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A Retrospective Analysis of Anti-nuclear Antibody Test Results Sent to the Medical Microbiology Laboratory of Uşak Training and Research Hospital and Compatibility with the Immunoblot ANA Profile Test Concordance

Uşak Eğitim ve Araştırma Hastanesi Tıbbi Mikrobiyoloji Laboratuvarı'na Gönderilen Antinükleer Antikor Testi Sonuçlarının ve İmmünblot ANA Profil Testi ile Uyumunun Retrospektif Analiz

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ABSTRACT

Aim: In our study, we retrospectively analyzed samples sent from various clinics with antinuclear antibody indirect immunofluorescence (ANA-IIF) test requests, aiming to evaluate the distribution of ANA-IIF patterns and the concordance between ANA-IIF and anti-extractable nuclear antigens (anti-ENA) immunoblot test results.

Materials and Methods: A total of 6.980 samples submitted with ANA-IIF test requests from various clinics to the Medical Microbiology Laboratory of Uşak Training and Research Hospital between January 1, 2023, and December 31, 2024, were retrospectively evaluated due to suspected autoimmune disease. The ANA-IIF test results of these samples, along with the anti-ENA immunoblot test results—if performed—were assessed. The anti-ENA immunoblot test panel includes the detection of autoantibodies against the following antigens: dense fine speckled 70 (DFS70), histone, nucleosome, Sjögren's syndrome antigen-A, Sjögren's syndrome antigen-B, Mi-2, Ku, Ro-52, Scl-70, PM-Scl100, centromere protein-B, Sm, nRNP/Sm, proliferating cell nuclear antigen, Jo-1, M2, and ribosomal P protein.

Results: ANA positivity was detected in 34.1% (2,380/6,980) of the samples, with various patterns and titers. The most common patterns were fine speckled (AC-4, 22.23%), DFS (AC-2, 14.12%), and nucleolar (AC-8, AC-9, AC-10; 10%). The highest concordance between ANA and ENA results was observed in centromere (AC-3) and topoisomerase I-like (AC-29) patterns. In the immunoblot test, DFS70 and Mi-2 antigens were the most frequently detected targets.

Conclusion: DFS70 autoantibodies were found to be prevalent among samples with suspected autoimmune disease. Therefore, panels including DFS70 and Mi-2 antigens are valuable; however, further studies incorporating clinical data are necessary to better clarify their diagnostic relevance and appropriate usage.

Keywords: Antinuclear antibody, anti-ENA immunoblot, DFS70, Mi-2

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ÖZ

Amaç: Çalışmamızda antinükleer antikor indirekt immünofloresan (ANA-İİF) test istemiyle çeşitli kliniklerden gönderilen numuneleri retrospektif olarak tarayarak, ANA-İİF patern dağılımı, ANA-İİF ile anti-ekstrakte edilebilir antijen (anti-ENA) immünoblot testleri arasındaki uyumu değerlendirmeyi hedefledik.

Gereç ve Yöntem: Otoimmün hastalık şüphesiyle Uşak Eğitim ve Araştırma Hastanesi Tıbbi Mikrobiyoloji Laboratuvarı'na 01.01.2023-31.12.2024 tarihleri arasında ANA-İİF test istemiyle çeşitli kliniklerden gönderilen 6980 numune verisi retrospektif olarak değerlendirmeye alınmıştır. Bu numunelerin ANA-İİF testleri ve beraberinde çalışıldıysa anti-ENA immünoblot test sonuçları değerlendirilmiştir. Anti-ENA immünoblot test panelinde yoğun ince benekli 70 (DFS70), histon, nükleozom, Sjögren sendromu antijeni-A, Sjögren sendromu antijeni-B, Mi-2, Ku, Ro-52, Scl-70, PM-Scl100, sentromer protein-B, Sm, nRNP/Sm, proliferasyon hücre nükleer antijeni, Jo-1, M2, Ribozomal P antijenlerine karşı otoantikorlar araştırılmaktadır.

