

DOI: 10.5455/msm.2024.36.47-53

Received: Jan 10 2024; Accepted: Feb 25, 2024

© 2024 Georgios Dragoutsos, Christina Tsiggallou, Efthymios Oikonomou, Anastasia Bothou, Grigorios Trypsiannis, Konstantinos Nikolettos, Christos Damaskos, Nikolaos Garmpis, Dimitrios Kyriakou, Theopi Nalbanti, Georgios Iatrakis, Nikolaos Nikolettos, Panagiotis Tsikouras

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORIGINAL PAPER

Mater Sociomed. 2024; 36(1): 47-53

Association of Anti-Ro/SSA and Anti-La/SSB Autoantibodies With Pregnancy Outcome in the General Population

Georgios Dragoutsos¹, Christina Tsiggallou², Efthymios Oikonomou¹, Anastasia Bothou¹, Grigorios Trypsiannis³, Konstantinos Nikolettos¹, Christos Damaskos⁴, Nikolaos Garmpis⁵, Dimitrios Kyriakou¹, Theopi Nalbanti¹, Georgios Iatrakis¹, Nikolaos Nikolettos¹, Panagiotis Tsikouras¹

¹Department of Obstetrics and Gynecology, Democritus University of Thrace, Greece

²Department of Medical Microbiology-Immunology Laboratory of Hygiene and Environmental Protection Democritus University of Thrace, Greece

³Department of Medical Statistics, Democritus University of Thrace, Greece

⁴Experimental Surgery and Surgical Research, Medical School, National and Kapodistrian University of Athens, Greece and Renal Transplantation Unit, Laiko General Hospital, Athens, Greece

⁵Laboratory of Experimental Surgery and Surgical Research, Medical School, National and Kapodistrian University of Athens, Greece

Corresponding author: Panagiotis Tsikouras. Professor of OB/GYN. Department of Obstetrics and Gynecology, Democritus University of Thrace, Greece E-mail: tsikouraspanagiotis@gmail.com. ORCID ID: //http://www.orcid.org/0000-0000-0000-0000.

ABSTRACT

Background: Autoimmune diseases encompass a diverse array of disorders that disturb the optimal functioning of the immune system, which is to eliminate the 'foreign or/and dangerous' to mistakenly target the body's own tissues. **Objective:** The aim of this research is to evaluate the most effective approach to managing autoimmune diseases within the framework of pregnancy. **Methods:** The exact causes and etiologies of these diseases are multifactorial and mostly still unclear. Ro/SSA autoantibodies and La/SSB, could be found in Sjögren's disease (SJ), systemic lupus (SLE) and other autoimmune disorders. Smoking, stress, UV exposure, vitamin D deficiency, and other genetic and environmental factors have been identified as risk factors for rheumatic diseases. **Results:** Over the years, an ever-increasing incidence of these diseases has been observed in the general population, with the female sex being at increased risk for their occurrence. This fact raises the question of what should be the management of these pathological entities during pregnancy. Taking into account the very significant impact on the quality of patients'daily life and the seemingly augmented prevalence of autoimmune diseases, as well as their preference in the female population, the reasonable question arises as to what should be the optimal management of these diseases in the context of pregnancy. **Conclusion:** Given the limited data of the global medical

community regarding the etiological factors and mechanisms that trigger the onset of rheumatic diseases, the management of pregnant women is a complex conundrum that health professionals are challenged to face and solve.

Keywords: anti-Ro/SSA, anti-La/SSB autoantibodies, pregnancy outcome, general population.

1. BACKGROUND

Anti-Ro/SSA antibodies are among the most frequently detected autoantibodies against extractable nuclear antigens and have been associated with systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS), subacute cutaneous lupus erythematosus (SCLE), and neonatal lupus erythematosus (NLE) (1-3). Anti-SS-A/Ro and anti-SS-B/La are autoantibodies raised against ribonucleoproteins. Ro and La antigens each consist of a protein complex with RNA molecules. The exclusive carrier of antigenicity is its protein part. The Ro and La proteins are presented as antigenic polypeptides of molecular weight 60 KD and 52 KD, respectively, while it has been reported that anti-Ro antibodies recognize in human tissues a second polypeptide with different antigenic properties from the first and with a lower molecular weight (52KD) (4-6). The detection of the frequency of anti-Ro/SSA and anti-La/SSB autoantibodies in pregnant

women in the general population and in pregnant women with immune disorders have a direct correlation with the perinatal outcome and fetal development (1-3). These autoantibodies are also found in 0.2–0.44% of healthy blood donors. (1) In pregnancy pathology, the involvement of antibodies against ribonucleoproteins (anti-SSA/Ro and anti-SSB/La) plays an important role because these antibodies are found in rheumatic diseases and can pass through the placenta into the fetal circulation creating in the newborn manifestations of neonatal lupus erythematosus (4-6). The autoimmune condition known as Sjögren's syndrome, which affects the body's salivary and lacrimal glands and frequently has a major impact on the kidneys, neurological system, lungs, and other organ systems, is most commonly associated with these autoantibodies. Exocrine gland destruction, resulting in conjunctival and mucous membrane dryness (dry mouth, dry eyes) (4-6).

