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Chapter 4

SLE, An Overlooked Disease: Possibilities for Early Rescue by Early Diagnosis

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Abstract

Systemic lupus erythematosus (SLE) is a progressive autoimmune disease associated with widespread organ damage that can eventually cause death. Worldwide prevalence of SLE is difficult to report mainly due to difficulty in diagnosis as a result of its heterogeneous nature and nonspecific protean manifestations. Currently, circulating anti-DNA antibodies are the most specific diagnostic biomarkers for SLE where many detection assays are being employed in clinical practice. However, the diagnostic value of these techniques is challenged by the detection of only subpopulations of these antibodies with varying sensitivity and specificity. This is mainly attributed to differences in the antigen source and presentation and in the employed reaction conditions. This chapter will thoroughly discuss the technology, advantages, and limitations of each assay in addition to a special focus on the recently developed diagnostic technologies and novel biomarkers. Moreover, SLE will be presented as a disease model highlighting the importance of personalized medicine.

Keywords: systemic lupus erythematosus, autoimmune disease, complex pathogenesis, challenging diagnosis, anti-DNA antibodies, novel biomarkers, state-of-the-art diagnostic technologies, personalized therapy

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic multiorgan autoimmune disease with many unresolved questions regarding its pathogenesis, causes, best approaches for proper diagnosis and therapy [1–3]. It is perhaps the most heterogeneous human disease where SLE patients exhibit clinical manifestations that hugely vary on the levels of organ involvement and severity that are accompanied by differential release of autoantibodies and other serological biomarkers

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[1, 4]. This is because the disease pathogenesis is highly chaotic and strikes any of the multiple stages of the immune cascade resulting in extremely wide-ranging and difficult-to-predict clinical and serological manifestations among SLE patients [1, 4]. As demonstrated through this chapter, SLE challenges the clinical community in its diagnosis, prediction of the course of the disease, extracting and monitoring reliable biomarkers, designing studies in clinical trials, and developing new therapeutics [1, 3–5]. Owing to this highly heterogeneous nature of the disease, SLE presents an ideal model for a disease that desperately calls for new developments in the state-of-the-art diagnostic technologies that can detect highly specific and reliable new biomarkers for disease diagnosis and prognosis, and it exemplifies the urge for personalized medicine that can target specific subsets of patients or specific organ involvement.

1.1. Epidemiology

Reporting on epidemiological data for SLE is not coherent among all countries, and the best informative data are obtained from North America and Europe, while less documentation has been received from Africa, Asia, Australia, and South America [1, 6]. Nevertheless, SLE is a global disease in which its incidence, prevalence, time of onset, and mortality are highly influenced by race and ethnicity [6]. For instance, in USA SLE has incidence and prevalence rates that show great variability ranging from 2 to 7.6 per 100,000 per year and 19 to 159 per 100,000 per year, respectively [7, 8]. These variations can originate from differences in ethnicity, race, and age within the studied SLE population and can also stem from differences in the employed diagnostic criteria for SLE. Similar variation trends were also observed in some European countries [9, 10].

One of the important characteristics of SLE is that it predominantly affects women more than men [11] with a disease onset that is influenced by ethnic background. For instance, in a different ethnic background-based study, it was found that the incidence of SLE in African-Caribbean females is higher at younger ages than in Asian or Caucasian females [12]. This age-specific incidence in females of different ethnicities was also seen in other studies performed in different countries [13–15]. However, the reason behind this characteristic SLE predominance in women more than in men is not entirely understood, but it was inferred to be related to hormonal factors. For example, serum prolactin was found at higher levels in SLE patients than a control group, but it is unknown how can prolactin be involved in SLE immune deregulation. Independent of gender, it was reported that generally people of African origin had a higher incidence of SLE than those of European origin [7, 8, 16].

Mortality risk is increased in SLE patients of Chinese, Hispanic, and African backgrounds with strong associations of renal damage [17, 18]. This, however, might relate to the levels of disease awareness and therapeutic adherence that might be different among different populations [6].

1.2. Pathophysiology

The chaos in biology associated with SLE, the involvement of multiple body organs along with the release of a wide array of autoantibodies has definitely challenged the advancement in understanding the disease pathophysiology. Such a comprehension is highly essential for the

identification of novel biomarkers, for efficient classification of SLE patients, and in exposing specific pathways prone for targeting to help guiding personalized therapies [1, 4, 5]. Nevertheless, great insights have been achieved through the use of mouse models of lupus and multiple genetic and epigenetic investigations [19–24] through which a mechanism for disease development has been proposed to proceed as illustrated in **Figure 1** [4].

Two important events set the basis for SLE pathophysiology: first is the loss of immune tolerance in which the immune system acquires autoantigen recognition and second the persistent release of autoantibodies that mainly target endogenous nucleic acids and associated proteins [1, 4]. A cascade of events has been postulated to underlie such two events and comprise impaired disposal of apoptotic cells, accumulation and immune recognition of nucleic acid material, deregulated lymphocyte signaling, and sustained production of interferons and other cytokines [1, 4].

Removal of apoptotic cell debris is normally a silent process. However, with impaired elimination of dead cells as in SLE, nucleic acid material becomes vastly accumulated and can acquire immunogenic properties through sustained exposure to the extracellular

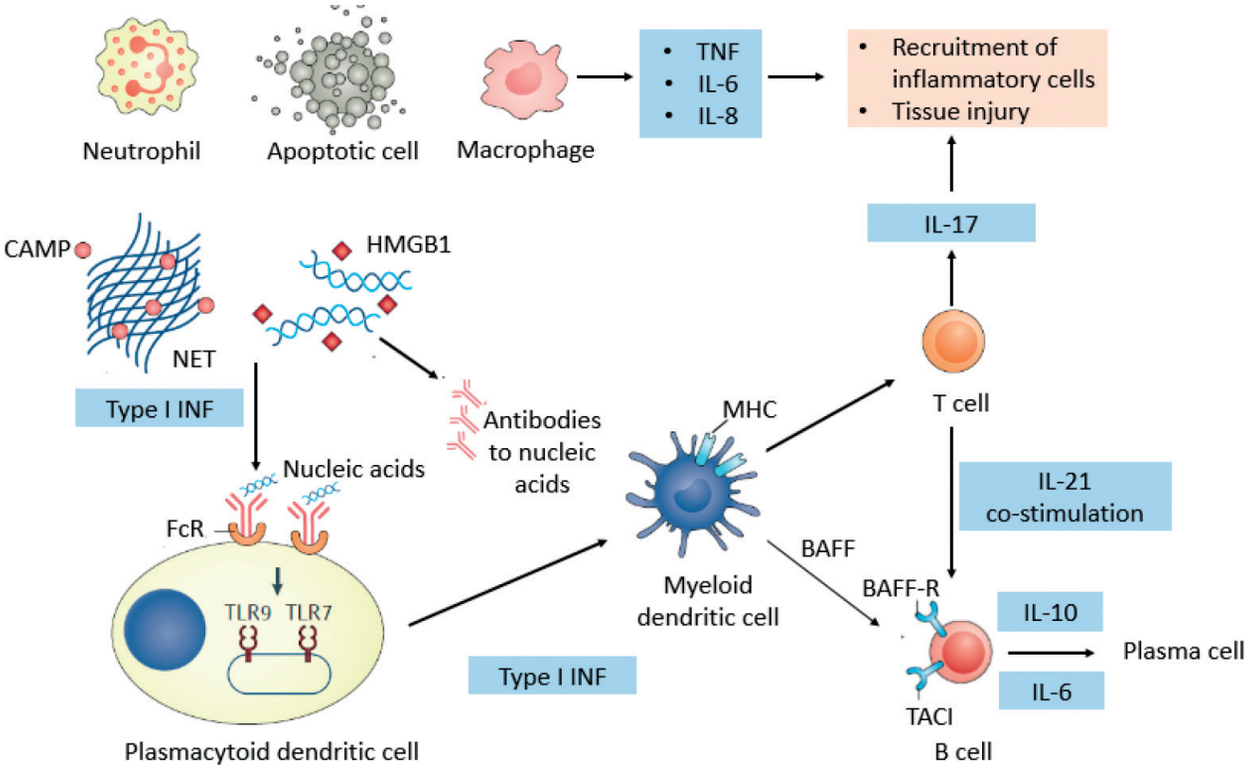


Figure 1. Underlying immune deregulation in SLE pathogenesis. Accumulated nucleic acid material containing DNA, RNA, and associated proteins that is released from apoptotic cells and neutrophils via NETosis can acquire immunogenic properties through their sustained availability in the extracellular surrounding in addition to the co-association with stimulatory molecules such as HGB1 protein. The accumulated nucleic acid material is engulfed by PCDs which in turn activate intracellular TLRs that stimulate potent release of type I INFs from PDCs which stimulate expression of BAFF that stimulates B-cell production of autoantibodies targeted against endogenous nucleic acids and associated proteins. T cells are important key players in SLE pathogenesis as they induce the release of inflammatory cytokines such as IL-17 and mediate tissue injury and destruction. Furthermore, T-cell and B-cell interactions stimulate B-cell differentiation and consequent release of pathogenic autoantibodies [4].

environment and the association with immune-stimulatory molecules such as IL-1 and the DNA-binding protein HMG-1 [25–27]. The innate immune cells plasmacytoid dendritic cells (PDCs) act by engulfing apoptotic remains containing nucleic acid material which stimulates the intracellular nucleic acid receptors Toll-like receptors (TLRs) particularly TLR7 and TLR9 [28–30]. When TLRs engage DNA or RNA, they elicit a strong release of type I interferons (IFNs) such as IFN-α which in addition to other cytokines induce autoantibody production through stimulating B-cell maturation and differentiation and can promote loss of immune tolerance and impaired immune responses [31, 32]. The activation of B cells via type I INFs has also been demonstrated by the increased expression of the B-cell-activating factor (BAFF) in response to IFN-α which stimulates B-cell activation and generation of autoantibodies [33–36]. Released autoantibodies bind their target antigens which are mainly nuclear components and form immune complexes that are recognizable by PDCs and further stimulate the release of type I IFNs amplifying INF signaling in a process known as "IFN signature" [1, 4, 27]. Many of the gene products that become actively expressed in response to type I INFs are engaged in immunoregulatory functions and were found to be highly elevated in many SLE patients with strong association of autoantibody release specifically those targeting RNA-binding proteins such as RNP, Sm, Ro, and La [37]. TLR7 specifically binds single-stranded RNA and was found to be strongly associated with the release of anti-Sm autoantibodies [38, 39]. On the other hand, TRL9 binds CpG-rich DNA sequences, was found to be highly expressed in B cells isolated from patients with severe SLE activity with an association of potent release of anti-double-stranded DNA (dsDNA) antibodies [38]. In addition to apoptotic cell death, neutrophil extracellular traps that are rich in DNA released from neutrophil cell death (NETosis) and other immunostimulatory molecules induce type I interferons through stimulating TLRs [40]. Impaired T-cell signaling is also a significant contributor to SLE pathogenesis where overly activated T cells of lupus were able to stimulate autoantibody production from B cells, activate dendritic cells, and stimulate the release of inflammatory cytokines and can thus mediate widespread tissue injury and inflammation [4, 41].

2. Biomarkers

A biomarker can be defined as an alteration in a cellular, molecular, genetic, epigenetic, biochemical, biological, or other body events that specifically accompanies a disease or condition and is amenable for quantitative and qualitative analysis. Therefore, a biomarker can be used for diagnostic, prognostic, and theranostic purposes, and the more specific it is for a disease, the more reliable it becomes [5, 42]. In SLE, the search for novel and specific biomarkers is highly crucial because as a disease of a huge range of clinical and serological manifestations, it is challenging in so many levels including accurate diagnosis, predicting disease progression, identification of disease flares, directing proper therapy, and for the discovery of new treatments [5]. Owing to this tremendous heterogeneity, it is therefore expected that no single biomarker for SLE can satisfy all the above purposes but the continuous efforts in understanding SLE pathogenesis should accumulate informative data for the discovery of novel and reliable biomarkers [43].