Bulgular: Numunelerin %34,1'inde (2380/6980) çeşitli patern ve titrelerde ANA pozitifliği saptanmıştır. En sık gözlenen paternler sırasıyla; ince benekli (AC-4, %22,23), DFS (AC-2, %14,12) ve nükleoler (AC-8, AC-9, AC-10; %10) paternlerdir. ANA ile ENA test sonuçları arasında en yüksek uyum sentromer (AC-3) ve topoizomeraz I-benzeri (AC-29) paternlerde gözlenmiştir. İmmunblot testinde en sık saptanan otoantikor hedefleri DFS70 ve Mi-2 antijenleridir.

Sonuç: Otoimmün hastalık şüphesiyle gönderilen örneklerde DFS70 otoantikorlarının belirgin oranda yer aldığı görülmüştür. Bu nedenle, DFS70 ve Mi-2 antijenlerini içeren test panelleri değerli olmakla birlikte, klinik verilerin de dahil edildiği daha kapsamlı çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Antinükleer antikor, anti-ENA immünoblot, DFS70, Mi-2

INTRODUCTION

Autoimmune diseases are conditions in which the state of "immunological tolerance" is disrupted, leading the organism to develop autoantibodies against its own antigens. In the United States of America (USA), the prevalence of autoimmune diseases has been reported to be around 7-8%. In industrialized countries, this rate is estimated to be approximately 5%[1]. A report from the USA also indicates that the national expenditure on autoimmune diseases is steadily increasing[2]. Autoimmune diseases are broadly classified into two categories: systemic and organ-specific. Systemic autoimmune diseases include conditions such as systemic lupus erythematosus (SLE), rheumatoid arthritis, juvenile idiopathic arthritis, scleroderma, systemic sclerosis (SSc), Sjögren's syndrome, polymyositis (PM), and dermatomyositis (DM). Autoantibodies play a crucial role in the diagnosis, treatment, and monitoring of these diseases. Antinuclear antibodies (ANA), which are commonly assessed in the diagnosis of autoimmune diseases, comprise a large group of autoantibodies targeting various antigens within the cell nucleus[1]. However, since the term "ANA" does not encompass autoantibodies directed against cytoplasmic and mitotic structures, the term "anti-cell antibody" is now preferred. To promote the use of this inclusive terminology and to standardize result reporting, the International Consensus on ANA Patterns (ICAP) has introduced the (AC) code system[3]. In the diagnosis of ANA, the gold standard method is the indirect immunofluorescence (IIF) assay. The ideal substrate used in this method is Hep-2 cells, which are derived from human laryngeal carcinoma. These cells are preferred because they express a wide range of nuclear, cytoplasmic, and mitotic structures, allowing for the detection of autoantibodies when present. In cases with a positive ANA result, the presence of specific autoantibodies should be investigated by testing for antibodies against extractable nuclear antigens (ENA). For this purpose, enzyme-linked immunosorbent assay (ELISA) or immunoblotting techniques may be employed. In certain clinical conditions, the ANA-IIF test may yield false-negative results. In such cases, especially when clinical suspicion remains high, anti-ENA testing can still be considered[1]. In our study, patient samples submitted to our laboratory with a request for ANA-IIF testing due to suspected autoimmune disease were retrospectively analyzed. The aim was to evaluate the distribution of ANA patterns and to assess the concordance between the results of the ANA-IIF test and the anti-ENA immunoblot test, which was either requested simultaneously or added reflexively.

MATERIALS AND METHODS

Sample Selection

In this study, data from 6980 samples submitted to the Medical Microbiology Laboratory of Uşak Training and Research Hospital between 01.01.2023 and 31.12.2024 with a request for ANA-IIF testing due to suspected autoimmune diseases were retrospectively analyzed. The study was approved by the Uşak University Faculty of Medicine Scientific Research Ethics Committee (decision no: 321-321-20, date: 15.02.2024).

ANA-IIF Assay

For the ANA-IIF test, samples were processed using the IIF assay kit (Euroimmun, Germany) with HEp-20-10 cells and monkey liver substrate at a 1:100 screening dilution, following the manufacturer's instructions. Evaluation was performed visually under a Eurostar III fluorescence microscope (Euroimmun AG, Lübeck, Germany) at 200× and 400× magnification. Reporting was conducted in accordance with ICAP standards, including the corresponding AC codes. Cells exhibiting staining in the nuclear region were reported along with the corresponding

positivity titer. In contrast, patterns showing no nuclear staining but displaying cytoplasmic and/or mitotic staining were reported as "ANA negative, see comment", with the observed pattern described in the comment section.

