Evolving Concepts in Systemic Lupus Erythematosus

Antonis Fanouriakis MD[1,2], Irini Gergianaki MD[2], Prodromos Sidiropoulos MD PhD[2], George Bertsias MD PhD[2,3] and Dimitrios T. Boumpas MD PhD[1,3,4,5]

[1]4th Department of Internal Medicine, Attikon University Hospital, Medical School, National and Kapodestrian University of Athens, Athens, Greece
[2]Department of Rheumatology, Clinical Immunology and Allergy, University Hospital of Heraklion, Heraklion, Greece
[3]Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology-Hellas, Voutes, Heraklion, Greece
[4]Laboratory of Autoimmunity and Inflammation, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[5]Joint Academic Rheumatology Program, Medical School, National and Kapodestrian University of Athens, Athens,Greece

KEY WORDS: systemic lupus erythematosus (SLE), treat-to-target (T2T), physician global assessment (PGA)

IMAJ 2016; 18: 749–753

Systemic lupus erythematosus (SLE) is the prototype human systemic autoimmune disease [1]. With the creation of the medical school in Crete in the 1990s and operation of the University Rheumatology Clinic at the University Hospital in Heraklion, we pursued the study of SLE by taking advantage of the unique aspects of the island. Crete has a genetically homogeneous population of approximately 600,000 inhabitants with minimal mobility outside the island (for work or medical reasons), a single tertiary referral center with excellent connectivity with primary and secondary care on the island, and internationally renowned institutes for biomedical research (University of Crete and the Foundation of Research and Technology in Hellas). Thus, Crete offers the opportunity to study the epidemiology, etiopathogenesis and natural history of complex diseases such as SLE.

SLE in the community may be more prevalent than previously believed. Strict adherence to SLE classification criteria can result in significant delays diagnosing the disease

EARLY DIAGNOSIS

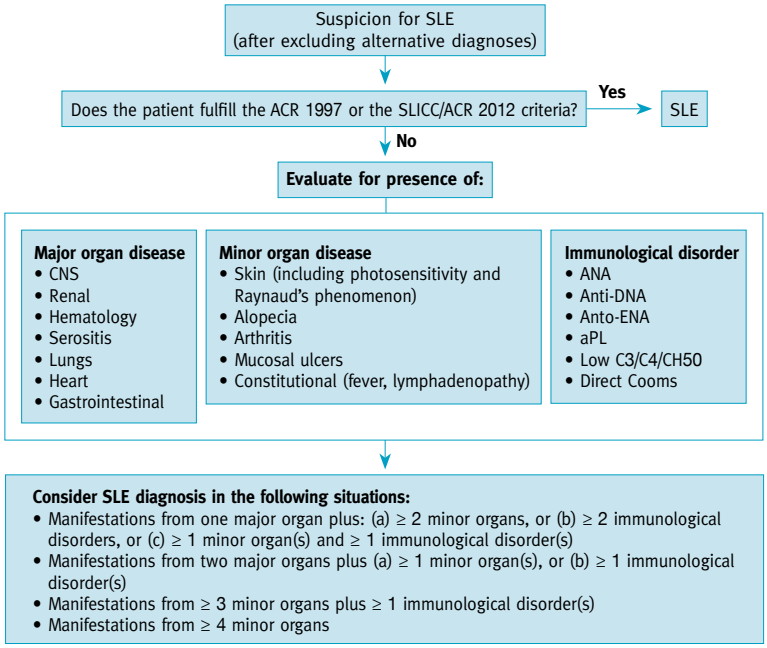
SLE is a multi-organ disease with protean manifestations. Being uncommon and heterogeneous, its diagnosis can pose a considerable challenge, especially for clinicians with limited expertise in the disease. This is more evident at the early stages of the disease when the number of features to secure the diagnosis are insufficient, and in patients presenting with atypical or uncommon features that can nonetheless be severe and require prompt treatment [2]. The suboptimal performance of immunological testing in patients referred for possible SLE is an additional problem, further delaying diagnosis. As a result, SLE remains largely a clinical diagnosis after excluding alternative possibilities. Classification criteria for SLE – namely those of the ACR (American College of Rheumatologists) [3] or the more recent SLICC (Systemic Lupus International Collaborating Clinics) [4] – are often used for diagnostic purposes, but strict adherence to these criteria could delay diagnosis in 20–30% of patients [5]. Importantly, the SLICC criteria have introduced the possibility of organ-dominant disease, in the case of nephritis. While waiting for diagnostic criteria, we have proposed interim potential solutions to facilitate its diagnosis [2] [Figure 1].

