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The most complex skin rashes: an evaluation of current and future diagnostics of Systemic Lupus Erythematosus

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Abstract

Systemic lupus erythematosus (SLE), the name being first suggested by the French dermatologist Pierre Cazenave in 1833, is the most common type of lupus. The disease is most prevalent amongst women between 20-40 years of age. SLE is characterized by malar or discoid rashes on patients' facial areas; it is a classic example of a type III hypersensitivity reaction, in which aberrant autoimmune responses are induced by the aggregation of immune complexes. Several parts of the patients' bodies are affected including the skin, kidney, GI tract, and joints; presenting with inflammation and tissue damage. This corresponds to some of the most common comorbidities of SLE such as nephritis, rheumatoid arthritis, and pleuritis. Given the various manifestations of SLE at various anatomical sites, the disease has historically been arduous to diagnose. Some immunological markers of SLE have been identified such as antinuclear antibodies, but high instances of false or delayed diagnoses still persist, contributing to devastating outcomes. This review will outline and evaluate current immunological and haematological diagnostics for SLE and propose an evidence-based diagnostics strategy. Where available, possible future directions or assays for SLE disease markers will also be proposed.

Keywords

Erythematosus, Systemic lupus erythematosus, Malar rashes, Autoimmune, Antinuclear antibodies, Classical complement pathway, Type III hypersensitivity, Antigen presenting cells, Immune complex

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Introduction

Imagine if "blisters" start to accumulate and form peculiar butterfly shapes on your face; accompanied with fever and severe organ pain. These are symptoms of Systemic lupus erythematosus (SLE), a complicated systemic autoimmune illness that causes widespread inflammation and tissue damage, affecting both the internal organs, and the epidermis. The name was first suggested by the French dermatologist Pierre Cazenave in 1833, and it is the most common type of lupus. SLE is characterized by malar or discoid rashes on patients' facial areas. It is a classic example of a type III hypersensitivity reaction, in which aberrant autoimmune responses are induced by the formation of antigen-antibody immune complexes. Several parts of the patient's body are affected including the skin, kidney, GI tract, and joints. Given the various manifestations of SLE, historically it has proven challenging to diagnose. Some immunological markers of SLE have been identified, however high instances of false or delayed diagnoses still exist; contributing to devastating outcomes. This review will outline and evaluate current immunological diagnostics for SLE and propose an evidence-based diagnostics strategy. In addition, possible future directions or assays for SLE disease markers will also be discussed.

History

The word "lupus" is attributed to its Latin roots, meaning "wolf". Interestingly, the lesions and rashes patterns present on SLE individuals' skin were reminiscent of wolf bites. The official discovery and identification of lupus dates back to early 19th century; the lesions now described as the "disc" shaped rashes were first derived from the term "Erythema centrifugum" and noted by Cazenave. In the same period, the "butterfly" rashes of SLE were described by the Austrian physician Ferdinand von Hebra in 1846. The two rashes were initially thought to be distinct conditions, it was not until the later part of the 19th century that the two rashes were linked together as the symptoms of one systemic disease. As the study on the rashes expanded, in 1872, Moriz Kaposi first described the systemic nature of the disease. He identified several symptoms characterizing the disease, including subcutaneous nodules (rashes), arthritis in both small and large joints, lymphadenopathy (infection of the lymph nodes), anemia, weight loss, and fever. The next breakthrough was the discovery of lupus erythematosus cells (LE cells) in 1948 by Malcolm Hargraves. LE cells were first observed in patients with acute disseminated lupus erythematosus, another form of acute lupus characterized by facial erythema similar to SLE. The cells were initially hypothesized to be either macrophages or neutrophils in the bone marrow. Further investigation demonstrated that LE cells were in fact myeloid cells, but they differed in that they had phagocytosed denatured nuclear material resulting in a "water balloon-like" appearance under light microscopy (1). LE cells are also prevalent in other disorders involving the bone marrow and joints including Rheumatoid arthritis, which is one of the prominent manifestations of SLE. Hargraves's discovery opened up possibilities of differential diagnostics for individuals with SLE specifically rather than a generalized diagnosis of inflammatory skin disease. It was the first instance in history where a direct potential cause of SLE was discovered, which laid the foundations of more accurate molecular level

research into SLE to proceed. More specifically, subsequent research focused on the Human Lymphocyte Antigen Class II genes (HLA genes), which will be discussed further in detail in the "Complexities of Diagnosis" and "Etiology" sections (2).

