DETECTION OF ANTI-CELL MEMBRANE DNA ANTIBODIES IN SLE PATIENTS WITH INDIRECT IMMUNOFLUORESCENT AND ELISA TECHNIQUES

By

FATEN NURUL AMIRA AWANG KECHIK

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DETECTION OF ANTI-CELL MEMBRANE DNA ANTIBODIES IN SLE PATIENTS WITH INDIRECT IMMUNOFLUORESCENT AND ELISA TECHNIQUES

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FATEN NURUL AMIRA BINTI AWANG KECHIK

December 2021

Chair : Hasni Mahayidin , MD, MPath
Faculty : Medicine and Health Sciences

Systemic lupus erythematosus (SLE) is known for its wide range of clinical manifestations. The diagnosis of SLE remains a challenge and to a great extent depends on multiple serum autoantibodies such as anti-nuclear antibody (ANA), anti-double stranded (ds) DNA antibody and anti-Smith antibody. ANA is a very sensitive but not specific marker and primarily use for SLE screening. Anti-dsDNA and anti-Sm are SLE-specific autoantibodies with lower sensitivity of 80% and 30% for SLE, respectively. A substantial percentage of SLE patients were found to be persistently negative for SLE-specific autoantibodies. It was reported to be as high as 51.2% for anti-dsDNA and 62.4% for anti-Sm. This impediment can delay the establishment of diagnosis and treatment. Therefore, researchers continue to search for other biomarkers that are better or able to complement the available standard SLE investigations. Cell membrane DNA (cmDNA) was identified as a specific target for autoantibodies in SLE patients. Autoantibodies towards cmDNA (anti-cmDNA) were shown to have promising value as an SLE biomarker. This study evaluated the potential of serum anti-cmDNA antibodies detection using indirect immunofluorescent (IIF) technique as a diagnostic marker in SLE. This study included serum samples of 83 SLE, 86 other connective tissue diseases (OCTD) and 61 healthy subjects. The OCTD samples were 56 rheumatoid arthritis, 12 scleroderma, 10 Sjogren's syndrome and eight mixed connected tissues disease (MCTD). All samples were analysed by both IIF technique utilising Raji cells as substrate and ELISA for the presence of anti-cmDNA. For IIF, anti-cmDNA was reported as positive if there was presence of cell membrane continuous or punctate fluorescent ring. For ELISA, anti-cmDNA positivity was determined according to the cut-off value identified using ROC curve analysis. Serums from SLE patients were also tested for anti-dsDNA and anti-Sm antibodies using enzyme-immunoassays. These findings showed that anti-cmDNA positivity by IIF was the highest in SLE (55.4%) than in OCTD (9.3%) and healthy subjects (0%). Detection of anti-cmDNA using IIF technique showed high

specificity in differentiating between SLE from healthy subject (100%) and OCTD (90.7%). The sensitivity of anti-cmDNA in differentiating between SLE from both groups was the same (55.4%). Anti-cmDNA was shown to be significantly associated with arthritis (p=0.019). However, no significant associations were found between anti-cmDNA and other SLE clinical presentations (mucocutaneous, serositis, lupus nephritis, neurological and haematological involvement). Despite, SLE-associated autoantibodies (ANA, anti-dsDNA and anti-Sm) were also more frequently seen in anti-cmDNA positive SLE, they were not statistically significant. In SLE with negative specific autoantibody, anti-cmDNA was detected in up to 52.1% of SLE patients with negative anti-Sm, 36.8% of SLE patients with negative anti-dsDNA and 31.3% of SLE patients with negative both anti-Sm and anti-dsDNA. Anti-cmDNA detection using ELISA was found to be more sensitive at 95.2% and 97.6% but less specific at 88.5% and 86.0% in differentiating SLE from healthy subjects and OCTD, respectively. In summary, IIF technique provided a high specificity for anti-cmDNA detection which makes it an excellent confirmatory tool for SLE diagnosis. ELISA technique on the other hand, is more suitable as a screening tool because it has better sensitivity. Anti-cmDNA also has the potential as a new additional biomarker to the current standard SLE autoantibodies especially in SLE with negative anti-dsDNA and/or anti-Sm antibodies.

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PENGESANAN ANTIBODI ANTI-SEL MEMBRAN DNA DALAM KALANGAN PESAKIT SLE MENGUNAKAN TEKNIK *INDIRECT IMMUNOFLUORESCENT* DAN *ELISA*

Oleh

FATEN NURUL AMIRA BINTI AWANG KECHIK

Disember 2021

Pengerusi : Hasni Mahayidin, MD, MPath
Fakulti : Perubatan dan Sains Kesihatan

Lupus eritematosus sistemik (SLE) terkenal dengan kepelbagaian manifestasi klinikal. Penyiasatan bagi SLE masih merupakan suatu cabaran dan banyak bergantung kepada kehadiran pelbagai jenis autoantibodi serum seperti antibodi anti-nuklear (ANA), antibodi anti-*double stranded* (ds) DNA dan antibodi anti-Smith. ANA adalah penanda biologi yang sangat sensitif namun tidak spesifik, dimana ia digunakan terutamanya sebagai ujian saringan SLE. Anti-dsDNA dan anti-Sm adalah autoantibodi spesifik-SLE namun mempunyai sensitiviti yang lebih rendah, masing-masing 80% dan 30%. Selain daripada itu, kajian mendapati bahawa terdapat sebahagian pesakit SLE yang kekal negatif untuk autoantibodi spesifik-SLE. Anti-dsDNA telah dilaporkan negatif sehingga 51.2%, manakala anti-Sm sehingga 62.4% dalam kalangan pesakit SLE. Cabaran ini boleh menyebabkan kelewatan dalam membuat diagnosis serta rawatan. Oleh sebab itu, para penyelidikan terus berusaha untuk mencari penanda biologi baharu yang lebih baik atau boleh melengkapi ujian autoantibodi SLE yang sedia ada. *Cell membrane* DNA (cmDNA) telah dikenal pasti sebagai sasaran spesifik untuk autoantibodi dalam pesakit SLE. Autoantibodi terhadap cmDNA (anti-cmDNA) didapati mempunyai nilai potensi sebagai penanda biologi untuk SLE. Kajian ini menilai potensi pengesanan antibodi serum anti-cmDNA menggunakan teknik *indirect immunofluorescence* (IIF) sebagai penanda diagnostik untuk SLE. Kajian ini menggunakan sampel serum daripada 83 SLE, 86 *other connective tissue disease* (OCTD) dan 61 subjek sihat. Sampel OCTD merangkumi 56 *rheumatoid arthritis*, 12 *scleroderma*, 10 *Sjogren's syndrome* dan lapan *mixed connective tissue disease* (MCTD). Semua sampel dianalisis dengan teknik IIF menggunakan sel Raji sebagai substrat dan ELISA untuk mengenal pasti kehadiran anti-

