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Research Article / Araştırma Makalesi

The Anti-Cytoplasmic and Anti-Mitotic Autoantibodies; Are These Antibodies Associated with Diseases?

Anti-Sitoplazmik ve Anti-Mitotik Otoantikorlar; Bu Antikorların Hastalıklarla İlişkisi Var Mı?

[1]Nilgün Kaşifoglu, [2]Nazife Şule Yaşar Bilge,[2]Timuçin Kaşifoğlu

[1]Department of Medical Microbiology, Eskisehir Osmangazi University, Faculty of Medicine, Eskisehir, Turkey

[2]Department of Internal Medicine, Division of Rheumatology, Eskisehir Osmangazi University, Faculty of Medicine, Eskisehir, Turkey

Abstract

Examination of antinuclear antibody (ANA) is used in diagnosis of systemic autoimmune diseases, and the indirect immunofluorescence (IIF) assay using HEp-2 cells is the gold standard method. HEp-2 allows the detection of multiple target antigen-directed autoantibodies. The guide "The International Consensus on ANA Patterns (ICAP)", characterizes the patterns into three groups: nuclear, cytoplasmic, and mitotic. The majority of these are associated with autoimmune diseases, but some are rarely seen in autoimmune diseases or may be associated with conditions other than autoimmune disease. There is no consensus on how to report cytoplasmic and mitotic patterns-negative or positive. We aimed to examine the characteristics of patients that had cytoplasmic or mitotic staining in ANA evaluation by IIF. In our Medical Microbiology Laboratory, 18985 ANA tests of 16940 patients were studied between 01.01.2015-31.12.2019. Cytoplasmic or mitotic pattern was detected in 393 (2.07%) tests belonging to 385 patients. Cytoplasmic patterns suggestive of anti-mitochondrial antibody (AMA), anti-smooth muscle antibody (ASMA), anti-Jo-1 and anti-ribosomal P-protein were not included. The most common patterns were anti-midbody, anti-spindle fibers, and anti-vimentin patterns. There were 66 rheumatology patients that were negative for ANA but had cytoplasmic or mitotic staining. There was no statistically significant difference between the diagnosis and patterns of these patients. We suggest that the ANA should be reported as "negative" in case of cytoplasmic or mitotic pattern unless the term anti-cell antibody is used. It should be noted in the description part of the report in order to distinguish significant cytoplasmic patterns and give an idea for some specific conditions.
Keywords: Anti-nuclear antibody; anti-cytoplasmic pattern; anti-mitotic pattern; autoantibody; indirect immunofluorescence assay

Özet

Sistemik otoimmün hastalıkların tanısında antinükleer antikor (ANA) incelemesi yapılır ve HEp-2 hücrelerini kullanan indirekt immünfloresan (IIF) test altın standart yöntemdir. HEp-2, çok sayıda hedef antijene yönelmiş otoantikorların saptanmasına imkân verir. "Antinükleer Antikor (ANA) Paterninde Uluslararası Uzlaşı" rehberi, paternleri üç gruba ayırır: nükleer, sitoplazmik ve mitotik. Bunların çoğu otoimmün hastalıklarla ilişkilidir, ancak bazıları otoimmün hastalıklarda nadiren görülür veya otoimmün hastalık dışındaki durumlarla ilişkili olabilir. Sitoplazmik ve mitotik paternlerin nasıl raporlanacağı konusunda- negatif veya pozitif- bir fikir birliği yoktur. IIF ile ANA değerlendirmesinde sitoplazmik veya mitotik boyanma olan hastaların özelliklerini incelemeyi amaçladık. Tıbbi Mikrobiyoloji Laboratuvarımızda 01.01.2015-31.12.2019 tarihleri arasında 16940 hastaya ait 18985 ANA testi çalışılmıştır. 385 hastaya ait 393 (%2.07) testte sitoplazmik veya mitotik patern tespit edildi. Anti-mitokondriyal antikor (AMA), anti-düz kas antikoru (ASMA), anti-Jo-1 ve anti-ribozomal P-proteini düşündüren sitoplazmik paternler çalışmaya dahil edilmedi. En sık görülen paternler anti-midbody (hücreler arası köprü), anti-spindle fibers (iğsi iplikçikler) ve anti-vimentin paternleriydi. Altmış altı romatoloji hastasında ANA negatifti ancak sitoplazmik veya mitotik boyanma saptandı. Bu hastaların tanı ve paternleri arasında istatistiksel olarak anlamlı bir fark bulunamadı. Anti-hücre antikoru terimi kullanılmadıkça, sitoplazmik veya mitotik patern olması durumunda ANA'nın "negatif" olarak rapor edilmesini öneriyoruz. Bu boyanma, önemli sitoplazmik paternleri ayırt etmek ve bazı spesifik durumlar hakkında fikir vermek için raporun açıklama kısmında belirtilmelidir.
Anahtar Kelimeler: Anti-nükleer antikor; anti-sitoplazmik patern; anti-mitotik patern; otoantikor; indirekt immünfloresan test