Demographics

SLE is deadly; mortality in patients is approximately 2-3 times higher than that of the general population. SLE is most prevalent in women between the ages of 20-40 and in certain ethnic groups. However, the estimated incidence and prevalence varies greatly among different geographical locations (3). For example, a study conducted with the intention of analyzing the demographics of SLE and lupus nephritis among US adults with medical coverage between 2000-2004 demonstrated significant differences in SLE incidence between ethnic groups. Out of 24 million adults surveyed; 34000 individuals were identified as having SLE. This prevalence is about 143 people per 100,000 for the entire US population. The SLE sample was further stratified based on their sociodemographic status (e.g., race, gender, location, age, etc.) Among these 34000 individuals, the prevalence of SLE was approximately 112 people per 100,000 for African Americans. However, the prevalence of SLE in Caucasians was significantly greater at 223 people per 100,000 (Figure1).

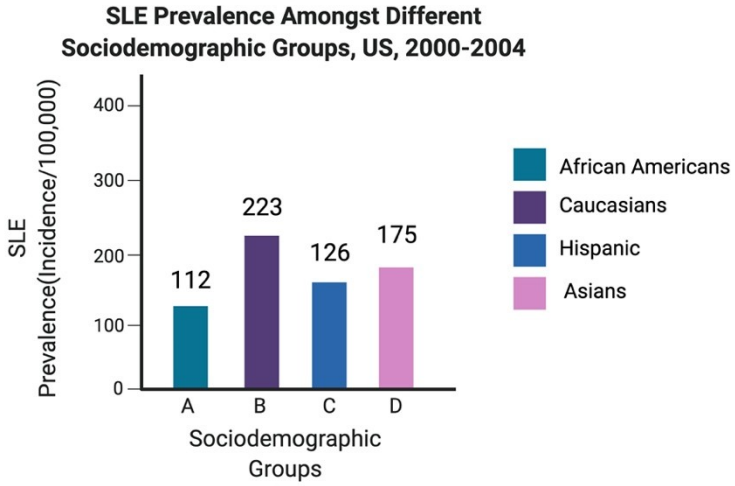

Figure 1. SLE Prevalence of Different Sociodemographic Groups in the US, Created with BioRender.com

These two ethnic groups represent the majority of cases of SLE in the United States; a major difference of prevalence between these two groups is enough to demonstrate the variations in different ethnic groups. When analyzing groups based in different geographical locations, this difference increased significantly: approximately 163.5 and 125.2 people per 100,000 were identified with SLE in the north and south of the USA respectively (4,5). Furthermore, not only does the incidence of SLE vary among different countries, there is a considerable intra-country variation as well. Another study conducted in Alberta, Canada, between 2001 and 2005 found that the overall prevalence of SLE was about 47.99 per 100,000 people. This number increased to 90 in 2015, but was still significantly lower than that in the USA (143 per 100,000). In both these studies, the number of women with SLE was 6 times higher than that of men (6). Some possible environmental factors such as UV light, air pollution, and pesticides are associated with the increased risk of SLE. For example, industrialized states such as California may contribute to the increased incidences of SLE in that region. The difference in terms of access to healthcare services (7), healthcare awareness, and overall hygiene can also affect the SLE incidence across various geographical regions.