The laboratory investigation for anti-SS-A (Ro) and anti-SS-B (La) autoantibodies in everyday routine is done for the evaluation and differential diagnosis of patients with symptoms of connective tissue diseases and in particular symptoms compatible with Sjögren's syndrome, erythematosus lupus and mixed connective tissue disease.

Anti-Ro antibodies are mainly found in patients with systemic lupus erythematosus (SLE) (35-50%) and Sjögren's syndrome (60%), but also in other connective tissue diseases with a lower frequency. Their presence in systemic lupus erythematosus is associated with hypoxic cutaneous lupus, neonatal lupus (7-9). Of particular value is the search for antibodies against antigenic epitopes (Ro52/Ro60) in the prognosis of atrioventricular block or fetal death in the context of neonatal lupus. Anti-La antibodies have been described in Sjögren's syndrome (40-60%) and systemic lupus erythematosus (frequency 10-15%). They are usually found in serum together with anti-Ro antibodies (7-9). The presence of anti-SS-A (Ro) and anti-SS-B (La) autoantibodies is linked to an earlier onset and prolonged duration of Sjögren's syndrome. Additionally, these antibodies are associated with clinical manifestations such as splenomegaly, lymphadenopathy, and vasculitis, thereby heightening the likelihood of developing extraglandular manifestations of the disease. In patients with systemic lupus erythematosus, the detection of anti-Ro and anti-La antibodies (with normal values being Ro: Negative, La: Negative) precedes the onset of clinical manifestations, on average preceding the diagnosis of the disease by 3.4 years (7-9).

Neonatal lupus erythematosus occurs at an incidence of 1 in 12,500 births. It is not exclusive to neonates born to mothers symptomatic with rheumatic diseases during pregnancy but also affects a substantial percentage (50%) of neonates born to mothers with conditions like SLE, even when the mothers are asymptomatic during pregnancy (10-13). The most severe neonatal complications of lupus erythematosus include cardiomyopathy and atrioventricular block. In the early stages, characterized by the onset of atrial and ventricular arrhythmias, these complications can be addressed with medication,

specifically therapeutic administration of dexamethasone and β -globulin. However, in stage 3, the condition becomes complete and irreversible, necessitating a pacemaker in 60% of cases (10-13).

2. OBJECTIVE

The purpose of the present study is to detect autoantibodies in the general population of pregnant women and the correlation with pregnancy contracts in order to certify asymptomatic pregnant women and to monitor the pregnant women found as well as symptomatic of the aforementioned rheumatic diseases.

3. MATERIAL AND METHODS

The monitoring protocol involves a comprehensive assessment, including extensive information gathering, laboratory checks for kidney function, screening for antiphospholipid syndrome, and fetal ultrasound between 18 and 24 weeks. From 24 weeks onward, check-ups occur every two weeks until 34 weeks, complemented by monthly assessments by a rheumatologist. Blood samples are collected from patients in vacutainer tubes without anti-coagulant. After immediate centrifugation at 4,000 rpm for 10 minutes, the extracted serum is stored in aliquots and frozen at -70°C until needed. All patients undergo testing for antinuclear autoantibodies (ANAs) using conventional indirect immunofluorescence assay (IIF) with HEp-2 cells (A.MENARINI Diagnostics) at dilution ratios of 1/80, 1/160, 1/320, and 1/640, with a cutoff for positivity set at 1/80. ANA patterns are determined based on the respective AC, following the guidelines of the International Consensus of Autoantibody Patterns (ICAP) (www.anapatterns.org). For anti-dsDNA, an indirect immunofluorescence assay (IIF) is conducted using Chrithidia lusiliae substrate at a screening dilution ratio of 1/10, with positivity determined accordingly.