Correspondence:
Nilgün KAŞİFOĞLU
Department of Medical Microbiology, Eskisehir Osmangazi University, Faculty of Medicine, Eskisehir, Turkey
e-mail: nkasifoglu@ogu.edu.tr

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1. Introduction

The indirect immunofluorescence (IIF) assay using HEp-2 cell substrate is still the gold standard method in the examination of antinuclear antibody (ANA) (1). HEp-2 is an epithelial cell line from human laryngeal carcinoma, consisting large cells with high number of mitotic cells. The high rate of mitosis provides the expression of a large number of different antigens specific to the cycle in cells, thus allowing the detection of multiple target antigen-directed autoantibodies (2). By using the HEp-2 cells, cytoplasmic and mitotic cell patterns besides nuclear patterns, can also be recognized (3). Therefore, the term "anti-cellular antibodies" has been suggested to meet the wider variety of these autoantibodies (4). In the guide referred as "The International Consensus on ANA Patterns (ICAP)", patterns are characterized into three major groups: nuclear, cytoplasmic, mitotic (5). In this guide, the patterns are numbered from AC (anti-cellular) -1 to AC-29 (6). Recently, AC-0 was added to refer the negative result. The nomenclature and representative 29 patterns are available online at the ICAP website: www.anapatterns.org.

The majority of these autoantibodies are associated with autoimmune diseases, but some of them are rarely seen in autoimmune diseases or may be associated with other conditions. Nevertheless, many autoantibodies that have not been proven to be disease-specific so far cause staining in these cells, so interpretation of them by the clinician can be confusing. There is no consensus on how to report cytoplasmic and mitotic patterns-negative or positive (7). The EASI (The European Autoimmunity Standardization Initiative) and IUIS (International Union of Immunological Societies) recommend that cytoplasmic and mitotic patterns should be reported and specified when possible (4).

In this study, patients who applied to the rheumatology clinic and were found to have only cytoplasmic or mitotic patterns were evaluated in terms of demographic characteristics, diagnoses, treatments and concomitant diseases.

2. Materials and Methods

In Medical Microbiology Department of Eskisehir Osmangazi University Faculty of Medicine, 18985 ANA tests of 16940 patients were studied between 01.01.2015-31.12.2019. ANA was tested by IIF on HEp-2 and primate liver cells with fluorescence microscopy according to the instructions of the manufacturer (Euroimmun AG, Luebeck, Germany). The patterns and titers according to the fluorescence intensity compared with the controls were recorded.

This study was approved by Eskişehir Osmangazi University Non-Interventional Clinical Research Ethics Committee (30.04.2019/25). This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Statistical analysis

SPSS statistics program (IBM SPSS Statistics for Windows, Version 23.0 Armonk, NY) was used for data analysis.

3. Results

Among 18985 serum samples, 6506 (34.27%) were positive for ANA. Cytoplasmic or mitotic pattern was detected in 393 (2.07%) tests belonging to 385 patients, but these patterns are not reported as ANA positive. Patients who had cytoplasmic patterns such as anti-mitochondrial antibody (AMA), anti-smooth muscle antibody (ASMA), anti-Jo-1, anti-ribosomal P-protein specific to an autoimmune disease were not included in this group.

Of these 385 patients, 250 (64.9%) were female and 135 (35.1%) were male. The mean age of these group was 46.11 (min 1-max 88). Ninety-two (23.9%) patients were from rheumatology clinics, 242 (62.9%) were from other adult clinics except rheumatology and 51 (13.2%) were from pediatric clinics.

The number and ratio of cytoplasmic or mitotic patterns among all tests in five years are shown on Table 1.