Complexities of Diagnosis

SLE is a complex systemic disease arising from multiple causes and risk factors. Both genetic and external factors contribute to the formation of SLE at specific locations in the patient's body. Genome wide association studies (GWAS) have identified approximately 100 risk loci. For example, one of the already identified gene variants is Apolipoprotein1 (APOL1), from among a total of 12 polymorphism loci associated with lupus nephritis. The sheer amount of identified and unidentified contributing gene variants is the main reason why SLE is hard to diagnose (8). AI classifier algorithms have had some success in predicting and identifying previously unknown genetic components of SLE. Using genotype data from the Immunochip® from Illumina, which consists of about 200,000 Single-nucleotide polymorphisms (SNPs) related to diseases of the immune system, a random forest classifier identified SLE related SNPs using two criteria: disease probability, the genetic risk for SLE; and gene importance score, the indicator of a gene region which contains alleles that confers the risk of SLE. After compiling data from 1160 patients and 2711 healthy controls, the classifier predicted 12 new genes that were previously associated with other autoimmune diseases such as: Proteasome Assembly Chaperone 1 (PSMG1), Prostaglandin E Receptor 4 (PTGER4), and Cytoplasmic Polyadenylation Element Binding Protein 4 (CPEB4). Three new genes, Zinc Finger Protein 804 A (ZNF804A), Ankyrin 3 (ANK3) and Dedicator of Cytokinesis 3 (DOCK3), that were previously not associated with any autoimmune diseases were also identified as risk alleles of SLE. Functionally associated genes for the latter two were identified to be related to SLE: Cyclin Dependent Kinase1(CDK1), the regulatory gene associated with cyclin dependent cytokine in mitosis and apoptosis, and Mesencephalic Astrocyte derived Neurotrophic factor (MANF), the promoter of neuron survivability (9-11).

External factors also add to the complexity of diagnosis. For example, malar rashes are hallmark indicators of SLE and are readily apparent on lighter skin tones. However, difficulties arise when discerning these rashes on darker skin tones or skin of different conditions (texture, patients with existing skin disorders) where redness and scaling is not as easy to visualize. The unremarkable rashes could be indicators of early stage SLE, or they could be merely sunburn. It is difficult to decide if the presentation is merely a cutaneous level infection or a full systemic autoimmune condition.

Similarly, another complication regarding diagnostics lies in the fact that SLE is similar to several other inflammatory skin diseases including cutaneous lupus erythematosus (CLE), acne vulgaris, and hidradenitis suppurativa. CLE is the milder form of SLE, and merely affects the skin. Acne vulgaris affects the pilosebaceous units such as the hair follicles. Hidradenitis suppurativa is a rash affecting those areas where there is skin to skin abrasion (12). All of these diseases cause damage and can form nodules. Since SLE is systemic, it can also create red blisters on any area of the skin which may overlap with the affected areas of the three other inflammatory skin diseases. The complexity involved in differentiating SLE from other diseases manifesting with similar external presentations signifies the necessity for finding biomarkers specific to SLE.

Lastly, diagnostic tools are rated in terms of their sensitivity (ability to identify all true SLE cases as being SLE positive) and their specificity (ability to identify only true SLE cases as being SLE positive). Depending on the mechanism and accuracy of the test, there is usually a trade-off between the two. Consequently, a high sensitivity test may incorrectly identify non-SLE cases as being SLE positive, whereas a high specificity test may not identify some true SLE cases as being SLE positive. Therefore, either multiple diagnostic tests, with a holistic approach, must be performed for a correct diagnosis (13) or ratings/weights need to be assigned to different diagnostic tests and tools so that manual calculation or AI classifier algorithms can eventually achieve the greatest sensitivity and selectivity. This evolving set of criteria culminating in the current ‘gold standard’ of diagnosis; the EULAR-ACR-2019 criteria, is discussed in the ‘current diagnostics’ section below.

Etiology

SLE is an autoimmune disease characterized by inappropriate innate and adaptive immune responses. A loss of self-tolerance in B-cells results in the formation of excessive auto-antibodies and immune complexes: an aggregate of antigens & immunoglobulins, being produced, both of which are characteristics of a classical type III hypersensitivity reaction. A type III hypersensitivity reaction is defined as the accumulation of immune complexes in the circulation. This overabundance of immune complexes subsequently triggers activation of the classical complement pathway, which is an innate immune mechanism that provides an immediate defensive response (8). One of the functions of such a response is the direct lysis of the surface of a target pathogen as a defense mechanism. It is believed that a dysfunctional C-Type Lectin Domain Containing 16A (CLEC16A) gene in antigen presenting cells

(APCs) in SLE patients leads to targeting host cells for lysis as well. APCs "display" specific antigen bound via surface proteins coded by the HLA Class II genes. HLA is the "human specific" version of the major histocompatibility complex, which is responsible for the display of cell surface proteins. These proteins are usually responsible for displaying to the antigen-specific T-lymphocytes, which targets to engage as part of the immune response. However, variations in these genes shape the central and peripheral T-cell repertoires towards autoimmune tendencies. This is reflected in the false signals that APCs end up providing to the host's innate immune units (14). The antibodies that are responsible for this "friendly fire" are called antinuclear antibodies (ANA), one of the most prominent hallmarks of current SLE diagnostics. These falsely launched attacks at the host cell tissues results in inflammation in the skin, GI tract and joints. This inflammation is subsequently manifested in the form of joint pain, skin rashes, and swollen glands, among other visible and non-visible symptoms of the disease.