cmDNA. Bagi teknik IIF, anti-cmDNA akan dilaporkan positif jika terdapat cincin *flourecent* yang *continuous* atau *punctate* pada sel membran. Bagi ELISA, nilai *cut-off* yang dikenalpasti menggunakan *ROC curve* telah digunakan untuk menentukan bacaan positif anti-cmDNA. Serum pesakit SLE juga diuji untuk antibodi anti-dsDNA dan anti-Sm menggunakan *enzyme immunoassay*. Hasil kajian kami menunjukkan bahawa anti-cmDNA positif adalah paling tinggi ada pada SLE (55.4%) berbanding OCTD (9.3%) dan subjek sihat (0%). Pengesanan anti-cmDNA menggunakan teknik IIF menunjukkan spesifisiti yang tinggi dalam membezakan SLE dari subjek sihat (100%) dan OCTD (90.7%). Sensitiviti anti-cmDNA dalam membezakan SLE dari kedua-dua kumpulan adalah sama, iaitu 55.4%. Anti-cmDNA terbukti mempunyai hubungan yang signifikan dengan *arthritis* (p=0.019). Walaupun begitu, tiada hubungan yang signifikan di antara anti-cmDNA dan ciri-ciri klinikal SLE yang lain (mukokutaneus, serositis, nefritis lupus, penglibatan neurologi dan hematologi). Walaupun autoantibodi spesifik-SLE lebih kerap didapati pada pesakit SLE yang positif anti-cmDNA, hubungan mereka adalah tidak signifikan secara statistik. Dalam SLE tanpa kehadiran autoantibodi spesifik-SLE, anti-cmDNA dapat dikesan dalam sebanyak 52.1% SLE yang negatif anti-Sm, 36.8% SLE yang negatif anti-dsDNA and 31.3% SLE negatif kedua-dua autoantibodi anti-Sm dan anti-dsDNA. Pengesanan anti-cmDNA menggunakan ELISA didapati lebih sensitif, iaitu 95.2% dan 97.6% tetapi kurang spesifik iaitu 88.5% dan 86.0% dalam membezakan SLE dari subjek sihat dan OCTD, masing-masing. Kesimpulannya, teknik IIF memberikan spesifisiti tinggi dalam pengesanan anti-cmDNA yang menjadikannya alat pengesahan yang sangat baik untuk SLE. Manakala teknik ELISA lebih sesuai sebagai alat saringan kena ia mempunyai sensitiviti yang lebih baik. Anti-cmDNA juga merupakan penanda biologi baharu yang berpotensi dan sangat berguna terutamanya untuk SLE yang negatif kepada anti-dsDNA dan/atau anti-Sm.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the Degree of Master of Science. The members of the Supervisory Committee were as follows:

Hasni binti Mahayidin, MD, MPath
Medical Lecturer
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Chairman)

Maha binti Abdullah, PhD
Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

Masriana binti Hassan, PhD
Senior Lecturer
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 14 April 2022

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Name and Matric No.: Faten Nurul Amira Binti Awang Kechik

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Signature: _____
Name of Chairman of Supervisory Committee: Dr. Hasni Mahayidin

Signature: _____
Name of Member of Supervisory Committee: Prof. Dr Maha Abdullah

Signature: _____
Name of Member of Supervisory Committee: Dr. Masriana Hassan

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LIST OF ABBREVIATIONS

SLE	Systemic Lupus Erythematous
RA	Rheumatoid Arthritis
DN	Dermatomyositis
ANA	Anti-Nuclear Antibodies
Anti-dsDNA	Anti-Double Stranded Antibody
Anti-Sm	Anti-Smith Antibodies
EULAR	European League Against Rheumatism
ACR	American College Of Rheumatology
cm-DNA	Cell Membrane DNA
ss-DNA	Single Stranded DNA
IIF	Indirect Immunofluorescence Method
ELISA	Enzyme-Linked Immunosorbent Assay
AIRC	Autoimmune Laboratory
IMR	Institute For Medical Research
Anti-cmDNA	Anti- Cell Membrane DNA
CNS	Central Nervous System
GWAS	Genetic-Wide Association Studies
SLICC	Systemic Lupus International Collaborating Clinics
NSAIDS	Nonsteroidal Anti-Inflammatory Drugs
GC	Glucocorticoids
FITC	Fluorescein Isothiocyanate
RPMI	Roswell Park Memorial Institute Medium
HRP	Horseradish peroxidase

ACPAs	Anti-Citrullinated Protein Antibodies
RF	Rheumatoid Factor
SS	Sjogren's Syndrome
SSc	Systemic Sclerosis
ACA	Anti-Centromere Antibody
DNA	Deoxyribonucleic Acid
U1-RNP	U1 Ribonucleoprotein
ENA	Extractable Nuclear Antigens
PBS	Phosphate Buffer Solutions
EDTA	Ethylenediamine Tetraacetic Acid
ATCC	American Type Culture Collection
OCTD	Other Connective Tissue Disease
MCTD	Mixed Connective Tissue Disease
HCQ	Hydroxychloroquine
RNA	Ribonucleic Acid
TMB	3,3',5,5' Tetramethylebenzidine
SD	Standard Deviation
PPV	Positive Predictive Value
NPV	Negative Predictive Value
AUC	Area Under the Curve
ROC	Receiver Operator Characteristic

CHAPTER 1

INTRODUCTION

1.1 Introduction

Systemic lupus erythematous (SLE) is an autoimmune disease characterised by a multitude of immune responses as a consequence of host antibodies attacking own tissues. Previous studies have concluded that the clinical aetiology of SLE can be derived from multifarious genetic alterations and environmental factors (Ching *et al*, 2012). SLE has a broad range of pathophysiologic mechanisms and clinical presentations. SLE characteristics often overlapped with the other autoimmune diseases such as dermatomyositis (DM) and rheumatoid arthritis (RA) as they possess some similar symptoms and clinical features (Encinas & Kuchroo, 2002). It is imperative that to explore and establish an improved method of differentiating between SLE and other autoimmune diseases in prospect of an effective diagnosis-making process.

To date, SLE diagnosis is often carried out by detecting the presence of serum anti-nuclear antibodies (ANA), anti-double stranded antibody (anti-dsDNA) and anti-Smith antibodies (anti-Sm). This is following the guideline by European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR). ANA is identify in about 78% of SLE patients. However, the drawback of ANA testing is that, it not specific to SLE. ANA can be positive in other autoimmune diseases such as in 14% of rheumatoid arthritis, 74% of Sjogren's syndrome and 87% of scleroderma patients (Tan *et al*, 1997). Anti-dsDNA on the other hand, can only be detected in 75% of SLE patients (Conti *et al*, 2015). Despite its higher specificity, there is still substantial possibility of under-diagnosing SLE. Although highly specific for SLE, anti-Sm is present in only up to 30% of patients (Aganovic-Musinovic *et al*, 2012). Therefore, it is evident that anti-Sm cannot be solely depended upon for diagnosing SLE. The current study aims to further investigate on anti-cell-membrane DNA (anti-cmDNA) antibody which has been described to deliver comparable specificity but with higher sensitivity when compared to anti-dsDNA and anti-Sm antibodies in diagnosing SLE (Ru *et al*, 2015).