The most commonly employed SLE biomarkers are antinuclear antibodies (ANAs) and anti-DNA antibodies [43]. However, as will be described below, ANAs possess low specificity to SLE due to their coexistence in other autoimmune diseases as well as in some healthy individuals [44]. On the other hand, anti-DNA antibodies, despite being the most specific biomarkers for SLE [27], have poor predictive values as their levels do not always parallel disease activity [45]. Therefore, the search for novel biomarkers for SLE never ceased, and with the emergence of newer detection technologies and the advances achieved in understanding SLE pathogenesis, new biomarkers have emerged from collective efforts of genetic, epigenetic, transcriptomic, and proteomic studies as shall be described [46].

2.1. Antinuclear antibodies (ANAs)

ANAs are a large group of autoantibodies that target various nuclear antigens including DNA, RNA, proteins, or complexes of nucleic acid and proteins [44]. They can generally be categorized into two groups based on the targeted antigens, one group that recognizes DNA and DNA-associated proteins such as histones or DNA-protein complexes such as nucleosomes and another group that recognizes distinct proteins that exist in association with RNA and are thus called RNA-binding proteins (RBPs) including the small nuclear ribonucleoproteins (snRBPs) Sm, RNP, Ro, and La [47].

These nuclear antigens are normally enclosed within the nucleus. However, as described above, upon cell death, these antigens are released into the extracellular space where they elicit immune responses that lead to the generation of ANAs that target these antigens and form immune complexes that further stimulate the immune system [4]. In the context of SLE, ANA detection in patients' sera is an important diagnostic criterion where they are specified as a stand-alone criterion in SLE classification criteria that are used for SLE diagnosis [48–50] as will be described in more details in the next section.

Although, ANA positivity is detected in not less than 95% of SLE patients [44, 51], they exhibit low specificity to SLE as they are also detected in other autoimmune diseases including rheumatoid arthritis, Sjogren's syndrome, and mixed connective tissue disease [51, 52]. Furthermore, depending on the employed detection method, ANA positivity can be seen in 20–30% of healthy individuals in the general population for yet unknown reasons [44]. Therefore, the value of ANA testing for SLE diagnosis is a debatable issue because of this diminished specificity despite of its inclusion in SLE classification criteria [53].

2.2. Anti-DNA antibodies

Anti-DNA antibodies are a subgroup of ANAs that can recognize and bind cellular DNA, and their detection is almost exclusive to SLE making them the serological hallmark for the disease [27, 51, 54]. Whether anti-DNA antibodies are also released in the context of other human diseases is a question that remains unanswered [27]. Detection of anti-DNA antibodies in the sera of SLE patients has been included as a separate criterion in an optimized version of SLE classification criteria that were set to enhance the sensitivity of the old criteria, as will be described below, which highlights their importance for SLE diagnosis [50] as they exhibit 95%

specificity for SLE and are detected in at least 70% of SLE patients [45]. In addition, their value is not confined to their diagnostic role, but they are also quite valuable in probing the molecular basis of lupus autoreactivity [27] and in theranostic investigations where they can be used as deterministic factors for eligibility in clinical trials and in directing the clinical use of certain therapeutics [55, 56]. In normal immune responses, antibodies that target DNA can be seen in some cases such as that in response to viral or bacterial infections [54, 57]. However, anti-DNA antibodies in normal immunity differ from SLE anti-DNA antibodies in many aspects. In normal immunity, anti-DNA antibodies are of the IgM isotype that can recognize and bind single-stranded DNA (ssDNA) with low affinity and are nonpathogenic in nature. But in SLE pathogenesis, there is an isotype shift of the expressed anti-DNA antibodies from IgM to IgG antibodies which are detected in the majority of SLE patients. These class-switched IgG antibodies exhibit a high affinity toward double-stranded DNA that resulted from specific somatic mutations in the variable regions of these antibodies mostly in the complementarity-determining regions (CDRs) that generated positively charged amino acid residues such as asparagine, arginine, and lysine that promote enhanced binding affinity to the negatively charged DNA [27, 54].

The origin of such IgG anti-DNA antibodies was suggested to derive from antigen-specific B-cell clonal expansion where DNA was used as the selecting antigen [54]. However, the various studies that investigated the release of high-affinity anti-DNA antibodies in SLE settled on the prerequisite association of DNA with proteins to be able to elicit anti-DNA immune responses [54, 58]. This implied a role for T cells that are reactive against histones, the proteins constituting octamer complexes around which stretches of DNA are wrapped inside the nucleus, and nucleosomes which are the basic structural unit of chromatin [27, 54]. A mechanism was proposed to start with presenting DNA in complexation with a foreign protein antigen (e.g., viral or bacterial) to T cells specific to this antigen. However, in a way that is not yet fully characterized, a shift in recognition takes place toward the complexed DNA that triggers the activation of T cells specific to histones and nucleosomes [27, 54].

Various studies have demonstrated differences in selectivity patterns exhibited between anti-DNA antibodies of normal individuals and that of SLE patients. Normal anti-DNA antibodies were found to exhibit high specificity toward species-specific DNA which suggests that they bind at DNA regions that are not shared with human DNA. In contrast, SLE anti-DNA antibodies were found to nonselectively bind to a variety of investigated structurally different DNA antigens. This suggests that SLE anti-DNA antibodies might specifically bind the highly conserved phosphate backbone rather than specific nucleotide sequence [27, 54].

Therefore, as will be described later in the section of diagnostic technologies, assays that are able to detect high-affinity anti-DNA antibodies are described as assays with high specificity to SLE as it is inferred that such high-affinity anti-DNA antibodies are more reflective of SLE immune-deregulated responses [27].

Despite this high specificity of anti-DNA antibodies to SLE, their value in disease prognosis is compromised as they are poor predictors of disease activity [45]. This was demonstrated by the detection of tenacious levels of anti-DNA antibodies in SLE patients in remission [59–61] or

the presence of normal levels in patients with active disease [62]. Therefore, new biomarkers are continuously emerging, and some of the promising biomarkers are discussed below. For more detailed discussions, interested readers can be directed to these reviews [43, 46].

2.3. New spectrum of SLE biomarkers

2.3.1. Epigenetic biomarkers

Epigenetic biomarkers refer to the epigenetic changes that govern gene expression without changing the nucleotide sequence of the DNA and are specifically associated with disease development such as DNA methylation pattern, microRNA expression, and various histone modifications [5]. In the case of SLE, certain epigenetic changes are detected in SLE patients and can act as biomarkers such as the widespread DNA hypomethylation pattern in CD4 T cells [63, 64] and DNA hypomethylation of the promoters of certain genes that encode immune mediators and are associated with SLE pathogenesis such as CD40L [65], CD70 [66], perforin [67], IL-10, and IL-13 [68, 69]. With reduced DNA methylation which normally acts as a repressive signal, the affected genes become highly activated and consequently activate T and B immune cells [5].

Another potential epigenetic biomarker for SLE is the aberrant changes in histone proteins modification patterns that are normally quintessential for controlling gene expression [70, 71]. Specific changes in histone modifications have been observed in immune cells of SLE patients [5] such as the widespread hypomethylation of lysine 9 residues in H3 histone protein in CD4 T cells [72].

In addition to histone modifications, changes in miRNA expression profile are promising SLE biomarkers [5]. MiRNAs are short noncoding RNA sequences that regulate gene expression via targeting and inhibiting mRNA transcripts [73]. It was recently discovered that miRNAs play important roles in both innate and adaptive immune systems and are involved in the pathogenesis of several autoimmune diseases including SLE [74–77]. Several studies have aimed at profiling miRNA expression patterns in SLE and have identified several miRNAs that were underexpressed in CD4 T cells isolated from SLE patients such as miRNA-146a [78, 79] and miRNA-125a [80]. Reduced levels of these miRNAs were inversely correlated with SLE activity as they were associated with increased activation of type I interferon and inflammatory chemokines, respectively.

2.3.2. Cytokines as potential biomarkers

As mentioned earlier with SLE pathophysiology, type I interferons and other cytokines exhibit exacerbated activities in SLE and are key players in disease activity [1, 4]. Therefore, such mediators represent potential biomarkers and well-validated therapeutic targets in SLE.

Cytokines with the most characterized roles in SLE pathology are those of type I interferon pathway such as IFN-α and downstream-induced gene products. Aberrantly elevated levels of INF-α have been detected in cerebrospinal fluid of patients with neuropsychiatric disease in systemic lupus erythematosus in comparison to cerebrospinal fluid samples obtained from

patients with other autoimmune diseases [81] and were suggested to contribute to disease pathogenicity [82].

Using DNA microarray technology, the expression pattern of IFN-induced genes was found to be highly elevated in peripheral blood mononuclear cells obtained from SLE patients with severe disease state [83]. Positive correlation between IFN-induced genes and severity of SLE clinical manifestations and multiple organ damage was also observed in other studies [84, 85].

Among the most noticeable gene products that are regulated by type I interferons are IP-10 and sialic acid-binding Ig-like lectin 1 which were found to be associated with SLE pathogenicity and were detected in highly elevated levels in many SLE patients [86, 87].

Many other cytokines have been detected in elevated levels in the sera of many SLE patients and can thus represent potential biomarkers including IL-17 [88], IL-6, IL-10, IL-12, IL-15, and IL-21 and others [5, 43].

3. Diagnosis

Diagnosis of SLE, according to the majority of clinicians, is best established upon combinatorial approach. Thus, a combination of patients' clinical manifestations and laboratory investigations that can comprise autoantibody assays, blood tests, cell cultures, certain functional tests such as echocardiogram, or imaging such as neuroimaging must be adopted [1, 51, 89]. The decision upon any of the aforementioned investigations is guided by the patient's clinical presentations [51]. In addition, other important factors must be examined including patient's history, risk factors, SLE prevalence within the patient's demographic population, and other epidemiological data. Clinical expertise is also crucial as a highly heterogeneous disease such as SLE can be easily missed or misdiagnosed [1, 51, 89]. Some clinical manifestations are strongly associated with SLE and are therefore of high diagnostic significance such as alopecia, leukopenia, neurological involvement, oral ulcers, and serositis [51].

Nevertheless, until now no diagnostic criteria have been established for SLE due to extreme disease heterogeneity where no consistent clinical presentation or degree of disease severity appears within the cohort of SLE patients [51, 89]. Therefore, clinicians had to refer to a sort of guidelines to aid proper diagnosis and relied on SLE classification criteria such as the revised American College of Rheumatology (ACR) classification criteria for SLE for clinical diagnosis [48, 49].

The ACR classification encompasses a total of 11 criteria for defining SLE including an individual criterion for abnormally elevated titers of antinuclear antibodies that are detected by immunofluorescence immunoassay, which will be described later, or any other equivalent assay and another criterion specifying immunologic abnormalities that include the release of aberrant titers of anti-DNA antibodies or anti-Sm antibodies [48]. Based on ACR, at least 4 of the 11 criteria must be met for a patient to be classified with SLE disease [48]. However, ACR

criteria are more suited for severe cases of SLE and not patients with mild or moderate conditions as it includes the most pronounced manifestations and excludes some of the clinical presentations shown by patients at early or mid-stages which are considered important. Clinical presentations need to be taken in consideration as SLE is known to be a progressive disease that tends to exacerbate over time and establish many of the clinical and serological manifestations in an accumulating manner [1, 90]. Therefore, the ACR classification criteria have a specificity reaching 96% but with a suboptimal sensitivity of 83% [89]. Specificity here is defined as the percentage of individuals who are known to be devoid of the disease and test negative for it, while sensitivity refers to the percentage of patients who are known to have the disease and test positive for it [91]. With the aim of overcoming the limitations of the ACR classification, the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria have been set in 2012 to encompass many of the clinical manifestations overlooked by ACR to reach a total of 17 criteria instead of 11 where at least 4 out of the 17 criteria must be met for a patient to be classified with SLE [50]. In SLICC, the antinuclear antibody criterion was not changed. However, the immunologic abnormalities criterion in ACR has been separated into individual criteria including a separate criterion for abnormal titers of anti-DNA antibodies but with a more strict cutoff value and a separate criterion for anti-Sm antibodies highlighting the importance of such autoantibodies in the diagnosis of SLE [50]. Nevertheless, despite an increase in sensitivity to reach 97% in SLICC compared to 83% in ACR, the specificity has been reduced to 84% compared to 96% in ACR [50]. Moreover, SLICC criteria did not improve the inclusion of SLE patients at early stages except for patients with renal nephritis damage [92].

