Review paper

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Antinuclear antibodies in healthy people and non-rheumatic diseases – diagnostic and clinical implications

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Abstract

The presence of antinuclear antibodies (ANA) is mainly associated with connective tissue diseases (CTD). In addition, their presence is found in healthy people. These antibodies are more common in women and the elderly. Some drugs and xenobiotics are also important for the development of autoimmunity and ANA synthesis. Moreover, the deficiency of vitamin D in the body of patients correlates with occurrence of these antibodies. Unlike the healthy group, a positive ANA count was observed in patients with atopic dermatitis (AD) and in people with immune disorders. Antinuclear antibodies in low counts are also found in the course of chronic bacterial or viral infection and in patients with hematological malignancies. Also the possibility of false positive results, which may be caused by the choice of method used to determine antibodies, should be borne in mind. Taking into account all these factors, it is concluded that the ANA result itself has no diagnostic value.

Key words: antinuclear antibodies, autoimmune diseases, methods of detection.

Epidemiology

Antinuclear antibodies (ANA) are present not only in patients with connective tissue diseases (CTD) but also in healthy people. Recently, there is a hypothesis that the prevalence of ANA in the general population may be associated with immune disorders characteristic of a given species. The confirmation of this supposition is the fact that the process of autoimmunity occurs more often in humans than in animals [1]. The percentage of the population with ANA is approximately 25% by using indirect immunofluorescence microscopy performed on HEp-2 cells (IIFA on HEp-2 or HEp-2000) [2]. This method is the reference method for ANA screening (gold standard) [2, 3]. According to some reports, using IIFA, ANA in low counts may appear in up to 40% of healthy people [4]. Moreover, patients with CTD, such as systemic lupus erythematosus, scleroderma and dermatomyositis, are also frequently ANA positive [3, 5, 6]. Commonly positive antibody values may result from the high sensitivity of the methods used, which allows their presence to be confirmed already in the case of low counts and the presence of low luminescence during immunofluorescence [7].

Even though ANA has very high sensitivity, its specificity is quite low [6]. In order to avoid overdiagnosis of autoimmune diseases, most laboratories set the upper limit as the cut-off point for positive results [8]. Thus, the quantification of antibodies is carried out by successive dilutions of the examined serum, which makes it possible to determine their count. In clinical practice, the count means such dilution of the serum when they are still detectable. Since all systemic lupus erythematosus (SLE) patients are ANA positive, ANA testing by IIFA is an excellent screening tool, which is very useful in diagnosis of this disease [9]. According to the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) one of the criteria of SLE is positive ANA at a titer of 1 : 80 or greater [10].

The incidence of a significantly elevated ANA level in the general population is 2.5% [2]. Most people with a positive ANA count are not diagnosed with autoimmune disease, and the probability of future disease is low. This is supported by the fact that autoimmune diseases occur in the general population with a frequency of 5 to 7% [11] and the occurrence of SLE does not ex-

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ceed 0.1% of the general population [12]. Thus, positive ANA should not encourage extensive diagnosis of CTD in healthy subjects without clinical signs. Moreover, the presence of ANA in the human population suggests that antinuclear antibodies may be an important component of the normal immune response. On the other hand, it is worth remembering that the presence of antibodies may be noted in the serum many years before the diagnosis of autoimmune disease. This is the case of SLE and Sjögren's syndrome, where ANA are present even for many years before the onset of the first symptoms of the disease [13, 14]. Thus, it is also true that subjects who are in preclinical SLE stages are represented in the ANA positive healthy population and the measurement of these antibodies often enables quick diagnosis of this pathology [15].

Bearing in mind that antinuclear antibodies can be present in healthy subjects as well as those with non-rheumatic diseases, the monitoring of their amount and further rheumatologic diagnosis should be mentioned only in case of clinical symptoms. The presence of ANA without signs of CTD does not require periodic rheumatic check-ups. Many studies show that ANA-positive subjects in the general population do not have an autoimmune disease and are unlikely to develop one in the future [6, 9, 10, 15].