Anti-ENA Immunoblot Assay

Detection of anti-ENA was performed using the immunoblot method with the EUROLINE ANA profile test kit including Mi-2, Ku, dense fine speckled 70 (DFS70) (Euroimmun, Germany). This kit detects autoantibodies against the following antigens: DFS70, histone, nucleosome, Sjögren's syndrome antigen(SS)-A, SS-B, DNA helicase (Mi-2), DNA binding nuclear protein (Ku), SS (Ro-52), topoisomerase-1 (Scl-70), histidine tRNA synthetase (PM-Scl100), centromere protein-B (CENP B), Smith antigen nuclear ribonucleoprotein (nRNP/Sm), proliferating cell nuclear antigen (PCNA), cytoplasmic histidyl tRNA synthetase (Jo-1), pyruvate dehydrogenase complex-E2 (M2), and ribosomal P. Since both ANA-IIF and ELISA tests are used in our laboratory for the detection of double-stranded DNA autoantibodies (anti-dsDNA), the results of these antibodies were not included in the study.

Statistical Analysis

Descriptive statistics were used to summarize the data. The number and percentage of each ANA-IIF and anti-ENA immunoblot result were calculated, and the findings were presented in tables. Ninety-five percent confidence intervals (CIs) for proportions were calculated using SPSS version 27. The Wilson method was applied for patterns with sufficient sample size, while the Clopper-Pearson (exact) method was used for patterns with small sample sizes or rare events. The agreement between ANA and ENA test results was evaluated using Cohen's kappa (κ) coefficient, based on a 2×2 contingency table constructed from the positive and negative results of both tests. Cohen's kappa was calculated using the following formula:

$$\kappa=(P_o-P_e)/(1-P_e),$$

where P_o represents the observed agreement and P_e denotes the expected agreement by chance. Interpretation of κ values was based on the widely accepted Landis and Koch classification: κ<0.00 = poor, 0.00-0.20 = slight, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 = substantial, and 0.81-1.00 = almost perfect agreement. In addition, the chi-square (χ^2) test was performed to assess the statistical association between ANA and ENA positivity. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 27 (IBM Corp., Armonk, NY, USA).

RESULTS

In our study, 6980 samples submitted to the Medical Microbiology Laboratory of Uşak Training and Research Hospital with a request for ANA testing were retrospectively analyzed. Among the patient samples examined, ANA-IIF positivity was detected in 34.1% (2380/6980) at various patterns and titers. The distribution of positive results according to pattern type is as follows: single patterns 73.70% (1754/2380), double patterns 23.28% (554/2380), and triple patterns 3.02% (72/2380). Among those with a single pattern: nuclear 55.17% (1313/2380), cytoplasmic 13.19% (314/2380), and mitotic 5.34% (127/2380). The most frequently reported pattern among samples with single pattern positivity was "fine speckled (AC-4)", accounting for 22.23% (529/2380) of all positive samples. This was followed by the "DFS (AC-2)" and "nucleolar (AC-8, 9, 10)" patterns. The most commonly reported cytoplasmic and mitotic patterns were "ANA-negative, cytoplasmic fine speckled (AC-20)" and "ANA-negative, intercellular bridge (midbody)", respectively. Dual patterns were observed in 23.28% (554/2380) and triple patterns in 3.02% (72/2380) of the positive samples. The numerical data and proportions of other reported positive patterns are presented in Table 1. Among the 6980 samples, anti-ENA immunoblot testing was performed on 4069 samples due to concurrent or reflex test requests. Of these 4069 samples, 1994 were reported as ANA-IIF positive, while 2075 were ANA-IIF negative. The ANA patterns associated with the antigens included in the anti-ENA immunoblot test used in our laboratory, along with the concordance and CIs between ANA-ENA tests, are presented in Table 2. The patterns showing the highest concordance between ANA and ENA were "centromere (AC-3)" and "topoisomerase I-like (AC-29)", followed by "pleomorphic PCNA (AC-13)" and "ANA-negative cytoplasmic reticular (AC-21)" patterns. Among the 1994 ANA-IIF-positive samples tested with the anti-ENA immunoblot testing, 1106 were found to be positive for anti-ENA antibodies and were therefore considered ANA-ENA compatible. Of the 2075 samples with a negative ANA-IIF test and a concurrent anti-ENA immunoblot test request, 1883 were found to be negative and were assessed as ANA-ENA compatible. ANA-ENA concordance rates for patients with concurrent ANA-IIF and anti-ENA immunoblot test requests are presented in Table 3. A total of 4069 serum samples were evaluated for the compatibility between ANA and ENA test results. A significant association was observed between ANA and ENA positivity [χ^2 (1, n=4069)=1267.5, p<0.001, φ=0.56]. Cohen's kappa coefficient was 0.46 (95% CI: 0.42-0.50), indicating a moderate level of agreement between the two tests. While the chi-square test demonstrated a statistically significant relationship, the kappa value suggested that the overall concordance between ANA and ENA results was moderate, implying that these two assays are not fully interchangeable in diagnostic evaluation. Among the 4069 samples tested for anti-ENA, a total of 1917 autoantibodies were detected, including cases of multiple autoantibody positivity observed in all samples that were either ANA-IIF test positive or negative. All autoantibodies