However, it should be noted that relying on classification criteria for diagnosis is actually problematic as both disease classification and diagnosis do not generally share the ultimate aim [89, 91]. Diagnosis aims at identifying a patient's illness in terms of its causes and nature and is based on a set of diagnostic criteria that include a number of clinical symptoms and investigations that are used routinely for guiding the clinical care of patients [91]. Therefore, diagnostic criteria should have nearly 100% specificity and sensitivity [89, 91]. On the other hand, classification criteria are basically established to define a total population of patients having a specific disease that can be recruited for clinical research. They, therefore, encompass the most prominent and prevalent manifestations dropping out the rarer or less common symptoms and thus typically enjoy high specificity but at the expense of sensitivity. Consequently, using classification criteria for diagnosis can easily miss or overlook patients with the disease [1, 89, 91].

With the emergence of new biomarkers that are strongly associated with SLE pathogenesis as described above, it is highly suggested that these biomarkers will reserve their places in future developments and optimization in diagnostic criteria next to autoantibodies which will not only aid accurate diagnosis but will significantly guide patients' clinical care and management. The increasingly accumulating data in SLE biomarkers will need to be paralleled with the development of new sensitive and reliable detection technologies that are able to simultaneously detect disease biomarkers in a rapid, cost-effective, and sensitive manner [93]. In the next section, current and new trends in diagnostic technologies for SLE will be discussed.

4. Diagnostic technologies

Abnormally elevated titers of antinuclear antibodies are one of the important factors involved in SLE pathogenesis [4] and are set as stand-alone criterion in both ACR and SLICC classification criteria used for SLE diagnosis [48, 50]. Out of these antinuclear antibodies, antibodies that target double-stranded DNA are highly specific to SLE and are considered the serological hallmark for SLE [27]. Numerous technologies have been described for the detection of such autoantibodies in the sera, plasma, or other body fluids of SLE patients where some of them date back to the 1950s, and until now various state-of-the-art technologies are being described.

In principle, all assays assess the formation of immune complexes between the autoantibodies and the test antigens which can comprise isolated DNA, complex nuclear mixtures, or an array of purified, recombinant, or synthetic proteins or peptides [27, 44]. These assays, however, differ in many factors including the source of the antigenic substrate, the presentation of such substrates to the autoantibodies where they could be immobilized on solid surfaces or move freely in solution, the methodology encountered for detecting binding and the employed reaction conditions [27, 54]. The variations among the detection assays happened to give conflicting results for the same patient sample where one assay gave positive detection and another had it negative [94–96]. The most important causation of such result variability amongst the different assays is the affinity of autoantibodies towards the test antigen which is highly influenced by the reaction conditions. For example, some assays, as will be described shortly, favor the recovery of low-affinity antibodies such as ELISA, while others assays favor the recovery of high-affinity antibodies such as the Farr assay [27, 97].

Another important aspect with regards to the conflicting results obtained from the distinct assays is the diffidence in the cutoff values set by different assay for considering autoantibody levels detected significant making data from different assays difficult to compare [27]. This confusion actually roots from the vague definition of antinuclear or anti-DNA antibody positivity described in SLICC [50]. The criteria state that an anti-DNA antibody test result is considered positive if it is higher than the laboratory's reference range except for ELISA in which a test result is only positive if it is two times or more the value of the laboratory reference range [27]. That said, the criteria did not define specific tests and did not refer to accepted levels for assay sensitivity or specificity [50, 98].

These discrepancies add to the confusion of diagnosticians who already face many challenges with SLE diagnosis and urge the need for standardization among the different assays. Simplicity, time, and costs are important factors for an assay to be chosen for routine clinical use in laboratories [27].

Amid all the available assays and newly developed technologies that are described for detecting anti-DNA antibodies as the most prominent biomarkers for SLE, an important question is issued of which assay to choose and is there a gold standard to refer to? It has been reported that assays that can specifically detect high-affinity antibodies as being reflective of mature immune responses are preferred by many investigators such as the Farr assay or CLIFT [27, 54]. However, the contribution of high-affinity and low-affinity anti-DNA antibodies to

SLE pathogenesis is not yet known, and therefore looking for other biomarkers that can arise from the multiple pathways contributing to SLE pathogenicity is highly needed to complement the value of anti-DNA antibodies in diagnosis and prognosis of SLE [27, 54].

In this section, the most prominent techniques employed for the detection of antinuclear antibodies and anti-DNA antibodies that have been described decades ago till today will be presented, in addition to some investment on the futuristic highly promising state-of-the-art technologies. However, before proceeding with the discussion of the different assays, a description of the DNA substrate antigenic properties and the factors that influence such antigenicity will be discussed as they are important dynamics in assaying anti-DNA antibodies.

4.1. DNA substrate

There are general characteristics of DNA that made it possible to employ DNA isolated from distinct sources including viral, bacterial, mammalian, and even flagella as the antigen substrates in multiple anti-DNA antibody detection assays [27, 54]. For instance, DNA is generally a highly charged polymeric molecule with repetitive charges that constitute an important factor of DNA epitopes that are targeted by anti-DNA antibodies. In addition, all double-stranded DNA isolated from natural origins predominantly exist in the B-form conformation rendering them recognizable by anti-DNA antibodies [27].

That said, there are some factors that can influence DNA antigenicity rendering it more or less antigenic and can thus consequently influence the performance of detection assays [94]. For instance, single-stranded DNA has a much more flexible structure than DNA double helix which tends to be more rigid, and thus targeted epitopes are more exposed in the single-stranded forms making it more antigenic [94]. Another factor that can influence DNA antigenicity is size. Anti-DNA antibodies bind DNA through a mechanism called monogamous bivalency in which both Fab fragments of the same antibody bind the same polynucleotide chain to ensure stable binding [27]. The distance between two Fab sites is 136 angstroms which corresponds to a stretch of 40 bp (**Figure 2**) [27]. Therefore, short oligonucleotides can be inefficient for binding anti-DNA antibodies, and longer DNA substrates can be essential for binding [27, 94]. Furthermore, certain isolated DNA such as that from *Crithidia luciliae* protozoal cells display a bent conformation resembling that of nucleosomes allowing binding only a subset of autoantibodies [27].

4.2. Immunofluorescence assay (IFA)

IFA (also known as indirect IFA) is an assay that dates back to the 1960s that was developed with the aim of providing sensitive and reliable means for the detection of ANAs [99]. As mentioned earlier, ANA positivity is one of the classification criteria described for SLE diagnosis, and IFA was mentioned by name in the ACR classification for detecting ANA [48]. Moreover, it is considered the gold standard for ANA testing [44, 48, 49, 90]. IFA involves fixation of culture cells on a slide to serve as the source of antigens targeted by ANAs, and

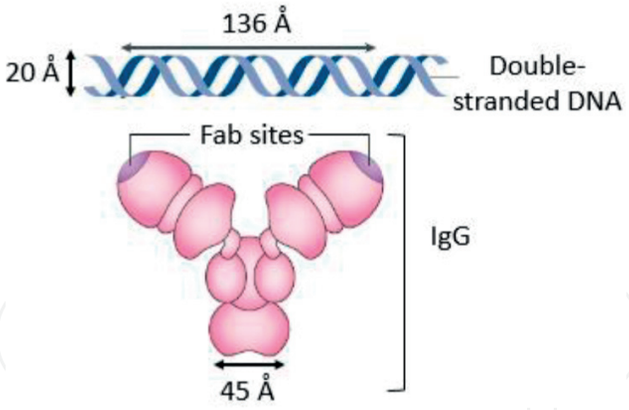


Figure 2. Monogamous bivalency binding of anti-DNA antibodies to DNA. Anti-DNA antibodies bind to the DNA via a mechanism known as monogamous bivalency in which both Fab sites bind the same stretch of polynucleotides. The distance between two Fab sites is 136 angstroms which corresponds to 40 base pairs. Accordingly, DNA fragments of shorter sizes are not efficient as substrates in anti-DNA antibody assays [27].

HEp-2 cells are currently employed for this purpose as they express a wide spectrum of antigens (**Figure 3**) [44]. Fixed cells are then incubated with serial dilutions of the patient's serum or plasma to promote the formation of immune complexes which is then followed by washing off unbound antibodies. The formed immune complexes are then detected by adding an anti-IgG antibody that is conjugated with a fluorescent agent and are visualized by fluorescence microscopy [44]. Positivity is assigned through determining the endpoint titer which is defined as the reciprocal of the last dilution that gave a fluorescence signal above the cutoff value [44, 100]. The specificity of binding can be inferred from examining the staining pattern which reflects the relative location of the antigen [44].

However, IFA is challenged by certain limitations that can compromise its specificity. First, whole cells are used as the source of test antigens which leads to the detection of other

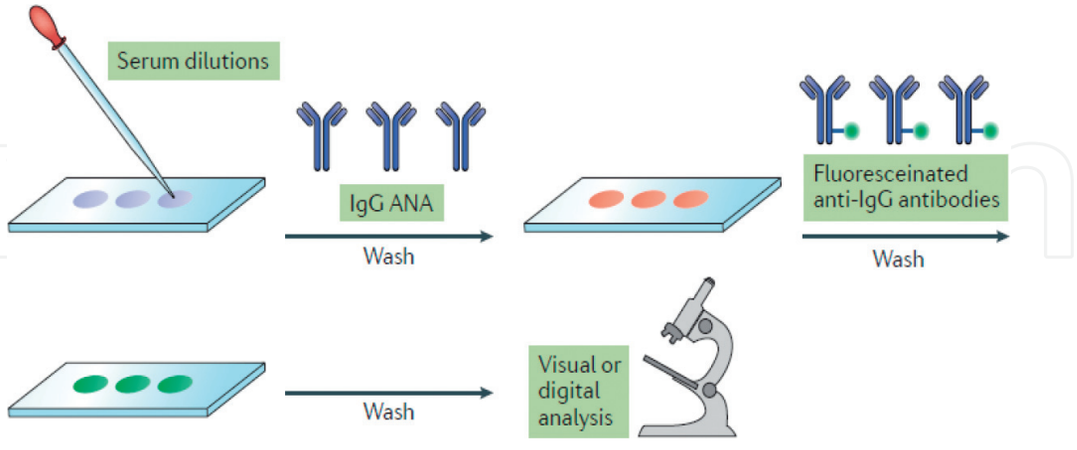


Figure 3. Immunofluorescence assay (IFA). IFA starts by fixation of culture cells on a glass slide which serves in providing the nuclear antigens that are targeted by ANAs in the patient's serum. Next, different dilutions of the patient's serum are incubated with the cells and are followed by a washing step to remove unbound antibodies. Immune complexes are detected by adding fluorescently labeled anti-IgG antibodies which are followed by a second washing step to remove unbound anti-IgG antibodies. Immune complexes are then visualized and analyzed through fluorescence microscopy [44].

antibodies targeting cytoplasmic and mitotic antigens that complicate the analysis [44]. Furthermore, certain antigens can be underexpressed in these cells which limit the detection of certain autoantibodies [101]. Moreover, IFA execution is subject to variability in experimental conditions including cell fixation protocols, concentrations of assayed cells, different assays of the commercially available kits, and the specificity of the anti-IgG antibodies [44]. Therefore, many efforts have been devoted at formulating other assays that can detect ANAs in a more robust and less technically demanding fashion and capable of high-throughput screening such as enzyme-linked immunosorbent assay (ELISA).