Methods for antinuclear antibody determination

The antinuclear antibody count can be determined using various methods (Fig. 1). As mentioned above, the gold and recommended standard in the immunological diagnosis of systemic CTD is IIFA [3] based on the reaction of antigens with fluorescently labeled specific antibodies. At the site of labeled antibody binding to the antigen, characteristic illumination is observed in a fluorescence microscope equipped with a lamp emitting UV radiation using a marker. This method allows one to demonstrate multiple ANA that react with various antigens. For this reason, IIFA is used as a preliminary study to assess the presence of autoantibodies [16]. This method is time-consuming and requires experienced personnel. False positive results in immunofluorescence tests most often result from non-specific adsorption of antibodies and the presence of several related antigens with similar epitopes [17]. The count of ANA measured by the IIFA method in the liver in healthy subjects is 1/20, and in 1% of subjects it can reach 1/160. Also, the prevalence of ANA is higher among females, African Americans, latent infections, inflammatory liver diseases, some medications and older individuals [16, 18–21]. Aging is associated with development of autoimmunity and higher ANA prevalence, which can be explained by loss of B cell tolerance with age [22, 23]. The study of van der Geest et al. [22] showed that pro-inflammatory B cells capable of producing TNF-α were retained in the elderly, whereas B cells capable of IL-10 production were decreased in old subjects [22]. Some studies confirmed the association of autoimmunity and higher prevalence of ANA in older people [20, 21], while other observations do show such a relation [24, 25].

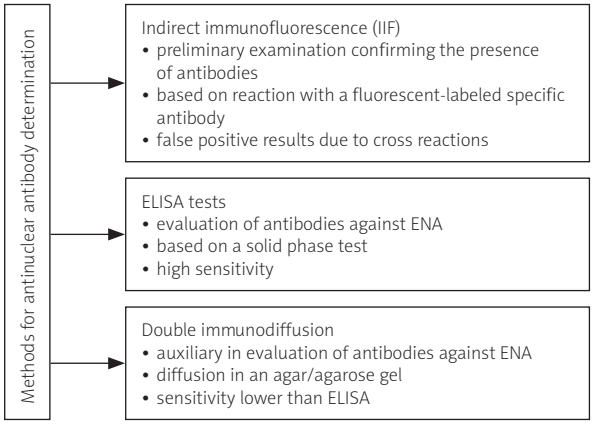

Fig. 1. Methods for antinuclear antibody determination.

Increased age and the presence of some CTD is strongly associated with cancer risk overall. For example, the presence of anti-RNA polymerase III is an independent marker of coincident cancer and scleroderma at any age. Such an association is also observed in patients with anti-topoisomerase I antibodies [26].

If the type of fluorescent spot is found or if no ANA is present and clinical symptoms suggest a systemic CTD, the presence of extractable nuclear antigen antibodies (ENA) should be determined. The enzyme-linked immunosorbent assay (ELISA), double gel diffusion and immunoblot methods are used for this purpose [16].

Immunoenzymatic methods, i.e., ELISA tests, allow qualitative and quantitative determination of antibodies in all classes of immunoglobulins. They can be used to evaluate the dynamics of anti-DNA antibodies in the course of the therapy of SLE [3, 27]. ELISA tests are characterized by simple, but time-consuming execution. They have high sensitivity and specificity due to the use of highly purified antigens on solid media (solid phase) and the use of labeled antigens for detection, combined with a substance whose amount or activity can be measured. The advantage of ELISA tests is the elimination of the subjective factor during the determination [17, 28]. The disadvantage of this method is the presence of minimal contamination with additional antigens that cause false positive results [17].

Both antibodies and antigens have the ability to diffuse into an agar or agarose gel. The double immunodiffusion method allows evaluation of the diffusion of the examined serum and the extract of tissue rich in nuclear material, which is the source of antigens in the agarose gel. An antigen-antibody complex is formed at the meeting point of the antigen and the antibody specific for it. The resulting complexes are precipitated and are visible in the form of white lines. The lines of the examined sera are compared to the reference lines containing the antibody. It is a time-consuming method and requires a lot of experience in the interpretation of results, yet it is used due to its simple implementation. It is characterized by high specificity due to the use of non-denatured antigens, but its sensitivity is lower compared to ELISA tests [17, 28].