Table 1. The number and ratio of cytoplasmic or mitotic patterns among all ANA tests

Cytoplasmic and/or mitotic patterns and AC codes	The number and ratio of patterns detected in all ANA tests n, % (n/18985)
Anti-midbody (AC-27 Intercellular bridge)	118, 0.62
Anti-spindle fibers (AC-25 Spindle-fibers)	87, 0.46
Anti-vimentin (AC-16 Cytoplasmic fibrillar filamentous)	66, 0.34
Anti-centrosome (AC-24 Centrosome)	42, 0.22
Anti-golgi-like (AC-22 Polar/Golgi-like)	35, 0.18
Rods and rings (AC-23 Rods and rings)	26, 0.14
Anti-tropomyosin-like (AC-16 Cytoplasmic fibrillar filamentous)	10, 0.05
Anti-mitotic coat (AC-28 Mitotic chromosomal)	6, 0.03
Anti-lysosome-like (AC-18 Cytoplasmic discrete dots/GW body-like)	3, 0.02
TOTAL	**393 tests**

There was no statistically significant difference between the cytoplasmic or mitotic pattern groups according to their ages. The most common cytoplasmic or mitotic pattern was anti-midbody pattern. There was no statistically significant difference between distinct patterns and the clinics of the patients ($p>0.05$). In addition, no statistically significant difference was found between gender and cytoplasmic or mitotic patterns ($p>0.05$).

Ninety-two of the patients with cytoplasmic or mitotic staining were rheumatology patients. Among them ANA was positive with different patterns and titers in 24 patients, and 2 patients were weak positive with speckled pattern. Since it was aimed to examine patients who were ANA negative but had cytoplasmic or mitotic staining, remaining 66 patients were evaluated in detail. Table 2 shows the cytoplasmic or mitotic patterns among these 66 patients.

Table 2. The number and ratios of cytoplasmic or mitotic patterns among rheumatology patients

Cytoplasmic and/or mitotic patterns (AC codes)	The number and ratio of patterns detected in rheumatology patients n, %
Anti-midbody (AC-27 Intercellular bridge)	20, 30.3
Anti-vimentin (AC-16 Cytoplasmic fibrillar filamentous)	14, 21.2
Anti-spindle fibers (AC-25 Spindle-fibers)	9, 13.6
Anti-centrosome (AC-24 Centrosome)	9, 13.6
Anti-golgi-like (AC-22 Polar/Golgi-like)	6, 9.1
Rods and rings (AC-23 Rods and rings)	4, 6.1
Anti-tropomyosin-like (AC-16 Cytoplasmic fibrillar filamentous)	3, 4.6
Anti-mitotic coat (AC-28 Mitotic chromosomal)	1, 1.5
Anti-lysosome-like (AC-18 Cytoplasmic discrete dots/GW body-like)	-
TOTAL	**66 patients, 100**

These 66 patients were evaluated in terms of the complaints at the initial referral to the clinics, the diagnoses (Table 3), the treatments they received, the course of their disease and the presence of accompanying diseases, retrospectively. There was no statistically significant difference between the diagnosis and patterns of 66 rheumatology patient.

Table 3. The diagnosis of rheumatology patients with cytoplasmic or mitotic pattern

	NRD	Fb	RA	SNRA	SS	SLE	CV	AS	BD	OA	FMF	PMR	GPA	HFA	Total
Anti-midbody	7	4	2	-	1	-	1	2	1	-	1	-	-	1	20
Anti-vimentin	7	4	1	1	-	-	-	-	-	-	-	-	1	-	14
Anti-spindle fibers	6	-	-	-	-	1	1	-	1	-	-	-	-	-	9
Anti-centrosome	5	1	-	-	1	-	-	-	-	1	1	-	-	-	9

Anti-golgi-like	1	2	-	1	-	-	1	-	-	-	-	1	-	-	6
Rods and rings	2	1	-	-	-	-	-	-	-	1	-	-	-	-	4
Anti-tropomyosin-like	-	-	1	1	-	-	-	1	-	-	-	-	-	-	3
Anti-mitotic coat	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1
TOTAL	28	12	4	4	2	1	3	3	2	2	2	1	1	1	66

NRD: no rheumatologic disease, Fb: fibromyalgia, RA: rheumatoid arthritis, SNRA: seronegative rheumatoid arthritis, SS: Sjögren's syndrome, SLE: systemic lupus erythematosus, CV: cutaneous vasculitis, AS: ankylosing spondylitis, BD: Behçet's disease, OA: osteoarthritis, FMF: familial Mediterranean fever, PMR: polymyalgia rheumatica, GPA: polyangiitis with granulomatosis, HFA: hereditary familial amyloidosis

