JOURNAL OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

E-ISSN: 2808-9405

JCMID, Vol1. No 1 2021

Prof. Kuntaman

Editor in Chief JCMID

CURRENT ISSUE

Vol. 3 No. 2 (2023): Available online: December 2023

ORIGINAL ARTICLES

Google Scholar

About Us

DOI: https://doi.org/10.51559/jcmid.v2i2

ORIGINAL ARTICLES

Limit of Detection (LOD) of in-house N1N2 CDC real-time RT-PCR assay and commercial kits to detect SARS-CoV-2

pdf

Simon Yosonegoro Liem, Fera Ibrahim, Andi Yasmon
Abstract 276 | pdf Downloads 196 | DOI https://doi.org/10.51559/jcmid.v2i2.23
Page 31-34

Characteristics of candidiasis patients and Candida species antifungal sensitivity patterns in tertiary referral hospitals, Indonesia

pdf

Grisye Sahetapy, Fidriati Olivia Manongga, Ni Nyoman Sri Budayanti, I Nengah Tony Rustawan
Abstract 140 | pdf Downloads 291 | DOI https://doi.org/10.51559/jcmid.v2i2.27
Page 35-38

REVIEW

Microbial co-infection of COVID-19 and therapeutic implications: literature review

pdf

Wani Devita Gunardi, Kris Herawan Timotius
Abstract 94 | pdf Downloads 83 | DOI https://doi.org/10.51559/jcmid.v2i2.31
Page 39-43

Modifications in Adenoviral vectors to enhance its tropism: a literature review

pdf

Erly Sintya, Ni Luh Putu Harta Wedari, Ni Nyoman Sri Budayanti, Ni Luh Made Mirah Rahayu
Abstract 100 | pdf Downloads 168 | DOI https://doi.org/10.51559/jcmid.v2i2.32

Implementation of antinuclear antibodies in autoimmune diagnostic tests: a literature review from immunological aspects

pdf

Ni Luh Putu Harta Wedari, Ni Nyoman Sri Budayanti, I Dewa Made Sukrama, I Putu Bayu Mayura
Abstract 166 | pdf Downloads 230 | DOI https://doi.org/10.51559/jcmid.v2i2.33
Page 27-30

REVIEW

Journal of Clinical Microbiology and Infectious Diseases (*JCMID*) 2022, Volume 2, Number 2: 27-30

Published By : the Indonesian Society for Clinical Microbiology

Implementation of antinuclear antibodies in autoimmune diagnostic tests: a literature review from immunological aspects

Ni Luh Putu Harta Wedari[1*], Ni Nyoman Sri Budayanti[2], I Dewa Made Sukrama[2], I Putu Bayu Mayura[2]

[1]Clinical Microbiology Specialist Program, Faculty of Medicine, Universitas Udayana, Prof. Dr. I.G.N.G. Ngoerah Hospital, Bali, Indonesia;
[2]Clinical Microbiology Department, Faculty of Medicine, Universitas Udayana, Bali, Indonesia;

*Corresponding to:
Ni Luh Putu Harta Wedari;
Clinical Microbiology Specialist Program, Faculty of Medicine, Universitas Udayana, Prof. Dr. I.G.N.G. Ngoerah Hospital, Bali, Indonesia;
hartawedari@gmail.com

Received: 2022-09-22
Accepted: 2022-11-20
Published: 2022-12-08

ABSTRACT

Antinuclear antibodies (ANA) test is mainly used in confirming autoimmune disorders such as systemic lupus erythematosus (SLE) and connective tissue diseases e.g., Sjogren's Syndrome and rheumatoid arthritis. ANA test is often being used as a screening tool for further serological examination. This review aims to explore immunological aspects of anti-nuclear antibodies implementation in autoimmune diagnostic tests. Fluorescent antinuclear antibody (FANA) tests are often being applied since they have high sensitivity and are pretty simple to perform, however, this test has low specificity in diagnosis. In doing this method, patient samples are first diluted then incubated with Hep-2 cells or mouse kidney in glass slides in order to proceed specific binding of antinuclear antibodies. Roughly, around 2% of healthy people and 75% of elderly are positive for FANA test. In contrast, around 5% of people suffering from SLE are negative. Even though it is only seen in 50% up to 70% of SLE patients, ds-DNA antibodies are still the main confirmatory diagnostic gold standard for SLE, particularly in the low amount of C3 complement. Beside ANA, the other diagnostic tests considerably applied are complete blood count test, level of muscle enzyme serum, CXCL4 serum level. Paediatric patients with PM-scleroderma overlapping have been revealed to possess strong positive ANA; anti-Ro/SSA antibody is considered to be the most frequent myositis associated antibody (MAA) in myositis patients.