LIMITATIONS OF THE CLASSIFICATION CRITERIA FOR DIAGNOSIS

There are some caveats in the application of the ACR or the SLICC criteria for diagnostic purposes in patients with early disease. Some systems, such as the mucocutaneous, are over-represented in the criteria, and generally, all features (with the exception of biopsy-proven nephritis in the SLICC criteria) contribute equally to classification without any weighting based on sensitivity or specificity for each individual criterion. Some patients, including those with major organ involvement, may have SLE disease manifestations for years before fulfilling the classification criteria. In individuals with typical features of SLE but low-positive or negative antinuclear antibody (ANA) results, the clinician should not hesitate to establish the diagnosis of SLE after excluding other diseases [6]. Importantly, the diagnostic utility of some tests or features of the disease might be reduced when applied to patients presenting to primary care centers with vague complaints resembling connective tissue disease. In our experience, an important clinical problem that diagnostic criteria could address is the diagnosis of patients with major organ involvement who are seen by clinicians who are not experts in SLE. In such patients, substantial delays in the initiation of treatment due to strict adherence to classification criteria could affect the outcome. For these cases, we propose an approach that takes into consideration the strengths of both classification systems and incorporates common sense and clinical experience [Figure 1]. On the other hand, a considerable number of patients with features suggestive of SLE might never develop the disease.

This paper was presented at the 1st Israel-Greece Meeting on Advances in Rheumatology and Autoimmunity in Haifa in October 2015

Figure 1. Proposed algorithm for the diagnosis of SLE (modified from [2])



In such cases, we recommend use of the term undifferentiated connective tissue disease (uCTD). These patients should be reassured that their prognosis is usually excellent.

PATTERNS OF DISEASE ACTIVITY, BURDEN AND COST ASPECTS

The economic crisis that began in 2008 has posed significant challenges to public health policies. This is more evident in southern European countries, where policy makers are under pressure to cut public spending on health and/or reallocate resources within the health system, sparing funds for innovative drugs covering unmet needs of conventional therapy. Analyses of the medical and economic burden of chronic disorders, such as SLE, are valuable for clinical and health policy decisions. We recently performed a national chart-based review of 215 adult SLE patients with active autoantibody-positive disease at a predefined ratio of 30% severe (involvement of major organs requiring treatment) and 70% non-severe disease, followed in seven mixed secondary/tertiary hospital centers in Greece [7]. In an ancillary study, we reviewed 318 SLE patients consecutively registered over 3 months. Disease activity, organ damage, flares, and health care resource utilization were recorded, with costs assessed from the third-party payer perspective. Severe SLE patients exhibited a chronic active disease pattern more frequently (22.4% versus 4.7%), higher average SLEDAI (10.5 vs. 6.1) and SLICC damage index (1.1 vs. 0.6) than non-severe patients. Medications, inpatient stays, laboratory investigations, day hospitalizations, biopsies/imaging tests, and specialist visits represented 51.7%, 33.8%, 7.9%, 2.7%, 2.6%, and 1.2% of the total medical cost respectively [7]. The mean annual direct medical cost was €3741 in severe versus €1225 in non-severe patients [7]. As expected, severe flares, active renal disease, and organ damage were independent cost predictors. In the sub-study, 19% of unselected patients were classified as severe SLE, and 30% of them had chronic active disease. These data indicate a significant clinical and financial burden of Greek SLE patients with active major organ disease, with 30% displaying chronic activity despite standard-of-care treatment with glucocorticoids, antimalarials and immunosuppressive agents. We are currently addressing the impact of biologic therapy with belimumab or rituximab on the management of these patients.