The development of new diagnostic technologies has contributed to the introduction of multiplex methods, automated to various degrees and capable of simultaneously measuring numerous antinuclear antibodies. They are often equipped with expert programs in the field of digital image analysis. Automation of the IIFA method can significantly improve the standardization of ANA determination and help to reduce intra-laboratory variability [29]. On the other hand, it should be taken into account that ANAs are molecules with a high charge and can bind to other charged molecules in so-called cross reactions. Therefore, multiplex tests and the solid phase method may give a distorted picture of seropositivity in relation to currently used methods. Older methods, i.e., the immunodiffusion method, require a high concentration of antibodies, and hence they are not very sensitive [7]. In turn, multiplex tests and solid phase tests are sensitive and allow for high throughput, but their interpretation requires caution in the case of preclinical and subclinical disease in which the measured responses may be low, which is why immunofluorescence ANA tests should remain the gold standard for ANA testing [30, 31].

Female gender and antinuclear antibody count

Antinuclear antibody counts are higher in women and increase with age [32]. Sex hormones (especially estrogens) play a significant role in the development of autoimmune diseases and predispose the female sex to more frequent occurrence of these diseases [33]. Estrogens induce an immune response towards T-helper 2 lymphocytes and interact through receptors found on many cells of the immune system. Their effect depends on local concentration and inflammation [34, 35]. The genetic factor also plays an important role. Gene products located on a not fully inactivated X chromosome can avoid presentation in the thymus and cause a break in immune tolerance [36].

It is worth taking into account the fact that pregnancy may induce the appearance of ANA in healthy women [16]. During the course of pregnancy, extensive exposure to nuclear antigens may occur. In addition, there is an increase in inflammatory activity that may also affect the immune response [37, 38].

Regulation at the gene level and antinuclear antibody count

Another interesting observation is the determination of the ANA relationship not only with female sex, but also with a certain demographic dependency. In a study conducted by Li et al. [39], the highest counts were found in non-Hispanic women. At the same time, it was noted that in the case of Afro-American women and men, the ANA counts were higher than in non-Afro-Americans. Furthermore, the presence of the TGM2 gene that is responsible for transglutaminase-2 and the incidence of celiac disease were noted in healthy individuals with high ANA counts [39].

Important information was also provided by a study determining the presence of anti-DSF 70 antibodies (dense fine speckled antigen) [24, 40]. The DSF70 antigen is the most recognizable antigen to which antinuclear antibodies react. They occur mainly in people with allergic diseases (AD) and are not associated with autoimmune diseases [41, 42]. A study conducted among Social Insurance Chukyo Hospital employees aimed to determine the prevalence of anti-DSF70 in a group of healthy people [24]. The presence of positive ANA was confirmed in 20% of people in the analyzed group, and anti-DSF70 antibodies were detected in more than half of this group (54%). Anti-DSF70 was more frequent in women (86%). Although the presence of anti-DSF70 is rare in patients with autoimmune diseases, the screening stage can exclude people who will not develop such diseases [24]. This fact was confirmed by a study conducted by Brazilian researchers. The speckled luminescence characteristic of anti-DSF70 was diagnosed only in a group of healthy people who did not develop any CTD 4 years after the end of the study [18].

Xenobiotics and antinuclear antibody presence

There are scientific reports confirming an effect of some drugs on autoimmunity development. These include gold salts, sulfasalazine, intravenous immunoglobulins and TNF-α blockers (infliximab) as well as procainamide, which can induce ANA production [16]. In addition to drugs, the development of autoimmunity is

affected by other environmental factors such as tobacco smoke, silica and various chemicals [43]. The latter group includes polychlorinated biphenyls and hexachlorobenzene [44, 45]. Persistent organic pollutants (POPs) can also cause hormonal imbalance, leading to an increase in ANA in healthy people and the development of SLE [46]. An effect of benzene, asbestos and mercury on the presence of ANA has also been proven [47–49].

