manner. COX-2 is also constitutively expressed (in a more restricted manner), but it is highly inducible and can be upregulated in a more than 20-fold manner during inflammation and tissue damage. It appears in the brain, kidneys and reproductive system (Gan, 2010).

NSAIDs can be classified either based on their chemical structure or based on their mechanism of action (MofA):

Based on their chemical structure the are classified as (Guerevitz et al., 2013):

- *Salicylates*: acetylsalicylic acid, salicylates, diflunisal
- *Propionic acid derivatives*: ibuprofen, dexibuprofen, naproxen, (dex)ketoprofen
- *Acetic acid derivatives*: diclofenac, indomethacin, sulindac, ketorolac, aceclofenac
- *Enolic acid derivatives (oxicam)*: meloxicam, piroxicam, lornoxicam
- *Anthranilic acid derivatives (fenamates)*: mefenamic acid, tolfenamic acid
- *Sulfonanilides*: Nimesulide

- *Selective COX-2 inhibitors:* celecoxib, rofecoxib (withdrawn), parecoxib (withdrawn), etoricoxib (licensed in EU, not FDA approved)

Due to the introduction of selective COX-2 inhibitors and for easier clinical assessment, NSAIDs are nowadays mostly classified according to their MofA (Sostres et al., 2010):

- *Nonselective COX inhibitors*: acetylsalicylic acid, diclofenac, naproxen, ibuprofen, dexibuprofen, sulindac, mefenamic acid, tolfenamic acid, ketorolac.
- *Preferential COX-1 inhibitors*: indomethacin, piroxicam
- *Selective COX-2 inhibitors*: celecoxib, rofecoxib (withdrawn), parecoxib (withdrawn), etoricoxib (licensed in EU, not FDA approved), Nimesulide (preferential), meloxicam (preferential)

In the figure below the action of NSAIDs in the arachidonic acid pathway is described:

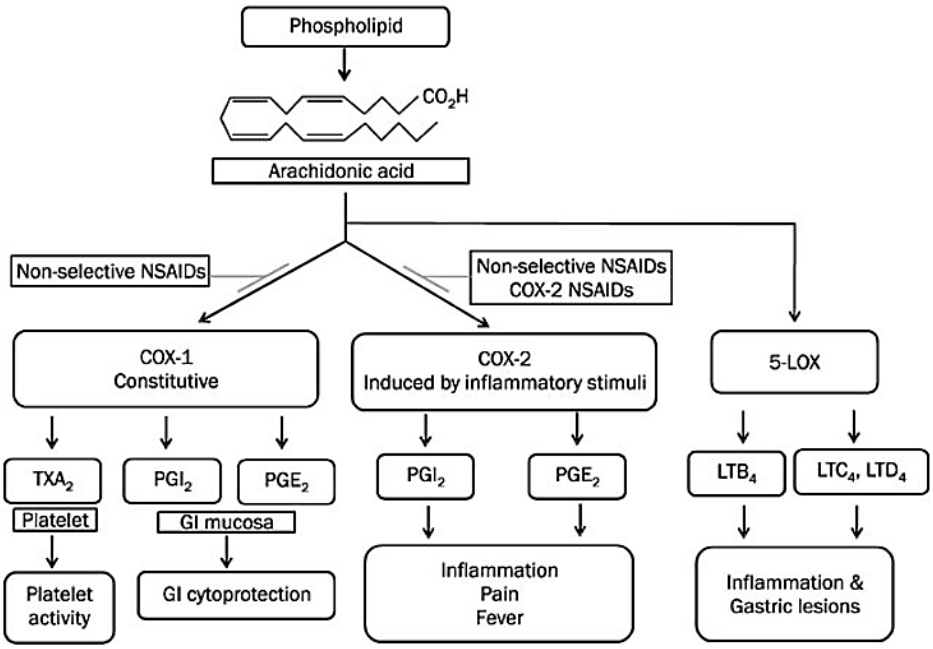


Figure 5 – Arachidonic acid pathway and the role of NSAIDs (5-LOX: 5-lipoxygenase, LT: Leukotriene, PG: Prostaglandin, TX: Thromboxane, GI: Gastrointestinal) (retrieved from Shim et al., 2016).

Adverse effects:

- **Gastrointestinal**: COX-1 inhibitors have more frequent and potent GI side effects compared to the selective COX-2 inhibitors. This is because COX-1 isoform which is expressed in most tissues, produces PGs that possess a significant protective role in the gut by the stimulation, synthesis and secretion of mucous and bicarbonate that increase the mucosal blood flow and promote epithelial proliferation. Therefore, when this enzyme is inhibited by NSAIDs the resulting gastric environment is more susceptible to topical attack by endogenous and exogenous factors (Gan, 2010). Additionally, by the inhibition of COX-1 the platelet production of thromboxane is also attenuated; thus, increased bleeding will occur when an active GI bleeding site is present. COX-2 isoform is induced in response to an inflammation. Prostaglandins derived from COX-2 can be generated at the ulcer margin and seem to have a significant role in ulcer healing by triggering cell proliferation, promotion of angiogenesis and restoration of mucosal integrity. Accordingly, we can assume that selective COX-2 inhibitors that have little to no effect on COX-1, can promote pain relief and anti-inflammatory effects with less GI adverse effects compared to the other NSAID classes (Horizon et al., 2004). The most frequent GI side effects presented in up to 40% of patients include abdominal pain, nausea, vomiting, heartburn and dyspepsia. More serious adverse effects include gastroduodenal bleeding, erosions and ulcers that can lead to perforation and obstruction (Sostres et al., 2010). In order to prevent or minimize GI side effects, the concomitant administration of gastroprotective agents (mainly proton-pump inhibitors and less effectively H_2-antagonists) is advised (Ardoin et al., 2006).
- **Renal**: COX-1 and COX-2 are expressed in the kidneys and their expression is regulated by various stimuli including vaso-regulatory mediators. Renal PGs are associated with the regulation and distribution of total renal blood flow, renin release

as well as sodium and water balance. Therefore, the inhibition of PGs can potentially cause disturbances of water, sodium and potassium homeostasis and GFR decline. Renal adverse effects are more common with indomethacin and much less with sulindac and nabumetone (Østensen et al., 2006). Among COX-2, rofecoxib is mostly related with sodium retention but not with GFR reduction which is more common in high doses of celecoxib. However, high doses of rofecoxib can result in the formation of edema (Horizon et al., 2004). LN is a risk factor for hemodynamically mediated NSAID-induces acute renal failure, however decreased GFR has also been observed in SLE patients with normal renal function exposed to NSAIDs. It has also been reported that intrarenal production of PGI_2 and TX_{A2} is altered in SLE patients. Rare idiosyncratic toxic reactions to NSAIDs include interstitial nephritis, papillary necrosis and nephrotic syndrome (Koutsoukeras et al., 2014).

- **Cutaneous**: It has been estimated that patients with SLE have a fourfold increased incidences of allergic skin reaction compared to patients with other forms of chronic arthritis, more frequently manifested by acetylsalicylic acid, sulindac and piroxicam (Lo et al., 2012). These reactions appear in the form of rashes (sulindac), urticaria, photosensitivity (piroxicam), pruritus (ibuprofen), purpura (acetylsalicylic acid), alopecia, exfoliative erythroderma as well as cutaneous vasculitis. SLE patients with a secondary Sjögren's syndrome are greater risk to develop these cutaneous reactions; especially photosensitivity (Østensen et al., 2006).
- **Hepatic**: Mild and reversible increasement of serum transaminases can be observed relatively fast in SLE patients consuming NSAIDs and are able to normalize after up to two weeks after the discontinuation of the medication. Severe hepatitis is rare and is mostly seen in patients on aspirin, nimesulide, sulindac, ibuprofen, indomethacin,

naproxen, fenoprofen and piroxicam. Impaired renal function in patients with LN, usually predisposes to hepatotoxicity (Sostres et al., 2010).

- **CNS**: The main CNS side effects include headache and dizziness, followed by more rare effects like neuropathy, tinnitus, vision disturbances, seizures and cognitive impairment. The most severe adverse effect is aseptic meningitis, which is more frequently encountered in SLE patients, and is associated mostly with ibuprofen, naproxen followed by diclofenac and sulindac (Gan, 2010).
- **Reproductive**: Since SLE is in a 9-fold manner more common in women and especially in the reproductive years, any adverse reaction involving fertility are of high importance. PGs are involved in ovulation and the initiation of parturition, while COX-2 is the enzyme expressed in the preovulatory follicles, inducing the maturation of the ovum. Various case reports described transient infertility following long-term administration of indomethacin, diclofenac, naproxen and piroxicam (Østensen et al., 2006).

2.9.2. Antimalarial agents

Antimalarials are widely and successfully used in the therapy of SLE as well as discoid lupus, possessing **disease-modifying** properties affecting inflammatory activity and thus, promoting a positive influence in the quality of life, morbidity and prognosis. The main antimalarial compounds used include **hydroxychloroquine**, **chloroquine** and **quinacrine** (ACR, 2018). Chloroquine is 2-3 times more potent than hydroxychloroquine although it has increased risk of retinopathy; thus, hydroxychloroquine is much more frequently used. Blood levels of hydroxychloroquine can independently predict a SLE exacerbation, therefore the measurements of hydroxychloroquine concentration in the whole blood concentration could aid in the identification of patients with increased risk. Due to hydroxychloroquine's long

elimination half-life, measurements of blood serum hydroxychloroquine concentration can serve as marker of therapy adherence. Furthermore, hydroxychloroquine has also protective role against renal damage and major infections (Wallace et al., 2012). Antimalarials have been widely used in the management of **cutaneous lupus** and they remain the first-line systemic agents for the therapy of widespread skin manifestations. Initially, the therapy is done with hydroxychloroquine where more than half of the patients respond efficiently; in case this therapy fails the addition of quinacrine should be considered (Jessop et al., 2017). It is believed that tobacco smoking reduces the efficacy of antimalarial medication in a dose-dependent manner (i.e. number of cigarettes smoked/day is inversely proportional to the degree of clinical response to antimalarials), especially in cutaneous lupus manifestations. Patients who ceased smoking tend to respond better to the therapy. However, this statement is not established yet, due to many conflicts between various studies (Wallace et al., 2012).

Mechanism of action

Antimalarials exert their action via multiple molecular pathways, some of them established whereas some of them are still under research.

- **Interference with lysosome function**. Hydroxychloroquine and chloroquine are weak bases with an affinity to the acidic lysosome (lysosomotropic action). They alter the endolysosomal pH and interfere with acidification of lysosomes. In T cells, lysosomes have the dual function of degradation of endocytosed material and participation the APCs apoptosis. Thus, if these functions are impaired, cytokine production (mainly IL-2, IL-6 and TNF) is inhibited. Hydroxychloroquine has been proven to have a long-lasting suppressive effect of IL-6. Antimalarials also downregulate the expression of mRNA of cytokines at the transcriptional level (Lee et al., 2011).
- **TLR-associated mechanism.** Antimalarials can block the activation of TLRs in various ways. They have been proven both *in vitro* and *in vivo* to antagonize immune

stimulation by CpG-DNA (a TLR9 ligand) and thus inhibit the CpG-DNA-induced synthesis of IL-6 and TNF. Moreover, *chloroquine* inhibits immune system stimulation by snRNA (TLR7 ligand) and subsequently, the production of IFN-α. The exact mechanism by which antimalarials antagonize TLR-mediated immune reactions and thus the synthesis of IFN and inflammatory cytokines, still remains unclear. It is estimated however that this could achieved either by a competitive (structural binding) or a noncompetitive (pH alteration) mechanism (Wallace et al., 2012).