Table 1. Distribution of samples with single pattern positivity (n=1754) in the anti-nuclear antibody indirect immunofluorescence (ANA-IIF) test

AC code	Pattern name	n (%)	AC code	Pattern name	n (%)	AC code	Pattern name	n (%)
Nuclear		1313 (55.17)	**Cytoplasmic**		314 (13.19)	**Mitotic**		127 (5.34)
AC-1	Homogenous	96 (4.03)	**AC-15**	Cytoplasmic fibrillar linear	15 (0.63)	**AC-24**	Centorosome	26 (1.09)
AC-2	Dense fine speckled	336 (14.12)	**AC-16**	Cytoplasmic fibrillar filamentous	33 (1.39)	**AC-25,26**	Spindle fibers	49 (2.06)
AC-3	Centromere	42 (1.77)	**AC-17**	Cytoplasmic fibrillar segmental	2 (0.08)	**AC-27**	Intercellular bridge (midbody)	52 (2.19)
AC-4	Fine speckled	529 (22.23)	**AC-15,16,17**	Cytoplasmic fibrillar	44 (1.85)	**AC-28**	Mitotic chromosomal	0 (0)
AC-5	Coarse speckled	22 (0.93)	**AC-18**	Cytoplasmic discrete dots	4 (0.17)			
AC-6	Multiple nuclear dots	6 (0.25)	**AC-19**	Cytoplasmic dense fine speckled	11 (0.46)			
AC-7	Few nuclear dots	7 (0.29)	**AC-20**	Cytoplasmic fine speckled	126 (5.29)			
AC-8,9,10	Nucleolar	238 (10)	**AC-21**	Cytoplasmic reticular	56 (2.35)			
AC-11,12	Nuclear membrane	17 (0.71)	**AC-22**	Cytoplasmic polar speckled	14 (0.59)			
AC-13	Pleomorphic PCNA	17 (0.71)	**AC-23**	Cytoplasmic rods and rings	9 (0.38)			
AC-14	Pleomorphic CENP-F	0 (0)						
AC-29	Topoisomerase-I like	3 (0.13)						

AC: Anti-cell, N: Number, P: Percentage, PCNA: Proliferating cell nuclear antigen, CENP-F: Centromere protein-F

Table 2. Autoantibodies corresponding to antigens in the extractable nuclear antigens (ENA) immunoblot test, corresponding antinuclear antibody indirect immunofluorescence (ANA-IIF) patterns, and ANA-ENA concordance rates