The serum of a group of healthy volunteers was tested as part of the National Health and Nutrition Examination Survey (NHANES). Based on the data collected in the years 1999–2004, there was a correlation between an increased concentration of triclosan in men and positive ANA [50]. Triclosan belongs to the group of phenols and is a germicide, commonly used in antibacterial soaps and toothpastes [51]. The direct mechanism responsible for the relationship between the presence of elevated triclosan concentration in the urine of men with the presence of elevated ANA is not known. One hypothesis indicates the stimulation of T lymphocytes as the cause of autoimmune diseases associated with exposure to environmental factors [52]. In turn, the study carried out by Dinse et al. did not confirm previous reports about the relationship between positive ANA and mercury concentration in the blood. Nevertheless, the influence of mercury on the development of autoimmunity cannot be excluded [50, 53].

In turn, the study conducted by Slight-Webb et al. [54] evaluated the coexistence of ANA with proinflammatory cytokines. In this study, serum of healthy subjects without the presence of ANA, in healthy subjects with the presence of ANA and in patients with SLE, was analyzed. Levels of the majority of proinflammatory cytokines, i.e., interferon-γ (IFN-γ), tumor necrosis factor (TNF), interleukin-17 (IL-17) and granulocyte colony-stimulating factor (GCS), were the highest in patients with SLE, lower in healthy ANA-positive individuals and the lowest in those without ANA. Interferons (INFs), IL-12p40 and stem cell factor/c-Kit ligand have been shown to be elevated in patients with SLE compared to healthy individuals with ANA. In turn, B lymphocyte stimulator (BLyS) is significantly higher in SLE patients and lower in healthy subjects with positive ANA [54].

Vitamin D and antinuclear antibody presence

Numerous scientific reports confirm that vitamin D deficiencies are associated with some autoimmune diseases – including SLE [55]. In addition, observations on mouse models have proven that supplementation with vitamin D leads to a reduction in symptoms in the course of lupus [56]. Vitamin D stimulates the production of B lymphocytes and immunoglobulins. Immune cells contain receptors for vitamin D and convert it to the active form [57], which stimulates Toll-like receptors (TLRs) responsible for increased activity of interferon-α (IFN-α) in patients with SLE [58]. Bearing in mind the above data, researchers from Helsinki compared the concentration of vitamin D in ANA-negative people, healthy people with ANA and patients with SLE. The relationship between vitamin D and B lymphocyte activation, antibody production and IFN-α activity in the analyzed groups was also evaluated. The results clearly indicate that vitamin D deficiencies occur mainly in healthy subjects with positive ANA and patients with SLE, which may be associated with a greater tendency to autoimmunity [59].

Conclusions

Determination of antinuclear antibody count and profile facilitates the diagnosis of CTD. However, it is worth remembering that ANA are often present in healthy people who will not develop autoimmune disease. Due to this fact, the positive ANA result alone has no diagnostic value. Nonetheless, supported by an ap-