Ultraviolet light protection is a TLR-independent mechanism where antimalarials absorb the UV light; significant protection as UV radiation is a potent risk factor inducing local inflammation, cell injury to keratinocytes resulting in cell death and the development of cutaneous lupus lesions. Chloroquine epidermal concentration in up to 15 times higher compared to the dermis, facilitating local anti-inflammatory effects like the inhibition of antigen presentation and cytokine synthesis. Moreover, antimalarials can activate the transcription of the c-Jun gene which seems to be a constituent of the early protective response to UV (Ruiz-Irastorza et al., 2008).

Further, TLR-independent effects of antimalarials include **antilipidemic effects** (by regulating enzyme activity through the lipid receptors and possibly TLRs; reducing VLDL, LDL and cholesterol, upregulating HDL), **antithrombotic effects** (through the inhibition of platelet aggregation, blockage of the interactions between platelets and coagulation factors, reduction of thrombotic manifestations and possible participation in primary thrombophylaxis in APS and SLE) (Kalia et al., 2007), **antiangiogenic effects** (reduction of the epidermal expression of vascular endothelial growth factor (VEGF), *in vitro* anti-proliferative and anti-apoptotic effects in endothelial cells and plausible effect in discoid lupus), **BAFF inhibition** (by the reduction of maturation and survival of B cells, including autoreactive B cells), **phospholipase A_2 inhibition** (through cell membrane stabilization,

inhibition of AA pathway and downstream synthesis of inflammatory mediators) and **MMP-TIMP modulation** (through the inhibition of the expression of MMP-1, -2, -8 and -9, regulation of extracellular matrix breakdown. Additionally, hypoglycemic (mainly with hydroxychloroquine) and cardiac quinidine-like effects can also occur. Due to the increased risk of atherosclerosis in SLE patients, antimalarials are also beneficial as they can decrease atherogenesis that have been shown to be influenced by TLRs (Wallace et al., 2012).

Side effects

Antimalarials are generally relatively safe, non-toxic and well-tolerated. General side effects include nausea, vomiting, pruritus, maculopapular rashes, skin and mucosal pigmentation, insomnia and nervousness. Less commonly tinnitus, neuropathy, psychosis, seizures and vestibular alterations may occur. Rarely, leucopenia, anemia, liver dysfunction, porphyria, hair depigmentation and hair loss, diarrhea and porphyria are observed (Lee et al., 2011).

The main concerning adverse effect profile induced by the antimalarial therapy is ocular toxicity. These side effects include keratopathy in the form of corneal deposits, bull's eye maculopathy, cycloplegia and cataracts observed in more than 50% in patients on chloroquine, 10% in patients on hydroxychloroquine and 5% in patients on quinacrine. Keratopathy is most of the times completely reversible with no residual corneal damage, while retinopathy is less frequent and will mostly occur in patients receiving chloroquine (Wozniacka et al., 2006, Ben-Zvi et al., 2012). Chronic eye toxicity is represented in **bull's eye maculopathy** (bilateral occurrence is more common), although the patient can have an excellent visual acuity during the initial stages; in later stages, the usually reversible damage can be irreversible. Therefore, annual screening is recommended. Additional caution should be considered in patients with pre-existing macular disease, elderly, obese and in those with hepatic or renal disorders as they have an increased risk of earlier manifestation of

retinopathy. Among those with pre-existing retinopathy the drug of choice is quinacrine (Wallace et al., 2012).

2.9.3. Systemic corticosteroids

Systemic corticosteroid administration in SLE is still a golden standard therapeutic intervention in patients with all but the mildest SLE forms. They are administered when NSAIDs and antimalarials fail to eliminate manifestations like arthralgia or rash effectively. Generally, they are best used in acute SLE flares; due to their serious long-term adverse effects it is essentials to implement steroid-sparing medications for long-term therapy. Corticosteroids are used alone or in combination with other immunosuppressants for patients with significant organ involvement or refractory symptoms. Patients requiring long-term corticosteroid therapy should be monitored for complications of hypertension, diabetes, myopathy, psychosis and cataracts (Gurevitz et al., 2013). General systemic SLE manifestations like fever, fatigue, lymphadenopathy, acute cutaneous lupus and unintended weight loss can be treated with 20-100mg prednisolone/day. In case the systemic symptoms occur in isolation, low-medium dose corticosteroids are used in combination with hydroxychloroquine. If the symptoms are persistent and recurring with taper of steroid despite the administration of hydroxychloroquine, immunosuppressants are necessary (Thamer et al., 2009). For the most serious SLE manifestations like life-threatening myocarditis, extreme cytopenias, CNS disease and alveolitis “pulse” corticosteroids are usually administered, i.e. methylprednisolone 1000mg as daily slow-releasing (at least 1 hour to avoid risk of severe cardiac arrhythmias) intravenous infusion for three consecutive days, followed by prednisone 1mg/kg. These extraordinarily high doses are able to achieve a unique effect on T cells and engage cytoplasmic corticosteroid receptors; very rapid improvements are detected after such dosing. However, the very-high but short-term

corticosteroid administration can be related with risks like avascular necrosis and psychosis; continuous monitoring is required (Parker et al., 2007).

Prednisone is a corticosteroid which also elicits mild mineralocorticoid activity and has a moderate anti-inflammatory activity. It prevents the inflammation by suppressing the migration of polymorphonuclear leukocytes (PMNs) and fibroblasts, as well as by controlling protein synthesis rate and stabilize lysosomes at cellular level (Kasturi et al., 2016). **Methylprednisolone** is a potent glucocorticoid with minimal to no mineralcorticoid activity. It is preferred over prednisone in hepatic impairment, because prednisone must be converted to prednisolone in liver (Mosca et al., 2011).

Mechanism of action

Corticosteroids are powerful SLE therapeutics due to their ability to target multiple pathogenic pathways due to their ability to **activate numerous anti-inflammatory genes**, as well as to **suppress a variety of inflammatory genes**. Additionally, they can modulate inflammation by additional post transcriptional mechanisms (Yap et al., 2012).

In order to activate various anti-inflammatory genes, corticosteroids diffuse across the cell membrane to bind to the glucocorticoid receptor (GR) in the cytoplasm. As a result, the GR conformation changes and is released from a variety of chaperone proteins (e.g heat shock protein 90), and GR enters the nucleus by binding to nuclear import proteins, like importin α (Kasturi et al., 2016). Corticosteroids bind to the ligand-binding domain of GRα, rather than GRβ which interacts with DNA but not with corticosteroids. The GR can homodimerize and bind to the glucocorticoid response element (GRE), which is a DNA recognition site in the promoter region of steroid-responsive genes. When GR binds to a GRE, gene transcription is switched on through the interaction with a transcriptional coactivator molecule, like CREB (cAMP response element binding protein)-binding protein, which has intrinsic histone acetyltransferase activity, leading to the acetylation of core histones

physically associated with DNA. This leads to the recruitment of chromatin remodeling engines by histones and RNA polymerase II, resulting in gene activation. SLE patients have decreased numbers of GRs in peripheral blood mononuclear cells compared to healthy individuals. The role of for endogenous corticosteroids in SLE pathogenesis is thus suggested; receptor levels are inversely related with disease activity and therapy. (Agusti, 2005)

Genes that are switched on by corticosteroids include genes encoding β2-receptors, leucine zipper (anti-inflammatory glucocorticoid-induced proteins), and mitogen-activated protein kinase phosphatase 1 (MAKP-1) which is able to inhibit the mitogen-activated protein kinase (MAPK) pathways, and thus contribute to the anti-inflammatory effects of corticosteroids. GR interaction with negative GREs, is also able to suppress gene transcription, mediating the side effects of steroids, including inhibition of osteocalcin, playing a key role in the bone synthesis (Rhen et al., 2005).

The main action of corticosteroids in the suppression of inflammation is to switch off activated inflammatory genes that encode for chemokines, adhesion molecules, cytokines, inflammatory enzymes and receptors. These inflammatory genes are activated by pro inflammatory transcription factors, including NF-κB, and activator protein 1 (AP-1). Activated GRs are able to reverse histone acetylation through the recruitment of HDAC2, and thus attenuate NF-κB-associated coactivator activity, leading to the suppression of inflammatory genes (Mosca et al., 2011).

Other mechanisms that contribute to the anti-inflammatory effects of corticosteroids include their potent inhibitory effects of MAPK signaling pathways by the induction of MKP1, leading to the inhibition of a variety of inflammatory genes. Corticosteroids can reverse the stimulation of inflammatory genes like TNF-a, either by the inhibition of inflammatory mediators that stabilize inflammatory genes, or by the induction of RNAses

that can rapidly degrade their mRNA and reduce the inflammatory protein secretion (Gurevitz et al., 2013).

Corticosteroids **immunosuppressive** effect can result by three plausible mechanisms (Kasturi et al., 2016):

- **Inhibition of IL-2/IL-2R activity** which is either achieved by the direct suppression of IL-2R mRNA levels through transrepression, or the upregulation of GILZ and mechanisms of NF-κB inhibition, or by the inhibition of IL-2 signaling by the blockage of STAT-5 activation, all resulting in the modulation T-cell activation.
- **Inhibition of DC function** by the impairment of their antigen-presenting function, or the decreased expression of costimulatory molecule. This is also achieved by the suppression of IL-12 production and the induction of apoptosis of the dermal/interstitial DC precursors.
- **Preferential enhancement of Th2-type cytokine profile** is achieved by the downregulation of IL-12 activity either by the downregulation of IL-12 receptor resulting in the reduction of inflammatory cytokines or by the rapid inhibition of STAT-4 phosphorylation.

Side effects

Corticosteroids have **suppressive effects on the hypothalamo-pituitary-adrenal (HPA) axis**, causing a negative feedback that attenuates the release of cortisol from the adrenal cortex. This effect of corticosteroids can be problematic during and a relatively long period of the discontinuation of the therapy, or if a higher cortisol response is needed during the introduction of a stressful stimuli (e.g. infection, trauma, surgery). Rarely, iatrogenic Cushing's syndrome (moon-shaped face, buffalo hump, central obesity), adrenal insufficiency and growth inhibition can occur in a dose-dependent and patient-specific manner (Schäcke et al., 2002).

Osteoporosis is highly associated with the use of systemic corticosteroids; characterized by an increased risk of bone fracture in a dose and time dependent manner. Sadly, high evidence of underdiagnosis of corticosteroid-induced osteoporosis is estimated. The fracture risk is also related with the female gender, age and body weight. Corticosteroids inhibit bone formation through the suppression of osteoblast proliferation, the increased osteoblast and osteocyte apoptosis, the decreased GI Ca^{2+} absorption and the increased urinary Ca^{2+} excretion. Moreover, the reduction of adrenal sex hormones and the suppression of growth hormone, insulin-like growth factor-1 and TGF-β are also implicated in the development of osteoporosis (Lo et al., 2012). In order to prevent or eliminate osteoporosis, patients should be administered calcium and vitamin D supplements, perform a bone mineral density scan frequently and if the T score is below -2.5 a bisphosphonate should be prescribed for increased bone protection, unless contraindicated (Tsokos et al., 2007).

Eyes are also influenced by the use of systemic and topical steroids. Cataracts, one of the leading causes of blindness worldwide, are well known complications of systemic steroids. Additionally, glaucoma can also occur, and rarely retinal emboli and maculopathy. The etiology of these effects is still unclear (Mosca et al., 2011).

Oral corticosteroids have been evidenced to **suppress cell-mediated immunity**, creating a favorable environment for pathogens (Gladman et al., 2005).

Skin thinning, and **increased bruising** have been reported in patients using high doses of corticosteroid therapy because corticosteroids can reduce collage synthesis by the skin (Panduya et al., 2014).

Topical and systemic corticosteroids can induce various **cutaneous** adverse effects, determined b the potency and the duration of the therapy. The most common side effects are the dermis and epidermis atrophy (thinning) and disrupted wound healing. Furthermore, hypertrichosis may develop, particularly facial; however, it is reversible and disappears after

the cessation of the therapy. Long-term usage of the local agents can induce erythema and telangiectasia. Corticosteroids suppress cutaneous cell proliferation and protein synthesis by fibroblasts, leading in collagen turnover (Kasturi et al., 2016).