AC code*	Pattern name	Associated antigen	Anti-ENA test performed (n)	ANA-ENA concordant (n)	ANA-ENA concordance (%)	95% CI
Positive single patterns			1450	736	50.76	0.48-0.53
Nuclear			1213	711	58.62	0.56-0.61
AC-1	Homogenous	Nükleosome, histone	83	31	37.35	0.28-0.48
AC-2	Dense fine speckled	DFS70	311	216	69.45	0.64-0.74
AC-3	Centromere	CENP-B	40	40	100	0.91-1.00
AC-4	Fine speckled	SS-A, SS-B, Mi-2, Ku	494	342	69.23	0.65-0.73
AC-5	Coarse speckled	nRNP/Sm, Sm	21	14	66.67	0.45-0.83
AC-8, 9, 10	Nucleolar	PM/Scl-100	225	49	21.78	0.17-0.28
AC-13	Pleomorphic PCNA	PCNA	17	16	94.12	0.73-0.99
AC-29	Topoisomerase-I like	Scl-70	3	3	100	0.29-1.00
Cytoplasmic			163	25	15.3	0.11-0.22
AC-19	Cytoplasmic dense fine speckled	Ribosomal P	7	1	14.29	0.00-0.58
AC-20	Cytoplasmic fine speckled	Jo-1	68	3	1.47	0.01-0.12
AC-21	Cytoplasmic reticular	M2	28	21	75	0.57-0.87
Positive double patterns			476	317	66.60	0.62-0.71
Positive triple patterns			68	53	77.94	0.67-0.86
Total			1994	1106	55.47	0.53-0.58

*Only ANA-IIF patterns (1841/1994) corresponding to the antigen in the anti-ENA immunoblot test are listed in the table.

AC: Anti-cell, ANA: Antinuclear antibody, ENA: Extractable nuclear antigen, PCNA: Proliferating cell nuclear, CENP-B: Centromere protein-F, CI: Confidence interval, SS-AA: Sjögren syndrome antigen-A, SS-B: Sjögren syndrome antigen-B, DFS70: Dense fine speckled 70, PM: Polymyositis

Table 3. The ANA-ENA concordance rate according to ANA-IIF positivity in patients with concurrent antinuclear antibody indirect immunofluorescence (ANA-IIF) and anti-extractable nuclear antigen (ENA) immunoblot test requests (n=4069)

	ENA positive	ENA negative	Total
ANA positive	1106 (55.5%-85.2%)	888 (44.5%-32.0%)	1994 (100%-49.0%)
ANA negative	192 (9.3%-14.8%)	1883 (90.7%-68.0%)	2075 (100%-51.0%)
Total	1298 (100%)	2771 (100%)	4069 (100%)

Values in each cell are presented as n (row %, column %)

ANA: Antinuclear antibody, ENA: Extractable nuclear antigen, IIF: Indirect immunofluorescence

Table 4. Antigen distribution of autoantibodies in samples that tested positive in the anti-ENA immunoblot test (n=1917)

	DFS70	Mi-2	Ro-52	PM-Scl100	Ku	SS-A	M2	Scl-70	Jo-1	RNP	SS-B	Centromere	Histone	PCNA	Ribosomal P	Nükleosome	Sm	Total
Number (n)	336	238	215	174	133	126	125	91	86	83	73	68	60	43	31	23	12	1917
Percent (%)	17.5	12.4	11.2	9.1	6.9	6.6	6.5	4.8	4.5	4.3	3.8	3.6	3.1	2.3	1.6	1.2	0.6	100

ENA: Extractable nuclear antigen, DFS70: Dense fine speckled 70, SS-A: Sjögren syndrome antigen-A, PCNA: Proliferating cell nuclear antigen, PM: Polymyositis

Table 5. Anti-ENA immunoblot results in ANA-negative (n=4600) samples

	Not requested	Negative	Ro-52	Jo-1	PM-Scl100	M2	Mi-2	Others	Total
Number (n)	2525	1883	39	33	22	16	15	67	4600
Percent (%)	54.9	40.9	0.9	0.7	0.5	0.3	0.3	1.5	100

ANA: Antinuclear antibody, ENA: Extractable nuclear antigen, PM: Polymyositis

identified in the anti-ENA tests are listed in Table 4. Among the 4600 samples that tested negative for ANA, 2525 did not have a request for anti-ENA testing. For the remaining 2075 samples, anti-ENA immunoblot testing was simultaneously requested by the clinicians along with the ANA test. Of these, 1883 (90.75%) were also negative for anti-ENA. However, in 192 samples (9.25%), various autoantibodies were detected as positive despite a negative ANA-IIF result. The numerical data and proportions of the top five autoantibodies identified in ANA-negative but anti-ENA-positive samples are presented in Table 5.