The ENA screen (Extractable nuclear antigens) involves Elisa measurements (Aesculisa, Aesku Diagnostics, Wendelsheim, Germany) for the following antigens: Anti-SSA(Ro), Anti-SSB(La), Anti-Sm, Anti-SmRNP, Anti-Jo-1, Anti- Scl-70. In case of positivity (cut-off value >1), specific ELISA kits for each autoantibody are employed. The aPL (anti-phospholipid abs) both IgG and IgM types (ACA), were measured by ELISA (Aesculisa, Aesku Diagnostics, Wendelsheim, Germany), and concentration above 18 GPL/MPL for all isotypes considered as a positive result. The RF(Rheumatoid factor) was measured by nephelometry by IMMAGE®®Immunochemistry Systems RF with positivity value >20 IU/ml according to the manufactures insert. We measured anti-TPO and anti-Tg in serum using electrochemiluminescence (Roche Cobas e411 analyzer; Roche Diagnostics, Mannheim, Germany). The reference ranges were less than 35 IU/mL for antiTpo and for anti-Tg <115 IU/ml. The study goals include the following correlations:

- a) incidence of positive ANA in immunofluorescence in general;
- b) categorization of diagnoses–identification of control group ??
- c) incidence of positive ANA in immunofluorescence

in total, in titer and by category;

d) positivity in specific autoantibodies as a reflex test in ENAscreen positive (i.e. ONE screen positive and one/a of the following). Note–screen includes Ro, La, Sm, Sn/RNP, Jo-1, Scl-70)

e) frequency of appearance per category of the other autoantibodies (BUT excel) and in relation to the previous ones

f) rate at which different types of autoantibodies coexist in the same patient The University Hospital in Alexandroupolis, Democritus University of Thrace (Alexandroupolis, Greece), ethics committee verified ethical approval for this prospective study (reference no. 17096/04/6/2020). Every patient who supplied written informed consent also gave written consent to be included in this study.

Statistical analysis

Statistical analysis of the data was performed using IBM Statistical Package for Social Sciences (SPSS), version 19.0 (IBM Corp., Armonk, NY, USA). All variables were qualitative and they were expressed as absolute and relative (%) frequencies. The prevalence of positive ANA between patients and healthy controls was compared using the chi-square test, while the odds ratio (OR) and its 95% confidence interval (CI) were estimated as a measure of the above association. All tests were two-sided and statistical significance was considered at a p-value <0.05.

4. RESULTS

In a 3-year prospective study (June 2020-June 2023) of the records of deliveries performed at the University Obstetrics-Gynecology Clinic Alexandroupolis of the Democritus University of Thrace during the above period and data were collected regarding the scientific study protocol.

Among the 79 patients included in the study, 11 (13.9%) were ANA-positive. According to the semi-quantitative method, 7 (8.9%) had titer 1/80, 3 (3.8%) 1/160 and 1 (1.3%) 1/320. Considering the ANA-pattern (type of immunofluorescence pattern) of the 11 ANA-positive patients, 5 (45.5%) had Homogeneous pattern (2 with Recurrent Spontaneous Abortions RSA; 1 with spontaneous abortion; 2 other disease), 4 (36.4%) had Dense Fine Speckled 70 (DFS-70) pattern (1 with missed abortion; 1 with spontaneous abortion; 2 with intrauterine fetus death) and 2 (18.2%) had fine speckled pattern (1 missed abortion; 1 RSA). From the 11 ANA-positive patients, 3 (27.3%) had RSA, 2 (18.2%) had missed abortion, 2 (18.2%) had spontaneous abortion, 2 (18.2%) had intrauterine death fetus and the last 2 (18.2%) had some other disease.

ANA-positivity was significantly more frequent among patients (11/79) than healthy controls (3/70) (13.9% vs 4.3%, p=0.044; OR=3.61, 95% CI=0.97-13.53). In relation to the diagnosis, ANA-positive was a more frequent event among patients with death fetus (2 of 5 patients, 40.0%) and RSA (3 of 11 patients, 27.3%) compared to patients with spontaneous abortion (2 of 11 patients, 18.2%), missed abortion (2 of 29 patients, 6.9%) or other disease

	No of subjects	Percentage (%)
ANA positive	11	13.9
ANA		
1/80	7	8.9
1/160	3	3.8
1/320	1	1.3
ENAscreen	3	3.8
anti-SSA	1	1.3
anti-SSB	-	-
anti-Sm	-	-
anti-Sm/RNP	1	1.3
anti-Jo-1	-	-
anti-Scl-70	-	-
anti-dsDNA	-	-
anti-TPO	3	3.8
anti-Tg	2	2.5
Rheumatoid Factor	6	7.6
ACA-G	5	6.3
ACA-M	3	3.8

Table 1. Results of ANA screening in the studied population. The titer of positivity in immunofluorescence started from cut-off 1/80. It depicted the ENA screen positivity and specific positivity included SSA, SSB, Sm, SmRNP, Jo-1, Scl-70.