Keywords: antinuclear, antibodies, autoimmune, immunology.
Cite This Article: Wedari, N.L.P.H., Budayanti, N.N.S., Sukrama, I.D.M., Mayura, I.P.B. 2022. Implementation of antinuclear antibodies in autoimmune diagnostic tests: a literature review from immunological aspects. *Journal of Clinical Microbiology and Infectious Diseases* 2(2): 27-30

INTRODUCTION

Antinuclear antibodies (ANA) are autoantibodies that are produced by the human's immune system when it unsuccessfully recognizes "self" and cannot distinguish it from "non self". To detect this phenomenon, ANA test has been widely used. ANA will react towards the body's healthy cell components causing various signs and symptoms e.g., inflammation in organ tissue and joint, fatigue, and muscle pain. ANA will target the substances those are found in the nucleus. ANA may not cause cell damage, however, it highly results in tissue damage due to reaction with nuclear substances by the time they are being released from cell injury. ANA test is mainly used in confirming autoimmune disorders such as systemic lupus erythematosus (SLE) and connective tissue diseases e.g., Sjogren's Syndrome and rheumatoid arthritis. People suffering from SLE commonly always have positive ANA results, however, a variety of the other autoimmune disorders also possess positive ANA. Roughly, around 3% up to 5% Caucasians people may have positive ANA and it may up to 30% in healthy people aged over 65 years old.[1-7]

Currently, fluorescent antinuclear antibody (FANA) tests are often being applied since they have high sensitivity and are pretty simple to perform, however, this test has low specificity in diagnosis. In doing this method, patient samples are first diluted then incubated with Hep-2 cells or mouse kidney in glass slides in order to proceed specific binding of antinuclear antibodies. Subsequently, to remove unbound antibodies, the slides are washed with phosphate buffered saline (PBS). Incubation with fluorescein labelled by anti-human immunoglobulin to be done afterwards. If the specific antibodies are present, they will be detected on a fluorescence microscope. Roughly, around 2% of healthy people and 75% of elderly are positive for FANA test.[7,8] In contrast, around 5% of people suffering SLE are negative. Therefore, the positive results are diluted to ascertain the titters. Being so, for instance if the FANA test shows positive at 1:40 dilution, eventually greater dilutions at 1:160 and 1:640 have to be performed. Staining patterns as well as the titters are reported to conclude any autoimmune diseases associated. Principally, people suspected with SLE firstly tested for ANA. ANA test is strongly fundamental as a diagnostic and management tool at primary health care settings. ANA test is often being used as a screening tool for further serological examination.[8-15] This review aims to explore the immunological aspects of anti-nuclear antibodies implementation in autoimmune diagnostic tests.

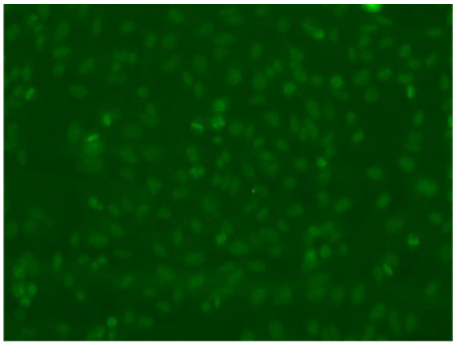

Figure 1. Homogenous ANA pattern.[3]

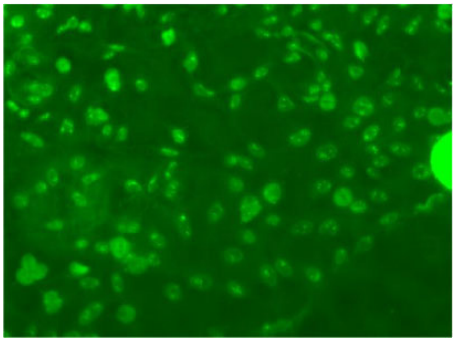

Figure 2. Nucleolar ANA pattern.[3]

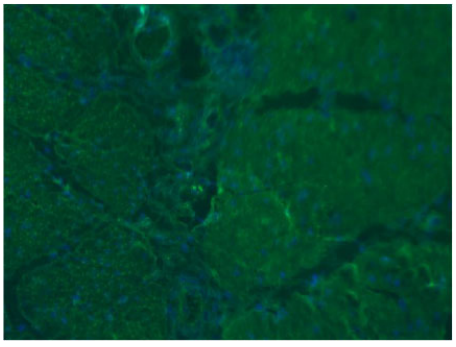

Figure 3. Positive staining result on EMA test at 1:10 dilution.[21]