DISCUSSION

The frequency of ANA positivity may vary considerably depending on the patient population and referral criteria. Previous studies evaluating ANA test results from patients referred for suspected systemic autoimmune diseases have reported positivity rates ranging from 15.4% to 41.2%[4-8]. The larger number of patients in our sample compared to other studies from our country in the literature increases the reliability of the results. In our cohort, the ANA positivity rate was 34.1%, which is consistent with these earlier findings. Among the ANA-positive samples, 55.17% exhibited a nuclear staining pattern, 13.19% showed a cytoplasmic pattern, and 5.34% displayed a mitotic pattern. These results align with previous studies. For example, Stinton et al.[9] identified nuclear patterns in 1102 (40.5%) and cytoplasmic patterns in 402 (14.8%) of 2724 ANA-IIF-tested samples. Similarly, Karakeçe et al.[4] reported the distribution of nuclear, nucleolar, mitotic, and cytoplasmic patterns as 56.2%, 16.2%, 14%, and 13.6%, respectively. The proportion of cytoplasmic staining among ANA-positive results has been reported to range from 6.4% to 21%[10]. In our study, the rate of isolated cytoplasmic staining was 13.19%, which also falls within this range. In studies involving patients tested for suspected systemic autoimmune diseases, the most common ANA-IIF pattern has been the speckled/granular type (AC-4, 5), followed by the homogeneous pattern (AC-1)[11,12]. The speckled pattern is seen in a wide range of autoimmune disorders, including SLE, SSc, mixed connective tissue disease, myositis, and Sjögren's syndrome. The homogeneous pattern is also frequently observed in SLE, RA, juvenile chronic arthritis, and Sjögren's syndrome. Aras et al.[13] reported the dense fine speckled pattern (AC-2) as the most frequent in their analysis of ANA tests from various clinics. Similarly, in a retrospective review of 3330 ANA-IIF results, Arslan and Togay[14] found the speckled (AC-4, 5), dense fine speckled (AC-2), and nucleolar (AC-8, 9, 10) patterns to be the most prevalent, with rates of 30.3%, 21.7%, and 19%, respectively. Our findings are in line with this, with the fine speckled (AC-4), dense fine speckled (AC-2), and nucleolar (AC-8, 9, 10) patterns being