Cell membrane DNA (cm-DNA) is derived from cell nucleus before being expressed on cell membrane of the surface of B-lymphocytes as a 17 kb polynucleotide chain and monocytes (Chen *et al*, 2008). The cm-DNA is different from other nuclear DNA in terms of its location, physical properties, and cell cycle synthesis (Lerner *et al*, 1971). Servais et al. in 1998 had demonstrated that cm-DNA can be the specific target for IgG antibodies in SLE and had shown that cm-DNA receptor epitopes are different from ds-DNA and single stranded DNA (ss-DNA) antibodies. The growing body of literature had suggested cm-DNA as the next highly potential biomarker to be used in diagnosing SLE. Anti-cmDNA was shown to have higher sensitivity and specificity compared to anti-dsDNA and anti-Sm antibodies (Tonutti *et al*, 2008; Servais et al, 1998).

1.2 Problem Statement

The clinical uses of serum biomarkers are indispensable for diagnosing SLE. However, the sensitivity of current standard SLE biomarkers are still lacking of their full functions. In addition, current standard SLE biomarkers can also be detected in other diseases, creating a tendency for misdiagnoses. Therefore, a new and enhanced biomarker needs to be explored and identified in order to provide superior diagnostic capacity for SLE. The studies of anti-cmDNA in SLE were rather scant and no similar studies have been done in the South East Asian countries including Malaysia. The SLE disease manifestations and the immunological profile are highly variable between patients of different population and ethnicity. It would be of great interest to see whether similar findings on the potential use of anti-cmDNA in diagnosing SLE can be found in Malaysian population.

This study would provide information on the usefulness of anti-cmDNA in diagnosing SLE. Previous studies have discovered that anti-cmDNA can be detected by IIF technique using Raji cell lines as substrate. This technique can be prepared in-house and compared to other cell lines, Raji cells have demonstrated the strongest expression of cm-DNA. It would be interesting to see if this IIF technique is able to consistently provide a reliable detection of anti-cmDNA and compare its sensitivity and specificity to the ELISA technique, which is relatively more expensive. Besides that, this study would also determine the diagnostic value of anti-cmDNA antibodies in diagnosing SLE compared to the standard autoantibodies (anti-dsDNA and anti-Sm) and determine the proportion of anti-cmDNA in SLE patients negative for SLE standard autoantibodies.

1.3 Objective

1.3.1 General Objective

To evaluate the potential of serum anti-cmDNA antibodies detected using indirect immunofluorescent (IIF) technique as diagnostic marker in systemic lupus erythematous (SLE).

1.3.2 Specific Objectives

1. To determine the proportion of serum anti-cmDNA antibodies among SLE, other connective tissue diseases and healthy subjects.
2. To determine the diagnostic accuracy (sensitivity and specificity) of anti-cmDNA antibodies in SLE.
3. To determine the associations between anti-cmDNA antibodies with SLE clinical manifestations.
4. To determine the proportion of anti-cmDNA antibodies among SLE with negative SLE standard autoantibodies (anti-dsDNA and anti-Sm).
5. To determine and compare the diagnostic accuracy (sensitivity and specificity) provided by IIF and ELISA techniques in detecting anti-cmDNA antibodies.

REFERENCES

Agmon-Levin, N., Damoiseaux, J., Kallenberg, C., Sack, U., Witte, T., Herold, M., Bossuyt, X., Musset, L., Cervera, R., Plaza-Lopez, A. (2014). International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *Ann. Rheum. Dis*. 73: 17–23.

Ahn, S., S., Jung, S., M. , Yoo, J., Lee, S., W., Song, J.,J., Park, Y.,B. (2019) Anti-Smith antibody is associated with disease activity in patients with new-onset systemic lupus erythematosus. *Rheumatol Int*. 39(11):1937-1944. doi: 10.1007/s00296- 019-04445-y. Epub 2019 Sep 24. PMID: 31552434.

Al Sawah, S., Zhang, X., Zhu, B. (2015). Effect of corticosteroid use by dose on the risk of developing organ damage over time in systemic lupus erythematosus the Hopkins Lupus Cohort. *Lupus Sci Med*. 2:e000066.

Arbuckle, M.R., McClain, M.T., Rubertone, M.V., Scofield, R.H., Dennis, G.J., James, J.A., Harley, J.B. (2003) Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med*. 349: 1526-1533.

Alarcón-Segovia, D & Cardiel, M., H. (1980). Comparison between 3 diagnostic criteria for mixed connective tissue disease. Study of 593 patients. *J Rheumatol*. 16 (3):328-34.

Aringer, M., Costenbader, K., Daikh, D. (2019) European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. *Ann Rheum Dis*. 78(9):1151-1159.

Arroyo-Avila, M., Santiago-Casas, Y., McGwin, G., Cantor, R., S., Petri, M., Ramsey-Goldman, R. (2015). Clinical associations of anti-Smith antibodies in PROFILE: a multi-ethnic lupus cohort. *Clin Rheumatol*. 34(7):1217- 23.

Alhajj, M., Farhana, A. (2021) Enzyme Linked Immunosorbent Assay. *StatPearls Publishing*. Avai from: https://www.ncbi.nlm.nih.gov/books/NBK555922/

Attar SM, Koshak EA. Medical conditions associated with a positive anti-double-stranded deoxyribonucleic acid. Saudi Med J. 2010 Jul;31(7):781-7. PMID: 20635012.

Amigues, J., M., Cantagrel, A., Abbal, M., Mazieres, B. (1996). Comparative study of 4 diagnosis criteria sets for mixed connective tissue disease in patients with anti-RNP antibodies. Autoimmunity Group of the Hospitals of Toulouse. *J Rheumatol*.(12):2055-62.

Alina, D., Jurcut, C., Baicus. (2018). The impact of anti-U1-RNP positivity: systemic lupus erythematosus versus mixed connective tissue disease. *Rheumatol Int*. 38(7):1169-1178.

Aganovic-Musinovic, I., Karamehic, J., Zecevic, L., Gavrankapetanovic, F., Avdagic, N., Zaciragic, A., Jukic, T., Grcic, N., & Svrakic, S. (2012). Evaluation of ENA-6 Profile by ELISA Immunoassay in Patients with Systemic Lupus Erythematodes. *Autoimmune diseases*, *2012*, 32:1614.

Bootsma, H., Spronk, P., E., Ter Borg, E., J. (1997). The predictive value of fluctuations in IgM and IgG class anti-dsDNA antibodies for relapses in systemic lupus erythematosus. A prospective long-term observation. *Ann Rheum Dis.* 56 (11): 661-666.

Bennett, J., C. (1996) Plum F, eds. Cecil Textbook of Medicine. 20th ed. Philadelphia, PA: W.B. Saunders Co. 1447.