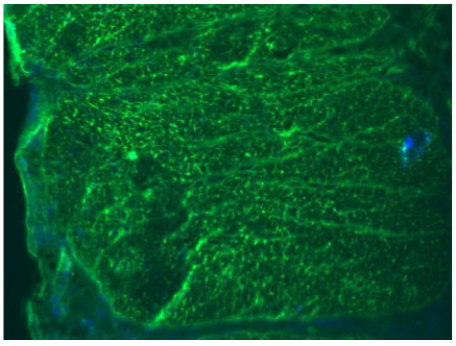

Figure 4. Positive control of EMA test.[21]

Homogenous ANA pattern

Laboratory finding below shows homogenous ANA test result pattern at titer 1:40 as total fluorescence in cell nuclear. The homogenous pattern was still strongly detected up to titer 1:640. Some diseases associated with homogenous ANA pattern are SLE, juvenile idiopathic arthritis, juvenile idiopathic arthritis, and mixed connective tissue disease.[9-17]

Following ANA test, further test of double stranded DNA (ds-DNA) antibodies is strongly suggested to perform as it will only appear if people are positively suffering from SLE. Its level is associated with SLE disease progression. Even though it is only seen in 50% up to 70% of SLE patients, ds-DNA antibodies are still the main confirmatory diagnostic gold standard for SLE, particularly in the low amount of C3 complement. Antibodies towards ds-DNA will assemble either peripheral or homogenous patterns in indirect immunofluorescence. Second main antibody that is found in lupus patient is anti-histone antibody.[7,8,9] That antibody could be detected in most patients with drug-induced lupus.[10] Approximately, 70% of SLE patients have surge amount anti-histone antibodies, however the titter is considerably low. Diagnosis of drug-induced lupus could be supported if merely anti-histone antibody is detected or in combination with antibody towards ds-DNA. High amount of anti-histone antibodies suggest severe SLE.[11,12] Besides, anti-Smith (anti-Sm) antibody is also specific in lupus patients as this antibody cannot be identified in other autoimmune disorders, but it is only detected in 15% until 30% of lupus patients. Anti-Sm antibodies typically will present a coarse speckled pattern in indirect immunofluorescence. Anti-Sm antibody also can be identified by immunoblotting, immunoprecipitation, or enzyme immunoassay (EIA).[18-23]

In SLE patients with cutaneous manifestation, antibodies to SS-B/La and SS-A/Ro have to be checked. Furthermore, anti-nuclear antibodies also can be recognized by immunodiffusion. It is performed in order to ascertain the specificity of FANA positive test results. Immunodiffusion is used to identify certain states of diseases and exclude healthy people who showed FANA positive test results. Nowadays, enzyme immunoassay has been widely used to detect anti ds-DNA and anti-histone antibodies. Anti-phospholipid antibodies also could be another further test as it is found in nearly 60% of lupus patients.[23-27]

Nucleolar ANA pattern

This laboratory finding shows nucleolar ANA test result pattern seen at titer 1:40 and remaining detectable up to titer 1:160. The fluorescent assembled in the nucleolus with a variety of size among the cells. Diseases associated with nucleolar ANA pattern are scleroderma and polymyositis.[3,5,7,8,9]

Scleroderma is disorder characterized by skin thickening and hardening as excess amount of tissue being deposited within the skin; Raynaud's phenomenon (unexpected constriction of blood vessels causing disruption of blood flow into extremities, mainly toes and fingers; muscle and joint pain, and stiffness. Beside ANA, the other diagnostic tests considerably applied are complete blood count test, level of muscle enzyme serum, CXCL4 serum level.[9,11,12] Patients suspected with polymyositis have symptoms such pain and stiffness of muscle, swallowing difficulty, if heart being affected will manifest as irregularity in heart rhythms.[13] To confirm polymyositis, further test should be performed such e.g., anti-synthetase autoantibodies in which the most common is anti Jo-1, a specific antibody usually found in people with idiopathic inflammatory myopathies; anti-signal recognition particle (SRP) that has high association to necrotizing myopathy and children suffering from muscle weakness or muscular dystrophy.[15] Anti-TIF1-γ antibody commonly found in juvenile dermatomyositis in paediatric patients; anti-nuclear matrix protein 2 (NXP2) in which increase significantly in patients with calcinosis; anti-PM/Scl that could be detected in patients with idiopathic inflammatory myositis and sclerodermatomyositis. Paediatric patients with PM-scleroderma overlapping have been revealed to possess strong positive ANA; anti-Ro/SSA antibody is considered to be most frequent myositis associated antibody (MAA) in myositis patients.[15,17,19]