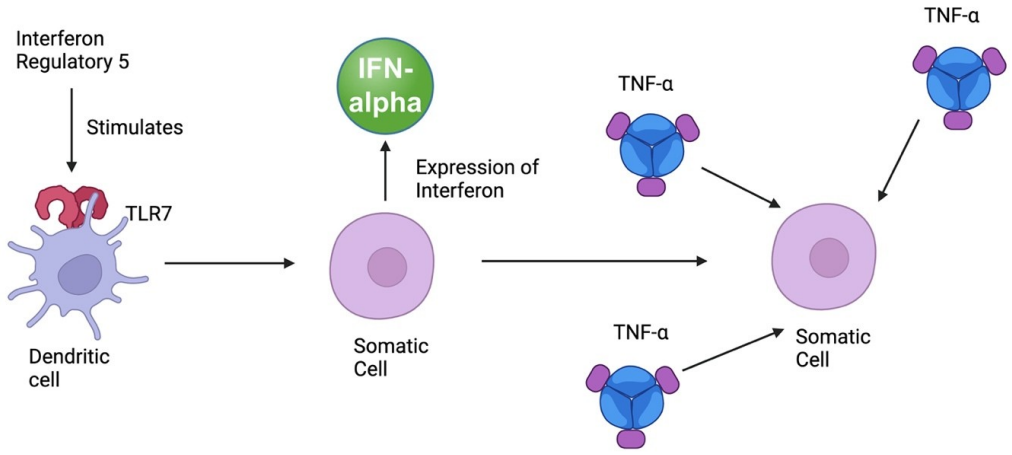

Figure 2. Inflammation Process induced by IRF5 Variant, Created with BioRender.com

On the other hand, within the adaptive component of the immune response, the most prominent risk factor shown to be associated with SLE are variants of the Interferon regulatory factor 5 gene (IRF5). The IRF5 gene is responsible for encoding a transcription factor called IFN regulatory factor 5, which, in turn, stimulates Toll-like receptors (TLR) present on myeloid cells such as macrophages and dendritic cells. Since the TLR proteins are encoded by their corresponding TLR gene, these genes are known as IFN-stimulated genes (ISGs). SLE patients commonly demonstrate a high expression of ISGs, this appears to be the primary driver of inflammation. The stimulation of three specific TLRs - TLR 7, 8,

and 9 - leads to the induction of Type I IFN and inflammatory cytokine genes. The former is commonly expressed by infected cells. In this case, the induction of interferon instructs the host that there is an infection that requires immediate mediation. Subsequently, large amounts of innate immune cells are recruited to the site of interferon expression – which, in the case of SLE, are healthy host cells - and cause an aberrant autoimmune inflammatory response. On the other hand, the inflammatory cytokine genes induce inflammatory responses as a defense mechanism (Figure 2). The autoimmune component of the inflammatory response is hence partly driven by the overexpression of the gene variants of IRF5 (15-19).

Current Diagnostics

Although commonly referred as a disease, according to more recent studies, SLE can be categorized more as a syndrome with multiple manifestations rather than a single disease with a definitive cause. All the signs and symptoms together characterize SLE as the archetypal systemic autoimmune condition. The most notable abnormalities in this condition, as discussed above in the etiology section, are associated with interferon regulated genes and the classical complement pathway. Depending on which of these gene(s) or signaling pathways are dysfunctional, patients may present with different manifestations and severity, which confounds diagnosis and treatment. Based on the signs and symptoms, diagnosis and treatment should become more individualized rather than merely focusing on the general autoimmune aspects of the syndrome (20). For example, if the patient's primary complaints are of presence of blood in the urine (hematuria) and pain below the rib cage, performing blood urine tests and kidney biopsies to confirm the presence of lupus nephritis benefits the patient more than treatment with corticosteroids; the 'catch all' option for SLE patients.