Attribution models can assist physicians in attributing neuropsychiatric manifestations to SLE or not

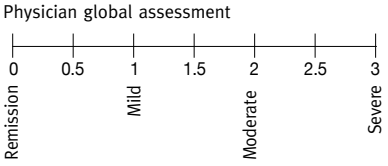
TARGETS OF THERAPY AND TREAT-TO-TARGET

Despite major improvements in care during the past three decades, SLE patients still experience nearly threefold increased mortality rates when compared with the general population, with irreversible organ dysfunction (damage) developing in almost 50% of SLE patients after 5–10 years of disease [8,9]. According to recent data, that damage is a powerful predictor of further damage accrual and mortality [10,11]. Disease flares, glucocorticoid use and persistent disease activity are major drivers of damage. Both major and mild-to-moderate flares contribute to organ damage accrual [12,13]. Chronic glucocorticoid intake – especially at dosage ≥ 7.5 mg/day of prednisone equivalent – is a major cause of organ damage in SLE. Important co-morbidities in SLE include infectious complications, cardiovascular disease, osteoporosis and malignancies. Because of pain, fatigue and depression, SLE patients experienced a reduced health-related quality of life.

In rheumatoid arthritis, well-designed controlled studies have shown improved long-term functional and structural outcomes with intensive management aiming at a pre-specified treatment target compared with usual care (treat-to-target, T2T). We participated in an international task force that has recently developed T2T recommendations for SLE patients following an evidence-based and expert-opinion approach [14]. A high level of agreement among experts was reached. In the recommendations, it is proposed that treatment of SLE should aim at remission or low disease activity, prevention of flares and damage accrual, improvement of health-related quality of life, minimization of glucocorticoid exposure, and prevention of antiphospholipid syndrome-related and other co-morbidities. Because of disease heterogeneity, measurable treatment targets for each different affected organ are needed [15]. With the exception of nephritis, such targets have not yet been clearly defined. Importantly, in extra-renal lupus, there is currently little evidence to suggest that targeting remission leads to significantly better outcomes when compared with low disease activity.

It is not yet clear whether application of T2T in routine practice should be based upon a battery of different treatment targets (i.e., one for each different organ) or a single, composite lupus activity index. Existing clinical tools for assessment of disease activity and damage suffer from inherent shortcomings and need optimization. Based on our experience, we recommend the use of SLEDAI (version 2K), coupled with the physician global assessment (PGA) in routine assessment of SLE patients [15] [Figure 2].

Figure 2. Proposed monitoring of SLE disease activity in routine clinical practice



SLEDAI-2K

8		Seizure	4		Proteinuria	4		Arthritis
8		Psychosis	4		Pyuria	4		Myositis
8		Organic brain syndrome	2		New rash	4		Urinary casts
8		Visual disturbance	2		Alopecia	4		Hematuria
8		Cranial nerve disorder	2		Mucosal ulcers	2		Increased DNA binding
8		Lupus headache	2		Pleurisy	1		Fever
8		CVA	2		Pericarditis	1		Thrombocytopenia
8		Vasculitis	2		Low complement	1		Leukopenia

NEUROPSYCHIATRIC LUPUS ASCERTAINMENT AND TREATMENT

Neuropsychiatric systemic lupus erythematosus (NPSLE) represents one of the most challenging aspects of the disease, owing to its wide heterogeneity, lack of specificity of certain clinical syndromes, and a paucity of high quality data to guide therapeutic decisions [16]. To better understand this entity, we have established a registry of SLE patients with neuropsychiatric manifestations (212 manifestations experienced by 178 patients, both attributed and non-attributed to SLE) to study issues regarding attribution, diagnosis and management. Attribution of a neuropsychiatric manifestation to lupus relies largely on physician judgment, based on various clinical, serologic and neuroimaging parameters. In centers with less experience in the disease, attribution models may be helpful in guiding physicians regarding attribution. Such models have been proposed by the SLICC group or, more recently, the Italian Study Group on NPSLE [17]. We compared attribution as per physician judgment to these models and found that the Italian model shows better correlation and could be used in centers with less experience in the disease [18]. A multicenter validation of this model is currently underway.