Furthermore, they are related with an **increased risk of developing diabetes mellitus** or **worsen the glycemic control** in patients already diagnosed with diabetes. Glucocorticoids can cause hyperglycemia either by increasing gluconeogenesis in the liver via the increment of glucagon release, or by reducing glucose uptake in the liver and adipocytes via the decreased insulin binding. SLE patients with diabetes in corticosteroid treatment, should be monitored closely for worsening of glycemic control (Rhen et al., 2005).

Steroid myopathy is a common side effect of long-term therapy with systemic corticosteroids by their catabolic effects on skeletal muscles and especially quadriceps and various pelvic girdle muscles. They inhibit the glucose uptake in skeletal muscles, leading in the breakdown of muscle proteins. One of the genes that is widely known to contribute in this process is glutamine synthetase (Schäcke et al., 2002).

Central nervous system: Mood swings, euphoria, depression and suicide attempts can occur in a previously stable person or can exacerbate psychiatric problems in a patient with existing psychiatric disorders. “Steroid psychosis” (i.e. mania, hallucinations and delusions) are reported mainly in women and manifest typically within 2 weeks of the initiation of the therapy, especially in doses > 40 mg/day of prednisolone (Chau et al., 2003). Furthermore, patients receiving acute and long-term administration of corticosteroids can develop psychiatric withdrawal symptoms like fatigue and depression. Corticosteroids also induce direct and reversible side effects on memory and cognition, as well as dose-dependent cerebral atrophy. The mechanism of these effects ranges from the disruption of cellular metabolism to an increased in the vulnerability of the hippocampal neurons and the augmentation of the extracellular glutamate. Abnormalities of the HPA-axis and the

suppression of the serotonin (5-HT) receptors (especially 5-HT_{1A} receptor) have a key role in the pathogenesis of depression (Bertsias et al., 2010).

2.9.4. Immunosuppressive agents

Immunosuppressive agents are mainly administered during the onset of active lupus with major organ implication.

2.9.4.1. Cyclophosphamide (CyX)

CyX is an alkylating agent causing cell death, originally used as a chemotherapeutic, has a strong, dose-dependent non-specific immunosuppressive effect resulting from its cytotoxic effect on rapidly dividing cell activated lymphocytes and/or granulocyte precursors. CyX is usually administered (monthly or biweekly) intravenously (i.v) in **lupus nephritis** with high-dose corticosteroids. The National Institutes of Health (NIH) suggest a dosing regime of 0.75-1 gram per square meter of body surface area given monthly for 6 months if nadir leukopenia (leukocytes < 2000/mm^3) is not achieved (Lo et al., 2012).

In spite that CyX used to be a golden standard the past decades, its usage is eliminated to the minimum due to its potent and serious side effects. Nausea and vomiting due to the stimulation of CNS vomiting receptors are common in approximately 12 hours after the administration of the drug. In the majority of patients antiemetics like oral ondansetron with dexamethasone are administered in order to minimize the GI effects (Mak et al., 2009). Furthermore, malaise, weight loss and hair loss occur usually with CyX. It is also well established as a **teratogen**; thus, effective birth control is crucial (Ginler et al., 2005).

CyX's cytotoxic effect is most commonly expressed by its effects on bone marrow function, especially leukocytes. **Peripheral leukopenia** is a dose-dependent issue, reaching

the nadir in 8-12 days after i.v administration or increased oral dose; numerous leukocyte counts should be obtained (Petri, 2004). Due to its immunosuppressive effect, CyX increases the patient's **susceptibility to bacterial, viral and fungal infections** as every drug of this category does, due to the induction of leukopenia, decreased antibody production and altered cellular immune function (Ruiz-Irastorza et al., 2009a). Prolonged CyX administration is also associated with ovarian failure and oligo/azoospermia leading in **infertility** due to the damage on the gonadal tissue. Specifically, serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are elevated, whereas estradiol levels are decreases. The estrogen deficiency leads to signs of menopause with amenorrhea, hot flashes, endometrial hypoplasia and vaginal epithelium atrophy, later causing follicle destruction and fibrosis in the interstitial areas. In males, the germinal epithelial lining layer of the seminiferous tubules is damaged and a decrease in testicular volume and oligo/azoospermia will exist. The recovery of ovarian function in women and spermatogenesis in men after the discontinuation of the drug is unpredictable; it has been observed that this procedure may take months or years (Ioannidis et al., 2002). CyX is uniquely related with **bladder toxicity** causing hemorrhagic cystitis, fibrosis and transitional and squamous cell carcinoma, associated slightly more with the drug oral administration. Oral CyX should be taken in the morning with increased fluid intake throughout the day to reduce the concentrations of its active metabolite acrolein in the bladder. Nonglomerular hematuria is the most useful marker of the tendency of a patient to develop bladder cancer (Appel et al., 2009). **Rare complications** of CyX include pneumonitis, myocardial necrosis, liver disorders, inappropriate antidiuretic hormone (vasopressin) syndrome and numerous hypersensitivity reactions (Lo et al., 2012).

2.9.4.2. Azathioprine

Azathioprine is an immunosuppressant used mainly as maintenance therapy -due to its slow onset of action- after the usage of more aggressive medication, in steroid-sparing or in patients with recurring flares. It is also claimed to be inferior as induction therapy for proliferative LN and useful as an alternative maintenance therapy to MMF or CyX (Feng et al., 2013).

Azathioprine is a purine analogue used for its enzymatic product **6-mercaptopurine**. Specifically, the metabolism of azathioprine in the body implicates two main pathways. Direct oxidation by the enzyme xanthine oxidase results in 6-thiouric acid, converted to uric acid. This pathway is blocked by allopurinol, a xanthine oxidase inhibitor and then, the metabolism is shifted to the second pathway; methylation of the sulfhydral group and subsequent oxidation. Mono-, di-, and triphosphate nucleosides of the methyltriopurine products accumulate, resulting in alterations of the cellular purine biosynthesis and DNA function. Metabolites of this pathway produce the immunosuppressive and toxic effects of 6-mercaptopurine. However, patients on azathioprine, using also allopurinol should have a decreased dosage (approximately by one-third) of azathioprine, due to the fact that allopurinol enhances the 6-mercaptopurine pathway (Gurevitz et al., 2013). Upon initiation of azathioprine the oral doses should be 25mg-50mg/day in order to determine acute toxicity/sensitivity. Afterwards, the dose can be increased by 0.5mg/kg/day every 4-6 weeks with a goal to reach 2-3mg/kg/day. A total hematologic test should take place every 1-2 weeks when the dose is still adjusting and then every 1-3 months, while liver enzymes should be monitored every 4 months. SLE patients on azathioprine have been documented to have stabilization or improvement of impaired renal function, decreased proteinuria and effective corticosteroid sparing. Disease exacerbations have been observed several months following azathioprine withdrawal, enhancing the evidence for the drug efficacy (Appel et al., 2009).

The most common side effects of azathioprine include malaise, fever, weight loss, anorexia, nausea, vomiting, diarrhea and abdominal pain. **Bone marrow toxicity** is also common, as azathioprine affects both erythroid and myeloid elements of the bone marrow. Upon vitamin B_{12} or folic acid deficiency, macrocytosis with megaloblastic erythroid changes of the bone marrow can occur, that can rarely result in selective erythroid hypoplasia. **Leukopenia** can also arise abruptly. Bone marrow toxicity is generally reversible after the reduction of the dose or the discontinuation of the therapy (Feng et al., 2013). **Hepatotoxicity** can be observed, indicated by the elevation of liver enzymes, particularly the pyruvic and glutamic oxaloacetic transaminases, and accompanied by fever, diffuse abdominal pain, diarrhea and maculopapular skin rash. The hepatic side effects are also usually reversible upon discontinuation of the medicine (Dooley et al., 2011). **Hypersensitivity reactions** indicated by fever, hypotension and oliguria have been also described. **Congenital defects** and evidence of **severe immunodeficiency** can occur in infants born from mothers taking azathioprine during pregnancy. Azathioprine can cause chromosome abnormalities and an increasement in sister chromatic exchanges. Finally, strong evidence suggests the **carcinogenic** effect of azathioprine in humans, especially for the development of solid tumors, leukemia, non-Hodgkin's lymphoma, reticlum cell carcinomas and a fourfold increase in cervical atypia (Bertsias et al., 2008).

2.9.4.3. Mycophenolate Mofetil (MMF)

MMF is widely used in transplantation medicine used in the prophylaxis of acute organ rejection, in combination with cyclosporin A and corticosteroids and has been proven to be superior than azathioprine. It is mainly used in the maintenance phase of LN (Mak et al., 2009). It is a potent inhibitor of inosine monophosphate dehydrogenase which is an enzyme essential for *de novo* production of guanine nucleosides and thus it has similar functional consequences like purine analogs. MMF is rapidly hydrolyzed to its active form

mycophenolic acid, which is selective for T cells and B cells due to the reliance of these cells on the *de novo* purine pathway rather than the hypoxanthine-guanine phosphoribosyl transferase salvage pathway for purine biosynthesis. MMF suppresses T cells and B cells proliferation, monocyte activation, antibody formation and recruitment of leukocytes in the inflammatory sites (Appel et al., 2009). It can be administered orally and parenterally and has exceptional bioavailability with a half-life of 17 hours. 1-2g are given orally twice a day, on an empty stomach, although the dose should be adjusted in patients with impaired renal function. Administration of MMF during pregnancy is not recommended due to its possible **teratogenic** effects. In general, it is a well-tolerated drug and very beneficial in patients with CyX refractory nephritis (Bertsias et al., 2008).

The side effects of MMF commonly include **GI intolerance**, **leukopenia**, **hepatotoxicity**, **hypersensitivity reactions**, susceptibility in opportunistic bacterial, viral (e.g. cytomegalovirus) and fungal **infections** that appears to improve with lower doses, and rarely **myelotoxicity** (Dooley et al., 2011).

2.9.4.4. Cyclosporine

Cyclosporine has been successfully used in the prevention of rejection in patients with heart, kidney and liver transplantation and later in RA. In SLE, it is mainly used in patients with major organ involvements (especially membranous nephritis) whose disease is unresponsive or poorly responsive to corticosteroids, cytotoxics, or both. Cyclosporine is a cyclic, lipophilic polypeptide produced as a metabolite by the fungus *Beauveria nivea*. It is an inhibitor of TCR-induced IL-2 transcription through inhibition of the calcium/calcineurin induced nuclear translocation of NF-ATc. Cytotoxic T cell production is also suppressed (Bertsias et al., 2012). Cyclosporin can improve LN; it reduces proteinuria (by a direct effect on the renal tubules) and cytopenias and slows the progression of renal insufficiency in

membranous forms of glomerulonephritis, although it is less effective in patients with steroid-resistance. Cyclosporine should be administered in lower doses of 2.5-5mg/kg/day due to its clear dose-dependent reduction in renal function yielding in hypertension and direct **nephrotoxicity** at higher doses which is only partially reversed upon dose-lowering or discontinuation of the agent. Other **side effects** include prominent hypertrichosis, GI intolerance, tremors, paresthesia, gingival hyperplasia and peculiar angioedema with decreased levels of C1 esterase levels (Moroni et al., 2008).