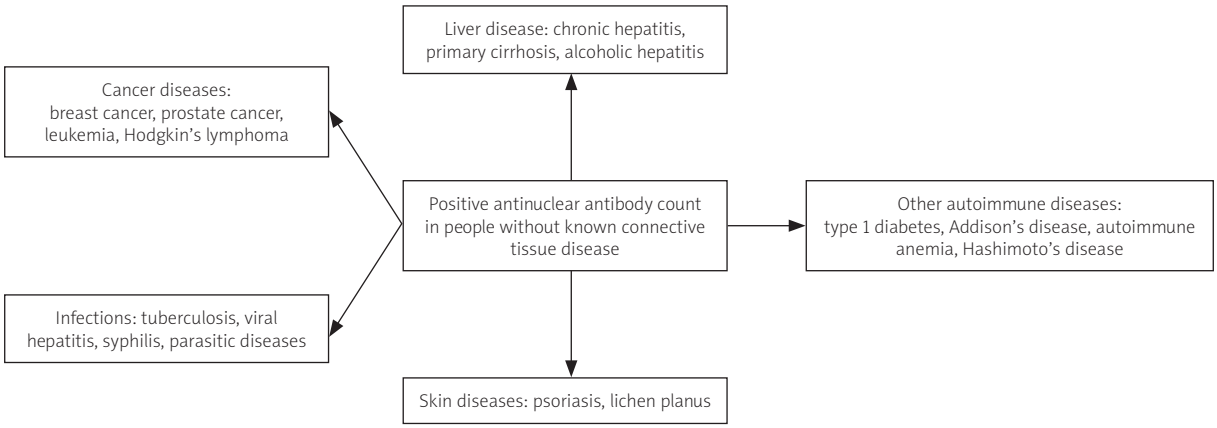

Fig. 2. The occurrence of antinuclear antibodies in patients without a diagnosed connective tissue disease.

propriate medical history, it makes it possible to make an accurate diagnosis. However, considering the fact of quite frequent prevalence of ANA in the general population, its positive titer without clinical symptoms should not be the reason for prolonging diagnosis of CTD. Nevertheless, the patient should be informed that in the case of occurrence of clinical symptoms they should consult the physician. In summary, it is worth noting that the ANA assay is helpful in the diagnosis of rheumatic diseases, although their occurrence frequently is not synonymous with the diagnosis of CTD (Fig. 2).

The authors declare no conflict of interest.