High-throughput "omics" technologies will enable the molecular taxonomy of SLE towards the application of targeted therapies

Demyelination may occur in SLE and its differential diagnosis from multiple sclerosis (MS) is often practically impossible. During the characterization of our NPSLE cohort, we identified nine patients fulfilling criteria for both diseases, SLE and MS [19]. In these patients, SLE generally followed a benign course and treatment decisions were mainly guided by the neurologic manifestations of MS. An accompanying systematic literature review confirmed the under-recognition of this overlap syndrome to date. The rarity of demyelinating syndrome in SLE in various cohorts, its resemblance to frank MS, and the increased sensitivity of the recent diagnostic criteria for the latter suggest that demyelinating syndromes in SLE may actually represent a coexistence of these two disorders [19].

The European League Against Rheumatism (EULAR) recommendations for NPSLE, which we coordinated and published in 2010, address multiple issues regarding diagnosis and management using the combination of an evidence-based approach and expert consensus [20]. Since guidelines may not capture all aspects of clinical practice, we compared routine clinical care with the EULAR recommendations in two European centers, with the help of Cristina Pamfil, a EULAR scholar from Cluj-Napoca, Romania. We found, overall, good concordance rates (60–70%) between our practice and the recommendations, with no difference regarding the period before or after their issuance [21]. This observation supported the feasibility and pragmatism of the recommendations and ensured that management of NPSLE – at least in centers with expertise – is mostly in accordance with the state of the art. In terms of therapy (largely empirical in NPSLE), we published our retrospective experience regarding the use of cyclophosphamide (CYC) in this setting. Among a total of 50 neuropsychiatric manifestations, almost 85% of patients experienced partial or complete response of the symptoms that prompted the use of CYC [22].

PATHOGENESIS OF SLE

- **Innate immune mechanisms, TLRs, neutrophils and immune regulation**

While T cell-mediated help via cell-cell contact or soluble factors predominantly drives the autoantibody production in lupus, nucleic acids released by apoptotic cells may further increase their production via Toll-like receptors (TLRs) [23]. We found increased TLR-9 expression and function in memory CD27+ B cells of patients with active lupus, which correlated with anti-DNA antibodies production [24,25]. Importantly, increased TLR expression was also detected in kidney biopsies of lupus nephritis patients, suggesting that stimulation of these TLRs may amplify inflammatory responses within the

kidney [26]. In other studies, we also documented aberrant expression and function of the T cell negative co-stimulatory molecule programmed death-1 (PD-1) in SLE patients and we identified PD-1 gene polymorphisms that decrease its transcriptional activity and suppressive function [27]. Notably, we found increased expression of PD-1 ligands (PD-L1, PD-L2) in renal tubular epithelial and mesangial cells in patients with active proliferative lupus nephritis, suggesting an important role of PD-1/PD-1 ligands in regulating local immune responses. On the other hand, increased interleukin (IL)-21 production by activated CD4+ T cells in SLE may enhance B cell differentiation into Ig-secreting plasma cells [28]. More recently we showed elimination of granulocytic myeloid-derived suppressor cells (MDSCs) in lupus-prone mice due to inflammation-driven reactive oxygen species-dependent extracellular trap formation [29]. Together, these data provide a cellular and molecular basis for the defective immune tolerance in SLE.

- **The transcriptomic architecture of SLE**

Genome-wide association studies (GWAS) have identified more than 40 disease-associated loci, together accounting for less than 20% of disease heritability. Gene expression represents the intermediate phenotype between DNA and disease phenotypic variation and provides insights regarding genetic and epigenetic effects [30,31]. Deregulation of genes involved in type I interferon signaling is a consistent finding in the peripheral blood of active and severe SLE patients [32]. Up-regulation of granulocyte-specific transcripts especially in bone marrow mononuclear cells (BMMCs), and of myeloid lineage transcripts in lupus nephritis, provide evidence for a pathogenic role of these cell subsets. Gene network analysis in BMMCs identified central gene regulators, which could represent therapeutic targets and a high similarity between SLE and non-Hodgkin lymphoma, providing a molecular basis for the reported association of the two diseases [33]. Gene expression abnormalities driven by deregulated expression of certain microRNAs (miRs) in SLE contribute to interferon production, T and B cell hyperactivity, DNA hypomethylation, and defective tissue response to injury. Methylation arrays have revealed alterations in white blood cell DNA methylation in SLE, suggesting an important role of epigenetics and the environment.