Diagnosis	No of subjects	% of subjects with this pathology
Missed abortion	2	6.9
RSA	3	27.3
Spontaneous abortion	2	18.2
Intrauterin fetus death	2	40.0
Other disease	2	8.7

Table 2. ANA positive in relation to diagnosis. There are 4 prevalent diagnoses (missed abortion, RSA, spontaneous abortion and intrauterine death fetus) as well as other diseases category.

	No of subjects	Pregnancy contracts
ANA + RF	1	Missed abortion
ANA + RF + ENAscreen	1	Intrauterin fetus death
ANA + RF + ENAscreen + ACA_G	1	RSA
ANA + ENAscreen	1	Missed abortion
ANA + ACA_G	1	Spontaneous abortion
ANA + antiTPO	1	RSA
ANA only	6	

Table 3. Multiple positivity in ANA positive. It depicts the coexistence of ANA positive subjects with other specificities or other type of autoantibodies and the results of pregnancy.

(2 of 23 patients, 8.7%). However, these differences did not reach the statistical significance (p=0.174).

The screening for the existence of rheumatic thyroid disease revealed anti TPO and anti Tg autoantibodies in 3 and 2 patients respectively with percentage of 3.8% for the first and 2,5% for the latter.

Likewise, Rheumatoid factor was found positive in 6 patients (7,6%) and antiphospholipid antibodies were positive as follows: ACA-G, ACA-M 5 AND 3 patients with percentages 6,3%, 3,8 respectively.

5. DISCUSSION

Autoimmune diseases can appear at any age, but each disease may have its own characteristic age of

onset. Of particular interest is the increased incidence of Sjogren's syndrome in northwestern Greece. In the majority of patients, the prevalence is increased in first-degree relatives and is even higher in monozygotic twins. The female gender appears to be at increased risk for a higher incidence of rheumatic diseases, with the female to male ratio varying as high as 10:1, the exception being Crohn's disease, with a ratio of 1:1.2 and others like TDI with a ratio of 1:1 (14-15). The racial predisposition of rheumatic diseases requires special attention and given the possibility of the female sex to conceive, special knowledge is needed by health professionals dealing with motherhood of the needs, risks and demands involved in pregnancies, when these are complicated by a rheumatic disease (14-15).

The immune system is responsible for protecting the body from infectious agents. However, there are two potential pathways through which the immune system can lead to pathology- overreaction and under-reaction. On the one hand, immunodeficiency is a condition in which one or more factors that make up the lines of immune action are unable to respond to the invasion of a pathogen, while on the other hand when the immune system turns against structures of the organism itself autoimmune diseases arise. The failure of the immune system to distinguish the body's own molecules from invaders is the basis of these diseases. Although rheumatic diseases were for many years considered rare pathological entities, epidemiological studies have shown that 3-5% of the world's population suffers from an autoimmune disease, with these percentages following an increasingly upward trend (16-17). A very recent study of Nathalie Conrad et.al 2023 revealed that currently Rheumatic diseases affect approximately 10% of the general population, and their burden for public health continues to increase. Environmental factors are crucial players in disease pathogenesis (18).

In recent years, thanks to the significant progress of molecular immunology technology and sophisticated clinical laboratory tests, significant advances have been made in the diagnosis, prognosis and classification of autoimmune diseases. The immune system is made up of two parts, the innate and adaptive immune systems and provides three lines of defense against pathogenic invaders, as the first line is the epithelial barrier, namely the skin and mucous membranes. When there is a rupture or discontinuity in the epithelial tissue and microorganisms enter the host, the immune system takes action to fight them (16-17).

The innate immune system is nonspecific and consists of a system of hematogenous macromolecular substances, complement, Toll like Receptors TLR (family of PRRs Pattern recognition receptors), macrophages, neutrophils, and natural killer (NK) cells. The innate immune system responds quickly but lacks immune memory and specificity. TLRs are receptors located on the cell membranes of innate immune cells, they work in pairs and recognize repetitive molecular sequences of various pathogenic microorganisms (PAMPs Pathogen associated molecular patterns, MAMPs Microbe asso-