2.9.4.5. Methotrexate (MTX)

MTX is an antimetabolite and the cornerstone of RA therapy. In SLE it is used for polyarthritis, fever, subacute cutaneous lupus, serositis and leukocytoclastic vasculitis, suppression of the disease activity, corticosteroid taping, and has been shown to be useful as adjunctive therapy for selected cases of childhood SLE. It is administered weekly, orally or parenterally, in the range of 7.5-15mg/week, similar to the doses administered in RA (Gurevitz et al., 2013). After entering the body, MTX is converted by the Kupfer cells in its polyglutamate form. MTX polyglutamates are partly responsible for both therapeutic and toxic effects. MTX is an antifolate, leading to adenosine release and inhibition of methylation reactions, yielding in the anti-inflammatory effects in SLE and numerous autoimmune diseases. MTX polyglutamates inhibit the enzyme 5-aminoimidazole-4-carbonxamide ribonucleotide (AICAR) transformylase resulting in AICAR intracellular accumulation that inhibits AMP deaminase, so adenosine is accumulated and subsequently released in the extracellular space. The interaction between adenosine and its A_2 receptor can inhibit the secretion of toxic oxygen metabolites as well as lymphocyte proliferation to mitogens and induce suppresser phenotype and function. Furthermore, adenosine can inhibit TNF_{α} and IL-8 production and increase the IL-10 production (Tsokos et al., 2007, Fortin et al., 2008).

MTX **side effects** include malaise, GI intolerance, increased transaminases and mucositis, however concomitant administration of folic acid could reduce some of these effects without affecting the drugs efficacy. Moreover, MTX is **teratogenic** and can lead to **fetal death**. Pregnancy should be avoided if the drug is administered in either partner in a minimum of 3 months after discontinuation of the therapy for males and one ovulary cycle for females (Lo et al., 2012).

2.9.4.6. Tacrolimus

Tacrolimus (previously known as FK506) is a potent macrolide immunosuppressant, T-cell specific calcineurin inhibitor, 10-100 times as potent as cyclosporine and although they are chemically unrelated, they have a similar mechanism of inhibition of T cell activation (Brennan et al., 2005). It is effective in the therapy of autoimmune, inflammatory and allergic skin diseases through 3 mechanisms. Firstly, tacrolimus suppresses T cell activation by the inhibition of the expression of early T cell response genes. Secondly, it has a potent antipruritic activity and inhibits IgE-mediated histamine release from mast cells, reduce transcriptional IL-3 and IL-5 activation and leukotriene expression. Finally, it inhibits IgE expression on Langerhans antigen presenting cells in the epidermis and inhibit their capability to stimulate autologous lymphocytes. Furthermore, production IL-2, IL-4, IL-5, IFN-γ and TNF-α are all reduced (Hannah et al., 2016).

Tacrolimus can be used “off-label” in LN as maintenance therapy in combination with corticosteroids. It has been related with a notably greater decrease in proteinuria (a potent predictor of a fair long-term renal outcome) and improved serum creatinine and GFR levels (Lee et al., 2011). Among many clinical trials, interesting was the multicenter randomized clinical trial of Chen et al., which compared the therapeutic outcomes of the calcineurin inhibitors tacrolimus and azathioprine, either combined with prednisolone. Tacrolimus had

a much more **favorable safety profile**, especially in the critical issue of leukopenia, as well as in nephrotoxicity, arterial hypertension, hyperlipidemia and other calcineurin-inhibitor-related side effects. Moreover, tacrolimus and azathioprine demonstrated similar low rates of renal relapse for maintaining remission of active LN. However, more clinical trials are needed in order to establish the promising role of tacrolimus in LN.

One more "off-label" tacrolimus effect can be also beneficial in topical preparations of 0.1% ointment, for different cutaneous lupus erythematosus subtypes. It can decrease edema and erythema in cutaneous LE skin lesions most potently. An increased response in early inflammatory skin lesions without prominent hyperkeratosis can be achieved in LE tumidus and acute cutaneous LE. 0.1% tacrolimus ointment is also effective in discoid lupus erythematosus, but in a lower rate (Kuhn et al., 2011).

2.9.5. Biologic agents

Biologic agents are widely used in the past two decades in the therapy of autoimmune diseases like RA, psoriasis, Crohn's disease and multiple sclerosis. They are large protein molecules derived with hybridoma and/or DNA recombinant methodologies and are designed to target a signaling molecule in the inflammatory pathways or a cell surface marker (Bertsias et al., 2012).

2.9.5.1. Belimumab

Belimumab is the only biological agent currently approved in the SLE therapy. It is a genetically engineered fully human monoclonal antibody which binds to BAFF. After bound, BAFF is not able to engage its receptor and B cell activation is diminished. Thus, belimumab allows more B cells to undergo apoptosis which inhibits their survival, including

autoreactive B cells and decreases the differentiation of B cells into Ig-producing plasma cells. It is indicated for patients with active, autoantibody-positive SLE patients who are receiving standard therapy. However, its effect is not evaluated in patients with severe active LN or severe active CNS lupus. It is administered as a 1-hour i.v infusion firstly as 3 infusions at a 2-week interval, then as 1 infusion every 4 weeks and finally in a subcutaneous form weekly (van Vollenhoven et al., 2012). Two phase III trials (BLISS-52 and BLISS-76) were able to demonstrate the positive effects of belimumab in patients with increased SLE activity at baseline, who were anti-dsDNA positive, had hypocomplementemia and required corticosteroid treatment. Patients with active LN and NPSLE were excluded from the trials. The patients who participated had improved disease activity, decreased rate of SLE flares and demonstrated a mild corticosteroid-sparing effect. Furthermore, there was a potential effect of belimumab on renal parameters in patients receiving MMF at baseline, showing a renal improvement after 52 weeks**.**

Side effects included opportunistic infections, hypersensitivity reactions including anaphylaxis and progressive multifocal leukoencephalopathy represented as new-onset or deteriorating neurological signs. Finally, a minor increase in depression and suicidality was observed (Lee et al., 2011, Chen et al., 2012, Dooley et al., 2013).

2.9.5.2. Rituximab

Rituximab is a chimeric monoclonal antibody directed against CD20, a surface marker of mature B cells. It induces depletion of circulating B cells. It was originally developed for the therapy of non-Hodgkin's lymphoma and remains a significant part of therapeutic regimes in various lymphomas and chronic lymphocytic leukemia. Later it was approved in the therapy of many autoimmune diseases like RA, ANCA-associated vasculitis, autoimmune hemolytic anemia, multiple sclerosis, ITP, Sjögren's syndrome etc. The results referring in rituximab's effect on SLE are controversial; some claiming it has a positive effect in the reduction of severe flares with significant response rates and improvement of the serologic

marker, whereas others are claiming no beneficial effect in SLE. (Willems et al., 2006, Kersh et al., 2018) Furthermore, Benham et al., stated that rituximab can have a beneficial effect in SLE patients with shrinking lung syndrome. However, numerous studies and clinical trials are necessary in order to evaluate the actual role of rituximab in SLE. Possible **side effects** of rituximab include prominent acute infusion reactions, transient hypotension, cardiac arrhythmias, serum sickness, progressive multifocal leukoencephalopathy, renal toxicity etc. (Hansel et al., 2010).

In an overall aspect, the American College of Rheumatology committee has suggested the following strategies in the SLE treatment recommendations (ACR, 2018):

- **Mild SLE**
  I. NSAIDs
  II. Antimalarials
  III. Low-dose oral corticosteroids
- **Serious, life-threatening, or organ-threatening SLE**
  I. High-dose corticosteroids
  II. Immunosuppressive/cytotoxic agents

2.9.6. Novel therapeutic approaches

B cells possess critical role in the pathogenesis of SLE, by a combination of antibody-mediated and antibody-independent manners as discussed in *chapter 2.4*. Their strong association with SLE made them possible targets in the disease pharmacotherapy and various researches take place focused on them.

Epratuzumab

Epratuzumab is a humanized monoclonal antibody targeted against CD22. The glycoprotein co-receptor CD22 is essential for B cell development and survival by the interaction with signaling molecules and the B cell receptor complex. It is located on mature B cells and is lost upon plasma B cell formation. Epratuzumab is a *B cell immunomodulator*, in contrast with rituximab which induces B cell depletion. Its antibody dependent cellular cytotoxic mechanisms include internalization of the CD22 receptor and thus downregulation of BCR signaling with a naïve and transitional cell preference, causing a significant decrease in the number of auto-B cells (Kamal et al., 2014). In 2006, Dörner et al., clinically evaluated the effect of epratuzumab in patients with moderate SLE; observing a minimus 50% attenuation in BILAG scores in 100% of the patients posttreatment and subsequent clinical improvement in almost all body systems; more than 90% of these patients maintained decreased BILAG scores for >4.5 months. They also reported no serious adverse effects. Due to the fact that epratuzumab is a *humanized* monoclonal antibody, it is less likely to cause the immune-mediated reactions related with the chimeric monoclonal antibody rituximab (Harvey et al., 2013). Furthermore, reduction of the corticosteroid usage and improvement of the health quality is suggested by epratuzumab administration (Lo et al., 2016).

Atacicept

Atacicept is a fully humanized fusion protein combining the Fc portion IgG and TACI which binds BAFF and APRIL. It has the ability to decrease B cell survival by inhibiting these B cell stimulating factors, providing a broader action compared with the action of belimumab. A phase II/III trials examining the administration of atacicept along with MMF in LN were halted due to increased serious opportunistic infections. More trials take place

to examine its efficacy in BILAG A or B flare after disease control. However, in order to acknowledge its possible benefits in SLE, more clinical trials are necessary (Harvey et al., 2013).

Targeting BCR signaling pathway is also thought to be a potent mechanism to reduce B-cell hyperactivity of the immune system. **Spleen tyrosine kinase (Syk)** is a key mediator of Fc receptor signaling and can also affect inflammatory cells like neutrophils, macrophages and DCs. Moreover, **antibodies to CD79**, a transmembrane protein related with BCR, represent an alternative way to target B cells through depletion and interruption of BCR signaling. Another possible B cell targeting mechanism is the blockade of either TLR or INF type I stimulation, affecting SLE through B cell inhibition (Sanz et al., 2010). **TLR inhibitors** are the most potent and specific among any other available or developing SLE therapeutic medication. Additionally, an anti-IFN-α humanized monoclonal antibody named **rontalizumab** is still under research for its effects on moderate-severe active SLE (Kamal et al., 2014).

Double negative (DN) T cells represent a subset of proinflammatory T cells, able to produce IL-17 and proliferate strenuously after anti-CD3 stimulation. IL-17 is also overproduced by CD_4^+ T cells. DN T cell subset in SLE patients demonstrates a pathologically expanded T cell subset, maintaining its proliferation and cytokine production characteristics. It has been proposed that attention should be paid in the signals that drive DN T cell expansion in SLE (Crispín et al., 2008).

While many trials on biologic agents (e.g. tocilizumab) as SLE therapeutics take place, many of them were halted either due to ineffective results or threatening side effects, attention is upon SLE pathogenesis mediators like B cells, T cells, interferon-α, cytokines etc that remain attractive targets and various studies are under development (Lo et al., 2013).

3. AIM

The aim of this diploma thesis was to analyze the pharmacological and non-pharmacological therapy approaches of systemic lupus erythematosus. Moreover, the systemic autoimmune processes influencing the disease progress are examined, as well as the prevalence of existing comorbidities that have similar genetic background and symptomatology that contribute to the disease morbidity.

4. METHODS

In order to successfully progress and integrate the former review, numerous scientific literature sources from ScienceDirect, ResearchGate etc., as well as medical and pharmacological textbooks were thoroughly examined. American College of Rheumatology and Lupus Research Alliance were also significantly useful data resources, as systemic lupus erythematosus is their main concern.