Bertsias, G.K, Salmon, J.E, Boumpas, D.T. (2010). Therapeutic opportunities in systemic lupus erythematosus: state of the art and prospects for the new decade. *Ann Rheum Dis*. 69:1603–11.

Bertsias, G., Fanouriakis, A., Boumpas, D., T. (2017). Treatment of Systemic Lupus Erythematosus. Firestein GS, Budd RC, Gabriel SE, MacInnes IB, O'Dell JR, eds. Kelley and Firestein's Textbook of Rheumatology., *Pa: Elsevier Saunders*. 10th ed. Philadelphia. 13:68-88.

Bach, M., K. (1962). The incorporation of tritiated thymidine into a microsomal fraction from HeLa cells during short time exposures. *Proc. Nat. Acad. Sci. U.S.A*. 48:1031-1055.

Broder, A., Khattri, S., Patel, R., Putterman, C. (2011). Undertreatment of Disease Activity in Systemic Lupus Erythematosus Patients with Endstage Renal Failure Is Associated with Increased All-cause Mortality. *J Rheumatol*. 38(11) :2382-9.

Borchers, A., T., Naguwa, S., M., Keen, C., L., Gershwin, M., E. (2003). Immunopathogenesis of Sjögren's syndrome. *Clin Rev Allergy Immunol.* 25(1)*:89-104.*

Bennett, R.M., Davis, J., Merritt, M. (1986) Anti DNA expressed on the surface of monocytes and B lymphocytes.*J Rheumatol*. 13:679–85.

Barr, S., G., Zonana-Nacach, A., Magder, L., S. (1999). Patterns of disease activity in systemic lupus erythematosus. *Arthritis Rheum*. 42: 2682.

Brahms, H., Raker, V., A., Van Venrooij, W., J., Luhrmann, R. (1997). A major, novel systemic lupus erythematosus autoantibody class recognizes the E, F, and G Sm snRNP proteins as an E-F-G complex but not in their denatured states. *Arthritis Rheum*. 40(4):672-82.

Bennett, R., M., Cornell, K., A., Merritt, M., J., Bakke, A., C., Hsu, P., H., Hefeneider, S., H. (1991). Autoimmunity to a 28–30 kD cell membrane DNA binding protein: occurrence in selected sera from patients with SLE and mixed connective tissue disease (MCTD). *Clin Exp Immunol*. 86:74–9.

Bolster, M., B., Silver, R., S. (2011). Clinical features of systemic sclerosis. In: Hochberg *et al*. *Editors. Rheumatology*. 5th ed. Philadelphia: Mosby, Elsevier. 1373– 1386.

Barsotti, S., Stagnaro, C., d'Ascanio, A & Della Rossa, A. (2016). One year in review 2016: systemic sclerosis. *Clin Exp Rheumatol*. (34 Suppl) 100 (5):3-13.

Barnes, J., Mayes, M., D. (2012). Epidemiology of systemic sclerosis: incidence,prevalence, survival, risk factors, malignancy, and environmental triggers. *Curr Opin Rheumatol*. 24(2):165-70.

Bono L, Cameron JS, Hicks JA. (1999). The very long-term prognosis and complications of lupus nephritis and its treatment. *Q J Med.* 92:211–8.

Beaufils, M., Kouki, F., Mignon, F., Camus, J., P., Morel-Maroger, L., Richet, Gabrie. (1982). Clinical Significance of Anti-Sm Antibodies in Systemic Lupus Erythematosus. *The American Journal of Medicine.* (74).

Brito-Zerón, P., Baldini, C., Bootsma, H., Bowman, S., J., Jonsson, R., Mariette, X., Sivils, K., Theander, E., Tzioufas, A., Ramos-Casals, M. (2016). Sjögren syndrome. *Nat Rev Dis Primers.* 2:16047. doi: 10.1038/nrdp.2016.47. PMID: 27383445.

Bagby, G., C., Jr., Rigas, V., D., Bennett, R., M., Vandenbark, A., A., Garewal, H., S. (1981). Interaction of lactoferrin, monocytes, and T lymphocyte subsets in the regulation of steady-state granulopoiesis in vitro. *J Clin Invest.* 68(1):56-63. doi: 10.1172/jci110254. PMID: 6972953; PMCID: PMC370772.

Conti, F., Ceccarelli, F., Perricone, C., Massaro, L., Marocchi, E., Miranda, F., Spinelli, F., R., Truglia, S., Alessandri, C., Valesini, Guido. (2015). Systemic Lupus Erythematosus with and without Anti-dsDNA Antibodies: Analysis from a Large Monocentric Cohort. *Mediators of Inflammation*.. (6) Article ID 328078, .

Cooper, G., S., Miller, F., W., Pandey, J., P. (1999). The role of genetic factors in autoimmune disease: implications for environmental research. *Environ Health Perspect.* (Suppl 5) 107 :693–700.

Cooper, G., S., Parks, C., G., Treadwell, E., L., St Clair, E., W., Gilkeson, G., S., Cohen,P., L., Roubey, R., A., Dooley, M., A. (2002). Differences by race, sex and age in the clinical and immunologic features of recently diagnosed systemic lupus erythematosus patients in the southeastern .*United States. Lupus*. 11(3):161-7. doi: 10.1191/0961203302lu161oa. PMID: 11999880.

Carson, V., Lee, E., Y., Hewitt, L. *et al.* (2017). Systematic review of the relationships between physical activity and health indicators in the early years (0-4 years). *BMC Public Health.* 17: 854. https://doi.org/10.1186/s12889-017- 4860-0

Chai, H., C., Phipps, M., E., and Chua, K., H. (2012). Genetic Risk Factors ofSystemicLupus Erythematosus in the Malaysian Population: A Mini review. *Clinical and Developmental Immunology*. (9), Article ID 963730, doi:10.1155/2012/963730.

Cervera, R., Khamashta, M., A., Font, J., Sebastiani, G., D., Gil, A., Lavilla, P., Doménech, I., Aydintug, A., O., Jedryka-Góral, A., de Ramón, E. (1993) Systemic lupus erythematosus: clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. The European Working Party on Systemic Lupus Erythematosus. *Medicine (Baltimore).* 72(2):113-24.

Chifflot, H., Fautrel, B., Sordet, C., Chatelus, E & Sibilia J. (2008). Incidence and prevalence of systemic sclerosis: a systematic literature review. *Semin Arthritis Rheum*. 37(4):223-35.

Cozzani, E,. Drosera, M., Gasparini, G., and Parodi, A. (2014). Serology of lupus erythematosus: correlation between immunopathological features and clinical aspects. *Autoimmune Diseases*. 13.

Cojocaru, M., Cojocaru, I., M., Silosi, I., Vrabie, C., D. (2011). *Maedica (Buchar)*. 6 (4):330-6.