4.3. Enzyme-linked immunosorbent assay (ELISA)

ELISA is one of the most renowned techniques that enjoy a high versatility that allowed its use in a variety of biomedical applications including the detection of ANAs and anti-DNA antibodies in patients with SLE and other autoimmune diseases [102–107]. ELISA is based on coating a solid surface with the antigens of interest such as DNA substrates or an array of nuclear antigens so that they are tightly bound and can withstand subsequent washing steps. After immobilization, the test sample containing autoantibodies such as a patient's serum is added to promote binding of autoantibodies with their respective antigens, and the formed immune complexes can be detected through the addition of an anti-IgG antibody that is either conjugated with a peroxidase or alkaline phosphatase enzyme or a fluorophore [44, 94].

ELISA has high sensitivity and can detect a wide spectrum of antibodies owing to the efficient exposure of surface-immobilized DNA substrates making them readily available in high concentrations for binding and can thus capture both high- and low-affinity antibodies [27, 44, 94]. Therefore, ELISA can be a good choice for initial screening [27]. Moreover, it is easy to perform and allows quick, quantitative, and high-throughput analysis of autoantibodies [44]. However, ELISA mediates the recovery of low-affinity anti-DNA antibodies which compromises its specificity to SLE [54]. This can be due to a variety of reasons that can relate to the reaction conditions and the structure and source of the DNA substrate [54]. In addition, the increased sensitivity of ELISA leads to the generation of false-positive results due to cross-reactivity [27, 44].

4.4. Farr radioimmunoassay assay

Farr assay has been first introduced in 1968 for the detection of anti-DNA antibodies [108], and until now it is preferred by many clinicians for assaying anti-DNA antibodies as it mediates the selective recovery of high-affinity antibodies which have been described to be reflective of mature immune responses [27]. The principle of the Farr assay is based on the incubation of a solution of radiolabeled DNA such as ^{14}C-DNA with patient's serum sample to promote the formation of immune complexes between anti-DNA antibodies and the DNA substrate. After incubation, immune complexes are precipitated with a saturated solution of ammonium sulfate, and the fraction of the initial radiolabeled DNA that has precipitated with autoantibodies is used to indicate the amount of anti-DNA antibodies in the serum sample [109]. With the ability to only recover high-affinity antibodies, Farr assay exhibits high specificity for SLE, but consequently its sensitivity is not the best when compared with other assays such as ELISA

that is able to detect both high- and low-affinity antibodies [27]. In addition, because of the hazardous radioactive material used in DNA labeling and the troublesome associated with its disposal, researches aimed at the development and optimization of other assays including ELISA which at the time suffered inconsistencies with the results owing the lack of standardization protocols with antigen immobilization [93]. However, as will be discussed below, ELISA has been eventually optimized and became one of the popular biomedical techniques in assaying autoantibodies [44].

4.5. PEG precipitation assay

In this assay, the same principle of the Farr assay is applied where a solution of radiolabeled DNA is used as the test antigen for anti-DNA antibodies in the serum sample. However, instead of using ammonium sulfate for precipitation, polyethylene glycol is used as the precipitating agent, which mainly leads to the recovery of low-affinity anti-DNA antibodies in contrast to the Farr assay [110].

4.6. Crithidia luciliae immunofluorescence test (CLIFT)

CLIFT is an assay that was first introduced in 1975 [111], and it is similar to the IFA assay described above except for the source of the used antigenic substrate. The assay employs *Crithidia luciliae* protozoal cells as the source of antigenic DNA substrate as they possess a giant mitochondrion called kinetoplast that contains a giant mass of mitochondrial DNA. The kinetoplast was considered as a good substrate for the detection of anti-DNA autoantibodies because it is unlikely to be associated with nuclear antigens and can thus serve as a source of naked double-stranded DNA [111]. The assay proceeds exactly as IFA where the *Crithidia luciliae* cells are fixed on a glass slide and a series of dilutions of the patient's serum is incubated with the cells and detection is mediated through the addition of fluorescently labeled anti-IgG antibodies. CLIFT has been described to be highly specific to SLE similar to the Farr assay [97]. However, DNA of *Crithidia luciliae* was described to have a bent conformation similar to nucleosomal DNA which can result in the recovery of only a subset of anti-DNA antibodies, and thus the assay was described to have a low sensitivity [27, 97].

4.7. Multiplex assays

Multiplex assays refer to the technologies that permit the simultaneous profiling of a repertoire of antigens in just a single test [112]. In the context of SLE where patients can express as many as 200 distinct antibodies targeting multiple antigens [44], such multiplex technologies provide concomitant determination of antibody specificities in a high-throughput, rapid, and cost-effective manner and can be highly advantageous in the discovery of novel biomarkers and monitoring disease activity [52, 93, 112]. Multiplex assays involve different settings in which some of them are already implicated in clinical use such as LINE immunoassays and microbeads assays, while other newer multiplex technologies are also rapidly evolving such as microfluidics and nanobarcodes [93, 112].

4.7.1. LINE immunoassays (LIA)

In LIA, selected distinct antigens that can be synthetic, recombinant, or purified proteins or peptides are immobilized onto a nylon membrane or other protein-binding surfaces. Individual antigens are attached in parallel "lines" where each line represents a specific antigen. These lines are then cut from the membrane forming thin strips. The same serum sample is then added to each individual strip to identify all autoantibodies reactive against the panel of strips. The formed immune complexes are detected through the addition of a secondary antibody that is conjugated to an enzyme or a fluorescent label. Therefore, LIA allows the simultaneous detection of multiple autoantibodies in the same patient sample [44, 112].

4.7.2. Addressable laser bead immunoassays

Addressable laser bead immunoassays (**Figure 4**) [112] are based on coupling distinct antigens (up to 100) onto microbeads that come in multiple laser reactive colors where each antigen is coupled to a specific color of beads creating an "addressable" color code that is used for the identification of each antigen [93]. After antigen coupling, all beads are stabilized, collected into a microtiter well, and incubated with the sample, and the formed immune complexes are detected by a fluorescently tagged secondary antibody. Samples are then analyzed using a dual laser system that utilizes flow cytometry and digital signal processing. Each bead is hit with two laser beams where one detects the specific color code or address of the microbead that is used to identify the specific antigen, and the other laser beam detects and quantifies the fluorescence signal coming from the secondary antibody. The generated data provides quantitative and qualitative analysis of each autoantibody [93].

Both LIA and addressable laser bead immunoassays use defined sets of identified antigens which allows the detection of specific autoantibodies associated with a certain disease such as anti-DNA antibodies in SLE which is of high diagnostic value and can thus overcome one of the main limitations of IFA and ELISA in which assigning absolute specificities to an

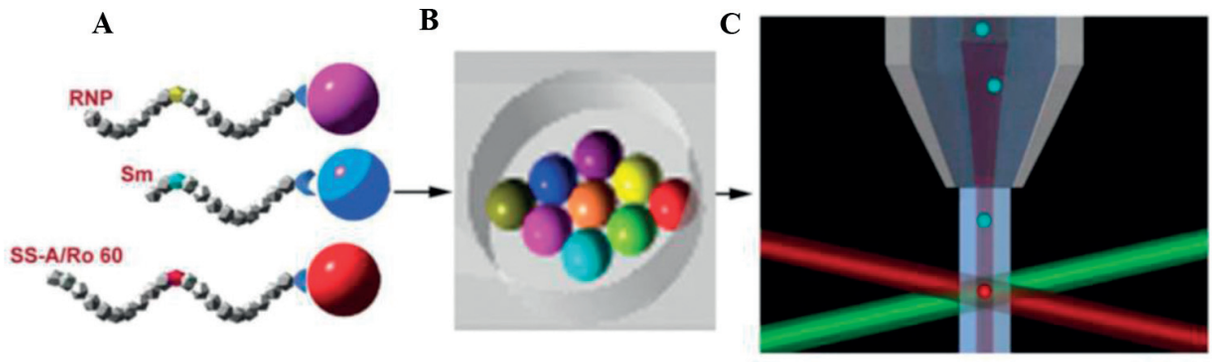


Figure 4. Addressable laser bead immunoassay (ALBIA). (A) ALBIA involves coupling of each individual antigen onto microbeads of laser active color so that antigens can be "addressable" by the color of the beads. (B) After coupling, all beads are combined into the microtiter well where they are incubated with the test sample such as patient's serum sample to promote formation of immune complexes which are detected by a secondary antibody that is fluorescently labeled. (C) Autoantibody-antigen complexes that are coupled to the beads are analyzed using a dual laser system where one detects the specific color code of the microbead and can thus be used to identify the specific antigen and the other laser beam detects and quantifies the fluorescence signal coming from the bound secondary antibody [112].

ANA-positive result is not possible. Furthermore, these assays are less time-consuming, are amenable for automation, and allow high-throughput analysis. However, this specificity stemming from the use of defined set of antigens can come at the expense of sensitivity where many autoantibodies can be missed. For example, SLE patients can release up to 200 different autoantibodies where only few of them are detected [113].

4.7.3. Autoantigen microarrays

Autoantigen microarrays are high-throughput assays that allow simultaneous detection of different autoantibodies in autoimmune diseases including SLE (**Figure 5**) [52]. The technique involves printing a distinct array of antigens into a surface that is coated with substrate that becomes covalently attached to the antigens. Printed antigens on the surface of microarrays are performed in a way that retains their reactivity with other molecules. In SLE, the array of antigens can include nuclear antigens including RNA and DNA and associated proteins, other cellular proteins, and specific targeted epitopes where the immobilized antigens can be purified or recombinant proteins or synthetic peptides. Different samples can be screened including serum, plasma, or other biological fluids such as cerebrospinal fluids or saliva for simultaneous detection of autoantibodies. Identification of immune complexes is mediated through the addition of secondary antihuman antibodies that are fluorescently labeled. Distinct isotypes of

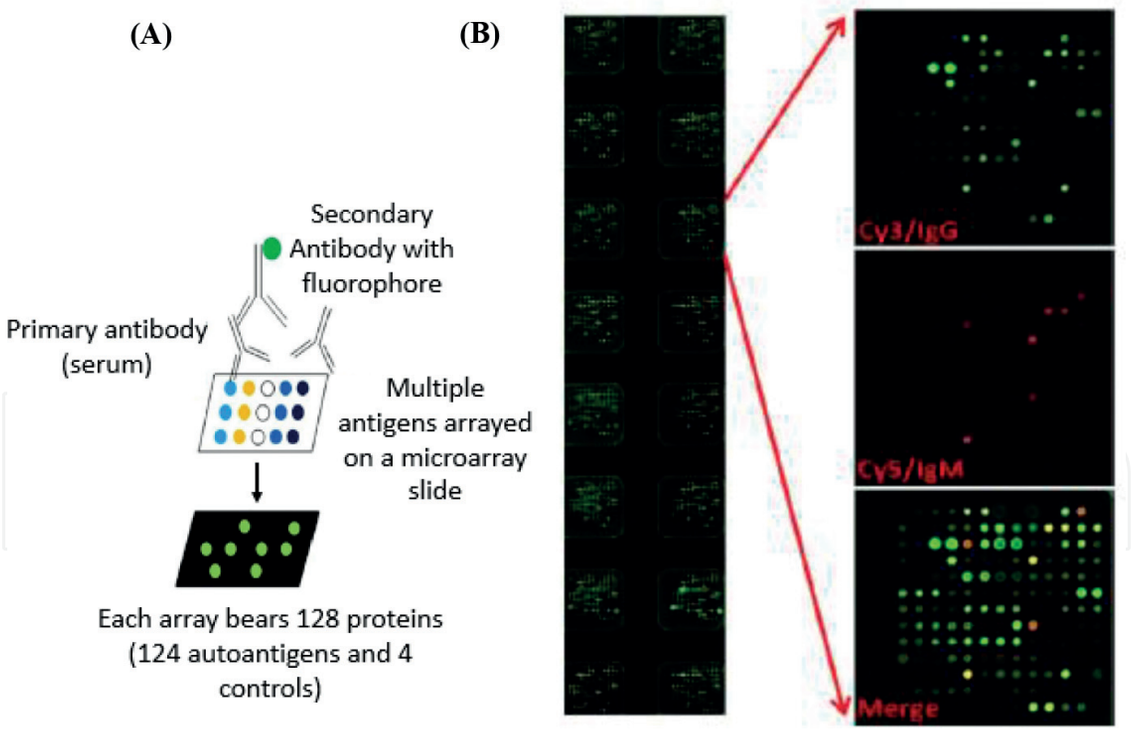


Figure 5. Simultaneous detection of autoantibodies using autoantigen microarrays. (A) Multiple autoantigens are printed onto a microarray surface and are incubated with patients' serum sample that contains autoantibodies. The formed immune complexes are identified through the addition of secondary antibodies that are labeled with a fluorophore so that each spot with a positive reaction produces a fluorescent signal. (B) Different isotypes of autoantibodies such as IgG and IgM can be visualized using secondary anti-IgG and anti-IgM that are labeled with differently colored fluorophores such as Cy5 and Cy3. Merging the images will show the different isotypes in the same sample [52].

autoantibodies such as IgG, IgM, or others can be specified by using secondary antibodies that are conjugated to different colored fluorophores. Autoantigen microarrays are not only useful in showing the differential detection of autoantibodies, but specific associations of certain autoantibodies with certain SLE manifestations can be made in addition to the identification of novel biomarkers. However, microarray chips can be challenging to be produced in consistent and reproducible manner for commercialization.