ciated molecular patterns, DAMPs Damage associated molecular patterns). It is worth noting that underactivity of TLRs can lead to immunodeficiency, while their overactivity can be responsible for certain autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis (19). The adaptive immune system depends on the action of T and B lymphocytes, responds more slowly than the innate system, but possesses immune memory. This system has a few more features, specificity, diversity, immune tolerance, memory, and self- and hetero-recognition. Immune tolerance is of particular importance, as thanks to it the immune system is able to recognize molecules that belong to the body and not trigger an immune response against them. The mechanism of tolerance is based on the elimination or inactivation of cells that can react against the organism itself. The loss of immune tolerance results in the dysfunction of the immune system and, by extension, the appearance of autoimmune diseases (20). Autoantibodies recognizing the Ro and La proteins of the small ribonuclei are frequently detected in the sera of patients with autoimmune rheumatic diseases and are particularly relevant in primary Sjögren's syndrome. The existence of these antibodies has been known since 1961 and since then the frequency with which they are detected in various diseases and the connection they show with specific clinical manifestations and laboratory disorders have been extensively studied. At the same time, sera containing antibodies of these specialties were valuable reagents for the characterization of Ro and La antigens, the existence of which was not known until then (21-23).

The methods by which these antibodies have been classically studied are contrast immunoelectrophoresis and similar double immunodiffusion. Antibodies that have precipitate properties are detected with these techniques. In recent years, the immunoblotting method has also started to be used for the detection of autoantibodies. This method is easy applied, shows great sensitivity and provides additional information regarding the specificity of the antibodies. Antinuclear antibodies ANA belong to the category of non-organ-specific autoantibodies, recognizing structures common to all types of cells. They are a heterogeneous group of autoantibodies and bind to a wide range of cell components such as DNA, RNA, proteins. Although today we know that many of the antinuclear antibodies, if not most, recognize structures that are also found in the cytoplasm or others are associated with molecules of the cell membrane, nevertheless, the name "antinuclear" has prevailed (21-23).

Antigens recognized by antinuclear antibodies show special characteristics. These are usually macromolecules that perform essential cellular functions and show broad conservation across species.

In fact, there are some indications that autoantibodies bind preferentially to epitopes that are part of the biologically active parts of these antigens. However, it is not known if these autoantibodies bind *in vivo* to the antigens they recognize, thereby interfering with their normal function. Also, with few exceptions, autoantibodies have not been shown to participate in the pathogenesis of the

tissue damage seen in autoimmune diseases (24-26).

Detection of antinuclear antibodies contributes to better assessment of patients with autoimmune rheumatic diseases. In fact, some of them have been characterized as indicators of specific diseases or as indicators of disease activity. The most important of these include antibodies against DNA, Scl-70, Jo-1, Sm, U1RNP, Ro, La, anti-centromeric, anti-histone and anti-ribosomal antibodies (24-26).

The Ro antigen was characterized simply as an acidic macromolecule, while the La antigen was found to be degraded after incubation with proteolytic enzymes and DNase and was thought to consist of a complex of RNA and protein molecules. Both antigens were detected by the double immunodiffusion method in a wide range of tissues of different origins but the Ro antigen was not detected in rodent tissues. Also, sera with antiRo activity were found to be negative in the test for antinuclear antibodies by the method of indirect immunofluorescence using various substrates. This fact was attributed to a low tissue concentration of the Ro antigen. AntiRo and antiLa antibodies were detected in these studies, exclusively in SLE patient sera, so they were considered as specific for this condition. It was also reported that serums with AntiLa specificity was almost always also antiRo one (24-26).

Patients exhibiting signs and symptoms of connective tissue sickness, particularly those consistent with Sjogren's syndrome or lupus erythematosus, are evaluated by antinuclear antibody (ANA) testing. These antibodies are checked for using SS-A and SS-B antibodies (20-22). Sjogren's syndrome is characterized by reduced secretion and eventual destruction of the exocrine glands, leading to dryness of the mucous membranes and conjunctiva. These tests are utilized in the differential diagnosis of Sjogren's syndrome, SLE, and mixed connective tissue disease. Laboratory test results constitute a crucial parameter for both the diagnosis and ongoing monitoring of various pathological conditions. Approximately 70-80% of diagnostic decisions hinge on the information derived from laboratory tests. It is essential to recognize that the accurate interpretation of these results enables physicians to discern between a state of "health" and the presence of "disease." It's important to note that laboratory test results should not be viewed merely as numerical outcomes from individual analyses; instead, their comprehensive interpretation is vital for a nuanced understanding of a patient's health status (24-26).