Chen, S., Jia, R.L., Han, L et al. (2001) DNA-associated autoanti- bodies to cell membrane for rapid detection of systemic lupus erythematosus. *Chin J Rheumatol*. 5:227–229.

Chua, K. H., Kee, B. P., Tan, S. Y., and Lian, L. H. (2008). Genetic polymorphisms of interleukin-4 third intron region in the Malaysian patients with systemic lupus erythematosus. *Journal of Medical Sciences.* 8(4): 437–442,

Chen, H.Y., Guo, J.L., Li, Z.G. (2008) Significance of anti-cell membrane-associated DNA (mDNA) antibodies in systemic lupus erythematosus. *Clinical Rheumatology*. 27:183–187.

Ching, K.H., Burbelo, P.D., Tipton, C., Wei, C., Petri, M., et al. (2012) Two Major Autoantibody Clusters in Systemic Lupus Erythematosus. PLOS ONE 7(2): e32001. doi: 10.1371/journal.pone.0032001.

Combe, B. (2009). Progression in early rheumatoid arthritis. *Best Pract Res Clin Rheumatol*. 23:59–69.

Cherie, H., Howard, & Susan L., M. (2006). The Pharmacist's Role in the Treatment of Systemic Lupus Erythematosus, *US Pharm*. 5:39-48.

Dieker, J., W., Van der Vlag, J., Berden, J., H. (2004). Deranged removal of apoptoticcells: its role in the genesis of lupus. *Nephrol. Dial. Transplant*. 19 (2): 282–5.

Didier, K., Bolko, L., Giusti, D., Toquet, S., Robbins, A., Antonicelli, F., & Servettaz, A. (2018). Autoantibodies Associated With Connective Tissue Diseases: What Meaning for Clinicians?. *Frontiers in immunology*, 9:541. https://doi.org/10.3389/fimmu.2018.00541

Damoiseaux, J., G., M, C., and Cohen Tervaert, J., W. (2006). From ANA to ENA: how to proceed?. *Autoimmunity Reviews.* 5 (1):10-17

Encinas, J.A., & Kuchroo, V.K. (2000). Mapping and identification of autoimmunity genes. *Curr Opin Immunol.* 12: 691–697.

El Miedany, Y., Youssef, S., Mehanna, A.,N & El Gaafary, M. (2008). Development ofa scoring system for assessment of outcome of early undifferentiated inflammatory synovitis. *Joint Bone Spine.* 75:155–62.

Egner, W. (2000). The use of laboratory tests in the diagnosis of SLE. *J. Clin. Pathol*. 53 (6): 424–32.

Edwards, C. J., Cooper, C. (2006). Early environmental exposure and the development of lupus. *Lupus*. 15: 814–819.

Fanouriakis, A., Kostopoulou, M., Alunno, A., *et al*. (2019). Update of the EULAR recommendations for the management of systemic lupus erythematosus. *Annals of the Rheumatic Diseases.* 78:736-745.

Eric, L., Greidinger, M., D. (2013). Mixed Connective-Tissue Disease. *Medscape Reference*. http://emedicine.medscape.com/article/335815-overview.

Font, J., Cervera, R., lRamos-Casals, Manue., García-Carrasco, Mario., Sentís, Juan., Herrero, Carme., Olmo, J., A., Darnell, A., Ingelmo, M. (2004). Clusters of clinical and immunologic features in systemic lupus erythematosus: analysis of 600 patients from a single center. *Seminar in Arthritis and Rheumatism*. 33(4): 217-230.

Frank, A. O., (1980). Apparent predisposition to systemic lupus erythematosus in Chinese patients in West Malaysia. *Annals of the Rheumatic Diseases.* 39(3): 266–269.

Fanouriakis, A., Kostopoulou, M., Alunno, A. (2019). Update of the EULAR recommendations for the management of systemic lupus erythematosus. *Ann Rheum Dis*. 78:736.

Finckh, A., Liang, M., H., van Herckenrode, C., M., de Pablo, P. (2006). Long-term impact of early treatment on radiographic progression in rheumatoid arthritis: A meta-analysis. *Arthritis Rheum.* 55:864–72.

Gossec, L., Combescure, C., Rincheval, N. (2010). Relative Clinical influence of Clinical, Laboratory, and Radiological Investigations in Early Arthritis on the Diagnosis of Rheumatoid Arthritis. Data from the French Early Arthritis Cohort ESPOIR. *J Rheumatol.* 37:2486–92.

Guo, J., L., Bi, L., Q., Li, Z., G. (2006). Significance of anti-cell membrane associated DNA antibodies in diagnosis of systemic lupus erythematosus. *Chin J Lab Med*. 9:787–90.

Guo, Q., Wang, Y., Xu, D., Nossent, J., Pavlos, N., J., & Xu, J., K. (2018). Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. *Bone Res*, 6:15, https://doi.org/10.1038/s41413-018-0016-9

Greidinger, E., L. (2016). Overlap Syndromes, In: Systemic Lupus Erythematosus: Basic, Applied, and Clinical Aspects, Tsokos G Ed., *New York: Elsevier*.

Gaubitz, M. (2006). Epidemiology of connective tissue disorders. *Rheumatology (Oxford)*. 45(iii): 3–4.

Grygiel-Górniak, B., Rogacka, N., & Puszczewicz, M. (2018). Antinuclear antibodies in healthy people and non-rheumatic diseases - diagnostic and clinical implications. *Reumatologia*, 56(4): 243–248.

Gladman, D., D., Urowitz, M., B., Keystone, C., C. (1979). Serologically active clinically quiescent systemic lupus erythematosus. *Am J Med.* 66:210–215.

Gunnarsson, R., Molberg, O., Gilboe, I., M., Gran, J., T. (2011). The prevalence and incidence of mixed connective tissue disease: a national multicentre survey of Norwegian patients. *Ann Rheum Dis*. 70 (6):1047-51.

Helmick, C.G., Felson, D.T., Lawrence, R.C., Gabrie,l S., Hirsch, R., Kwoh, C.K et al. (2008). Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. *Arthritis Rheum*. 58: 15-25.

Holborrow, E., Weir, D., Johnson, G. (1957). A serum factor in lupus erythematosus with affinity for tissue nuclei. *Br Med J.* 2:732 – 4.

Hefeneider, S., H., Bennett, R., M., Pham, T., Q., Cornell, K., McCoy, S., L., Heinrich, M., C. (1990). Identification of a cell-surface DNA receptor and its association with systemic lupus erythematosus. *J Invest Dermatol* . 94(6):79S–84S.

Hahn BH. (2005). Systemic lupus erythematosus. In: Kasper D, ed. Harrison's Principles of Internal Medicine. 16th ed. New York: McGraw-Hill.1960-1967.

Hall, M. R., Meinke, W., Goldstein, D. A. & Lerner, R. A. (1971). Synthesis Of cytoplasmic membrane associated DNA in the phocyte nucleus. *Nat. New Biol*. 234:227-229.