Diagnostic tests of coeliac disease

Coeliac disease is chronic enteropathy that can affect all ages resulting from abnormal reaction of the immune system

to gluten. It is characterized by response of autoimmune in people those susceptible genetically leading to injury of intestinal mucosal, hence developing malabsorption finally resulting in malnutrition along with its effects such as vitamin deficiencies, anaemia, and even osteoporosis.[18,19] Constant ceasing intake of gluten diet is commonly able to heal the damage of small intestine mucosal and enhance nutrition absorption.[18] Gluten-free intake is usually effective and sufficient enough as coeliac disease management in most of patients in which improvement can be seen in few weeks, however, around 2-5% coeliac patients aged over 50 years old showed no response to gluten-free diet and experiencing refractory coeliac disease (RCD).[21] Various external factors associated with coeliac disease namely early introduction of gluten during childhood, infectious vectors, socioeconomic status, genetic discrepancy in non-human leukocytes antigen (HLA) genes, and HLA-DQ2 or HLA-DQ8 haplotypes.[23,27]

Coeliac disease has become the most prevalent food intolerance in Western countries and appears to be one of emerging issues in health care units. In 1970, worldwide prevalence was 0.03%, however, currently it increases to 1% with a probability of about 0.5%-1.26% in European countries and the United States. Even though its real case rate has been underestimated over decades, the disease's occurrence is constantly increasing. First peak of clinical presentation happened at the age of 6 or 7 years old, however, it may be earlier when gluten was introduced, whereas the second peak happened when patients aged 40 or 50 years old. In Australia, it affects 1 in 70 people, but up to 80% was undiagnosed.[11,13,17,19]

In the 1970s, gastrointestinal endoscopies to take biopsy samples routinely were introduced which brought positive effect in regard to diagnostic approach and case findings globally. Subsequently, in late 1980 two HLA have been identified to be associated with coeliac disease namely HLA-DQ2 and HLA-DQ8. Progression to sensitive and specific serological test has been significantly important to do screening to obtain the actual case prevalence and do prompt treatment afterwards.[13,15] Extra-intestinal chief complaints from the patient could be associated with coeliac disease in all age groups hence histological abnormalities detection has important role to improve diagnosis.[17] Being said that, even though advancement of screening, coeliac disease remains undiagnosed. In 1980, ratio between coeliac patients have been diagnosed accurately and those never been diagnosed ranged from 1:5.5 to 1:10, however decades later diagnostic approach has been improved and less invasive.[19] Endomysial antibody (EMA) test was performed to detect IgA autoantibody towards endomysium of monkey oesophagus tissue substrate by indirect immunofluorescence (IF). Specimen was diluted into 1:10, 1:40, and 1:160. Positive staining result appeared weakly on dilution 1:10 (Figure 3) under IF microscope compared to positive control (Figure 4).[22,25]

Currently, serological tests to assess the presence of IgA autoantibody towards EMA and tissue transglutaminase (IgA anti-TG2) are the most sensitive and specific diagnostic tool for coeliac disease. IgA anti-TG2 antibody would be measured, subsequently if the antibody detected, it should be confirmed with anti-EMA antibody test.[25] However, IF for IgA anti-EMA is subjective compared to TG2-ELISA. Nevertheless, EMA remains more sensitive and specific than anti-gliadin assay. Studies peer reviewed in 1985 to 1999 showed EMA test sensitivity was 74% up to 94%, while the specificity was 64% up to 99%. However, post-test probability depends on coeliac disease prevalent in the study population. Hence, if the disease prevalence is lower, post-test probability becomes lower, vice versa.[17,19,21,23,25]