4. Discussion and Conclusion

The usage of HEp-2 cells as a substrate for ANA IIF has raised awareness that cytoplasmic and mitotic cell patterns can be recognized as well as nuclear patterns (3). The terms 'anti-nuclear antibodies' (ANA) and 'extractable nuclear antigens' (ENA) are no longer technically correct and they do not cover all of the autoantibodies targeted against mitotic spindle apparatus, cytosol or cytoplasmic organelles. (4). Therefore, ICAP actually recommends using the definition of anti-cell (AC) antibody instead of ANA. When these autoantibodies are reported as ANA negative, they can be overlooked by the physician even if additional information is stated in the explanation section (3). According to some reports, clear cytoplasmic or mitotic apparatus reactivity should be reported as ANA IIF positive (5), but some literatures opposed to report cytoplasmic or mitotic patterns as ANA positive (3,8,9). Cytoplasmic patterns have been reported as ANA positive for more than a decade in Brazil (10).

Von Mühlen et al. reported an article based on practices of 118 laboratories in 68 countries, on how to report the ANA (anti-cell antibodies) with recommendations from ICAP. Fifty-five percent of the laboratories reported cytoplasmic patterns as ANA positive (11). In fact, since cytoplasmic patterns are not exactly ANA, the Brazilian Consensus recommends using "anti-cell antibodies" to cover anticytoplasmic patterns, rather than calling them ANA, as per ICAP's recommendation. They also suggest to report the mitotic patterns (AC-24 to AC-28) as positive (12). In our laboratory, we report these patterns as ANA negative and write additional information in the explanation section.

In routine ANA tests, cytoplasmic pattern is reported in different rates as 6.4–21.8% (13-17). In a study, cytoplasmic patterns were detected in 21.8% of 670 ANA positive cases, and the frequency of cytoplasmic patterns was reported to increase with age (17). Stinton et al. reported a positivity rate of 40.5% nuclear pattern and 15% of cytoplasmic pattern out of 2724 sera (14). In our laboratory, cytoplasmic or mitotic patterns, other than significant patterns for autoimmune diseases such as AMA and Jo-1 account for about 2.07% of all ANA tests.

Anti-midbody antibody

We detected the anti-midbody pattern most frequently among cytoplasmic or mitotic patterns, with a rate of 0.62% (118/18985). In ICAP, it is defined and coded as intercellular bridge, AC-27 pattern. Betancur et al. reported anti-midbody positivity as 0.32% in 113491 sera. Among those anti-midbody positive patients, 43% had connective tissue disease (mostly Sjögren's syndrome, rheumatoid arthritis-RA and systemic lupus erythematosus-SLE) and 6% had malignancy. They reported sensorineural hearing loss in 36.6% of patients (18). Vermeersch and Bossuyt reported anti-midbody positivity rate as 0.13% in 68128 consecutive patients in a 14-year period (19). In 1980's anti-midbody antibodies were described in patients with systemic sclerosis and Raynaud's syndrome (20,21). There are case reports reporting the association of systemic sclerosis and anti-midbody antibody (22,23).

Twenty of 66 rheumatology patients were positive for anti-midbody pattern. No

rheumatological disease was considered in 7 of them while four of them were followed-up with fibromyalgia. Only one patient was diagnosed as systemic sclerosis, and the remaining 8 patients were diagnosed with different rheumatological diseases.

Anti-spindle fibers antibody

Anti-spindle fiber antibodies, one of the components of the mitotic apparatus, were the second most common cytoplasmic or mitotic pattern among all ANA tests, and were seen in 9 of 66 rheumatology patients. The two main autoantigens of the anti-mitotic spindle apparatus antibodies are nuclear mitotic apparatus protein 1 (NuMa1) and the kinesin HsEg5 (NuMa2) (19,24,25). In our study, we did not include NuMA1 patterns that are spindle fiber staining accompanied by nuclear speckled staining on interphase cells. ICAP identified spindle fiber (AC-25) and NuMA-like patterns (AC-26) under different codes. The AC-25 spindle fibers pattern is reported to have low positive predictive value for any disease and to be found infrequently in a routine serology diagnostic setting (26). Vermeersch and Bossuyt reported the prevalence of anti-spindle fiber (NuMA2) pattern as 0.06% among 9268 ANA-positive patients (19). Szalat et al. reported 13 anti-NuMA2 positive patients among 36498 sera. One of them was presented with an antiphospholipid syndrome (27).