The fact that SLE could be more of a syndrome than a disease significantly impacts current diagnostic considerations. Both treatment and diagnosis are trending toward specialization. Among the considerations, one syndrome that has an intimate link to SLE is Antiphospholipid syndrome (APS), a disorder commonly known for its ability to increase the risk of blood clots. Patients diagnosed with APS may or may not present characteristics of SLE simultaneously. Those individuals who do not, are categorized as primary APS (PAPS) patients, while those who do are called secondary APS (SAPS) patients. PAPS patients show signs of organ damage, and up to 10% have their condition evolving into SLE with more severe inflammation. Some of defining characteristics of SLE, such as anti-nuclear antibodies, a dysfunctional complement pathway, and renal failure, are also found in PAPS patients. Alongside the previously mentioned skin disorders, the similarity between APS patients and SLE patients suggest that they could be part of the "SLE Syndrome" instead of being classified as a comorbidity. The fact that SLE seems to be an "aggregation of diseases" suggests new future directions as well as challenges for its diagnosis (21-23).

Various medical associations have listed standards and criteria to enable SLE diagnosis. The first of these standards was proposed in 1997 by the College of Rheumatology (ACR). The 11-point criteria (24) involves the definition of various symptoms such as malar

rashes, oral ulcers, and hematologic disorders. Out of these, the most important "hallmark" for current diagnosis of SLE is the identification of antinuclear antibodies in the patient's blood. A revised version of these criteria was proposed by the Systemic Lupus International Collaborating Clinics (SLICC) association in 2012. The revised version added hypocomplementemia and APS antibodies tests and expanded up to 17 clinical representations (25). Currently, for a patient to be diagnosed with SLE, there are two different minimal requirements. The patient must either present with at least four out of the seventeen clinical representations, including at least one clinical and one immunological criterion, or present symptoms of lupus nephritis as the sole clinical representation as well as testing positive for ANA or anti-dsDNA antibodies in the blood. The more the clinical and/or immunological conditions a patient presents with that are above the minimum requirement, the more likely it is that diagnosis will be highly sensitive/selective. Renal damage (lupus nephritis), presence of ANA, and a deficiency in the complement system are the three most defining characteristics of SLE. Aside from these three, all other criteria are associated equally with SLE (25,26). These criteria certainly provide valuable starting points for diagnosis, but are not usually capable of producing diagnostic results with high sensitivity and specificity. In order to address this concern, another diagnostic standard was proposed in 2019 by both the ACR and the European League Against Rheumatism (EULAR). The most recent EULAR-ACR-2019 standard graduated from inspecting clinical manifestations and checking boxes, and instead attempted a more holistic and quantitative approach based on a scoring system. Similar to its predecessors, the presence of ANA still remains the minimal entry criterion of a likely positive diagnosis, albeit of high sensitivity and low selectivity (27). Since most SLE patients are characterized with ANA, this still yields an accurate result in positive diagnosis. The 2019 standard weighted the different criteria based on their level of contribution to SLE. The new classification standard, with refined definition for each clinical and immunological representation, consists of 22 criteria. For a full list with definitions for each criterion, see Table1 in reference 27. The 22 criteria are further classified into seven clinical domains (constitutional, hematological, neuropsychiatr ic, mucocutaneous, serosal, musculoskeletal, and renal) and three immunological domains (antiphospholipid antibodies, complement proteins, SLE-specific antibodies). For a full list classified by domains, see Table 2 in reference 27. Each criterion is attributed to SLE unless otherwise explainable. Each individual criterion was weighted and given a score between 2 and 10 indicating its level of association. Under the premise of being detected to have ANA (entry criterion), a patient is diagnosed with SLE if the cumulative score equals or exceeds 10. When attempting to diagnose, only the highest-weighted criterion from each domain is counted. For each domain, it is not necessary for a patient to simultaneously fulfill all criteria in that domain, and occurrence of a criterion on a single occasion (does not require signs of remission) is sufficient for diagnosis. For example, if the patient presents with fever, leukopenia, and autoimmune induced haemolysis, they will receive a score of 6 (2 from having fever and 4 from autoimmune haemolysis since that ranks higher than

leukopenia in the haematological domain). Use of the 2019 standard resulted in significant increases in sensitivity and specificity of 96.1% and 93.4% respectively. A complete flow diagram of this refined classification process can be found in Figure 2 of reference 27. However, there are still shortcomings to this classification method. For example, one of the drawbacks of utilizing the presence of ANA as the entry criterion is that the classification proceeds under the premise that all patients of SLE are ANA positive. However, there exists a small subset of SLE patients who persistently show ANA negative results. Although these patients can be potentially distinguished by low cytokine levels and lack of efficacy with immunomodulatory treatments such as corticosteroids, it is still challenging to trace these patients with an effective diagnosis measure. The size of this subset may vary among different populations, and requires further investigation. By creating a more inclusive classification method, SLE diagnosis is anticipated to become more precise and effective (27-29).