References

1. Rai E, Wakeland EK. Genetic predisposition to autoimmunity – what have we learned? Semin Immunol 2011; 23: 67-83.
2. Wandstrat A, Carr-Johnson F, Branch V, et al. Autoantibody profiling to identify individuals at risk for systemic lupus erythematosus. J Autoimmun 2006; 27: 153-160.
3. Agmon-Levin N, Damoiseaux J, Kallenberg C, et al. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. Ann Rheum Dis 2014; 73: 17-23.
4. Marin GG, Cardiel MH, Cornejo H, et al. Prevalence of Antinuclear Antibodies in 3 Groups of Healthy Individuals. J Clin Rheumatol 2009; 15: 325-329.
5. Petri M, Orbai AM, Alarcón GS, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum 2012; 64: 2677-2686.
6. New Lupus Classification Criteria Presented at ACR/ARHP Annual Meeting. Susan Bernstein. the-rheumatologist.org/article/new-lupus-classification-criteria-presented-acrarhp-annual-meeting
7. Tan EM. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol 1989; 44: 93-151.
8. O'Sullivan M, McLean-Tooke A, Loh R. Antinulcear antibody test. Aust Fam Physician 2013; 42: 718-721.
9. Karamehic J, Subasic D, Gavrankapetanovic F, et al. The incidence of antinuclear antibodies (ANA) detected by indirect immunofluorescence assay (IFA) method. Med Arh 2007; 61: 16-19.
10. Pisetsky DS, Lipsky PE. The Role of ANA Determinations in Classification Criteria for SLE. Arthritis Care Res (Hoboken) 2018 Mar 26. doi: 10.1002/acr.23559.
11. Davidson A, Diamond B. Autoimmune diseases. N Engl J Med 2001; 345: 340-350.
12. Chakravarty E, Bush T, Manzi S, et al. Prevalence of adult systemic lupus erythematosus in California and Pennsylvania in 2000: estimates obtained using hospitalization data. Arthritis Rheum 2007; 56: 2092-2094.
13. Heinlen LD, McClain MT, Merrill J, et al. Clinicalcriteria for systemic lupus erythematosus precede diagnosis, and associated autoantibodies are present before clinical symptoms. Arthritis Rheum 2007; 56: 2344-2351.
14. Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med 2003; 349: 1526-1533.
15. Li QZ, Karp DR, Quan J, et al. Risk factors for ANA positivity in healthy persons. Arthritis Res Ther 2011; 13: R38.
16. Puszczewicz M, Białkowska-Puszczewicz G, Majewski D. Znaczenie autoprzeciwciał w rozpoznaniu chorób reumatycznych. Post Nauk Med 2012; 2: 156-163.
17. Kątnik-Prastowska I. Immunochemia w biologii medycznej. PWN, Warszawa 2009.
18. MarizHA, Sato EI, Barbosa SH, et al. Pattern on the antinuclear antibody-HEp-2 test is a critical parameter for discriminating antinuclear antibody-positive healthy individuals and patients with autoimmune rheumatic diseases. Arthritis Rheum 2011; 63: 191-200.
19. Satoh M, Chan EK, Ho LA, et al. Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. Arthritis Rheum 2012; 64: 2319-2327.
20. Candore G, Grimaldi MP, Listi F, et al. Prevalence of nonorgan-specific autoantibodies in healthy centenarians. Arch Gerontol Geriatr Suppl 2002; 35: 75-80.
21. Nilsson BO, Skogh T, Ernerudh J, et al. Antinuclear antibodies in the oldest-old women and men. J Autoimmun 2006; 27: 281-288.
22. van der Geest KS, Lorencetti PG, Abdulahad WH, et al. Aging-dependent decline of IL-10 producing B cells coincides with production of antinuclear antibodies but not rheumatoid factors. Exp Gerontol 2016; 75: 24-29.
23. Nilsson BO, Skogh T, Ernerudh J, et al. Antinuclear antibodies in the oldest-old women and men. J Autoimmun 2006; 27: 281-288.
24. Watanabe A, Kodera M, Sugiura K, et al. Anti-DFS70 antibodies in 597 healthy hospital workers. Arthritis Rheum 2004; 50: 892-900.
25. Hayashi N, Koshiba M, Nishimura K, et al. Prevalence of disease-specific antinuclear antibodies in general population: estimates from annual physical examinations of residents of a small town over a 5-year period. Mod Rheumatol 2008; 18: 153-160.
26. Shah AA, Hummers LK, Casciola-Rosen L, et al. Examination of autoantibody status and clinical features associated with cancer risk and cancer-associated scleroderma. Arthritis Rheumatol 2015; 67: 1053-1061.
27. Ząbek J. Wsparcie diagnostyczne w rozpoznaniu schorzeń z autoimmunizacją. Medyk, Warszawa 2013.
28. Odrowąż-Sypniewska G. Diagnostyka laboratoryjna wybranych chorób reumatycznych. Med Pharm Polska, Wrocław 2011.
29. Tozzoli R, Bonaguri Ch, Melegari A, et al. Current state of diagnostics technologies in the autoimmunology laboratory. Clin Chem Lab Med 2013; 51: 129-138.
30. Copple SS, Martins TB, Masterson C, et al. Comparison of three multiplex immunoassays for detection of antibodies to extractable nuclear antibodies using clinically defined sera. Ann NY Acad Sci 2007; 1109: 464-472.
31. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. Ann Rheum Dis 2010; 69: 1420-1422.
32. Wiik AS, Bizzaro N. Missing links in high quality diagnostics of inflammatory systemic rheumatic diseases. It is all about the patient! Autoimmun Highlights 2010; 3: 35-49.