The search for anti-SS-A (Ro) and anti-SS-B (La) autoantibodies in clinical practice is done for the evaluation and differential diagnosis of patients with symptoms of connective tissue diseases and in particular symptoms compatible with Sjogren's syndrome, erythematosus lupus and mixed connective tissue disease. The Ro and La proteins are presented as antigenic polypeptides of molecular weight 60 KD and 50 KD, respectively, while it has been reported that anti-Ro antibodies recognize in human tissues a second polypeptide with different antigenic properties from the first and with a lower molecular weight (52KD) (24-26).

Anti-Ro antibodies are mainly found in patients with systemic lupus erythematosus (SLE) (35-50%) and Sjogren's syndrome (60%), but also in other connective tissue diseases with a lower frequency.

Merz et al 2022 studied autoimmune diseases during pregnancy, including systemic lupus erythematosus (26) SLE is associated with severe maternal, neonatal and obstetric complications. The most important point of attention is the planning of the pregnancy, so that the disease is in an inactive state for at least 6-12 months. During this Antiphospholipid syndrome APS occurs in about 20% of patients with SLE. The syndrome's antibodies are associated with a higher risk of blood clotting and complications such as late miscarriage and placental insufficiency. Depending on the clinical and serological data, the treatment consists of acetylsalicylic acid and heparin. In 2020 Davis-Porada et al investigated the incidence of SLE flares during pregnancy and delivery. According to the results of this study, a quarter of pregnant SLE patients are at increased risk of flare-ups, which in most cases were mild or moderate in intensity. During the period between the second and sixth month of childbirth, the risk of flare-up is greater. Pre-pregnancy disease status, age, and race can be prognostic factors. More specifically, inactive pre-pregnancy disease status, older age, and non-Hispanic white race appeared to be protective against SLE flares during pregnancy and delivery (27).

According to Dalal et al, fetal losses in patients with SLE have decreased significantly, with 80-90% of these pregnancies resulting in live births. Prematurity, preeclampsia, which is 2-3 times more common in patients with SLE, and pregnancy loss are among the most frequent complications of the disease. Contrary to the aforementioned Davis-Porada study, Dalal et al argue that pregnancy increases the incidence of disease flares, ranging from 25-65% (24) In the present study, 11% of included subjects were ANA positive by indirect immunofluorescence. This data depicts lower prevalence regarding previous study carried out in 1997 by Cubillos J, et.al which reported in patients with a history of miscarriage a rate of 31.8% (28). On the other hand only 3 out of 70 healthy controls 4.3% (without autoimmune disease or any infertility problem or pregnancy loss) presented with ANA positivity 1/79 of the pregnant women had anti-Ro antibodies (1.3%) and, ANA-positive was more prevalent among patients with fetal death (2 of 5 patients, 40.0%).

An important limitation is the small number of the women with pregnancy problems and also another limitation is the limited follow up of them. Therefore the results need to be confirmed by larger studies. It is proposed to follow an algorithm regarding further estimation and evaluation of the autoantibody positivity. Heading to a personalized way of addressing pregnancy issues demands accurate and cost-effective laboratory techniques. ANA by Indirect immunofluorescence is still considered gold standard for the diagnosis of autoimmune diseases but the use of solid phase assays is of added value especially regarding specificities of autoantibodies. All the laboratory and clinical infor-

mation should be combined and adjusted to guidelines and evidenced based algorithms targeting to accurate and on time diagnosis as well as effective treatment. Autoimmune diseases are pathological entities that show an increased frequency in recent years, with the female population being more prone to their occurrence. While their exact mechanism and etiology are not fully understood, it appears that there are certain genetic and environmental factors that lead the immune system to produce antibodies against the organism itself. Pregnancies complicated by such diseases are characterized as high risk and require specialized monitoring and care by specialized health professionals. Based on the literature review, it appears as a general rule that these pregnancies should be treated individually and that the counseling given to couples before conception, throughout pregnancy, plays a decisive role throughout the course of the pregnancy and also during the caesarean section, but also after childbirth (22-28).

Rheumatic diseases consist of a very wide range of diseases, each of which presents its own symptoms and requires specialized monitoring and treatment. Because of the limited data available on their etiology and pathogenesis, it is essential that people protect themselves and avoid environmental factors that appear to increase the risk of autoimmune diseases, such as UV radiation, certain chemicals, and bacterial or viral infections. In addition, smoking cessation and adopting healthy lifestyles, such as adequate intake of nutrients and in particular vitamin D, physical exercise and reduction of stress can be protective (22-28).

6. CONCLUSION

Taking into account that rheumatic diseases mostly affect women, the state and primary health care should ensure, through health promotion programs, timely information for women from a young age. Subsequently, an important role can be played by secondary prevention, which concerns the diagnosis of the disease as early as possible, in order to achieve better therapeutic results.