Another more recently proposed diagnostic measure that could possibly be complementary to the EULAR-ACR-2019 classification criteria is calculating the genetic risk scores (GRS) of a patient. Genetic risk score is an estimate of the overall contributions of genetic factors to a specific disease incidence. It has already demonstrated its potential in a set of patients with inflammatory arthritis (30). Instead of analyzing each individual risk loci among the 100 identified SLE risk loci, it is more efficient to look at their overall contribution. Physicians can utilize this measure to predict a patient's susceptibility to SLE which can significantly help determine if the patients' symptoms are in fact due to SLE. A comparison of the calculated prediction with previously established genome-wide significant variants ($p \leq 5 \times 10^{-8}$) results in determining the extent to which those genetic factors have contributed to the patient's symptoms. The closer the patients' genetic variants are to the identified risk loci, the more likely it is that their symptoms are a consequence of SLE, and the more confidence physicians can have in their diagnosis (30). Another study conducted more recently in 2020 suggested a different approach to assigning GRS to the different associated risk loci of SLE. The study involved 1001 patients and 2802 healthy controls. The inclusion criteria allowed for the inclusion of 57 previously identified SNPs that are associated with SLE, and cumulative GRS was assigned to each individual based on these SNPs. For each individual SNP, a statistical analysis of the comparison of the patient and control group yielded an odds ratio for SLE susceptibility, a number categorizing strength of association between the SNP and SLE. The natural logarithm of that ratio was then multiplied with the number of previously identified risk alleles in each individual. The resulting score was a predictor of the onset and outcomes of SLE accrual in an individual patient. Since the disease has several manifestations, the possible outcomes included the risk of organ damage, renal dysfunction, and all-cause mortality due to comorbidities. A higher GRS is directly associated with an increase in such risks and potentially, with a higher mortality rate (31).

However, one possible concern with both methods is that both are significantly based on Caucasian/European ancestry cohorts. As mentioned above in the demographics section,

there are significant differences between different ethnic groups and the prevalence of SLE among them. The diversities are caused by several factors such as their race-specific genetic characteristics, healthcare access, and geographical locations.

With an objective of finding common cross-ethnic diagnostic attributes, trans-ancestral genetic components associated with SLE were analyzed in 2017 to address the potential diagnosis variation between ethnicities. The study examined the data of 196,524 polymorphisms across patients of European ancestry (EA), African American ancestry (AA), and Hispanic (Amerindian) American ancestry (HA), and discovered novel association loci common across all three ancestries. The study characterized distinct associated regions into three different levels based on their statistical significance. Tier 1 regions with P-values of $< 5 \times 10^{-8}$; tier 2 regions with P-values between 5×10^{-8} and 1×10^{-6}; tier 3 regions with P-values greater than 1×10^{-6}. For people with EA, 6 novel tier 1 regions including: 4p16 (*DKGQ*), 6p22 (*SLC17A4* and *LRRC16A*), 6q23 (*OLIG3-LOC100130476*), 8p23 (*FAM86B3P*), 8q21 (*PKIA-ZC2HC1A*) and 17q25 (*GRB2*) were identified on chromosomes 4, 6, 8, and 17. For people with AA ancestry, only 3 novel tier 1 regions including: 5q33 (*PTTG1-MIR146A*), 6p21 (*UHRF1BP1-DEF6*) and 16q22 (*ZFP90*) were identified. Lastly, for people with HA ancestry, 2 novel tier 1 regions including: 14q31 (*GALC*) and 16p13 (*CLEC16A*) were identified. Although all these newly identified regions noted above no doubt advance precision diagnosis of patients with specific ancestries, only one of them is common across all three ancestries, viz. 16p13 on chromosome 16. Specific SNPs worth further investigation in that region are: rs8054198, rs12448240, and rs12925552. The CLEC16A gene associated with that region is a direct regulator of the Human Leukocyte Antigen (HLA) class II pathway in APCs. Content in the 16p13 region could be a pivotal breakthrough point at addressing the diagnosis variation between Caucasians and people of other races (32, 33).