33. Gołąb J, Jakóbisiak M, Lasek W, et al. Immunologia. PWN, Warszawa 2014.
34. Quintero O, Amador-Patarroyo M, Montoya-Ortiz G, et al. Autoimmune disease and gender: plausible mechanisms for the female predominance of autoimmunity. J Autoimmun 2012; 38: 109-119.
35. Cutolo M, Straub R. Insights into endocrine-immunological disturbances in autoimmunity and their impact on treatment. Arthritis Res Ther 2009; 11: 218
36. Sacks GP, Studena K, Sargent IL, Redman CWG. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. Am J Obstet Gynecol 1998; 179: 80-86.
37. Bianchi DW, Wataganara T, Lapaire O, et al. Fetal nucleicacids in maternal body fluids. Ann NY Acad Sci 2006; 1075: 63-73.
38. Quintero O, Amador-Patarroyo M, Montoya-Ortiz G, et al. Autoimmune disease and gender: plausible mechanisms for the female predominance of autoimmunity. J Autoimmun 2012; 38: 109-119.
39. Li Q-Z, Karp DR, Quan J, et al. Risk factors for ANA positivity in healthy persons. Arthritis Res Ther 2011; 13: R38
40. Mahler M, Hanly JG, Fritzler MJ. Importance of the dense fine speckled pattern onHEp-2 cells and anti-DFS70 antibodies for the diagnosis of systemic autoimmune diseases. Autoimmun Rev 2012; 11: 642-645.
41. Ochs RL, Muro Y, Si Y, et al. Autoantibodies to DFS 70 kd/transcription coactivator p75 in atopic dermatitis and other conditions. J Allergy Clin Immunol 2000; 105: 1211-1220.
42. Singh DP, Ohguro N, Kikuchi T, et al. Lens epithelium-derive growth factor: effects on growth and survival of lens epithelial cells, keratinocytes, and fibroblasts. Biochem Biophys Res Commun 2000; 267: 373-381.
43. Miller FW. Environmental agents and auto-immune diseases. Adv Exp Med Biol 2011; 711: 61-81.
44. Langer P, Kocan A, Tajtáková M, et al. Increased thyroid volume, prevalence of thyroid antibodies and impaired fasting glucose in young adults from organochlorine cocktail polluted area: outcome of trans generational transmission? Chemosphere 2008; 73: 1145-1150.
45. Daniel V, Huber W, Bauer K, et al. Associations of blood levels of PCB, HCHs, and HCB with numbers of lymphocyte subpopulations, in vitro lymphocyte response, plasmacytokine levels, and immunoglobulin auto-antibodies. Environ Health Perspect 2001; 109: 173-178.
46. Cooper GS, Dooley MA, Treadwell EL, et al. Hormonal, environmental, and infectious disease risk factors for the development of systemic lupus erythematosus. Arthritis Rheum 2004; 41: 1714-1724.
47. Cooper GS, Martin SA, Longnecker MP, et al. Associations between plasma DDE levels and immunologic measures in African- American farmers in North Carolina. Environ Health Perspect 2004; 112: 1080-1084.
48. Pfau JC, Sentissi JJ, Weller G, et al. Assessment of autoimmune responses associated with asbestos exposure in Libby, Montana, USA. Enviro Health Perspect 2005; 113: 25-30.
49. Bernhoft RA. Mercury toxicity and treatment: a review of the literature. J Environ Public Health 2012: ID 460508.
50. Dinse GE, Jusko TA, Whitt IZ, et al. Associations between selected xenobiotics and antinuclear antibodies in the national health and nutrition examination survey, 1999–2004. Environ Health Perspect 2016; 124: 426-436.
51. Fang JL, Stingley RL, Beland FA, et al. Occurrence, efficacy, metabolism, and toxicity of triclosan. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2010; 28: 147-171.
52. Selmi C, Leung PS, Sherr DH, et al. Mechanisms of environmental influence on human autoimmunity: a National Institute of Environmental Health Sciences expert panel workshop. J Autoimmun 2012; 39: 272-284.
53. Somers EC, Ganser MA, Warren JS, et al. Mercury exposure and antinuclear antibodies among females of reproductive age in the United States: NHANES. Environ Health Perspect 2015; 123: 792-798.
54. Slight-Webb S, Ritterhouse LL, Munroe ME, et al. Autoantibody-Positive Healthy Individuals Display Unique Immune Profiles That May Regulate Autoimmunity. Arthritis Rheumatol 2016; 68: 2492-2502.
55. Nagpal S, Na S, Rathnachalam R. Non calcemic actions of vitamin D receptor ligands. Endocr Rev 2005; 26: 662-687.
56. Kamen DL, Cooper GS, Bouali H, et al. Vitamin D deficiency in systemic lupus erythematosus. Autoimmun Rev 2006; 5: 114-117.
57. Crow MK, Kirou KA. Interferon-alpha in systemic lupus erythematosus. Curr Opin Rheumatol 2004; 16: 541-547.
58. Kamen DL, Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. J Mol Med 2010; 88: 441-450.
59. Ritterhouse LL, Crowel SR, Niewold TB, et al. Vitamin D deficiency is associated with an increased autoimmune response in healthy individuals and in patients with systemic lupus erythematosus. Ann Rheum Dis 2011; 70: 1569-1574.