Serological tests of coeliac disease

Prior to serological tests, patients are advised to consume foods containing gluten minimally a few days before the test is performed since half-life of antibodies serum is 30 – 60 days. Even though EMA test has high sensitivity (96.1%) and high specificity (97.4%), roughly about 5-10% of coeliac disease patient have no positive EMA test results.[20] Positive predictive value of EMA test is 83% while IgA anti-TG2 is 72%. TG2 ELISA could be performed in a clinical laboratory that has been standardized in which TG2 is the major auto-antigen of EMA. IgA anti-TG2 test has 93.1% sensitivity and 96.3% specificity as a diagnostic tool. Diagnostic by native gliadin antibodies assessment has lower specificity and sensitivity than IgA anti-TG2 and EMA test, whereas its positive predictive value ranges from 18%-31%. IgA anti-TG2 expressed by plasma cell is prominently expanded in duodenal mucosal in active coeliac disease patients and exhibit high affinity towards TG2 but has minimal adaptation against somatic mutation.[15-21]

Notably, surge cases with selective IgA deficiency occurred in 2% of coeliac disease patients, in which IgA autoantibody will be negative even though they are in active disease.[25] Therefore, to prevent false negatives from serological test results, continuous IgA serum levels should be monitored. Furthermore, in IgA deficiency cases, screening of IgG towards deamidated gliadin peptides (DGP) has to be performed since gliadin peptides only formed in small intestine mucosa in coeliac disease, but IgG anti-DGP has lower predictive value (<70%). Infrequently, in people who have been predisposed genetically, the coeliac antibodies will present. Furthermore, baby aged ≤24 months old has lower sensitivity to IgA antibodies towards endomysium, DGP, and TG2.[21,23,25] According to new diagnostic guidelines from European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) in 2012, if HLA-DQ2 and HLA-DQ8 present, coeliac disease should be confirmed without such an invasive biopsy if some of following criteria are met: classic manifestation of gastrointestinal disorder, IgA anti-TG2 increasing tenfold over cut off value, seropositive confirmation of anti-endomysium antibodies ≥1:5 in certified clinical laboratory, serological and clinical remission after gluten-free diet.[23-25]

CONCLUSION

Positive ANA test results should lead to further specific tests according to patients sign and symptoms. In contrast, negative ANA is supposed to rule out the other underlying autoimmune disorders.

Diagnostic approach of coeliac disease currently evolving rapidly and more focuses on serological testing that is often incorporated with HLA genotyping i.e., in samples with positive result of biopsy but seronegative of coeliac antibodies in blood sample. Serological tests are highly considered to be the main diagnostic tools since they are safe and non-invasive thus can be applied to all age groups of patients.

DISCLOSURES

Conflicts of interest

No conflicts of interest in this literature review.

Funding

All authors are responsible for funding in this study, there is no third party involved.

Ethical Statement

Not applicable.

Author contribution

All authors contributed equally for publication of this literature review.