Traditional lab tests still have a role to play in SLE diagnosis. The key lies in the fact that new biomarkers with higher sensitivity or specificity need to be utilized. The most common serological biomarker for SLE in use at the present moment is the ANA, which has a high sensitivity of 94% but a low specificity of 61%. The low specificity accounts for the presence of ANA as an entry criterion in the EULAR-ACR-2019 standard. Other common biomarkers are the anti-dsDNA and anti-Smith antibodies. Although they have a high specificity (both have reached 100% specificity in diagnosis), both biomarkers lack sensitivity. This suggests the necessity of including an array of biomarkers that may be utilized in conjunction for a precise diagnosis; such an aggregation of biomarkers is also known as a biomarker panel (34). One such panel proposed in 2017 involves peptidomic analysis from the urine samples of SLE patients. Urine samples are often good indications of possible lupus nephritis in the patient, and nephritis is often associated with SLE. In the initial stages of testing with the discovery cohort, this panel involving a total 65 peptide markers reached a 0.99 Area Under Curve (AUC) in the Recorder operating curve (ROC), an extremely high evaluation for a classification method. Although this number dropped to 0.80 during

testing with the multicentric validation cohort involving lupus nephritis and non-SLE patients, the panel has potential in diagnosing SLE (35). Aside from including biomarkers involved in comorbidities, those in the complement system also demonstrated effective levels of diagnosis accuracy. The C3 protein in a patient's immune system is often the most common biomarker derived from the complement system that is indicative of the state of the immune system. A more accurate biomarker known as C3dg, an opsonin generated after complement activation has recently been found. Since the hyperactivity of the complement system constitutes a large proportion of SLE's identity, C3dg is abundant in the plasma of SLE patients compared to that of healthy individuals. The AUC under the ROC when using C3 and C3dg as biomarkers for diagnosing SLE were 0.52 and 0.96 respectively. A high AUC implies that the classification method is effective between discriminating positive and negative results, as well as demonstrating high sensitivity and specificity (36,37). Judicious utilization of appropriate biomarker panels, has the potential to diagnose SLE with high sensitivity and specificity.

Future Directions for Diagnostics

As mentioned above, an internal-focused approach is more appropriate and efficient for clinical purposes of diagnosing SLE. Inappropriate activation of the classical complement pathway and hematological abnormalities are common comorbidities associated with SLE. When the complement pathway is activated, it forms membrane attack complexes (MAC), a lytic surface protein that binds to the surface of the pathogens. At the same time, another protein fragment from the complement system, iC3b, an opsonin phagocytosis signaling protein, recruits this lytic surface protein to the host tissue. As time progresses and complement is excessively activated in SLE patients, MAC starts to accumulate and generates a neoantigen on the iC3b protein; iC3b-NEO. This neoantigen is complex-specific, and it is not expressed on any other individual complement proteins (38). Similar to how ANAs are previously identified serological hallmarks of SLE, everything in this chain of events is specific to SLE, which is why analyzing the iC3b-NEO neoantigen can accurately diagnose the disease. The association between iC3b-NEO and SLE patients was first established in a research paper written in 1989, examining the clinical significance of iC3b-NEO neoantigen (39). Since iC3b is the result of the cleaved C3b protein in the complement system, the study utilized radioimmunoassay (RIA) techniques to quantitatively measure C3 fragments in the SLE patients' plasma. The study compared 40 untreated SLE patients to 36 randomly selected controls; the results indicated significantly higher average iC3b-NEO level in SLE patients than in controls. Although these results are dated and have not been further investigated to date, they provide valuable guidance for future SLE research.

Three different assays can be used to test for iC3b-NEO to more accurately diagnose SLE; testing for iC3b specific T cells, detection of iC3b-NEO neoantigen presented by APCs, and detection of iC3b-NEO neoantigen in the blood. To test whether patients have iC3b specific T cells, immortalized dendritic cells can be used as model APCs and loaded with iC3b-NEO neoantigen. Next, T lymphocytes from the blood of a patient are mixed with

these iC3b-NEO loaded DCs. Since T-cells are antigen specific, they will only bind onto their corresponding antigens. If the T-cells successfully bind with the transformed dendritic cells, it is an indication that the patients' bodies are actively producing T-cells for this neoantigen. Therefore, a positive SLE diagnosis can be reasonably made. (Figure 3).