Hoffman, R., W. (2007). Mixed connective tissue disease and overlap syndrome, in: D.J. Wallace, B.H. Hahn (Eds.), Dubois' Lupus Erythematosus, 7th ed., *Lippincott Williams and Wilkins, New York*., 975–991.

Hochberg, M., C. (1997). For the Diagnostic and Therapeutic Criteria Committee of the American College of Rheumatology. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. 40:1725.

Hahn, B., H., Tsao, B.,P. (2008). Pathogenesis of systemic lupus erythematosus. In: Firestein GS, Budd RC, Harris ED Jr., et al. *Kelley's Textbook of Rheumatology. Pa: Saunders Elsevier; Philadelphia*. *Eds.*, (74).

Isenberg D, Ramsey-Goldman R. (1999). Assessing patients with lupus: towards a drug responder index. *Rheumatology.* 38:1045–9.

Isenberg, D., A., Manson, J., J., Ehrenstein, M., R., and Rahman, A. (2007). Fifty years of anti-ds DNA antibodies: are we approaching journey's end?. *Rheumatology*,46(7): 1052–1056.

Jacobson, D.L., Gange, S.J., Rose, N.R., Graham, N.M. (1997) Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *ClinImmunolImmunopathol*. 84(3): 223-243.

Jacob, L., Lety, M., A., Bach, J., F., Louvard, D. (1986). Human systemic lupus erythematosus sera contain antibodies against cell surface protein(s) that share(s) epitope(s) with DNA. *Proc Natl Acad Sci USA*. 83:6970±4.

Jacob, L., Viard , J., P., Allenet, B., Anin, M., F., Slama, F., B., Vandekerkhove, J, *et al*. (1989). A monoclonal anti double stranded DNA autoantibody binds to a 94 kD cell surface protein on various cell types via nucleosomes or a DNA-histone complex. *Proc Natl Acad Sci USA*. 86:4669±73.

Jacobsen, S., Ullman, S., Shen, G., Q., et al. (2001). Influence of clinical features, serum antinuclear antibodies, and lung function on survival of patients with systemic sclerosis. *J Rheumatol*. 28:2454–2459

Knobler, R., Moinzadeh, P., Hunzelmann, N. (2017). European Dermatology Forum S1-guideline on the diagnosis and treatment of sclerosing diseases of the skin, Part 1: localized scleroderma, systemic sclerosis and overlap syndromes. *J Eur Acad Dermatol Venereol*. 31(9):1401-1424.

Katsumoto, T., R., Whitfield, M., L & Connolly, M., K. (2011). The pathogenesis of systemic sclerosis. *Annu Rev Pathol*. 6:509-37.

Kavanaugh, A., Tomar, R., Reveille, J., Solomon, D., H., Homburger, H., A. (2000). Guidelines for clinical use of the antinuclear antibody test and tests for specific autoantibodies to nuclear antigens. American College of Pathologists. *Arch. Pathol. Lab. Med*. 124 (1): 71–81.

Kuo, M. T., Meinke, W., & Saunders, G. F. (1975). Localization of cytoplasmic-membrane-associated DNA in human chromosomes. *Proceedings of the National Academy of Sciences of the United States of America*, *72*(12), 5004–5006. https://doi.org/10.1073/pnas.72.12.5004

Lisnevskaia, L., Murphy, G., Isenberg, D. (2014). Systemic lupus erythematosus. *The Lancet*. 384(9957), 1879-1888.

Lau, C., S., Yin, G., and Mok, M., Y. (2006) . Ethnic and geographical differences in systemic lupus erythematosus: an overview. *Lupus.* 15(11): 713–714.

Lerner, R.A., Meinke, W., Goldstein, D.A. (1971) Membrane-associated DNA in the cytoplasm of diploid human lymphocytes. *Proc Natl Acad Sci USA*. 68:1212–6.

Lehmeier, T., Foulaki, K., Luhrmann, R. (1990). Evidence for three distinct D proteins,which react differentially with anti-Sm autoantibodies, in the cores of the major snRNPs U1, U2, U4/U6 and U5. *Nucleic Acids Res.* 18(22):6475-84. 28.

Mosca, M., Neri, R., Bencivelli, W., Tavoni, A., Bombardieri, S. (2002).Undifferentiated connective tissue disease: analysis of 83 patients with a minimum follow up of 5 years. *J Rheumatol*. 29: 2345-9.

McCauliffe, D., P. (2001). Cutaneous lupus erythematosus. *Semin Cutan Med Surg.* 20(1):14-26.

Marks, S., D., Tullus, K. (2012). Autoantibodies in systemic lupus erythematosus. *Pediatric Nephrology.* 27(10):1855–1868.

Manole, C., & Inimioara, C., & Silosi, I., & Vrabie, C. (2011). Manifestations of Systemic Lupus Erythematosus. *Mædica*. 6. 330-6.

Moyer, J., P. (1979). The association ofDNA and RNA with membranes. Int. Rev. Cytol. 61:1-61.

Marin, G., G., Cardiel, M., H., Cornejo, H., Viveros, M., E. . (2009). Prevalence of Antinuclear Antibodies in 3 Groups of Healthy Individuals. *JCR: Journal of Clinical Rheumatology*. 15(7):325-329 doi: 10.1097/RHU.0b013e3181bb971b

Meinke, W., Hall, M.R., Goldstein, D.A., Kohne, D.E., Lerner, R.A. (1973) Physical properties of cytoplasmic membrane associated DNA. *Mol Biol* .78:43–56.

Mok, C.C., Lau, C.S. (2003) Lupus in Hong Kong Chinese. *Lupus*.12: 717–722.

Myrthe A.M. van Delft, Tom W.J. Huizinga. (2020). An overview of autoantibodies in rheumatoid arthritis. *Journal of Autoimmunity*. Volume 110,102392,ISSN 0896-8411,

Mohan, C., Alas, E., Morel, L., Yang, P., and Wakeland, E., K. (1998). Genetic Dissection of SLE Pathogenesis Sle1 on Murine Chromosome 1 Leads to a Selective Loss of Tolerance to H2A/H2B/DNA Subnucleosomes. *J. Clin. Invest.* 101: 6.

Mecoli, C., A., & Casciola-Rosen, L. (2018). An update on autoantibodies in scleroderma. *Curr Opin Rheumatol*. 30:000–000.

Nzeusseu Toukap, A., Galant, C., Theate, I., Maudoux, A., L., Lories, R., J., Houssiau, F., A., Lauwerys, B., R. (2007). Identification of distinct gene expression profiles in the synovium of patients with systemic lupus erythematosus. *Arthritis Rheum.* 56(5):1579-88.

Nishimura K, et al. (2007). Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. *Ann. Intern.* Med. 146:797–808.

Osio-Salido, E., and Manapat-Reyes, H. (2010). Epidemiology of systemic lupus erythematosus in Asia. *Lupus*, 19(12):1365–1373.