Having realized the complexity of the disease, when high-throughput technologies became available, we adopted a *bottom-down* approach in our work using a comprehensive transcriptomic analysis (cDNA and miR arrays and next-generation sequencing) in peripheral blood, bone marrow and renal tissue of lupus patients. In addition to genes related to the interferon pathway, our cDNA microarrays revealed a strong neutrophilic signature with genes related to granulopoiesis, apoptosis and autophagy [33]. Gene network analysis of BMMCs revealed activation of multiple kinase pathways in human SLE [34] including tpl2 kinase, whose inhibition in vivo in animal models ameliorated autoimmune thrombocytopenia [35]. MiR analysis in peripheral blood identified several miRs that strongly correlate with disease activity. Exploration of the top up-regulated miR-21 showed that it contributes to aberrant T cell responses in lupus, through down-regulation of PDCD4 expression [36]. Of note, over-expression of miR-21 in healthy T cells led to acquisition of the lupus-like phenotype with increased cell proliferation, CD40-ligand membrane expression, IL-10 secretion and promotion of B cell differentiation in mixed lymphocyte reactions. MiR analysis of kidney biopsies in human lupus nephritis demonstrated up-regulated miR-422a, which drives reduction of the renoprotective protein kallikrein-related peptidase 4, suggesting that its inhibition through antagomirs might have a beneficial effect in lupus nephritis [37]. Interestingly, increased intra-renal miR-422a levels correlated with the presence of active fibrinoid necrosis, a histological finding associated with poor renal survival and, therefore, could be used as a disease biomarker. Based on leads from the aforementioned studies, we are currently studying autophagy and NETosis in lupus neutrophils and their contribution to renal injury.

More recently, we have performed RNA sequencing in peripheral blood mononuclear cells (PBMCs) from 150 SLE patients with diverse clinical manifestations (renal, neuropsychiatric disease, etc). In the comparison of active versus inactive nephritis SLE patients, we identified several differentially expressed genes involved in patho/physiologically plausible pathways as interferon signaling, chemotaxis of monocytes, oxidative phosphorylation, dendritic cell maturation, angiogenesis and MAPK pathway. A deconvolution process from existing data and RNA sequencing studies in PBMC subsets (such as low density neutrophils, B cells, CD4+ T cells and monocytes) is in progress.

CONCLUDING REMARKS AND PERSPECTIVE

Both clinically and biologically, SLE is a heterogeneous disease. There is a currently unmet need to develop a taxonomy that integrates knowledge of immune and other effector pathways, the clinical and immunological expression of the disease, and its response to treatment. Novel technologies, such as next-generation sequencing, hold the promise of enabling the molecular taxonomy of the disease. This molecular taxonomy would facilitate the conduct of trials of targeted therapy for molecularly defined patient subsets.

Acknowledgments

We wish to thank all the rheumatologists in Crete who referred patients for the completion of this project (E. Kouroumali, M. Mamoulaki, C. Linardakis, J. Kalitsakis, A. Pompieri, N. Kougas, I. Papalopoulos, K. Androulakis, I. Lasithiotakis, M. Papadakis, E. Kteniadaki, P. Katzakis), the nurses of the Department of Rheumatology and the patients for their contribution to this work.

This paper is dedicated to the loving memory of the late Dr. Heraklis D. Kritikos (1952–2010), Associate Director of the Department of Rheumatology and Clinical Immunology, University Hospital of Crete, whose vision and dedication continue to inspire us

Correspondence
Dr. D.T. Boumpas
4th Department of Internal Medicine, Attikon University Hospital, Medical School, 1 Rimini St 124 62, National and Kapodestrian University of Athens, Greece
Phone: (30-210) 583-2021
email: boumpasd@uoc.gr