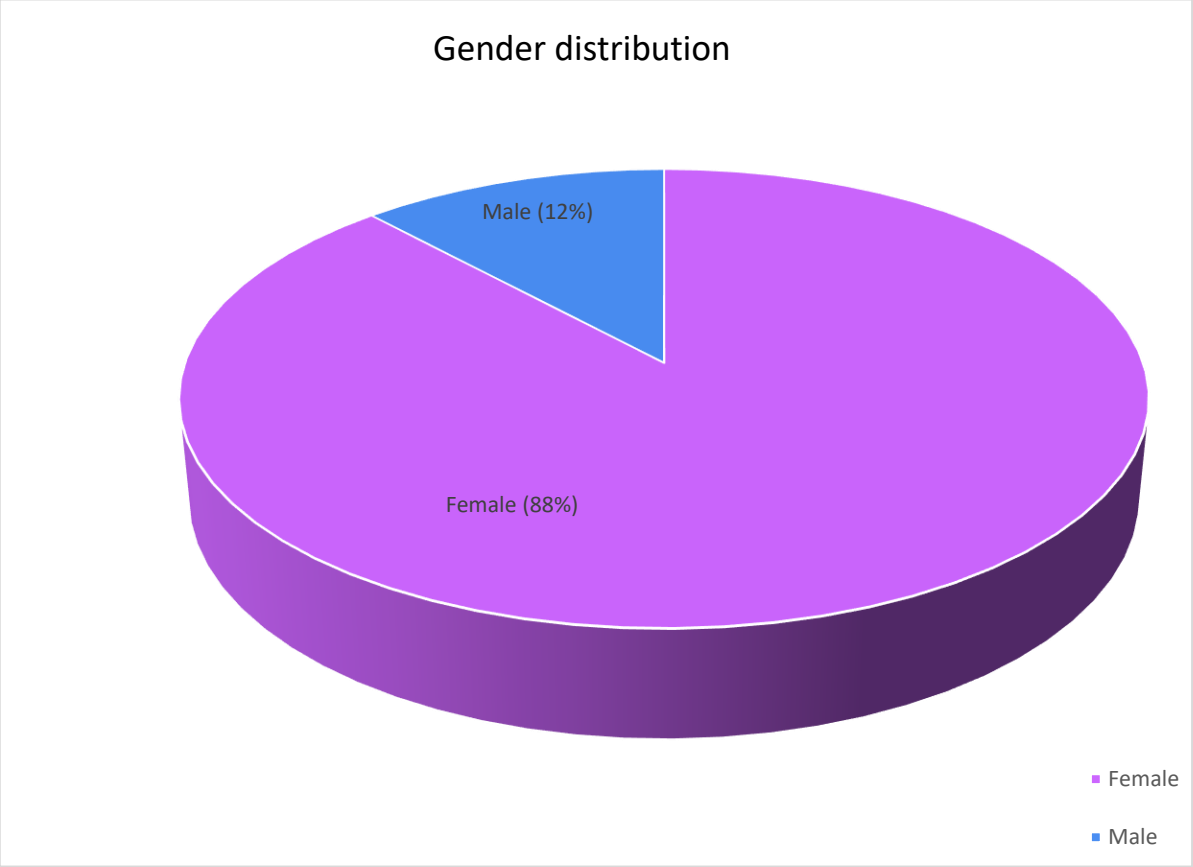
Referring to the practical part of this rigorous thesis, the cases of 42 patients who visited private rheumatologists at the onset of undiagnosed systemic lupus erythematosus are examined, as well as the progress of the disease throughout the years. The data were collected from the rheumatologists Evangelos Mandrakos and Theodoros Anagnostis. After the evaluation and examination of these data, statistical graphs were constructed using Microsoft Office Excel.

5. RESULTS

This research illustrates data from 42 patients, who visited two rheumatologists with symptoms of SLE.

GENDER

Graph 1, illustrates the gender distribution of the patients, demonstrating that out of the total 42 patients, 37 were female (88%) and 5 were male (12%).



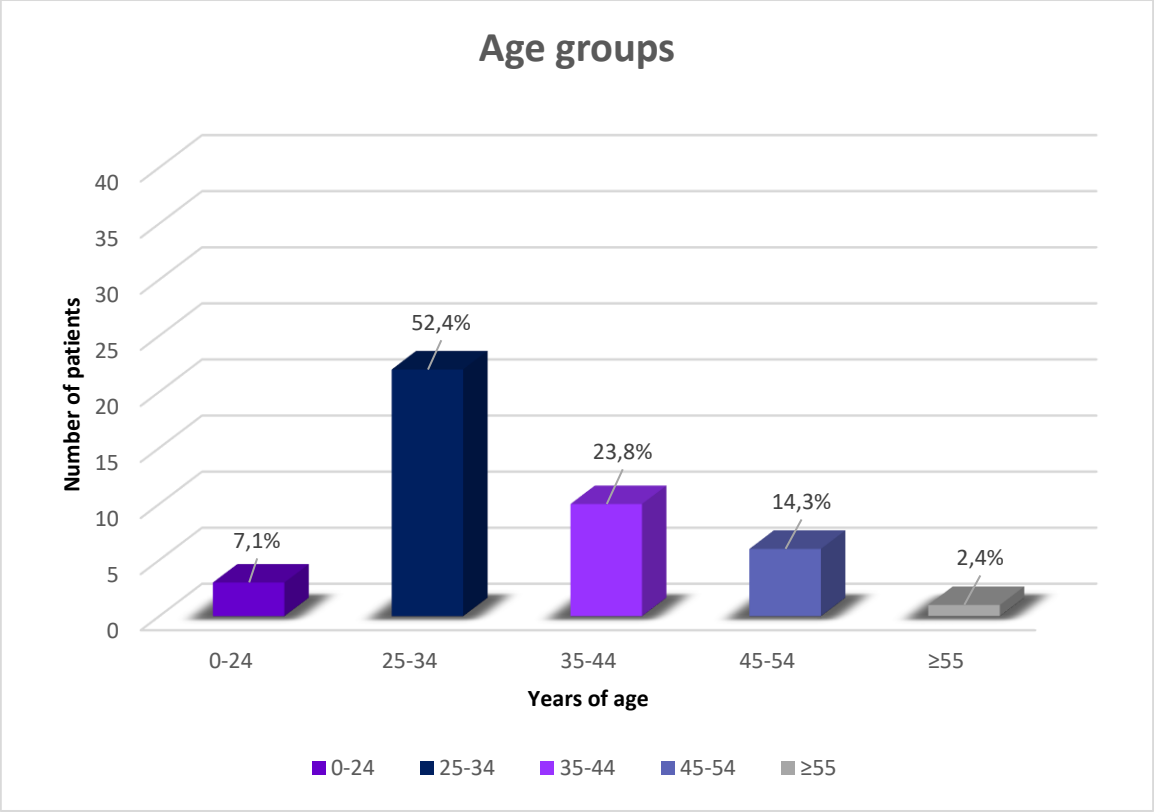
Graph 1 – Gender distribution among SLE patients

AGE

In Graph 2, the ages in which SLE was first manifested and diagnosed among the patients are illustrated. The median age of the 42 patients was 34,6 years of age. The youngest patient firstly diagnosed with SLE was a 18 years old female, whereas the oldest patient was a 58 years female. For a better representation of the disease age distribution, 5 age classes divisions were made:

- Younger than 25 years of age – 3 patients
- 25 – 34 years of age – 22 patients
- 35 – 44 years of age – 10 patients
- 45 – 54 years of age – 6 patients
- 55 years and older – 1 patient

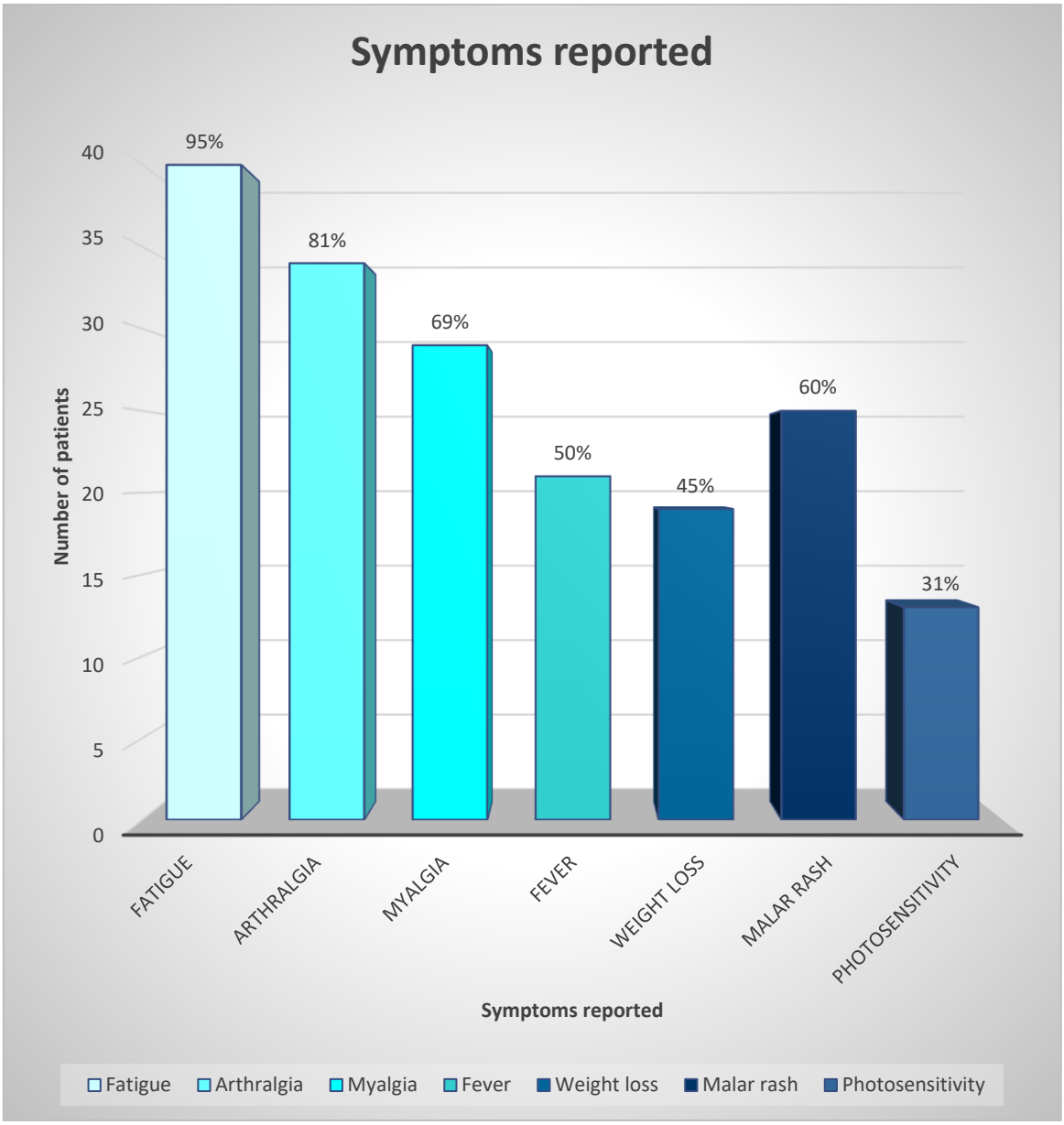
Age distribution is illustrated in Graph 2.