4.7.4. Microfluidics and nanobarcodes

With the aim of developing assays that are highly amenable for automation and point-of-care usage with the ability to concomitantly detect many analytes that are prepared in minute volumes with very high sensitivity, technologies such as microfluidics and nanobarcodes are implemented as bioassays for many molecules including the detection of autoantibodies in autoimmune diseases [112, 114, 115]. These assays are not yet implemented in clinical use, but they are highly promising as near-future diagnostics.

Microfluidics (also known as lab-on-a-chip microtechnologies) from its name is a technology that involves the analysis of ultra-low amounts of sample that are applied into specific devices that are fabricated to be in tens to hundreds of micrometers in dimensions [112]. These devices can be made in different configurations such as channels, pumps, pipes, or valves. The technology of microfluidics makes use of the flow characteristics of fluids in such micro-sized channels such as laminar flow and increased surface tension and capillary forces to move the sample through the microdevice [116]. In the detection of autoantibodies, the walls of such microdevices are coated with an array of antigens, and sample containing autoantibodies is applied to flow through the microdevice where immobilized antigens catch their respective antigens. A washing step is then applied to remove unbound antibodies followed by the application of a labeled secondary antibody, and signal can be detected by specific detectors [112, 114].

Nanobarcodes fall under the bigger science of nanotechnology in which systems, devices, or materials are fabricated in the nano-range to render them new and enhanced properties [117]. Unlike microfluidics that are fabricated at the micron scale, nanobarcodes are made even smaller to the nanoscale [117]. In the detection of autoantibodies, nanobarcodes consist of different stripes where each stripe is composed of a different metal such as gold, silver, platinum, or nickel that are electroplated into templates [115]. Different antigens are coupled to each metal stripe which are then incubated with the serum sample allowing the binding of each autoantibody to its respective coupled antigen. Detection of immune complexes is achieved via the addition of secondary antibody that is fluorescently labeled, and different antigens can be identified by fluorescence microscopy through the differential reflectivity of each stripe creating a pattern that resembles that of a barcode [112, 115, 117].

Microfluidics and nanobarcodes offer many advantages including the application of minute amounts of the sample and reagents which reduce costs and minimize chemical waste. Moreover, these devices are amenable to complete automation and maybe well applied as point-of-care diagnostics without the need of specialized labs and technical skills. In addition, they produce data that allow simultaneous comparison of the different analytes and at the same time reliable due to the inclusion of multiple internal controls [112, 115, 116].

5. Personalized therapy in SLE

SLE is actually a highly representative model for diseases that are in crucial need for personalized therapies as it is one of the highly heterogeneous and complex human diseases with chaotic pathogenesis [1, 4]. Although under the same disease umbrella, SLE patients are not homogenous cohorts that can be classified, treated, or managed equally as they show marked discrepancies in their responses to the same treatment, manifestations of disease severity, type and levels of circulating biomarkers, organ involvement, and the underlying pathogenic mechanisms that are highly influenced by genetic, environmental, and other risk factors [1, 118]. Currently, SLE patients are routinely treated with potent immunosuppressive agents that can cause adverse side effects which tend to be even more aggressive than the disease itself [41]. With the aim of achieving optimum management of SLE patients, it is therefore very wise to stratify these patients into subsets that share common pathogenic pathways which can be best accommodated with targeted or *personalized* therapeutic approaches that do not only increase treatment efficacy but also present safer alternatives to the nonselective immune-toxic steroids that are currently employed for the management of SLE patients [3, 118].

Recent gains in understanding SLE immunopathology have exposed certain deregulated immune trends that are now known to be common in subsets of SLE patients where some of these trends are now well characterized and paved the way for the exposure of various targets that are highly promising in personalized therapeutic approaches (**Figure 6**) [3]. Over the past 10 years, various medications made their way through preclinical and early clinical testing for the treatment of SLE patients, but unfortunately almost none of them was met with success during the later stages of clinical trials. This can be attributed to the highly heterogeneous nature of SLE patients which make study designs for clinical trials a very difficult mission [119, 120].

In this section of the chapter, highly promising therapeutic targets for specific subsets of SLE patients and recent therapeutic developments that hold a great potential in personalized medicine targeting specific cohorts of SLE patients will be discussed.

5.1. New potential therapeutic targets in SLE

As previously learnt, type I IFNs including IFN-α are greatly implicated in SLE pathogenesis and mediate a variety of downstream-deregulated immune responses including the release of autoantibodies [4]. However, highly elevated levels of type I INFs are only found in 40–50% of SLE patients constituting a subset of patients that can be particularly responsive to therapies targeting type I INFs and other mediators implicated in their pathway such as TLRs and IFN receptors [118, 121, 122]. One such agent is the anti-IFN-α monoclonal antibody sifalimumab which showed promising clinical activity in phase I and phase II clinical trials with high tolerance and safety profile [3, 123]. Other agents include the monoclonal anti-IFN-α antibodies rontalizumab [124] and AGS-009 which have finished phase II and phase I studies, respectively, with promising clinical results [3].

Another promising target is BAFF cytokine (also called BLyS) which is essential for B-cell maturation and function [4]. Differential circulating levels of this cytokine have been observed among SLE patients of different ethnic backgrounds where it was particularly elevated in

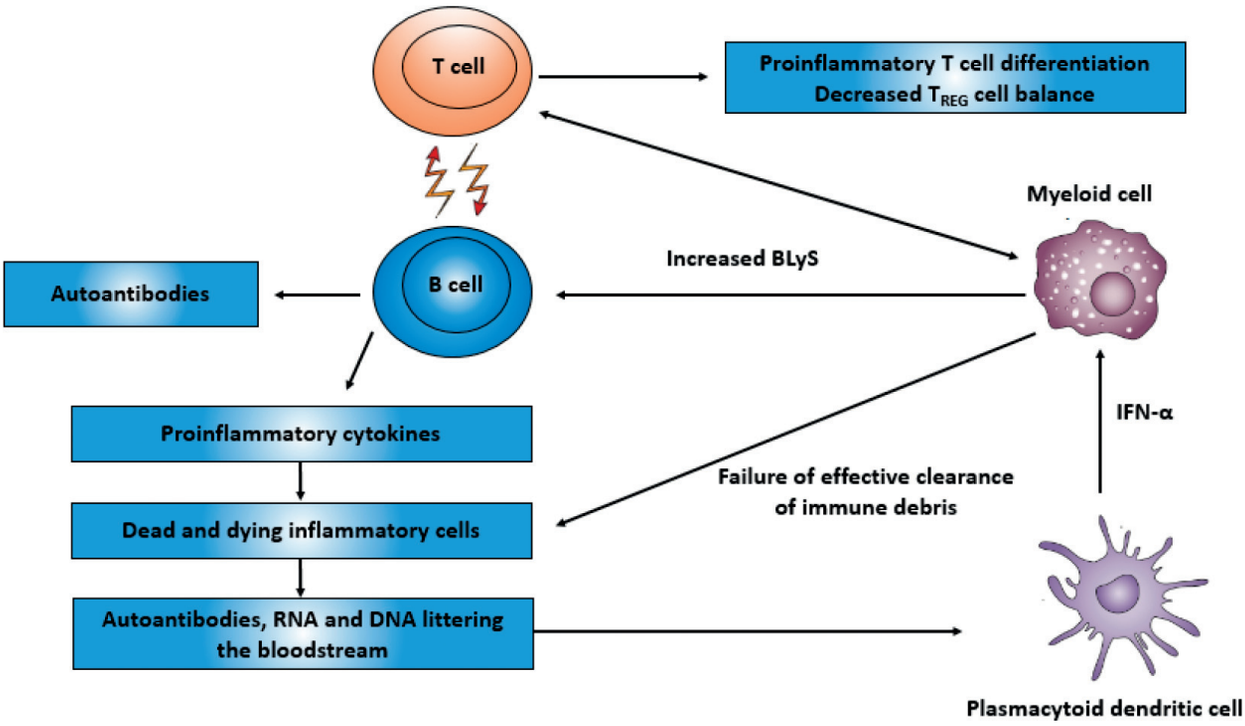


Figure 6. Several therapeutic approaches target distinct immune modulators in the pathogenesis of systemic lupus erythematosus. Deregulated immune cascade in SLE involves activation of TLRs in plasmacytoid dendritic cells mediated by internalized nucleic acids that are released from dead cells. Activated TLR induces potent release of type I INF such as INF-α which activates myeloid cells that act to release elevated amounts of the B-cell activator factor BAFF (also called BLyS). BLyS stimulates B-cell maturation and release of autoantibodies. Further activation of B cells is mediated via B-cell and T-cell interactions that can ultimately lead to loss of immune tolerance. Several agents have been described for targeting key players in the above immune cascade where some have paved their way in clinical use; others are still under clinical investigations, while some were not met with clinical successes [3].

patients of African background in comparison to patients of European background [125]. One of the agents that has gained approval in many countries for the treatment of SLE patients is the anti-BAFF monoclonal antibody belimumab [3] that has shown considerable efficacy and tolerability in randomized placebo-controlled phase III clinical trials in SLE patients against a control group [55, 126]. Many other agents that target BAFF are also still under clinical investigations and have shown promising results including blisibimod which has currently passed phase II clinical trials for SLE [3, 127]. Another agent that target B cells and showed highly encouraging early results for the treatment of SLE but has unfortunately failed in advanced stages of clinical trials is the chimeric antibody Rituximab [3]. Rituximab showed highly promising results in reducing SLE activity particularly in patients with lupus nephritis [128, 129] and has been largely prescribed to SLE patients all over the world with a decision that was mainly based on clinical experience and open-label studies [3]. However, in randomized placebo-controlled clinical trials, rituximab failed to provide efficacy in moderate to severe SLE patients with and without renal nephritis [130, 131]. Nevertheless, these results could be potentially misleading owing to some issues in the study design as it was shown that both test and control groups were receiving strong immunosuppressive agents including high doses of glucocorticoids [3, 118]. Nevertheless, rituximab is still prescribed by some clinicians, which also suggests the probability that this treatment works best at certain subsets of lupus patients, and further investigations should be implemented [118].

Finally, T cells and associated stimulatory pathways play a key role in the deregulated immune cascade in SLE pathogenesis [1, 4] and are thus highly promising therapeutic targets in SLE. Many attempts have been made in generating therapeutics targeting T cells including anti-CD40 ligand antibodies such as CDP7657 which is currently under clinical investigations in phase I study for SLE [3]. Other anti-T-cell approaches are being attempted including small molecule inhibitor drugs such as quinoline-3-carboxamide derivatives [132] and analogues of sphingosine-1-phosphate [133] which are still under clinical developments.