Pregnancies complicated by rheumatic diseases should be approached interdisciplinary, by teams of experienced professionals that should definitely include an obstetrician-gynecologist and a rheumatologist.

- **Author's contribution:** All authors were involved in all steps of preparation this article. Final proofreading was made by the first author.
- **Conflicts of interest:** There are no conflicts of interest.
- **Financial support and sponsorship:** None.

REFERENCES

1. Sheng X, Song X, Xiong Y. et al. Maternal and infant outcomes of pregnancy associated with anti-SSA/RO antibodies: a systematic review and meta-analysis. *Pediatr Rheumatol.* 2023; 21: <https://doi.org/10.1186/s12969-023-00803-0>
2. Wang L, Wang FS, Gershwin ME. Human autoimmune diseases: a comprehensive update. *J Intern Med.* 2015 Oct;278(4):369-95 PMID: 26212387
3. Tincani A, Biasini Rebaioli C, Frassi M, Taglietti M, Gorla R, Cavazzana I, Pregnancy Study Group of Italian Society of Rheumatology Pregnancy and autoimmunity: maternal treatment and maternal disease influence on pregnancy outcome *Autoimmun Rev.* 2005 Sep; 4(7): 423-428. doi: 0.1016/j.autrev.2005.03.001. Epub 2005 Apr 2. PMID: 16137607
4. Marder W, Littlejohn EA, Somers EC. Pregnancy and autoimmune connective tissue diseases. *Best Practice & Research Clinical Rheumatology.* 2016; 30(1): 63-80.
5. Ma K, Li J, Fang Y, Lu L. Roles of B Cell-Intrinsic TLR Signals in Systemic Lupus Erythematosus. *Int J Mol Sci.* 2015 Jun 9; 16(6): 13084-13105. doi: 10.3390/ijms160613084. PMID: 26068236
6. Marshak-Rothstein A. Toll-like receptors in systemic autoimmune disease. *Nat Rev Immunol.* 2006 Nov; 6(11): 823-835.
7. Andreoli L, Bertias GK, Agmon-Levin N, Brown S, Cervera R, Tektonidou M, et al. EULAR recommendations for women's health and the management of family planning, assisted reproduction, pregnancy and menopause in patients with systemic lupus erythematosus and/or antiphospholipid syndrome. *Ann Rheum Dis.* 2017 Mar; 76(3): 476-485.
8. Mavragani CP, Dafni UG, Tzioufas AG, Moutsopoulos HM. Pregnancy outcome and anti-Ro/SSA in autoimmune diseases: a retrospective cohort study. *Br J Rheumatol.* 1998 Jul; 37(7): 740-745. doi: 10.1093/rheumatology/37.7.740. PMID: 9714349
9. Brucato A, Doria A, Frassi M, Castellino G, Franceschini F, Faden D, et al. Pregnancy outcome in 100 women with autoimmune diseases and anti-Ro/SSA antibodies: a prospective controlled study. *Lupus.* 2002; 11(11): 716-721. doi: 10.1191/0961203302lu252oa. PMID: 12475001
10. Novak GV, Marques M, Balbi V, Gormezano NW, Kozu K, Sakamoto AP, et al. Anti-RO/SSA and anti-La/SSB antibodies: Association with mild lupus manifestations in 645 childhood-onset systemic lupus erythematosus. *Autoimmun Rev.* 2017 Feb;16(2):132-135. doi: 10.1016/j.autrev.2016.12.004. Epub 2016 Dec 14. PMID: 27988434
11. Mofors J, Eliasson H, Ambrosi A, Salomonsson S, Skog A, Fored M, et al. Comorbidity and long-term outcome in patients with congenital heart block and their siblings exposed to Ro/SSA autoantibodies in utero. *Ann Rheum Dis.* 2019 May;78(5):696-703. doi: 10.1136/annrheumdis-2018-214406. Epub 2019 Feb 26. PMID: 30808622
12. Knight CL, Nelson-Piercy C. Management of systemic lupus erythematosus during pregnancy: challenges and solutions. *Open Access Rheumatol.* 2017 Mar 10; 9: 37-53.
13. Savino F, et al. *Ital J Pediatr.* Neonatal lupus erythematosus: a cutaneous cases based update. 2016. PMID: 26743447.
14. Fairweather D, Frisancho-Kiss S, Rose NR. Sex differences in autoimmune disease from a pathological perspective, *Am J Pathol.* 2008; 173: 600-609.
15. Rottenstreich A, Shifman Z, Grisaru-Granovksy S, Mishael T, Koslowsky B, Bar-Gil Shitrit A. Factors Associated with Inflammatory Bowel Disease Flare During Pregnancy Among Women with Preconception Remission. *Dig Dis Sci.* 2021 Apr; 66(4): 1189-1194.
16. Lorenz G, Anders HJ. Neutrophils, Dendritic Cells, Toll-