Graph 2 – Age distribution among SLE patients

SYMPTOMS

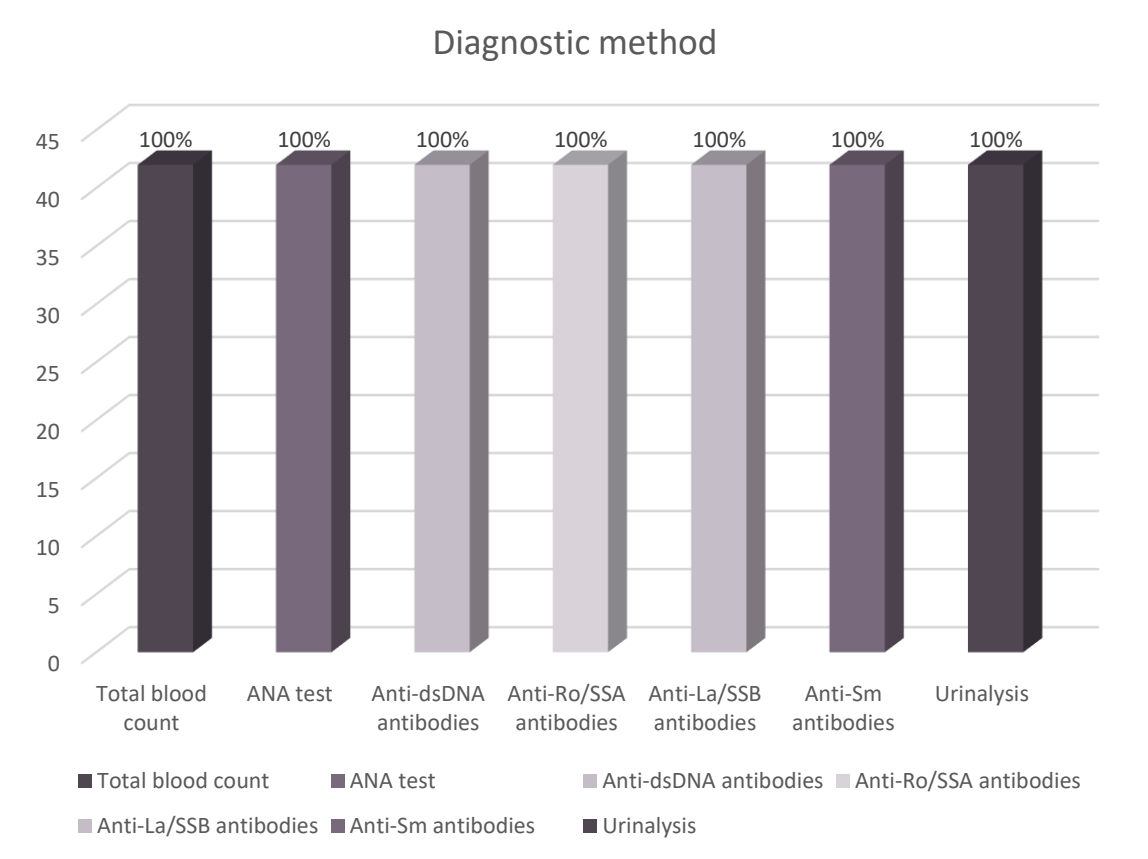
All of the patients visited the rheumatologist, complaining of symptoms indicating a SLE onset. The most prevailing symptom was fatigue, followed by arthralgia. Graph 3 illustrates the symptoms reported by the patients.



Graph 3 – Symptoms reported among SLE patients

DIAGNOSTIC METHODS

Graph 4 illustrates the diagnostic methods used in order to reassure the accurate SLE diagnosis. All patients went through the same diagnostic techniques. Two of these cases had to further undergo through renal biopsies are a strong kidney disorder was suggested through the urinalysis and total blood count measurements.

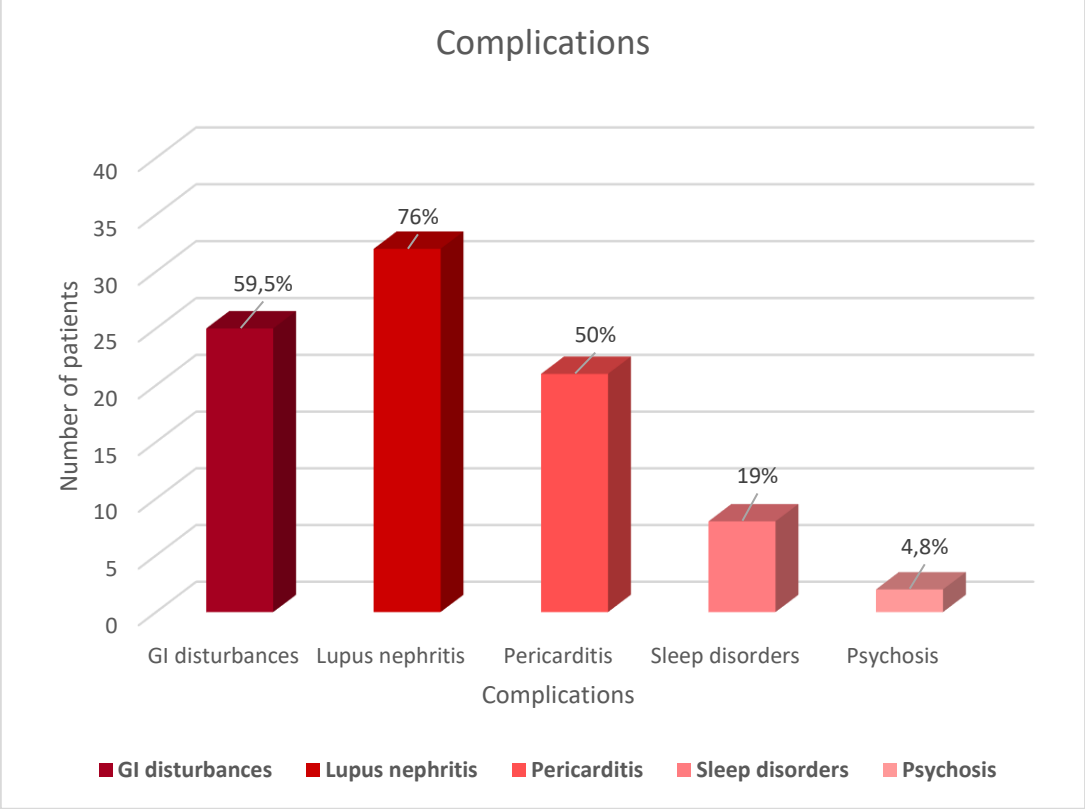


Graph 4 – Diagnostic methods used for SLE diagnosis

Among these tests, all of the patients had positive ANA test, anti-dsDNA antibodies, anti-Ro/SSA, anti-La/SSB, 95% (40 patients) had positive anti-Sm antibodies, 93% (39 patients) had elevated erythrocyte sedimentation rate (ESR) and 19% (8 patients) had proteinuria; 2 of them had prominent proteinuria and thus they underwent renal biopsy.

COMPLICATIONS

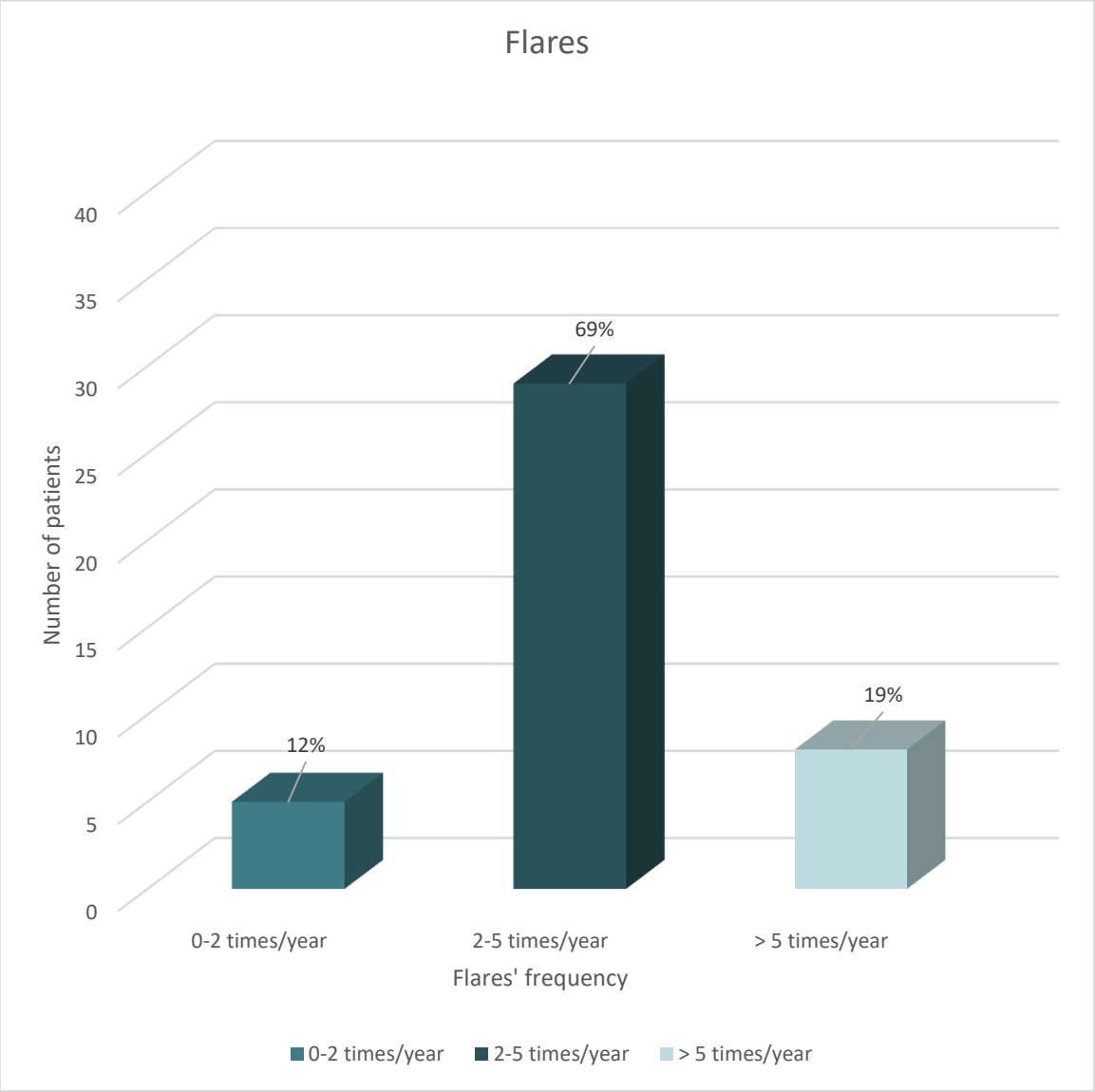
The patients of our study went through some certain SLE complications through the course of the disease. The most prevailing complication was lupus nephritis, developed in 76% of the patients, whereas psychosis was affecting only 4,8% of them. However, upon the manifestation of the psychotic symptoms, it was uncertain whether psychosis was a SLE manifestation or a corticosteroid side effect. The doctor, in order to distinguish the origin of the psychosis in each patient, increased the daily dose of prednisolone that was administered to them, with no improvement of the psychotic symptoms after approximately 2 weeks. Therefore, he concluded that the psychosis was drug-induced. In both cases, the corticosteroids were gradually caseated, and hydroxychloroquine was added. After the tapering of the orally administered corticosteroid, psychosis in both patients was absent, so any antipsychotic medication was unnecessary.



Graph 5 – Complications occurred among SLE patients

FLARE FREQUENCY

SLE is a disease characterized by periods of flares and remissions. In the graph below, the frequency of mild disease flares, characterized by the exacerbation of symptoms like arthralgia, myalgia/fibromyalgia, malaise, fatigue, continuous headaches, and less frequently tachycardia, is illustrated.