the most common in our study as well, observed in 22.23%, 14.12%, and 10% of cases, respectively. It is well established that ANA positivity can be found in up to 20% of healthy individuals, and nearly half of these cases are linked to anti-DFS70 antibodies[15]. The dense fine speckled pattern has also been observed in various non-autoimmune conditions, including chronic inflammatory diseases, cancer, human immunodeficiency virus (HIV) infection, alopecia areata, and atopic dermatitis[16]. Takeichi et al.[17] demonstrated in their study that DFS70 autoantibodies trigger proinflammatory cytokines in keratinocytes, thereby establishing a link between these antibodies and inflammation. Notably, studies without clinical preselection tend to report this pattern more frequently. Our results, which showed the dense fine speckled pattern in 14.12% (n=336) of patients, are consistent with this observation and support previous findings in the literature. In two previous studies evaluating the concordance between ANA-IIF and anti-ENA immunoblot tests, the highest agreement was observed with the centromere (AC-3) pattern, with concordance rates of 92% and 77.77%, respectively[13,14]. Similarly, in our study, this pattern showed the highest concordance, with a rate of 100%. The antigens associated with this pattern include CENP-A, CENP-B, CENP-C, and less commonly, CENP-D[1]. Among these, only CENP-B is included in the anti-ENA immunoblot test panel used in our laboratory. The perfect concordance observed suggests that the current panel is largely sufficient for detecting the autoantibody responsible for the centromere pattern in ANA-IIF. Interestingly, our study also demonstrated 100% concordance for the topoisomerase I-like staining pattern (AC-29) and 94.12% for the pleomorphic PCNA pattern (AC-13), which is higher than previously reported rates. This may be attributed to the high specificity of the associated autoantibodies. The concordance rate for the dense fine speckled (AC-2) pattern in our cohort was 69.45%. Previous studies have reported concordance rates of 85% and 37.98% for this pattern[13,14]. Gurbuz et al.[18] determined the concordance rate for the dense fine speckled (AC-2) pattern in the ANA-IIF test to be 81.9% in their study examining DFS-positive cases. When comparing the anti-ENA immunoblot test results of the 221 samples showing this pattern, they stated that they could not detect the DFS70 autoantibody in 40 samples, and that five different autoantibodies were detected in 7 of these. This variability supports the idea that the AC-2 pattern may be associated with a broader spectrum of autoantibodies and not exclusively with DFS70. In our study, the most frequently detected autoantibody among the samples tested with the anti-ENA immunoblot was anti-DFS70, identified in 17.5% of cases (336/1917). Similarly, in another study using an immunoblot panel that included DFS70, this autoantibody was also the most common, with a frequency of 20.65%[19]. The second most frequent antibody in our cohort was anti-Mi-2. Initially identified in 1976 in a patient with DM, anti-Mi-2 was originally considered a biomarker for DM. However, subsequent studies have shown its potential utility as a marker for PM as well[20]. While ANA positivity alone is a relatively weak biomarker for DM/PM, the presence of specific ENA antibodies can be more informative. In our study, the third most frequently detected antibody was anti-Ro-52, which is the most commonly observed ENA type in PM/DM and plays a valuable role in differential diagnosis[1]. The relatively high frequency of anti-Mi-2 and anti-Ro-52 in our results highlights the potential diagnostic benefit of incorporating disease-specific myositis panels concurrent with standard ENA immunoblot assays in routine clinical practice, particularly for the evaluation of systemic autoimmune diseases. Among the 2075 samples that underwent simultaneous ANA-IIF and anti-ENA immunoblot testing, 1883 (90.74%) were negative by both methods, while 192 (9.25%) were ANA-negative but positive for at least one autoantibody in the ENA panel. In ANA-negative samples, the most frequently detected autoantibodies were anti-Ro-52, anti-PM-Scl100, and anti-Jo-1. Notably, Gür Vural et al[7]. identified the same top three antibodies in ANA-negative samples in their study[19]. Ro-52 and SS-A autoantibodies are associated with Sjögren's syndrome, while the Jo-1 autoantibody is linked to inflammatory myopathies. Since SS-A, Ro-52, Jo-1, and ribosomal P antigens are not adequately expressed in Hep-2 cells, they may lead to false-negative results in ANA-IIF tests. Therefore, it is recommended to perform an anti-ENA immunoblot test even when the ANA-IIF test is negative, especially in cases with high clinical suspicion[1]. This may explain the detection of anti-Ro-52 and anti-Jo-1 in ANA-negative cases. However, further studies from multiple centers using different commercial kits and larger sample sizes are needed to clarify the behavior of anti-PM-Scl100 antibodies.

Study Limitations

One of the limitations of our study is the insufficient number of certain patterns within the sample population, which restricted the assessment of ANA-ENA concordance for those specific patterns. Another limitation is the inability to perform advanced characterization in some patterns, such as nucleolar (AC-8, 9, 10), nuclear membrane (AC-11, 12), and cytoplasmic fibrillar patterns (AC-15, 16, 17).

CONCLUSION

In conclusion, anti-ENA immunoblot testing should be reserved for specific patient groups when performed together with ANA-IIF, since routine testing of all patients may lead to unnecessary healthcare expenses. Our study identified high frequencies of anti-DFS70 and anti-Mi-2 antibodies. We believe that ENA panels containing these specificities, as well as disease-focused myositis antibody panels, may offer valuable support in the

diagnosis of autoimmune diseases. However, further studies with broader sample sizes and integrated clinical data are warranted to evaluate their full diagnostic utility.

Ethics

Ethics Committee Approval: The study was approved by the Uşak University Faculty of Medicine Scientific Research Ethics Committee (decision no: 321-321-20, date: 15.02.2024).

Informed Consent: Retrospective study.

Footnote

Authorship Contributions

Concept: B.G., Design: B.G., H.H.K., Data Collection or Processing: B.G., Analysis or Interpretation: B.G., H.H.K., Literature Search: B.G., H.H.K., Writing: B.G., H.H.K,

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