References

1. Lisnevskaia L, Murphy G, Isenberg D. Systemic lupus erythematosus. *Lancet* 2014; 384: 1878-88.
2. Bertsias GK, Pamfil C, Fanouriakis A, Boumpas DT. Diagnostic criteria for systemic lupus erythematosus: has the time come? *Nat Rev Rheumatol* 2013; 9: 687-94.
3. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
4. Petri M, Orbai AM, Alarcon GS, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012; 64: 2677-86.
5. Pons-Estel GJ, Wojdyla D, McGwin G Jr, et al. The American College of Rheumatology and the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus in two multiethnic cohorts: a commentary. *Lupus* 2014; 23: 3-9.
6. Olsen NJ, Karp DR. Autoantibodies and SLE: the threshold for disease. *Nat Rev Rheumatol* 2014; 10: 181-6.
7. Bertsias G, Karampli E, Sidiropoulos P, et al. Clinical and financial burden of SLE in Greece: a nation-wide, multi-centre study. *Ann Rheum Dis* 2015; 74: 571.
8. Sutton EJ, Davidson JE, Bruce IN. The systemic lupus international collaborating clinics (SLICC) damage index: a systematic literature review. *Semin Arthritis Rheum* 2013; 43: 352-61.
9. Urowitz MB, Gladman DD, Ibanez D, et al. Evolution of disease burden over five years in a multicenter inception systemic lupus erythematosus cohort. *Arthritis Care Res* (Hoboken) 2012; 64: 132-7.
10. Bruce IN, O'Keeffe AG, Farewell V, et al. Factors associated with damage accrual in patients with systemic lupus erythematosus: results from the Systemic Lupus International Collaborating Clinics (SLICC) Inception Cohort. *Ann Rheum Dis* 2015; 74 (9): 1706-13.
11. Rahman P, Gladman DD, Urowitz MB, Hallett D, Tam LS. Early damage as measured by the SLICC/ACR damage index is a predictor of mortality in systemic lupus erythematosus. *Lupus* 2001; 10: 93-6.
12. Nikpour M, Urowitz MB, Ibanez D, Gladman DD. Frequency and determinants of flare and persistently active disease in systemic lupus erythematosus. *Arthritis Rheum* 2009; 61: 1152-8.
13. Ugarte-Gil MF, Acevedo-Vasquez E, Alarcon GS, et al. The number of flares patients experience impacts on damage accrual in systemic lupus erythematosus: data from a multiethnic Latin American cohort. *Ann Rheum Dis* 2014; 74: 1019-23.
14. van Vollenhoven RF, Mosca M, Bertsias G, et al. Treat-to-target in systemic lupus erythematosus: recommendations from an international task force. *Ann Rheum Dis* 2014; 73: 958-67.
15. Fanouriakis A, Bertsias G. Treat-to-target in lupus: what does the future hold? *Int J Clin Rheumatol* 2015; 10: 461-77.
16. Sciascia S, Bertolaccini ML, Baldovino S, et al. Central nervous system involvement in systemic lupus erythematosus: overview on classification criteria. *Autoimmun Rev* 2012; 12: 426-9.
17. Bortoluzzi A, Scire CA, Bombardieri S, et al. Development and validation of a new algorithm for attribution of neuropsychiatric events in systemic lupus erythematosus. *Rheumatology* (Oxford) 2015; 54: 891-8.
18. Fanouriakis A, Pamfil C, Rednic S, et al. Is it primary neuropsychiatric systemic lupus erythematosus? Performance of existing attribution models using physician judgment as the gold standard. *Clin Exp Rheumatol* 2016; 34 (5): 910-17.
19. Fanouriakis A, Mastorodemos V, Pamfil C, et al. Coexistence of systemic lupus erythematosus and multiple sclerosis: prevalence, clinical characteristics, and natural history. *Semin Arthritis Rheum* 2013; 43: 751-8.
20. Bertsias GK, Ioannidis JP, Aringer M, et al. EULAR recommendations for the management of systemic lupus erythematosus with neuropsychiatric manifestations: report of a task force of the EULAR standing committee for clinical affairs. *Ann R*heum Dis 2010; 69: 2074-82.