6. Conclusion

SLE is a chronic and highly progressive autoimmune disease that carries a high risk of early death [1–3]. The incidence and prevalence of SLE are highly influenced by many factors such as race, ethnicity, age, gender, and patients' demographics [6]. Although informative reporting on SLE epidemiology is inconsistent among the different countries, it is recognized as a global disease that faces so many clinical challenges in its diagnosis, prognosis, monitoring, and management [1]. The challenging nature of SLE originates from its chaotic immunopathology in which the affected stage in the immune cascade and the extent of deregulation are highly variable among SLE patients who as a consequence express a wide array of nonhomogeneous highly protean clinical and serological manifestations making them impossible to be recognized as a single cohort of patients that can be managed equally [1, 4]. Consequently, setting specific diagnostic criteria is very challenging, and until now no defined diagnostic guidelines have been established for SLE [51, 89]. However, as a reference, clinicians have used SLE classification criteria for diagnostic purposes such as the ACR or the more recent SLICC [48, 49, 92]. Nevertheless, using classification criteria for diagnosis is problematic because they are set to include the most prevalent manifestations of the disease that occur during late or severe stages and neglect many of the early or mid-stage symptoms making them highly specific but not sensitive [89, 91]. This cannot be well suited for SLE owing to its progressive nature where many of its pathologies accrue overtime and therefore referring to classification criteria for diagnosis can dismiss many SLE patients specifically those at the early stages of the disease [1, 89, 91]. Another challenge facing SLE is the insufficient availability of reliable and specific biomarkers which are highly needed for the highly heterogeneous nature of SLE where it is highly improbable that only a single biomarker can be indicative of the wide array of manifestations [5]. Therefore, the search for distinct and specific biomarkers that can accurately mediate early diagnosis, predict disease development and emergence of disease flares, monitor disease activity, indicate specific organ damage, and guide therapies, guide reliable inclusion in eligibility criteria for conducting clinical trials, and evaluate patients' responses to novel therapeutics is highly needed and never ceased [5, 43]. Conventionally, ANAs and anti-DNA antibodies are routinely used as biomarkers for SLE as they occur in at least 70 and 95% of SLE patients, respectively, and their detection is included in the classification criteria for SLE [48, 50, 51]. However, ANAs are highly unspecific for SLE as they are detected in other autoimmune diseases in addition to a not so small proportion of the general population [44]. Anti-DNA antibodies are currently the most specific biomarkers for SLE, and their detection outside SLE is not yet found [27]. Nevertheless, anti-DNA antibodies are poor predictors for SLE

activity as it happens that elevated levels of anti-DNA antibodies accompany patients in remission while normal levels accompany flared disease activity [45, 59, 61, 62]. Many promising novel biomarkers are emerging such as type I INFs which are highly elevated in a subset of SLE patients or some epigenetic biomarkers that are associated with SLE progression such as DNA methylation pattern, microRNA expression, and various histone modifications [5]. However, efforts devoted to overcome the abovementioned challenges of SLE along those employed at the discovery of novel biomarkers will not be possible without being rivaled with developments in state-of-the-art technologies that can accurately detect and monitor biomarkers with high sensitivity and specificity and in a manner that is cost-effective, rapid, easy to perform, and amenable to high-throughput screening [27, 44]. Currently, many of the technologies available for SLE diagnosis and monitoring are set to detect ANAs and anti-DNA antibodies [27, 44]. According to many investigators, the Farr assay is considered the gold standard for being able to detect high-affinity autoantibodies which are described to be more reflective of mature immune responses [27]. Nevertheless, a variety of techniques are available that all set to measure the formation of autoantibody-antigen immune complexes but differ in the source of the antigenic substrates, the way that such substrates are being exposed to the autoantibodies in the sample, the employed reaction conditions, and in the principle of detection [27, 54]. These discrepancies in the setup of the distinct assays render the differential sensitivities and specificities toward the recovered or detected autoantibodies in which a particular autoantibody can be tested positive in one assay and negative in another for the same sample [94–96]. The most important contributor for such conflicting results is the affinity of autoantibodies where some assays employ reaction settings that favor the recovery of low-affinity antibodies such as ELISA, while others favor the recovery of high-affinity antibodies such as the Farr assay [27, 97]. Therefore, the results obtained from different assays are difficult to compare and add to the confusion already facing SLE diagnosis. With the emergence of novel biomarkers, the need for multiplex technologies that permit the simultaneous detection of many antigens in just a single test in a rapid, cost-effective, and high-throughput fashion intensifies to accommodate the multiple parameters introduced with the increased variability of detected biomarkers [112, 113, 134]. Many multiplex technologies such as autoantigen arrays, nanobarcodes, microfluidics, and addressable laser immunoassays are strongly emerging with a highly promising potential for clinical diagnosis and monitoring of SLE [93, 112]. Finally, SLE most accurately exemplifies a disease model that in crucial need for the development of personalized therapies owing to the highly versatile clinical manifestations of SLE patients who are not possible to be treated equally [3, 118]. Potent immunosuppressants are currently employed for the nonspecific management of SLE patients, but they are associated with many adverse side effects that can even be more aggressive than the disease itself [41]. The better understanding of SLE pathophysiology has helped in stratifying SLE patients into subsets that share common immune-pathologies and thus can guide many of the emerging highly promising personalized therapeutic approaches [3, 118].

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Kaul A, Gordon C, Crow MK, Touma Z, Urowitz MB, Van Vollenhoven R, et al. Systemic lupus erythematosus. Nature Reviews Disease Primers. 2016;**2**:1-21

[2] Fritzler M. Reflections on lupus 2013: Butterflies, wolves and prophecies. Lupus [Internet]. 2013;**22**(August):1092-1101. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23989735

[3] Thanou A, Merrill JT. Treatment of systemic lupus erythematosus: New therapeutic avenues and blind alleys. Nature Reviews Rheumatology. 2014;**10**:23-34

[4] Tsokos GC, Lo MS, Reis PC, Sullivan KE. New insights into the immunopathogenesis of systemic lupus erythematosus. Nature Reviews Rheumatology [Internet]. 2016;**12**(12): 716-730. DOI: 10.1038/nrrheum.2016.186%5Cnhttp://10.0.4.14/nrrheum.2016.186

[5] Liu C-C, Kao AH, Manzi S, Ahearn JM. Biomarkers in systemic lupus erythematosus: Challenges and prospects for the future. Therapeutic Advances in Musculoskeletal Disease [Internet]. 2013;**5**(4):210-233

[6] Kumar K, Chambers S, Gordon C. Challenges of ethnicity in SLE. Best Practice and Research: Clinical Rheumatology. 2009;**23**:549-561

[7] Lim SS, Bayakly AR, Helmick CG, Gordon C, Easley KA, Drenkard C. The incidence and prevalence of systemic lupus erythematosus, 2002–2004: The Georgia lupus registry. Arthritis & Rhematology. 2014;**66**(2):357-368

[8] Somers EC, Marder W, Cagnoli P, Lewis EE, DeGuire P, Gordon C, et al. Population-based incidence and prevalence of systemic lupus erythematosus: The Michigan lupus epidemiology and surveillance program. Arthritis & Rhematology. 2014;**66**(2):369-378

[9] Laustrup H, Voss A, Green A, Junker P. Occurrence of systemic lupus erythematosus in a Danish community: An 8-year prospective study. Scandinavian Journal of Rheumatology. 2009;**38**(2):128-132

[10] Rees F, Doherty M, Grainge M, Davenport G, Lanyon P, Zhang W. The incidence and prevalence of systemic lupus erythematosus in the UK, 1999–2012. Annals of the Rheumatic Diseases. 2016;**75**(1):136-141

[11] Pons-Estel GJ, Alarcón GS, Scofield L, Reinlib L, Cooper GS. Understanding the epidemiology and progression of systemic lupus erythematosus. Seminars in Arthritis and Rheumatism. 2010;**39**:257-268

[12] Johnson AE, Gordon C, Palmer RG, Bacon PA. The prevalence and incidence of systemic lupus erythematosus in Birmingham, England: Relationship to ethnicity and country of birth. Arthritis and Rheumatism. 1995;**38**(4):551-558

[13] Hochberg MC. The incidence of systemic lupus erythematosus in Baltimore, Maryland, 1970–1977. Arthritis and Rheumatism. 1985;**28**(1):80-86

[14] McCarty DJ, Manzi S, Medsger TA, Ramsey-Goldman R, LaPorte RE, Kwoh CK. Incidence of systemic lupus erythematosus. Race and gender differences. Arthritis and Rheumatism [Internet]. 1995;**38**(9):1260-1270

[15] Somers EC, Thomas SL, Smeeth L, Schoonen WM, Hall AJ. Incidence of systemic lupus erythematosus in the United Kingdom, 1990–1999. Arthritis Care and Research. 2007; **57**(4):612-618

[16] Sexton DJ, Reule S, Solid C, Chen SC, Collins AJ, Foley RN. ESRD from lupus nephritis in the United States, 1995–2010. Clinical Journal of the American Society of Nephrology. 2015;**10**(2):251-259

[17] Mok CC, Kwok RCL, Yip PSF. Effect of renal disease on the standardized mortality ratio and life expectancy of patients with systemic lupus erythematosus. Arthritis and Rheumatism. 2013;**65**(8):2154-2160

[18] González LA, Toloza SMA, Alarcón GS. Impact of race and ethnicity in the course and outcome of systemic lupus erythematosus. Rheumatic Disease Clinics of North America. 2014;**40**:433-454

[19] Wei S, Sun Y, Sha H. Therapeutic targeting of BET protein BRD4 delays murine lupus. International Immunopharmacology. 2015;**29**(2):314-319

[20] Fairhurst AM, Mathian A, Connolly JE, Wang A, Gray HF, George TA, et al. Systemic IFN-α drives kidney nephritis in B6.Sle123 mice. European Journal of Immunology. 2008; **38**(7):1948-1960

[21] Liu Z, Bethunaickan R, Huang W, Lodhi U, Solano I, Madaio MP, et al. Interferon-?? Accelerates murine systemic lupus erythematosus in a T cell-dependent manner. Arthritis and Rheumatism. 2011;**63**(1):219-229

[22] Kyttaris VC, Kampagianni O, Tsokos GC. Treatment with anti-interleukin 23 antibody ameliorates disease in lupus-prone mice. BioMed Research International. 2013;**2013** (861028):5

[23] Stohl W, Xu D, Kim KS, Koss MN, Jorgensen TN, Deocharan B, et al. BAFF overexpression and accelerated glomerular disease in mice with an incomplete genetic predisposition to systemic lupus erythematosus. Arthritis and Rheumatism. 2005;**52**(7): 2080-2091

[24] Keszei M, Detre C, Castro W, Magelky E, O'Keeffe M, Kis-Toth K, et al. Expansion of an osteopontin-expressing T follicular helper cell subset correlates with autoimmunity in B6.Sle1b mice and is suppressed by the H1-isoform of the Slamf6 receptor. The FASEB Journal. 2013;**27**(8):3123-3131

[25] Dieker J, Hilbrands L, Thielen A, Dijkman H, Berden JH, van der Vlag J. Enhanced activation of dendritic cells by autologous apoptotic microvesicles in MRL/lpr mice. Arthritis Research & Therapy. 2015;**17**(1):1-14

[26] Casciola-Rosen LA, Anhalt G, Rosen A. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. The Journal of Experimental Medicine. 1994;**179**(4):1317-1330

[27] Pisetsky DS. Anti-DNA antibodies - quintessential biomarkers of SLE. Nature Reviews Rheumatology. 2016;**12**:102-110

[28] Honda K, Ohba Y, Yanai H, Hegishi H, Mizutani T, Takaoka A, et al. Spatiotemporal regulation of MyD88-IRF-7 signalling for robust type-I interferon induction. Nature. 2005;**434**(7036):1035-1040

[29] Barton GM, Kagan JC, Medzhitov R. Intracellular localization of Toll-like receptor 9 prevents recognition of self DNA but facilitates access to viral DNA. Nature Immunology. 2006;**7**(1):49-56

[30] Savarese E, Steinberg C, Pawar RD, Reindl W, Akira S, Anders HJ, et al. Requirement of toll-like receptor 7 for pristane-induced production of autoantibodies and development of murine lupus nephritis. Arthritis and Rheumatism. 2008;**58**(4):1107-1115