Pan LT, Tin SK, Boey ML, Fong KY. (1998). The sensitivity and specificity of autoantibodies to the Sm antigen in the diagnosis of systemic lupus erythematosus. *Ann Acad Med Singap*. 27(1):21-3.

Puszczewicz, M., Białkowska-Puszczewicz, G., Majewski, D. (2012). The importance of autoantibodies in the diagnosis of rheumatic diseases, Implications of the autoantibodies in the diagnosis of rheumatic diseases. *Postępy Nauk Medycznych*. 156-163.

Pagalavan, L., & Ong, S., G. (2007), Demography, Clinical and Laboratory Features of Systemic Sclerosis in a Malaysian Rheumatology Centre. *Med J Malaysia*. 62:2.

Petri M. (1998). Treatment of systemic lupus erythematosus: an update. *Am Fam Physician*.57:2753-2760.

Pan , Q., J., Chen, X., Q., Liao, S., Z., Chen, X., C., Zhao, C., F., Xu, Y., Z., and Liu, H., F. (2019). Updated advances of linking psychosocial factors and sex hormones with systemic lupus erythematosus susceptibility and development, *PeerJ*. 7: e7179.

Pisetsky, D., S. (2012). Antinuclear antibodies in rheumatic disease: a proposal for a function-based classification. *Scand J Immunol*. 76(3):223-8.

Prockop, D., J., Bateman, J., F. (2014). Heritable Disorders of Connective Tissue. In: Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J. eds. *Harrison's Principles of Internal Medicine*, 19e New York, NY: McGraw-Hill.

Petri, M., Orbai, A., M., Alarcon, G., S., Gordon, C., Merrill, J., T., Fortin, P., R., Bruce, I., N., Isenberg, D., Wallace, D., J., Nived, O. (2012). Derivation and validation of the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum*. 64, 2677–2686.

Reichlin, M., & Harley, J.B. (2002) Antibodies to Ro/ SSA and La/SSB. In: Wallace DJ, Hahn BH, eds. Dubois' lupus erythematosus. *Philadelphia: Lippincott Williams & Wilkins*. 6:467-80.

Ru, J.L., Zhao, Y., Xie, X.X., Che, G.Z., Cheng, C.F., Zhao, H.M., Jin, Z.Y., Sun, H.P., Li, X.F. (2014) Clinical applications of the indirect immunofluorescence assay for detection of anti-cell membrane-associated DNA antibodies in Juvenile Systemic Lupus Erythematosus. *Pediatric Research*. 2(77): 376-380.

Ruiz-Irastorza, G., Ramos-Casals, M., Brito-Zeron, P. (2010). Clinical efficacy and side effects of antimalarials in systemic lupus erythematosus: a systematic review. *Ann Rheum Dis*. 69:20–8.

Raza, M., A., Khan, M., I. (2012). Systemic lupus erythematosus: disease manifestations in patients from central Punjab (Pakistan) *Int J Clin Rheumatol*. 7(6):607–614.

Robert, M., Bennett, M., D. (2014). Clinical manifestations of mixed connective tissue disease. *UpToDate*.

Reid, B., L., Charlson, A., J. (1979). Cytoplasmic and cell surface deoxyribonucleic acid with consideration of their origin.*Int Rev Cytol.* 60:25±52

Rekvig, O., P.(1989). Intrinsic cell membrane antigens recognized by antichromatin autoantibodies. *Scand J Immunol*.29. 7±13.

Sakthiswary, R., Suresh, E. (2014). Methotrexate in systemic lupus erythematosus: a systematic review of its efficacy. *Lupus*. 23:225–35.

Spencer-Green, G., Alter, D., Welch, H., G. (1997). Test performance in systemic sclerosis: anti-centromere and anti-Scl-70 antibodies. *Am J Med* . 103:242–248, (34).

Symmons, Deborah. (2002). Epidemiology of rheumatoid arthritis: determinants of onset, persistence and outcome. Best Pract. Res. *Clin. Rheumatol*. 16: 707-722.

Shahrir, M., Shahdan, M., Mohamed, S., Sulaiman, W., Mokhtar, A., M., Othman ,M. (2008). Multicentre survey of rheumatoid arthritis patients from Ministry of Health Rheumatology Centers in Malaysia. *Int. J. Rheum. Dis.* 11 (3): 287–292. 10.1111/j.1756-185X.2008.00379.x

Scott, D., L., Pugner, K., Kaarela, K., Doyle, D., V., Woolf, A., Holmes, J., Hieke, K. (2000). The links between joint damage and disability in rheumatoid arthritis, *Rheumatology*. 39(2):122 - 132, https://doi.org/10.1093/rheumatology/39.2.122.

Sherrer, Y., S., Bloch, D., A., Mitchell, D., M., Young, D., Y., Fries, J., F. (1986). The development of disability in rheumatoid arthritis. *Arthritis Rheum*. 29(4):494-500. doi: 10.1002/art.1780290406. PMID: 3707627.

Servais, G., Deans, S., Guillaume, M.P et al. (2002) Diagnostic specificities and sensitivities of anti dsDNA, anti-membrane DNA and anti-nucleosomes autoantibodies. *Scand J Clin Lab Invest*. 61 (235):61–67.

Servais, G., Guillaume,M.P., Dumarey, N., Duchateau, J. (1998) Evidence of autoantibodies to cell membrane associated DNA (cultured lymphocytes): a new specific marker for rapid identification of systemic lupus erythematosus. *Ann Rheum*. 57:606–13.

Saraux, A., Pers, J., O., Devauchelle-Pensec, V. (2016). Treatment of primary Sjögren syndrome. *Nat Rev Rheumatol.* 12:456–71.

Silver, R., M., Bogatkevich, G., Tourkina, E., Nietert, P., J., Hoffman, S. (2012). Racial differences between blacks and whites with systemic sclerosis. *Curr Opin Rheumatol*. 24(6):642-8.

Su, Y., Ru, L., J., Lei, H., Zhan, G., Li. (2007). Role of anti-nucleosome antibody in the diagnosis of systemic lupus erythematosus, *Clinical Immunology*. 122: 115– 120.

Su, K., Y,, Pisetsky, D., S. (2009). The role of extracellular DNA in autoimmunity in SLE. *Scand. J. Immunol*. 70 (3): 175–83.

Tonutti, E., Blasone, N., Visentini, D., Poletto, M., Villalta, D., Tozzoli, R., Bizzaro, N. (2008) Cell lines that express membrane-associated DNA for anti-DNA antibody detection.*Clinical Chemistry and Laboratory Medicine*. 4(46): 458- 462

Tan, E.M., Cohen, A.S., Fries, J.F., Masi, A.T., McShane, D.J., Rothfield, N.F., et al. (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. 25:1271–7. 7.

Tan, E., M. (1989). Antinuclear antibodies: Diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol.*. 44:93–151.

To, C., H., Petri, M. (2005). Is antibody clustering predictive of clinical subsets and damage in systemic lupus erythematosus?. *Arthritis Rheum.* 52(12):4003-10.