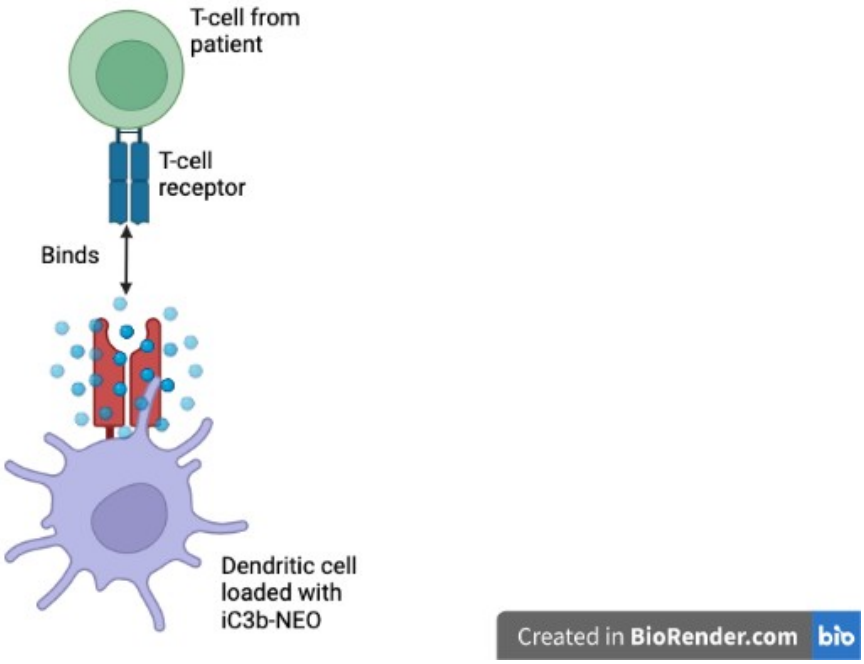

Figure 3. T-lymphocyte from patient binding with dendritic cell, Created with BioRender.com

A second possible test is to determine whether iC3b-NEO is presented by APCs from patients' blood. APCs are extracted from the patient's blood, and combined with immortalized iC3b specific T-cells. If this step of the examination process indicates that the patients' own APCs are also presenting iC3b specific entities, the case for a positive SLE diagnosis becomes stronger.

For both assays, the presence of iC3b-NEO can be determined by Fluorescence-Activated Cell Sorting (FACS) or by ELISA immunoassay (40,41). iC3b-NEO has a high correlation with SLE, and may be a biomarker warranting further research.

Conclusion

Accurately diagnosing SLE continues to be challenging despite decades of research. Although significant progress has been made with the adoption of the EULAR-ACR-2019 criteria, AI classification algorithms, biomarker panels, identification specific proteins and components of the complement pathway, and calculation of GRS from GWAS studies, there still remains a chance of false diagnosis. In this case, false diagnosis could mean not identifying SLE despite pathological,

immunological or other signs/symptoms and instead, assigning an incorrect diagnosis. The inflammatory skin diseases mentioned above are very similar to each other, and there are evidence suggesting they have similar etiology. An incorrect diagnosis is devastating as it can lead to inappropriate treatment and worsening or progression of the disease. It is certainly necessary to conduct more studies into differentiating different inflammatory skin diseases, including SLE, on a fundamental level with high sensitivity and selectivity. It is anticipated that in the near future, the utilization of genetic risk scores in conjunction with biomarker panels and EULAR-ACR-2019 criteria will significantly increase diagnostic accuracy. The presence of certain complement immune system proteins and/or their fragments or derivatives such as C3dg, iC3b and/or iC3b-NEO can be incorporated into biomarker panels. Cross-ethnic common diagnostic attributes such as polymorphisms at the 16p13 region on chromosome 16 can be exploited to accurately diagnose the disease regardless of patient ethnicity. Concurrent utilization of these diagnostic tools is expected to significantly increase the sensitivity and sepecificity of SLE diagnosis.

Author Contributions

E.Y. researched and wrote the paper. M.S.S. supervised the writing of the paper.

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