[31] Guerrier T, Youinou P, Pers JO, Jamin C. TLR9 drives the development of transitional B cells towards the marginal zone pathway and promotes autoimmunity. Journal of Autoimmunity. 2012;**39**(3):173-179

[32] Dörner T, Jacobi AM, Lee J, Lipsky PE. Abnormalities of B cell subsets in patients with systemic lupus erythematosus. Journal of Immunological Methods. 2011;**363**(2):187-197

[33] Liu Z, Davidson A. IFN?? Inducible models of murine SLE. Frontiers in Immunology. 2013;**4**:1-7

[34] Thien M, Phan TG, Gardam S, Amesbury M, Basten A, MacKay F, et al. Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches. Immunity. 2004;**20**(6):785-798

[35] Lesley R, Xu Y, Kalled SL, Hess DM, Schwab SR, Shu HB, et al. Reduced competitiveness of autoantigen-engaged B cells due to increased dependence on BAFF. Immunity. 2004; **20**(4):441-453

[36] Mackay F, Woodcock SA, Lawton P, Ambrose C, Baetscher M, Schneider P, et al. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. Journal of Experimental Medicine [Internet]. 1999;**190**(11):1697-1710

[37] Kirou KA, Lee C, George S, Louca K, Peterson MGE, Crow MK. Activation of the interferon-α pathway identifies a subgroup of systemic lupus erythematosus patients with distinct serologic features and active disease. Arthritis and Rheumatism. 2005;**52**(5): 1491-1503

[38] Lövgren T, Eloranta ML, Båve U, Alm GV, Rönnblom L. Induction of interferon-α production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. Arthritis and Rheumatism. 2004;**50**(6):1861-1872

[39] Christensen SR, Shupe J, Nickerson K, Kashgarian M, Flavell RAA, Shlomchik MJ. Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. Immunity. 2006;**25**(3):417-428

[40] Villanueva E, Yalavarthi S, Berthier CC, Hodgin JB, Khandpur R, Lin AM, et al. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. Journal of Immunology [Internet]. 2011; **187**(1):538-552

[41] Crispín JC, Kyttaris VC, Terhorst C, Tsokos GC. T cells as therapeutic targets in SLE. Nature Reviews Rheumatology. 2010;**6**:317-325

[42] Illei GG, Tackey E, Lapteva L, Lipsky PE. Biomarkers in systemic lupus erythematosus: I. General overview of biomarkers and their applicability. Arthritis and Rheumatism. 2004; **50**:1709-1720

[43] Ahearn JM, Liu C-C, Kao AH, Manzi S. Biomarkers for systemic lupus erythematosus. Translational Research [Internet]. 2012;**159**(4):326-342

[44] Pisetsky DS. Antinuclear antibody testing — Misunderstood or misbegotten? Nature Reviews Rheumatology [Internet]. 2017;**13**(8):495-502

[45] Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. Lupus [Internet]. 2004;**13**(5):290-297

[46] Liu C, Kao AH, Manzi S, Ahearn JM. Biomarkers in systemic lupus erythematosus: Challenges and prospects for the future. Therapeutic Advances in Musculoskeletal Disease [Internet]. 2013;**5**(4):210-233

[47] Tan EM. Antinuclear antibodies: Diagnostic markers for autoimmune diseases and probes for cell biology. Advances in Immunology. 1989;**44**(C):93-151

[48] Tan EM, Cohen AS, Fries JF, Masi AT, Mcshane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis and Rheumatism. 1982;**25**(11):1271-1277

[49] Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis and Rheumatism [Internet]. 1997;**40**(9):1725. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9324032

[50] Petri M, Orbai AM, Alarcõn GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and validation of the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. Arthritis and Rheumatism. 2012;**64**(8): 2677-2686

[51] Bertsias GK, Pamfil C, Fanouriakis A, Boumpas DT. Diagnostic criteria for systemic lupus erythematosus: Has the time come? Nature Reviews Rheumatology. 2013;**9**: 687-694

[52] Zhu H, Luo H, Yan M, Zuo X, Li QZ. Autoantigen microarray for high-throughput autoantibody profiling in systemic lupus erythematosus. Genomics, Proteomics and Bioinformatics. 2015;**13**:210-218

[53] Ippolito A, Wallace DJ, Gladman D, Fortin PR, Urowitz M, Werth V, et al. Autoantibodies in systemic lupus erythematosus: Comparison of historical and current assessment of seropositivity. Lupus. 2011;**20**(3):250-255

[54] Riboldi P, Gerosa M, Moroni G, Radice A, Allegri F, Sinico A, et al. Anti-DNA antibodies: A diagnostic and prognostic tool for systemic lupus erythematosus? Autoimmunity. 2005;**38**(1):39-45

[55] Furie R, Petri M, Zamani O, Cervera R, Wallace DJ, Tegzová D, et al. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. Arthritis and Rheumatism. 2011;**63**(12):3918-3930

[56] Van Vollenhoven RF, Petri MA, Cervera R, Roth DA, Ji BN, Kleoudis CS, et al. Belimumab in the treatment of systemic lupus erythematosus: High disease activity predictors of response. Annals of the Rheumatic Diseases. 2012;**71**(8):1343-1349

[57] Fredriksen K, Skogsholm A, Flaegstad T, Traavik T, Rekvig OP. Antibodies to dsDNA are produced during primary BK virus infection in man, indicating that anti-dsDNA antibodies may be related to virus replication in vivo. Scandinavian Journal of Immunology. 1993;**38**(4):401-406

[58] Rekvig OP, Nossent JC. Anti-double-stranded DNA antibodies, nucleosomes, and systemic lupus erythematosus: A time for new paradigms? Arthritis and Rheumatism. 2003; **48**:300-312

[59] Petri M, Genovese M, Engle E, Hochberg M. Definition, incidence, and clinical description of flare in systemic lupus erythematosus. A prospective cohort study. Arthritis and Rheumatism. 1991;**34**(8):937-944

[60] Walz LeBlanc BAE, Gladman DD, Urowitz MB. Serologically active clinically quiescent systemic lupus erythematosus predictors of clinical flares. The Journal of Rheumatology. 1994;**21**(12):2239-2241

[61] Esdaile JM, Abrahamowicz M, Joseph L, MacKenzie T, Li Y, Danoff D. Laboratory tests as predictors of disease exacerbations in systemic lupus erythematosus: Why some tests fail. Arthritis and Rheumatism. 1996;**39**(3):370-378

[62] Gladman DD, Hirani N, Ibañez D, Urowitz MB. Clinically active serologically quiescent systemic lupus erythematosus. The Journal of Rheumatology. 2003;**30**(9):1960-1962

[63] Richardson B, Scheinbart L, Strahler J, Gross L, Hanash S, Johnson M. Evidence for impaired T cell DNA methylation in systemic lupus erythematosus and rheumatoid arthritis. Arthritis and Rheumatism. 1990;**33**(11):1665-1673

[64] Hughes T, Webb R, Fei Y, Wren JD, Sawalha AH. DNA methylome in human CD4+ T cells identifies transcriptionally repressive and non-repressive methylation peaks. Genes and Immunity. 2010;**11**(7):554-560

[65] Lu Q, Wu A, Tesmer L, Ray D, Yousif N, Richardson B. Demethylation of CD40LG on the inactive X in T cells from women with lupus. Journal of Immunology [Internet]. 2007; **179**(9):6352-6358

[66] Lu Q, Wu A, Richardson BC. Demethylation of the same promoter sequence increases CD70 expression in lupus T cells and T cells treated with lupus-inducing drugs. Journal of Immunology [Internet]. 2005;**174**(10):6212-6219

[67] Kaplan MJ, Lu Q, Wu A, Attwood J, Richardson B. Demethylation of promoter regulatory elements contributes to perforin overexpression in CD4+ lupus T cells. Journal of Immunology [Internet]. 2004;**172**(6):3652-3661

[68] Lin SY, Hsieh SC, Lin YC, Lee CN, Tsai MH, Lai LC, et al. A whole genome methylation analysis of systemic lupus erythematosus: Hypomethylation of the IL10 and IL1R2 promoters is associated with disease activity. Genes and Immunity. 2012;**13**(3):214-220

[69] Zhao M, Tang J, Gao F, Wu X, Liang Y, Yin H, et al. Hypomethylation of IL10 and IL13 promoters in CD4+ T cells of patients with systemic lupus erythematosus. Journal of Biomedicine and Biotechnology [Internet]. 2010;**2010**:931018

[70] Snowden AW, Gregory PD, Case CC, Pabo CO. Gene-specific targeting of H3K9 methylation is sufficient for initiating repression in vivo. Current Biology. 2002;**12**(24):2159-2166

[71] Roh TY, Cuddapah S, Zhao K. Active chromatin domains are defined by acetylation islands revealed by genome-wide mapping. Genes & Development. 2005;**19**(5):542-552

[72] Hu N, Qiu X, Luo Y, Yuan J, Li Y, Lei W, et al. Abnormal histone modification patterns in lupus CD4+ T cells. The Journal of Rheumatology. 2008;**35**(5):804-810

[73] Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annual Review of Biochemistry [Internet]. 2010;**79**(1):351-379

[74] Baltimore D, Boldin MP, O'Connell RM, Rao DS, Taganov KD. MicroRNAs: New regulators of immune cell development and function. Nature Immunology. 2008;**9**:839-845

[75] Xiao C, Rajewsky K. MicroRNA control in the immune system: Basic principles. Cell. 2009;**136**:26-36

[76] Dai R, Ahmed SA. MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. Translational Research. 2011;**157**:163-179

[77] Shen N, Liang D, Tang Y, De Vries N, Tak PP. MicroRNAs-novel regulators of systemic lupus erythematosus pathogenesis. Nature Reviews Rheumatology. 2012;**8**:701-709

[78] Tang Y, Luo X, Cui H, Ni X, Yuan M, Guo Y, et al. MicroRNA-146a contributes to abnormal activation of the type I interferon pathway in human lupus by targeting the key signaling proteins. Arthritis and Rheumatism. 2009;**60**(4):1065-1075

[79] Luo X, Yang W, Ye D-Q, Cui H, Zhang Y, Hirankarn N, et al. A functional variant in microRNA-146a promoter modulates its expression and confers disease risk for systemic lupus erythematosus. PLoS Genetics [Internet]. 2011;**7**(6):1-11

[80] Zhao X, Tang Y, Qu B, Cui H, Wang S, Wang L, et al. MicroRNA-125a contributes to elevated inflammatory chemokine RANTES levels via targeting KLF13 in systemic lupus erythematosus. Arthritis and Rheumatism. 2010;**62**(11):3425-3435

[81] Santer DM, Yoshio T, Minota S, Moller T, Elkon KB. Potent induction of IFN- and chemokines by autoantibodies in the cerebrospinal fluid of patients with neuropsychiatric lupus. Journal of Immunology [Internet]. 2009;**182**(2):1192-1201

[82] Karageorgas TP, Tseronis DD, Mavragani CP. Activation of type I interferon pathway in systemic lupus erythematosus: association with distinct clinical phenotypes. Journal of Biomedicine and Biotechnology [Internet]. 2011;**2011**:273907

[83] Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 2003;**100**(5):2610-2615

[84] Nikpour M, Dempsey AA, Urowitz MB, Gladman DD, Barnes DA. Association of a gene expression profile from whole blood with disease activity in systemic lupus erythaematosus. Annals of the Rheumatic Diseases. 2008;**67**(8):1069-1075

[85] Feng X, Wu H, Grossman JM, Hanvivadhanakul P, FitzGerald JD, Park GS, et al. Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. Arthritis and Rheumatism. 2006;**54**(9):2951-2962

[86] Biesen R, Demir C, Barkhudarova F, Grün JR, Steinbrich-Zöllner M, Backhaus M, et al. Sialic acid-binding Ig-like lectin 1 expression in inflammatory and resident monocytes is a potential biomarker for monitoring disease activity and success of therapy in systemic lupus erythematosus. Arthritis and Rheumatism. 2008;**58**(4):1136-1145