21. Pamfil C, Fanouriakis A, Damian L, *et al.* EULAR recommendations for neuropsychiatric systemic lupus erythematosus vs usual care: results from two European centres. *Rheumatology* (Oxford) 2015; 54: 1270-8.
22. Fanouriakis A, Pamfil C, Sidiropoulos P, et al. Cyclophosphamide in combination with glucocorticoids for severe neuropsychiatric systemic lupus erythematosus: a retrospective, observational two-centre study. *Lupus* 2016; 25: 627-36.
23. Gianchecchi E, Fierabracci A. Gene/environment interactions in the pathogenesis of autoimmunity: new insights on the role of Toll-like receptors. *Autoimmun Rev* 2015; 14: 971-83.
24. Papadimitraki ED, Bertsias GK, Boumpas DT. Toll like receptors and autoimmunity: a critical appraisal. *J Autoimmun* 2007; 29: 310-18.
25. Papadimitraki ED, Choulaki C, Koutala E, et al. Expansion of toll-like receptor 9-expressing B cells in active systemic lupus erythematosus: implications for the induction and maintenance of the autoimmune process. *Arthritis Rheum* 2006; 54: 3601-11.
26. Papadimitraki ED, Tzardi M, Bertsias G, Sotsiou E, Boumpas DT. Glomerular expression of toll-like receptor-9 in lupus nephritis but not in normal kidneys: implications for the amplification of the inflammatory response. *Lupus* 2009; 18: 831-5.
27. Bertsias GK, Nakou M, Choulaki C, et al. Genetic, immunologic, and immunohistochemical analysis of the programmed death 1/programmed death ligand 1 pathway in human systemic lupus erythematosus. *Arthritis Rheum* 2009; 60: 207-18.
28. Nakou M, Papadimitraki ED, Fanouriakis A, et al. Interleukin-21 is increased in active systemic lupus erythematosus patients and contributes to the generation of plasma B cells. *Clin Exp Rheumatol* 2013; 31: 172-9.
29. Vlachou K, Mintzas K, Glymenaki M, et al. Elimination of granulocytic myeloid-derived suppressor cells in lupus-prone mice linked to reactive oxygen species-dependent extracellular trap formation. *Arthritis Rheum* 2016; 68: 449-61.
30. Frangou EA, Bertsias GK, Boumpas DT. Gene expression and regulation in systemic lupus erythematosus. *Eur J Clin Invest* 2013; 43: 1084-96.
31. Zhang Z, Zhang R. Epigenetics in autoimmune diseases: Pathogenesis and prospects for therapy. *Autoimmun Rev* 2015; 14: 854-63.
32. Bennett L, Palucka AK, Arce E, et al. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J Exp Med* 2003; 197: 711-23.
33. Nakou M, Knowlton N, Frank MB, et al. Gene expression in systemic lupus erythematosus: bone marrow analysis differentiates active from inactive disease and reveals apoptosis and granulopoiesis signatures. *Arthritis Rheum* 2008; 58: 3541-9.
34. Nakou M, Bertsias G, Stagakis I, et al. Gene network analysis of bone marrow mononuclear cells reveals activation of multiple kinase pathways in human systemic lupus erythematosus. *PLoS One* 2010; 5: e13351.
35. Kyrmizi I, Ioannou M, Hatziapostolou M, et al. Tpl2 kinase regulates FcgammaR signaling and immune thrombocytopenia in mice. *J Leukoc Biol* 2013; 94: 751-7.
36. Stagakis E, Bertsias G, Verginis P, et al. Identification of novel microRNA signatures linked to human lupus disease activity and pathogenesis: miR-21 regulates aberrant T cell responses through regulation of PDCD4 expression. *Ann Rheum Dis* 2011; 70: 1496-506.
37. Krasoudaki E, Banos A, Stagakis E, et al. Micro-RNA analysis of renal biopsies in human lupus nephritis demonstrates up-regulated miR-422a driving reduction of kallikrein-related peptidase 4. *Nephrol Dial Transplant* 2015; 31 (10): 1676-86.

"Those who can make you believe absurdities can make you commit atrocities"

Voltaire (1694-1778), French Enlightenment writer, historian, and philosopher famous for his wit, his attacks on the established Catholic Church, and his advocacy of freedom of religion, freedom of speech, and separation of church and state