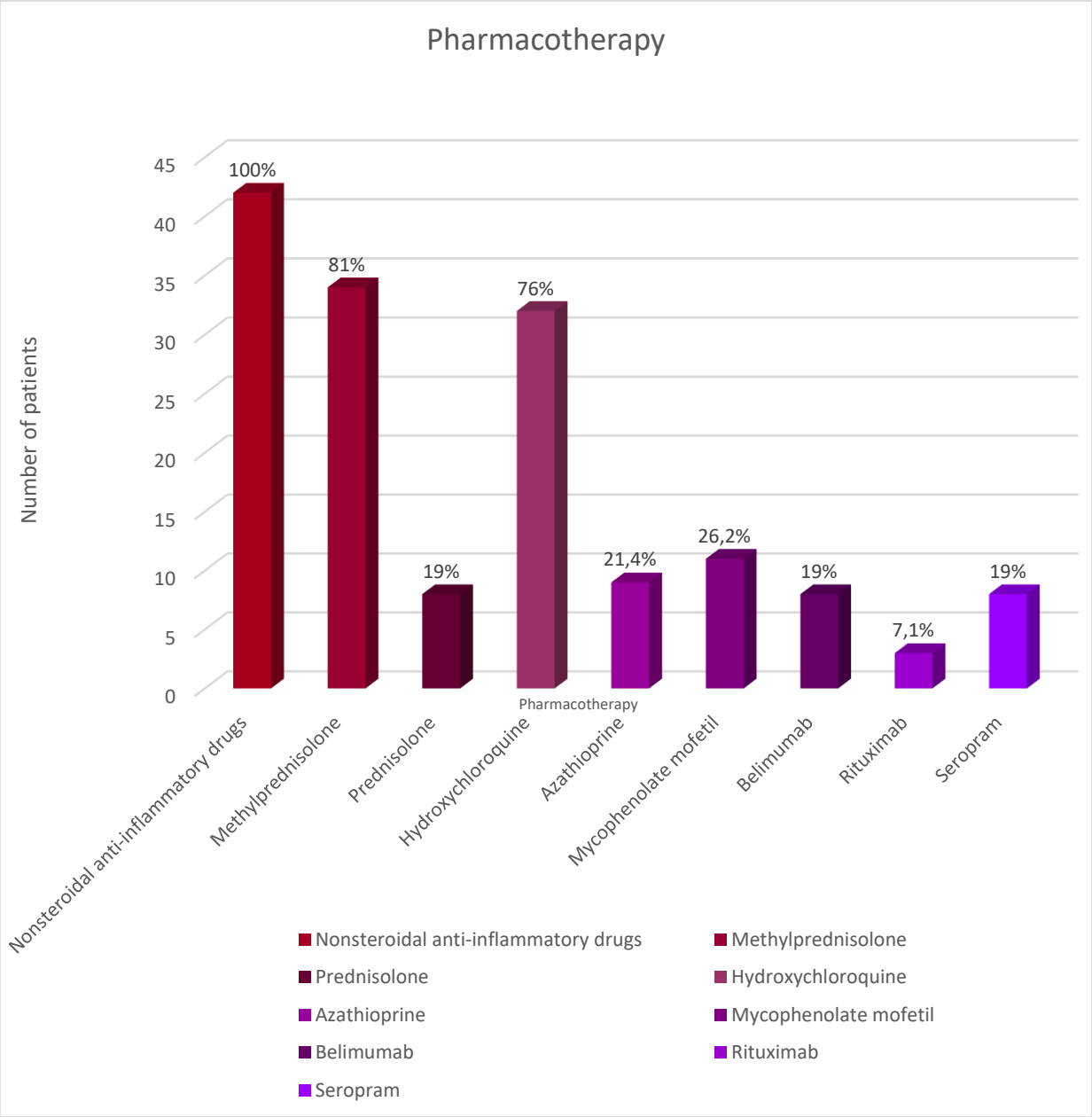
Graph 6 – Frequency of mild disease flares

PHARMACOTHERAPY

The medication prescribed in the patients included orally administered corticosteroids in 100% of the patients, either alone or in combination with antimalarials and/or immunosuppressants during disease flares or in patients with LN. Particularly, either methylprednisolone 16mg was administered once a day or prednisolone 5mg 2-3 times a day. “Pulse” corticosteroids were slowly (≈1h) administered i.v for 2-3 constitutive days in a 1000mg/day regime in case of acute flares like pericarditis, during hospitalization. In lupus nephritis a commonly used regime is cyclophosphamide (1mg/m^2 of body surface) in combination with oral corticosteroids (60mg/24h). Afterwards, azathioprine was frequently administered in an average of 4mg/kg/day combined with methylprednisolone 16mg/day. Furthermore, in patients more prone for disease flares, corticosteroids with hydroxychloroquine were administered. Hydroxychloroquine was given as 200mg once a day and was gradually (every 4 weeks) increased to the final dose of 200mg twice a day alone, or in the aforementioned combinations. The combined administration of corticosteroids, antimalarials and immunosuppressants was observed in more severe active lupus. Monotherapy could exist either with oral corticosteroids or antimalarials. MMF was less frequently used, usually during the maintenance phase after LN, together with cyclosporine (3-4mg/kg/day) and corticosteroids. Depending on the severity of the kidney disease, 500mg of MMF were administered once or twice a day. Biologic agents were administered in a therapy of corticosteroids in combination with antimalarials in more aggressively active disease. NSAIDs were administered in all of the patients in times of mild arthralgias and myalgias as adjuvates.

Furthermore, 8 of the patients demonstrated sleep disorders and especially insomnia. After further inspection, it appeared these disorders originated from general anxiety due to the frequent pain which made their everyday tasks (e.g. working) very difficult. Some of

them also admitted being frustrated from their lowered quality of life and were possessed with a constant fear of an acute flare or the possibility of early death. They were treated with seropram 20mg along with cognitive behavioral therapy, in order to alleviate this issue. None of these cases needed a hypnotic or an alternative antidepressant for the symptoms of anxiety. Out of the 42 patients, one developed fibromyalgia; thus, she was also administered with pregabalin 75mg twice a day. In graph 7, the medications administered in SLE patients are illustrated.



Graph 7 – Pharmacotherapy among SLE patients

5.1. CASE REPORT

Out of the 42 patients, particularly interesting was the case of a 40-year-old woman who was diagnosed with SLE at the age of 23 years old, although it is believed that lupus was manifested approximately 2 years before her diagnosis. At the age of 21, general symptoms associated with SLE were manifested, including fatigue, arthralgias, myalgias and sporadic unexplained low-grade fever. Some photosensitivity reactions were most probably present, however due to her light-skin color they were misjudged as sunburns. In the age of 21 and 23 she had two unexplained miscarriages. Her first visit to the rheumatologist was at the age of 23 where the musculoskeletal manifestations were very prominent, accompanied with fatigue, malaise, interfering with her ability to perform daily tasks, and an unexplained loss of approximately 10kg in a one-month period. Upon serologic examination she presented a positive test for ANA (+) 1:1240 with dot fluorescence, when a titer ≥ 1:80 is enough to indicate the existence of ANA (Abbles et al., 2013). Furthermore, she had anti-Ro/SSA (+) antibodies, anti-La/SSB (-) antibodies, antiSm (+) antibodies, anti-dsDNA (+) antibodies, hematocrit (Ht): 31% and normal leukocyte count. She was also presented with acute renal failure (> 4g albumin/24h). In her renal biopsy glomerulonephritis of minimal lesion was detected. She was treated with cyclophosphamide is a dose of $1g/m^2$ of body surface every 4 weeks for 6 months alongside with high-dose prednisolone (60mg/24h) perorally, with rapid response in the therapy, and no further LN or renal failure recurrence. Her maintenance therapy afterwards was azathioprine 50mg twice daily and methylprednisolone 16mg once daily. Despite the fact, that she rarely had disease flares of arthralgias, myalgias and fatigue, she has hospitalized 14 times with pericarditis up to today. In all of these occasions, she was treated with “pulse” i.v corticosteroid therapy for 3-4 subsequent days. At the age of 34 and 36 years, she carried through with 2 pregnancies and gave birth to two children without any presence of neonatal lupus, or any later complication. After her second childbirth, she

reported that her clinical features of arthralgias, myalgias and fatigue were significantly lower, and she currently is on NSAIDs only in the presence of mild arthralgias and fatigue.

6. DISCUSSION

Taking in account the prevalence of SLE among genders, in our study it was evidenced that 88% of the patients were female, whereas 12% were male (Graph 1), compatible with the established and widely accepted ACR data claiming that 90% of SLE patients are of female gender. Furthermore, a variety of studies have demonstrated that approximately 65% of newly-diagnosed SLE patients were among the age groups of 16-55 years suggesting the higher prevalence of the disease in childbearing years (Pons-Estel et al., 2010). In our study, 97,6% of the newly diagnosed patients belonged in this age group, with the highest prevalence of 52,4% met at the age of 25-34 years. These evidences strongly support the increased rates of SLE onset in childbearing year, however this significantly increased interval between the literature and our patient list can be explained by the limited number of patients in our study. Life span rates are quite impressive in our study, as 10 out of the 42 patients which are ≥40 years of age, have been diagnosed with SLE for approximately 20 years now, and the vast majority of them had transient and minimal disease complications (LN, pericarditis, persistent arthralgias) and an overall good quality of life. Ippolito et al., have also demonstrated an increased lifespan rate with 76% of patients achieving a 15-year survival.

Arnaud et al., as illustrated in chapter 2.6. has listed the most common symptoms during the onset of the disease. In our study, as illustrated in graph 3, the most prevalent symptom was fatigue followed by arthralgia, myalgia, malar rash, fever of unknown etiology, weight loss and photosensitivity, which are referred by the literature and ACR as the most common constitutional symptoms of new onset SLE. In Chapter 2.7. the ACR revised criteria for SLE diagnosis and classification are listed. Graph 4 demonstrates that all of the patients in our study had ANA test, anti-dsDNA antibody test, anti-ssDNA antibody test, anti-Ro/SSA antibody test, anti-La/SSB antibody test, anti-Sm antibody test, as well as a total blood count

test and uranalysis. However, 2 of the patients later had renal biopsies, due to prominent proteinuria. In general, our group had an overall frequency of disease flares, characterized by the exacerbation of symptoms like arthralgia, myalgia/fibromyalgia, malaise, fatigue, continuous headaches, and less frequently tachycardia. 12% of the patients had an occurrence of ≤ 2 flares per year, 69% had these symptoms 2-5 times per year whereas 19% of them had these flares ≥ 5 times per year. Clinical manifestations and complications derived from SLE are described in Chapter 2.6. In our study, the most apparent complication was LN occurred in 76% of patients followed by GI disturbances, pericarditis, sleep disorders and psychosis. The existence of GI disturbances could either arise by SLE itself, or as an adverse reaction of the pharmacotherapy. In most of these 42 patients, these disturbances (like stomach pain, nausea and vomiting) were induced from the ongoing medication, rather than the disease. In both cases, gastroprotective proton-pump inhibitors; omeprazole (20mg once or twice a day, on an empty stomach) or pantoprazole were administered to the patients. Interestingly, the psychosis caused in 2 patients was of uncertain cause, as the patients were on long-term oral corticosteroid administration which can cause such side effect (Chau et al., 2003). However, the psychosis could also be a NPSLE manifestation (Bertsias et al., 2010). The doctor, in order to distinguish the origin of the psychosis in each patient, increased the daily dose of prednisolone that was administered to them, from 10mg to 20mg per day, with no improvement of the psychotic symptoms after approximately 2 weeks. Therefore, he concluded that the psychosis was drug-induced. In both cases, the corticosteroids were gradually caseated, and hydroxychloroquine was added. After the tapering of the orally administered corticosteroid, psychosis in both patients was absent, so any antipsychotic medication was unnecessary.

In our study, referring to pharmacotherapy of SLE, the golden standard was the oral administration of corticosteroids. Particularly, 81% of patients were on methylprednisolone, whereas 19% were on prednisolone. Two of the female patients were previously diagnosed

with osteopenia and were already using bisphosphonates. The rest of the patients were administered supplements of calcium and vitamin D, due to the high potency of corticosteroids to induce osteoporosis (Lo et al., 2012). Among the antimalarial agents, hydroxychloroquine was only used, in combination with oral corticosteroids and/or immunosuppressants in patients with increased disease activity. Among the immunosuppressive agents, cyclophosphamide was mostly administered, followed by MMF and azathioprine. This is a paradoxical regime, as CyX is the agent with a potent bladder toxicity effect (Appel et al., 2009); however, due to the elimination of CyX usage only during acute LN, in combination with high doses of corticosteroids, not such side effects were reported. MMF was used when possible in the maintenance phase of LN, combined with cyclosporine and corticosteroid.