[87] Rose T, Grutzkau A, Hirseland H, Huscher D, Dahnrich C, Dzionek A, et al. IFNalpha and its response proteins, IP-10 and SIGLEC-1, are biomarkers of disease activity in systemic lupus erythematosus. Annals of the Rheumatic Diseases [Internet]. 2013; **72**(10):1639-1645

[88] Crispín JC, Tsokos GC. IL-17 in systemic lupus erythematosus. Journal of Biomedicine and Biotechnology [Internet]. 2010;**2010**:943254

[89] Larosa M, Iaccarino L, Gatto M, Punzi L, Doria A. Advances in the diagnosis and classification of systemic lupus erythematosus. Expert Review of Clinical Immunology. 2016;**12**:1309-1320

[90] Urowitz MB, Gladman DD, Ibañez D, Sanchez-Guerrero J, Romero-Diaz J, Gordon C, et al. American college of rheumatology criteria at inception, and accrual over 5 years in the slicc inception cohort. The Journal of Rheumatology. 2014;**41**(5):875-880

[91] Aggarwal R, Ringold S, Khanna D, Neogi T, Johnson SR, Miller A, et al. Distinctions between diagnostic and classification criteria? Arthritis Care & Research (Hoboken) [Internet]. 2015;**67**(7):891-897

[92] Amezcua-Guerra LM, Higuera-Ortiz V, Arteaga-García U, Gallegos-Nava S, Hübbe-Tena C. Performance of the 2012 systemic lupus international collaborating clinics and the 1997 American College of Rheumatology classification criteria for systemic lupus erythematosus in a real-life scenario. Arthritis Care and Research. 2015;**67**(3):437-441

[93] Fritzler MJ, Fritzler ML. Microbead-based technologies in diagnostic autoantibody detection. Expert Opinion on Medical Diagnostics. 2009;**3**(1):81-89

[94] Pisetsky DS. Standardization of anti-DNA antibody assays. Immunologic Research. 2013;**56**(2–3):420-424

[95] Venner AA, Ibañez D, Gladman DD, Urowitz MB, MacKinnon A, Blasutig IM, et al. Comparison of three anti-dsDNA assays: Performance and correlation with systemic lupus erythematosus disease activity. Clinical Biochemistry. 2013;**46**(4–5):317-320

[96] Enocsson H, Sjöwall C, Wirestam L, Dahle C, Kastbom A, Rönnelid J, et al. Four anti-dsDNA antibody assays in relation to systemic lupus erythematosus disease specificity and activity. The Journal of Rheumatology. 2015;**42**(5):817-825

[97] Žigon P, Lakota K, Čučnik S, Švec T, Ambrožič A, Sodin-Šemrl S, et al. Comparison and evaluation of different methodologies and tests for detection of anti-dsDNA antibodies on 889 Slovenian patients' and blood donors' sera. Croatian Medical Journal [Internet]. 2011; **52**(6):694-702

[98] Rekvig OP. Anti-dsDNA antibodies as a classification criterion and a diagnostic marker for systemic lupus erythematosus: Critical remarks. Clinical and Experimental Immunology. 2015;**179**(1):5-10

[99] Friou GJ. Antinuclear antibodies: Diagnostic significance and methods. Arthritis and Rheumatism. 1967;**10**(2):151-159

[100] Frey A, Di Canzio J, Zurakowski D. A statistically defined endpoint titer determination method for immunoassays. Journal of Immunological Methods 1998;**221**(1–2):35-41

[101] Hoffman IEA, Peene I, Veys EM, De Keyser F. Detection of specific antinuclear reactivities in patients with negative anti-nuclear antibody immunofluorescence screening tests. Clinical Chemistry 2002;**48**(12):2171-2176

[102] Kávai M, Bányai A, Zsindely A, Sonkoly I, Szegedi G. Enzyme-linked immunosorbent assay for antibodies to native DNA in sera of patients with SLE. Journal of Immunological Methods. 1982;**48**(2):169-175

[103] Stokes RP, Cordwell a TR a. A simple, rapid ELISA method for the detection of DNA antibodies. Journal of Clinical Pathology. 1982;**35**(5):566-573

[104] Rubin RL, Joslin FG, Tan EM. An improved ELISA for anti-native DNA by elimination of interference by anti-histone antibodies. Journal of Immunological Methods. 1983;**63**(3): 359-366

[105] Fenger M, Wiik A, Høier-Madsen M, Lykkegaard JJ, Rozenfeld T, Hansen MS, et al. Detection of antinuclear antibodies by solid-phase immunoassays and immunofluorescence analysis. Clinical Chemistry. 2004;**50**(11):2141-2147

[106] Stearns NA, Zhou S, Petri M, Binder SR, Pisetsky DS. The use of poly-L-lysine as a capture agent to enhance the detection of antinuclear antibodies by ELISA. PLoS One. 2016;**11**(9):1-25

[107] Tan EM, Smolen JS, McDougal JS, Butcher BT, Conn D, Dawkins R, et al. A critical evaluation of enzyme immunoassays for detection of antinuclear autoantibodies of defined specificities: I. Precision, sensitivity, and specificity. Arthritis and Rheumatism. 1999;**42**(3):455-464

[108] Wold RT, Young FE, Tan EM, Farr RS. Deoxyribonucleic acid antibody: A method to detect its primary interaction with deoxyribonucleic acid. Science [Internet]. 1968; **161**(3843):806-807

[109] Manthorpe R, Palif J, Bendexen G. Anti-DNA antibody in serum measured by radioimmunoassay (fair technique): Description of method and recommended procedure. Allergy. 1978;**33**(1):42-49

[110] Smeenk R, Aarden L. The use of polyethylene glycol precipitation to detect low-avidity anti-DNA antibodies in systemic lupus erythematosus. Journal of Immunological Methods. 1980;**39**(1–2):165-180

[111] Aarden LA, de Groot ER, Feltkamp TEW. Immunology of DNA. III. Crithidia luciliae, a simple substrate for the determination of anti-dsdna with the immunofluorescence technique. Annals of the New York Academy of Sciences. 1975;**254**(1):505-515

[112] Fritzler MJ. Advances and applications of multiplexed diagnostic technologies in autoimmune diseases. Lupus. 2006;**15**(7):422-427

[113] Satoh M, Tanaka S, Chan EKL. The uses and misuses of multiplex autoantibody assays in systemic autoimmune rheumatic diseases. Frontiers in Immunology. 2015;**6**(Apr):1-4

[114] Zubair A, Burbelo PD, Vincent LG, Iadarola MJ, Smith PD, Morgan NY. Microfluidic LIPS for serum antibody detection: Demonstration of a rapid test for HSV-2 infection. Biomedical Microdevices. 2011;**13**(6):1053-1062

[115] Freeman RG, Raju P A, Norton SM, Walton ID, Smith PC, He L, et al. Use of nanobarcodes particles in bioassays. NanoBiotechnology Protocols [Internet]. 2005;**303**:73-83

[116] Sackmann EK, Fulton AL, Beebe DJ. The present and future role of microfluidics in biomedical research. Nature. 2014;**507**:181-189

[117] Jain KK. Nanodiagnostics: Application of nanotechnology in molecular diagnostics. Expert Review of Molecular Diagnostics. 2003;**3**:153-161

[118] Sinicato NA, Postal M, Appenzeller S, Niewold TB. Defining biological subsets in systemic lupus erythematosus: Progress toward personalized therapy. Pharmaceutical Medicine. 2017;**31**(2):81-88

[119] Lo MS, Tsokos GC. Treatment of systemic lupus erythematosus: New advances in targeted therapy. Annals of the New York Academy of Sciences. 2012;**1247**(1):138-152

[120] Bruce IN, Gordon C, Merrill JT, Isenberg D. Clinical trials in lupus: What have we learned so far? Rheumatology. 2010;**49**(6):1025-1027

[121] Weckerle CE, Franek BS, Kelly JA, Kumabe M, Mikolaitis RA, Green SL, et al. Network analysis of associations between serum interferon-α activity, autoantibodies, and clinical features in systemic lupus erythematosus. Arthritis and Rheumatism. 2011;**63**(4):1044-1053

[122] Lichtman EI, Helfgott SM, Kriegel MA. Emerging therapies for systemic lupus erythematosus–focus on targeting interferon-alpha. Clinical Immunology [Internet]. 2012;**143**(3): 210-221

[123] Petri M, Wallace DJ, Spindler A, Chindalore V, Kalunian K, Mysler E, et al. Sifalimumab, a human anti-interferon-α monoclonal antibody, in systemic lupus erythematosus: A phase i randomized, controlled, dose-escalation study. Arthritis and Rheumatism. 2013; **65**(4):1011-1021

[124] Kalunian K, Merrill JT, Maciuca R, Ouyang W, McBride JM, Townsend MJ, et al. Efficacy and safety of rontalizumab (anti-interferon alpha) in SLE subjects with restricted immunosuppressant use: Results of a randomized, double-blind, placebo-controlled phase 2 study. Arthritis and Rheumatism [Internet]. 2012;**64**:S1111

[125] Ritterhouse LL, Crowe SR, Niewold TB, Merrill JT, Roberts VC, Dedeke AB, et al. B lymphocyte stimulator levels in systemic lupus erythematosus: Higher circulating levels in African American patients and increased production after influenza vaccination in patients with low baseline levels. Arthritis and Rheumatism. 2011;**63**(12):3931-3941

[126] Navarra SV, Guzmán RM, Gallacher AE, Hall S, Levy RA, Jimenez RE, et al. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: A randomised, placebo-controlled, phase 3 trial. Lancet. 2011;**377**(9767):721-731

[127] Furie RA, Leon G, Thomas M, Petri MA, Chu AD, Hislop C, et al. A phase 2, randomised, placebo-controlled clinical trial of blisibimod, an inhibitor of B cell activating factor, in patients with moderate-to-severe systemic lupus erythematosus, the PEARL-SC study. Annals of the Rheumatic Diseases. 2015;**74**(9):1667-1675

[128] Vigna-Perez M, Hernández-Castro B, Paredes-Saharopulos O, Portales-Pérez D, Baranda L, Abud-Mendoza C, et al. Clinical and immunological effects of Rituximab in patients with lupus nephritis refractory to conventional therapy: A pilot study. Arthritis Research & Therapy [Internet]. 2006;**8**(3):R83

[129] Díaz-Lagares C, Croca S, Sangle S, Vital EM, Catapano F, Martínez-Berriotxoa A, et al. Efficacy of rituximab in 164 patients with biopsy-proven lupus nephritis: Pooled data from European cohorts. Autoimmunity Reviews. 2012;**11**:357-364

[130] Rovin BH, Furie R, Latinis K, Looney RJ, Fervenza FC, Sanchez-Guerrero J, et al. Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: The lupus nephritis assessment with rituximab study. Arthritis and Rheumatism. 2012; **64**(4):1215-1226

[131] Merrill JT, Neuwelt CM, Wallace DJ, Shanahan JC, Latinis KM, Oates JC, et al. Efficacy and safety of rituximab in moderately-to-severely active systemic lupus erythematosus: The randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. Arthritis and Rheumatism. 2010;**62**(1):222-233

[132] Bengtsson AA, Sturfelt G, Lood C, Rönnblom L, Van Vollenhoven RF, Axelsson B, et al. Pharmacokinetics, tolerability, and preliminary efficacy of paquinimod (ABR-215757), a new quinoline-3-carboxamide derivative: Studies in lupus-prone mice and a multicenter, randomized, double-blind, placebo-controlled, repeat-dose, dose-ranging study in. Arthritis and Rheumatism. 2012;**64**(5):1579-1588

[133] Ando S, Amano H, Amano E, Minowa K, Watanabe T, Nakano S, et al. FTY720 exerts a survival advantage through the prevention of end-stage glomerular inflammation in lupus-prone BXSB mice. Biochemical and Biophysical Research Communications. 2010; **394**(3):804-810

[134] Op De Beéck K, Vermeersch P, Verschueren P, Westhovens R, Mariën G, Blockmans D, et al. Antinuclear antibody detection by automated multiplex immunoassay in untreated patients at the time of diagnosis. Autoimmunity Reviews. 2012;**12**:137-143

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