Ter Borg, E., J., Horst, G., Hummel, E., J. (1990). Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus. *Arthritis Rheum*. 33 : 634-643.

Tan, E., M., Feltkamp, T.,E., Smolen, J.,S., Butcher, B., Dawkins, R., Fritzler, M.,J., Gordon, T., Hardin, J.,A., Kalden, J., R., Lahita, R.,G., Maini, R.,N., McDougal, J.,S., Rothfield, N.,F., Smeenk, R.,J., Takasaki, Y., Wiik, A., Wilson, M., R., Koziol, J.,A. (1997). Range of antinuclear antibodies in "healthy" individuals. Arthritis Rheum.40(9):1601-11. doi: 10.1002/art.1780400909. PMID: 9324014.

Tobón, G., J., Youinou, P., & Saraux, A. (2009). The environment, geo-epidemiology, and autoimmune disease: Rheumatoid arthritis. *Autoimmun Rev*. (5).

Trager, J., Ward, M., M. (2001). Mortality and causes of death in systemic lupus erythematosus. *Curr Opin Rheumato.* ;13:345–51.

Takehara, K., and Sato, S. (2005). Localized scleroderma is an autoimmune disorder. *Rheumatology*. 44:274–279.

Tartar, D., M., Chung, L & Fiorentino, D., F. (2018). Clinical significance of autoantibodies in dermatomyositis and systemic sclerosis. *Clin Dermatol*. 36(4):508-524.

Tsokos, G., C. (2011) "Systemic lupus erythematosus," *The New England Journal of Medicine*, 365(22): 2110–2121.

Ungprasert, P., Crowson, C., S., Chowdhary, V., R., Ernste, F., C., Moder, K., G & Matteson, E., L. (2016). Epidemiology of Mixed Connective Tissue Disease 1985-2014: A Population Based Study. *Arthritis Care Res (Hoboken)*.

Vitali C, Bootsma H, Bowman SJ. (2013). Classification criteria for Sjogren's syndrome:we actually need to definitively resolve the long debate on the issue. *Ann Rheum Dis* .72:476-8.

Van den Hoogen, F., Khanna, D & Fransen J. (2013). classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. *Ann Rheum Dis*. 72(11):1747-1755.

van der Linden, M., P. (2010). Long-term impact of delay in assessment of patients with early arthritis. *Arthritis Rheum*. 62:3537–3546.

Voskuhl, R. (2011). Sex differences in autoimmune disease. *Biology of Sex Differences*. 2(1): 1.

Romão, V., C., Talarico, R., Scirè, C., A., Vieira, A., Alexander, T., Baldini, C., Mariette, X. (2018). Sjögren's syndrome : state of the art on clinical practice guidelines. *RMD OPEN*, 4(suppl. 1). https://doi.org/10.1136/rmdopen-2018- 000789

Weckerle, C., E., Niewold, T., B (2011). The unexplained female predominance of systemic lupus erythematosus: clues from genetic and cytokine studies. *Clin Rev Allergy Immunol*. 40(1):42-9. doi: 10.1007/s12016-009-8192-4. PMID: 20063186; PMCID: PMC2891868.

Wang, F., Wang, C.L., Tan, C.T., *et al*. (1997). Systemic lupus erythematosus in Malaysia: a study of 539 patients and comparison of prevalence and disease expression in different racial and gender groups. *Lupus* 6: 248-253.

Wong, S.N., Tse, K.C., Lee, T.L., Lee, K.W., Chim, S. (2006). Lupus nephritis in Chinese children–a territory-wide cohort study in Hong Kong. *Pediatr Nephrol* 21: 1104–1112.

Wei H, Lü Z, Ru J, et al. The detection of DNA-associated autoantibodies to cell membrane and its combination with other autoantibodies in the diagnosis of systemic lupus erythematosus. Chinese Journal of Rheumatology. 2010;14:185–187

Waterman, S., A., Gordon, T., P., Rischmueller, M. (2000). Inhibitory effects of muscarinic receptor autoantibodies on parasympathetic neurotransmission in Sjogren's syndrome. *Arthritis Rheum.* 43:1647–54.

Will, C., L., Luhrmann, R. (2001). Spliceosomal UsnRNP biogenesis, structure and function. *Curr Opin Cell Biol*.13(3):290-301. 26.

Yang, W., Shen, N., Ye, D. Q., Liu, Q., Zhang, Y., Qian, X. X., Hirankarn, N., Ying, D., Pan,H.F., Mok, C. C., Chan, T. M., Wong, R. W. S., Lee, K. W., Mok, M. Y.,Wong, S. N., Leung, A. M. H., Li, X. P., Avihingsanon, Y., Wong, C. M., Lee, T. L., Ho, M. H. K., Lee, P. P.W., Chang, Y. K., Li, P. H., Li, R. J., Zhang, L., Wong, W. Hi. S., Ng, I. O. L., Lau, C. S., Sham, P. C., Lau, Y. L. (2010). Genome-wide association study in Asian populations identifies variants in ETS1 and WDFY4 associated with systemic lupus erythematosus. *PLoS Genetics*. 6(2).

Yap, S.N., Phipps, M.E., Manivasagar, M., S.Y., and Bosco, J. J. (1999). Fc gamma receptor IIIB-NA gene frequencies in patients with systemic lupus erythematosus and healthy individuals of Malay and Chinese ethnicity. *Immunology Letters.* 68(2-3): 295–300.

Yeap, S., S., Chow, S., K., Manivasagar, M., Veerapen, K., and Wang,F. .(2001). Mortality patterns in Malaysian systemic lupus erythematosus patients. *Medical Journal of Malaysia.* 56(3): 308–312.

Yashwant, K., and Alka, B. (2014). Detection of antinuclear antibodies in SLE. *Methods Mol Biol*. 1134:37-45.

Yung, S., Chan, T., M. (2008). Anti-DNA antibodies in the pathogenesis of lupus nephritis--the emerging mechanisms. *Autoimmun Rev*. **7** (4): 317–21.

Yaniv, G., Twig, G., Shor, D., B., Furer, A., Sherer, Y., Mozes, O. (2015). A volcanic explosion of autoantibodies in systemic lupus erythematosus: a diversity of 180 different antibodies found in SLE patients. *Autoimmun Rev*.14 (1):75–9.

Yasuoka, H., Shirai, Y., Tamura, Y., Takeuchi, T., Kuwana, M. (2018). Predictors of Favorable Responses to Immunosuppressive Treatment in Pulmonary Arterial Hypertension Associated With Connective Tissue Disease. *Circulation Journal*. 82:546-554.

Zandman-Goddard, G., Solomon, M., Rosman, Z., Peeva, E & Shoenfeld, Y. (2011). Environment and lupus related diseases. *Lupus*.

Zieve, G., W., Khusial, P., R. (2003). The anti-Sm immune response in autoimmunity and cell biology. *Autoimmun Rev*.2(5):235-40.