REFERENCES

1. Li H, Zheng Y, Chen L. High titers of antinuclear antibody and the presence of multiple autoantibodies are highly suggestive of systemic lupus erythematosus. Sci Rep. 2022:1687-1695.
2. Tešija Kuna A, Đerek L, Drvar V, Kozmar A, Gugo K. Assessment of antinuclear antibodies (ANA): National recommendations on behalf of the Croatian society of medical biochemistry and laboratory medicine. Biochem Med. 2021;31(2):020502.
3. Marvin JF, May YC. Antinuclear Antibody Testing: Gold Standard Revisited. The Journal of Applied Laboratory Medicine. 2022;7(1):2771-2783.
4. Petri AM, Orbai GS, Alarcon C. Gordon JT, Merrill PR. Fortin M. Derivation and validation of systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012;64:2677–2686.
5. Mariz HA, Sato EI, Barbosa, SH, Rodrigues SH, Dellavance A, Andrade LE. 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.
6. So M, O'Rourke C, Bahnson HT, Greenbaum CJ, Speake C. Autoantibody Reversion: Changing Risk Categories in Multiple-Autoantibody-Positive Individuals. Diabetes Care. 2020;43(4):913-917.
7. Fritzler MJ, Choi MY, Satoh M, Mahler M. Autoantibody Discovery, Assay Development and Adoption: Death Valley, the Sea of Survival and Beyond. Front Immunol. 2021;12:679613.
8. Tozzoli R, Villalta D, Bizzaro N. Challenges in the Standardization of Autoantibody Testing: a Comprehensive Review. Clin Rev Allergy Immunol. 2017;53(1):68-77.
9. Conrad K, Roggenbuck D, Reinhold D, Sack U. Autoantibody diagnostics in clinical practice. Autoimmun Rev. 2012;11(3):207-11.
10. Olsen NJ, Choi MY, Fritzler MJ. Emerging technologies in autoantibody testing for rheumatic diseases. Arthritis Res Ther. 2017;19(1):172.
11. Håkansson Å, Andrén Aronsson C, Brundin C, Oscarsson E, Molin G, Agardh D. Effects of *Lactobacillus plantarum* and *Lactobacillus paracasei* on the Peripheral Immune Response in Children with Celiac Disease Autoimmunity: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. Nutrients. 2019;11(8):1925.
12. Gupta S, Banerjee A, Syed P, Srivastava S. Profiling Autoantibody Responses to Devise Novel Diagnostic and Prognostic Markers Using High-Density Protein Microarrays. Methods Mol Biol. 2021;2344:191-208.
13. Sahlström P, Hansson M, Steen J, Amara K, Titcombe PJ, Forsström B, Stålesen R, Israelsson L, Piccoli L, Lundberg K, Klareskog L, Mueller DL, Catrina AI, Skriner K, Malmström V, Grönwall C. Different Hierarchies of Anti-Modified Protein Autoantibody Reactivities in Rheumatoid Arthritis. Arthritis Rheumatol. 2020;10:1643-1657.
14. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, Harley JB. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med. 2003;349(16):1526-33.
15. Meroni PL, Bizzaro N, Cavazzana I, Borghi MO, Tincani A. Automated tests of ANA immunofluorescence as throughput autoantibody detection technology: strengths and limitations. BMC Med. 2014;12:38.
16. Bagnasco M, Grassia L, Pesce G. The management of the patient with unexpected autoantibody positivity. Autoimmun Rev. 2007;6(6):347-53.
17. Putranto TA, Wibisini D, Astoro NW, Yana ML, Prabowo ET, Irwansyah D, Nurhadiyanta, Rantung Y, Ikrar T, Fandrich F. Introducing the tolerogenic macrophage therapy as an alternative approach to manage systemic lupus erythematosus: a case series. Bali Medical Journal. 2019;8(3): 610-616.
18. Stohl W, Hiepe F, Latinis KM, Thomas M, Scheinberg MA, Clarke A, Aranow C, Wellborne FR, Abud-Mendoza C, Hough DR, Pineda L, Migone TS, Zhong ZJ, Freimuth WW, Chatham WW. Belimumab reduces autoantibodies, normalizes low complement levels, and reduces select B cell populations in patients with systemic lupus erythematosus. Arthritis Rheum. 2012;64(7):2328-37.
19. Trallero-Araguás E, Rodrigo-Pendás JÁ, Selva-O'Callaghan A, Martínez-Gómez X, Bosch X, Labrador-Horrillo M, Grau-Junyent JM, Vilardell-Tarrés M. Usefulness of anti-p155 autoantibody for diagnosing cancer-associated dermatomyositis: a systematic review and meta-analysis. Arthritis Rheum. 2012;64(2):523-32.
20. Tozzoli R, Villalta D. Profiling of patients with antiphospholipid syndrome using an automated multiplexed immunoassay system. Autoimmun Rev. 2014;13(1):59-63.
21. Caja S, Mäki M, Kaukinen K, Lindfors K. Antibodies in celiac disease: implications beyond diagnostics. Cell Mol Immunol. 2011;8(2):103-9.
22. Kavanaugh A, Tomar R, Reveille J, Solomon DH, Homburger HA. 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. 2000;124(1):71-81.
23. Adler BL, Boin F, Wolters PJ, Bingham CO, Shah AA, Greider C, Casciola-Rosen L, Rosen A. Autoantibodies targeting telomere-associated proteins in systemic sclerosis. Ann Rheum Dis. 2021;80(7):912-919.
24. Wu S, Liu F, Wang F, Huang J, Yin B, Huang M, Wang T, Wei N. Evaluation of the Automated Indirect Immunofluorescence Test for Antinuclear Antibodies. Ann Clin Lab Sci. 2020;50(2):282-286.
25. Marchionatti A, Woodhall M, Waters PJ, Sato DK. Detection of MOG-IgG by cell-based assay: moving from discovery to clinical practice. Neurol Sci. 2021;42(1):73-80.
26. Sack U, Conrad K, Csernok E, Frank I, Hiepe F, Krieger T, Kromminga A, Landenberg Pv, Messer G, Witte T, Mierau R. Autoantikörpernachweis mittels indirekter Immunfluoreszenz an HEp-2-Zellen. Dtsch Med Wochenschr. 2009;134(24):1278-82.
27. Lee H, Kim Y, Han K, Oh EJ. Application of anti-DFS70 antibody and specific autoantibody test algorithms to patients with the dense fine speckled pattern on HEp-2 cells. Scand J Rheumatol. 2016;45(2):122-8.