It is well established that pregnancy can carry many potential risks both for the mother and the fetus; meaning it should be well planned and well monitored during gestation and after childbirth. Women in higher risk of complications are those with a previous difficult or interrupted pregnancy, presence of aPL, and mainly LA (increased risk of maternal thrombosis, embryo/fetal loss and pre-eclampsia), anti-Ro/SSA and anti-La/SSB antibodies which can rarely cause (2%) congenital heart block, leading in a high chance of permanent pacemaker requirement or even mortality (Madazli et al., 2010). Furthermore, chronic renal failure is related with obstetric complications like hypertension and miscarriage; increasing sharply if creatinine > 3-4 mg/dl and the presence of proteinuria or urine sedimentation. Restrictive pulmonary diseases can aggravate in pregnancy, as the growing uterus results in thoracic compression. Likewise, the risk of heart failure is increased in pregnant women with heart disease due to volume overload (Ruiz-Irastorza et al., 2009b). Women with active or recently active SLE (6 months interval) should be discouraged from pregnancy, as well as women with symptomatic pulmonary hypertension, as a risk higher than 30% for maternal mortality during late pregnancy and puerperium exists (Lateef et al., 2013). The 40-year old

female discussed in chapter 5.1., had already positive anti-Ro/SSA antibody tests, a past event of renal failure and two miscarriages, repeated pericarditis and a slightly active disease. During her pregnancies, she went through frequent examinations and controls including total blood count, uranalysis and ultrasounds in order to assess the potential risks for both mother and fetus. During her first pregnancy, hydroxychloroquine was used to control SLE and to avoid flares, thrombosis and damage accrual. This medication is considered by many the golden standard in pregnancy due to its absolute safety profile in pregnant women (Ruiz-Irastorza et al., 2009b). Moreover, low-dose corticosteroids were used for short-term periods, in fear of a disease flare. During her second pregnancy, only hydroxychloroquine was used.

Despite the greater risk of SLE flares and numerous complications listed above, during her pregnancies, especially the second one, her clinical features related with SLE were minimal, with no later complications in neither of the children. Moreover, the patient could describe to experience a lupus-free life since then, with sporadic exceptions of minor arthralgias and fatigue. This case can lead in the hypothesis of a more complicated genetic, epigenetic, environmental and racial relation between SLE and pregnancy.

7. CONCLUSION

Systemic lupus erythematosus is a chronic autoimmune disease, with variable severity characterized by altering periods of flare and remission. It has a multisystemic character, affecting various body tissues and organs. SLE is predominately affecting women in a ratio 9:1 when compared with men and is mainly manifested during the reproductive years with disease peak incidence rates among 16 and 55 years of age. The main risk factor, apart from the aforementioned, is the genetic predisposition, enhanced by epigenetic and environmental factors.

By the end of 20th century the SLE diagnosis incidence was increased, while the prevalence of the disease was not significantly altered, indicating the better and earlier diagnosis due to the improved diagnostic methods and understanding of SLE pathophysiology. Differences in the prevalence of the disease are noted among different geographical locations and racial groups. Moreover, mortality rates have demonstrated encouraging data, due to the increased expected lifespan after SLE diagnosis, leading in a 15-year survival for 76% of the patients.

The most common SLE manifestations include fatigue, arthritis, malar rash, photosensitivity, leukopenia and in more severe occasions, active LN, pericarditis, myocarditis, pleuritis, serositis, autoimmune hemolytic anemia, Raynaud's phenomenon etc. Overlap syndrome with relevant connective tissue diseases like RA, SS, scleroderma and dermomyositis may occur. Diagnostic methods used in the identification of SLE include the Antinuclear Antibody (ANA) test, anti-DNA antibodies (anti-dsDNA, anti-ssDNA) , anti-ENA antibodies (anti-Ro/SSA and anti-La/SSB, anti-Sm antibodies), complement levels, antiphospholipid (aPL) antibodies (lupus anticoagulants, anticardiolipin antibodies etc). Furthermore, a total blood count should be monitored which may indicate an organ-involvement.

SLE pharmacotherapy is adjusted in the amount of the systems involved as well as the severity of the disease. NSAIDs are used to treat mild-moderate general symptoms. Corticosteroids are widely used orally alone or in combination in maintenance treatment and increased doses during disease flares, or parenterally in “pulse” high-dose therapies for SLE complications like LN. However, their long-term use should be avoided, due to potent dose-depended adverse effects. Antimalarials (mainly hydroxychloroquine) are also widely used due to their disease-modifying properties alone or in combination with corticosteroids and/or immunosuppressives in maintenance therapy, lupus flares and commonly in cutaneous lupus but it has been noted that tobacco smoking can reduce the efficacy of antimalarials in a dose-dependent manner. Immunosuppressive agents like cyclophosphamide, azathioprine, mycophenolate mofetil, cyclosporine, methotrexate and tacrolimus are mainly administered in the onset of active lupus with major organ implications, although caution and regular monitoring should take place due to some potent toxicities. Tacrolimus is also used in local preparations in lupus cutaneous disorders. Interesting is the insertion of biologic agents, used in patients with active, autoantibody-positive patients receiving standard therapy. So far, belimumab is the only biologic agent approved in SLE therapy. Rituximab has been also suggested to be effective, although it has not been yet approved as a therapeutic in lupus due to some controversial results in various clinical trials. There are yet many potent and plausible drugs that are under research, with promising results in SLE pharmacotherapy.

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10. LIST OF ABBREVIATIONS

1,25(OH)D3 – 1,25-dihydroxy vitamin D3

5-HT – 5-hydroxytryptophan

AA – Arachidonic acid

aCL – anticardiolipin antibodies

ACPA – Anti-citrullinated peptides

ACR – American College of Rheumatology

AID – Autoimmune disease

AIHA – Autoimmune hemolytic anemia

ALP – Alkaline phosphatase

ALT – Alanine transaminase

ANA – Antinuclear antibody

Anti-Ribo P – Antiribosomal P

AP-1 – Activator protein 1

APA – Antiphospholipid antibodies

aPL – Antiphospholipid

APC – Antigen-presenting cell

APRIL – A proliferation-inducing ligand

APS – Antiphospholipid syndrome

aPTT – activated partial thromboplastin time

AST – Aspartate transaminase

ATG – Autophagy protein

BAFF – B-cell activating factor

BANK1 – B-cell scaffold protein with ankyrin repeats 1

BCMA – B cell maturation antigen

BCR – B-cell receptor

BILAG – British Isles Lupus Assessment Group

BLK – B-lymphocyte kinase

CLIFT – Crithidia luciliae immunofluorescence test

CNS – Central nervous system

CpG – Cytosine-phosphate-guanosine

COX – Cyclooxygenase

CPK – Creatinine phosphokinase

CREB – cAMP response element binding protein

CSF – Cerebrospinal fluid

CT – Computer tomography

CTD – Connective tissue diseases

CTL – Cytotoxic T-lymphocytes

CVD – Cardiovascular disease

CyX- Cyclophosphamide

DAMP – Damage-associated molecular pattern

DC – Dendritic cell

DFS70 – Dense fine speckled 70

DIL – Drug-induced lupus

DM – Dermomyositis

DNA – Deoxyribonucleic acid

DNMT – DNA methyltransferase

DN T cells – Double-negative T cells

dRVVT – Dilute Russell viper venom time

dsRNA – double-stranded RNA

EBV – Epstein-Barr virus

ECG - Electrocardiography

ELISA – Enzyme-linked immunosorbent assay

ENA – Exactable nuclear antigens

ER – Estrogen receptor

ESKD – End-stage kidney disease

FCγR – FCγ receptor

FCGR$_2$A – Low affinity immunoglobulin gamma Fc region receptor II-a

FSH – Follicle-stimulating hormone

GADD45α – Growth-arrest and DNA damage inducible protein 45α

GC – Germinal center

GGT – Gamma glutamyl transpeptidase

GIT – Gastrointestinal tract

GFR – Glomerular filtration rate

GR – Glucocorticoid receptor

GRE – Glucocorticoid response element

HDL – High density lipoprotein

HEp2 tissue – Human epithelial 2 tissue

HHV4 – Human herpesvirus 4

HDAC – Histone deacetylase

HPA axis – hypothalamo-pituitary-adrenal axis

HPV – Human Papilloma virus

HRT – Hormone replacement therapy

IC – Immune complex

IFN – Interferon

Ig – Immunoglobulin

IL – Interleukin

IRF – Interferon regulatory factor

IRAK2 – Interleukin-1 receptor-associated kinase 2

ISN – International Society of Nephrology

ITIM – Immunoreceptor tyrosine-based inhibitory motif

ITGAM – Integrin-a_M

ITP – Idiopathic thrombocytopenic purpura

LA – Lupus anticoagulants

LD – L-lactate dehydrogenase

LDL – Low density lipoprotein

LFA – Lupus Foundation of America

LH – Luteinizing hormone

LN – Lupus nephritis

LOX – Lipoxygenase

LPS – Lipopolysaccharide

LT – Leukotriene

MAPK – Mitogen activated protein kinase

Mcl-1 – Myeloid cell leukemia-1

mDC – Myeloid dendritic cell

MECP2 – Methyl CpG binding protein 2

MHCI – Major histocompatibility complex class I

MHCII – Major histocompatibility complex class II

MI – Myocardial infraction

MMF – Mycophenolate mofetil

MAPK – Mitogen-activated protein kinase

MAPKP-1 – Mitogen-activated protein kinase phosphatase 1

MRI – Magnetic resonance

MTB – Mycobacterium tuberculosis

MTX – Methotrexate

MZ – Marginal zone

NF- Nuclear factor

NK – Natural killer

NPSLE – Neuropsychiatric systemic lupus erythematosus

NSAID – Nonsteroidal anti-inflammatory drug

OCP – Oral contraception

PAMP – Pathogen-associated molecular pattern

pDC – Plasmacytoid dendritic cell

PDCD1 – Programmed cell death 1 gene

$PEDM_1$ – PR domain zinc finger protein 1

PM – Polymyositis

PRR – Pattern recognition receptors

PTPN22 – Protein tyrosine phosphatase, non-receptor type 22

PXK – PX domain containing serine/threonine kinase

RNA – Ribonucleic acid

RA – Rheumatoid arthritis

RP – Raynaud's phenomenon

ROS – Reactive oxygen species

RPS – Renal pathology society

SLE – Systemic lupus erythematosus

SLEDAI – Systemic lupus erythematosus disease activity index

SLO – Secondary lymphoid organ

SMA – Smooth muscle antibodies

snRNP – Small nuclear ribonucleoprotein

SPECT – Single-photon-emission computer tomography

ssDNA – Single stranded DNA

SS – Sjögrens's syndrome

STAT – Signal transducer and activator of transcription

STS – Serologic test for syphilis

T_{FH} – Follicular helper T cells

T_H cells – T helper cells

TACI – Transmembrane activator and cyclophilin ligand interactor

TAP1 – Transporter 1, ATP binding cassette subfamily B member

TAP2 – Transporter 2, ATP binding cassette subfamily B member

TCR – T cell receptor

TGF – Transforming growth factor

TLR – Toll-like receptor

TNF – Tumor necrosis factor

$TNFAIP_3$ – Tumor necrosis factor, alpha-induced protein 3

$TNFSF_4$ – Tumor necrosis factor superfamily member OX40L

TREX1 – Transcription export

TTP – Thrombocytopenic purpura

TX – Thromboxane

VEGF – Vascular endothelial growth factor

VLDL – Very low density lipoprotein

VDR – Vitamin D receptor

WHO – World Health Organization

UBE2L3 – Ubiquitin-conjugating enzyme E2 L3