- Like Receptors, and Interferon- γ in Lupus Nephritis. *Semin Nephrol.* 2015 Sep; 35(5): 410-426. doi: 10.1016/j.semnephrol.2015.08.003. PMID: 26573544
17. Zhang X, Zhang J, Pan Z, Zhang Y, Xu X, Sheng Y, et al Transcriptome sequencing reveals novel molecular features of SLE severity. *Front Genet.* 2023 Jul 24; 14: 1121359. doi: 10.3389/fgene.2023.1121359. eCollection 2023. PMID: 37554401
 18. Conrad N, Misra S, Verbakel JY, Verbeke G, Molenberghs G, Taylor PN, et al . Incidence, prevalence, and co-occurrence of autoimmune disorders over time and by age, sex, and socioeconomic status: a population-based cohort study of 22 million individuals in the UK. *Lancet.* 2023 Jun 3; 401(10391): 1878-1890. doi: 10.1016/S0140-6736(23)00457-9. Epub 2023 May 5. PMID: 37156255.
 19. Amirchaghmaghi E, Taghavi SA, Shapouri F, Saeidi S, Rezaei A, Aflatoonian R. The role of toll like receptors in pregnancy. *Int J Fertil Steril.* 2013 Oct; 7(3): 147-154. Epub 2013 Sep 18. PMID: 24520479; PMCID: PMC3914489.
 20. Valeff NJ, Ventimiglia MS, Diao L, Jensen F. Lupus and recurrent pregnancy loss: the role of female sex hormones and B cells. *Front Endocrinol (Lausanne).* 2023 Oct 3; 14: 1233883. doi: 10.3389/fendo.2023.1233883. PMID: 37859991; PMCID: PMC10584304.
 21. Kyriakidis NC, Kockum I, Julkunen H, Hoxha A, Salomonsson S, Meneghel L, et al European families reveal MHC class I and II associations with autoimmune-mediated congenital heart block. *Ann Rheum Dis.* 2018 Sep; 77(9): 1381-1382. doi:10.1136/annrheumdis-2018-212953. Epub 2018 Feb 16. PMID: 29453219
 22. Skog A, Eliasson H, Tingström J, Källberg H, Salomonsson S. Swedish Congenital Heart Block Study Group, Sonesson SE, Wahren-Herlenius M. Long-term growth of children with autoantibody-mediated congenital heart block. *Acta Paediatr.* 2013 Jul; 102(7): 718-726. doi: 10.1111/apa.12248. Epub 2013 Apr 22. PMID: 23551183
 23. Eliasson H, Sonesson SE, Salomonsson S, Skog A, Wahren-Herlenius M, Gadler F; Swedish Congenital Heart Block Study Group. Outcome in young patients with isolated complete atrioventricular block and permanent pacemaker treatment: A nationwide study of 127 patients. *Heart Rhythm.* 2015 Nov; 12(11): 2278-2284. doi: 10.1016/j.hrthm.2015.06.028. Epub 2015 Jun 18. PMID: 26096611
 24. Dalal DS, Patel KA, Patel MA. Systemic Lupus Erythematosus and Pregnancy: A Brief Review. *J Obstet Gynaecol India.* 2019 Apr; 69(2): 104-109.
 25. Yamamoto Y, Aoki S. Systemic lupus erythematosus: strategies to improve pregnancy outcomes. *Int J Womens Health.* 2016 Jul 8; 8: 265-272.
 26. Merz WM, Fischer-Betz R, Hellwig K, Lamprecht G, Gembruch U. Pregnancy and Autoimmune Disease. *Dtsch Arztebl Int.* 2022 Mar 4; 119(9): 145-156.
 27. Davis-Porada J, Kim MY, Guerra MM, Laskin CA, Petri M, Lockshin MD, et al . Low frequency of flares during pregnancy and post-partum in stable lupus patients. *Arthritis Res Ther.* 2020 Mar 19; 22(1): 52.
 28. Cubillos J, Lucena A, Lucena C, Mendoza JC, Ruiz H, Arango A, et al Incidence of autoantibodies in the infertile population. *Early Pregnancy.* 1997 Jun; 3(2): 119-124. PMID: 9429853.