In our study, the positivity rate of anti-spindle-fiber antibody was 0.46%. Among rheumatology patients, 9 had anti-spindle fiber pattern. No rheumatologic disease was considered in 6 of these 9 patients. One of the remaining patients was diagnosed as cutaneous vasculitis currently in remission, the other was a SLE patient, and the last one was a Behçet's disease patient with uveitis.

Anti-vimentin antibody

Autoantibodies that target vimentin, one of the cytoskeletal filaments, and other microtubules and intermediate filaments cause cytoplasmic filamentous staining. The pattern, encoded and defined as AC-16 cytoplasmic fibrillar filamentous in ICAP, was detected in 66 (0.34%) of 18985 serum samples. It is reported in various diseases but is not typical for a systemic autoimmune rheumatologic disease.

Studies have shown that anti-vimentin antibodies may indicate tissue damage (28) and it can be produced after injury from infection and/or trauma (29), but whether anti-vimentin antibodies accelerate or accentuate tissue damage is less certain (28). Anti-vimentin antibodies may be produced as a signal of chronic injury in organ transplant recipients (30-32) and can be implicated in rejection and poor outcome in solid organ transplantations (32,33). Increased vimentin levels and anti-vimentin antibodies have also been reported in patients with idiopathic pulmonary fibrosis and non-specific interstitial pneumonia, suggesting they occurred after lung injury (34,35).

Kotaska et al. studied anti-vimentin antibodies of 131 children and adolescents with neurofibromatosis type 1 and in control group of 40 individuals, and reported the anti-vimentin antibodies as relevant markers for monitoring the disease (36).

Anti-vimentin pattern was detected in 14 rheumatology patients. Only two of these patients were male. No rheumatologic disease was considered in 7 patients, one of whom was a 56-year-old woman diagnosed with interstitial lung disease. Four of the patients were fibromyalgia patients, one was RA, one was seronegative RA and the other was granulomatosis with polyangiitis (GPA).

Anti-centrosome antibody

Centrosome is major microtubule-organizing center of the cell (37) and is located in the cytoplasm usually close to the nucleus. It consists of two centrioles. Centrioles are needed to organize the assembly of microtubules in mitosis (38). In ICAP, anti-centrioles and anti-centrosome antibodies are coded as AC-24 and defined as distinct centrioles in cytoplasm of interphase and at the poles of metaphase cells. In our study, anti-centrosome antibody was detected in 42 serum samples with a ratio of 0.22%.

Betancur et al. reported anti-centrosome antibody positivity rate as 0.17% in 113491 sera, at a rate similar to ours (18). Vermeersch and Bossuyt reported anti-centrosome antibody positivity rate as 0.08% in 68128 consecutive patients in a 14-year period (19).

After being first described by Brenner et al. in 1980 (39), the antibody against centrosome or centrioles was reported in patients with Raynaud's phenomenon, localized scleroderma, systemic sclerosis, SLE and RA (40-43). In addition to these diseases, anti-centrosome antibodies are described in children with *Mycoplasma pneumonia* infection (44) and in malignancies especially in breast cancer (45).

When anti-centrosome antibody is searched in the literature, especially systemic sclerosis and breast cancer draw attention (42,43,45,46). Hamaguchi et al. reported pulmonary arterial hypertension in 4 of 5 systemic sclerosis patients with anti-centrosome antibody (42). It is stated that anti-centrosome antibody occurs early in breast carcinogenesis (46) or begins in the pre-malignant phase (45).

Anti-centrosome pattern was detected in 9 rheumatology patients that were ANA negative. No rheumatologic disease was considered in 5 of them. The other four patients were diagnosed as fibromyalgia, Sjögren's syndrome, osteoarthritis and familial Mediterranean fever. There were no patients diagnosed with systemic sclerosis. In accordance with the information stated in the literature, one of our patients, a 39-year-old female patient with no rheumatologic disease, had a metastatic breast cancer.

Seven of 9 patients with anti-centrosome were women and one of them was diagnosed with metastatic breast cancer. No information about breast cancer has been found in the records of other patients. However, since it is stated that it may occur in the early breast cancer or premalignant phase, it may be important to follow these women in terms of breast cancer.

Anti-golgi antibody

As anti-golgi antibody has a typical discontinuous speckled or granular perinuclear staining, IIF staining alone may be sufficient for morphological detection (47).

In two studies that screened patients with connective tissue or rheumatic disease, the anti-golgi antibody rate was found 0.1% (48,49). Three different studies reported anti-golgi antibody positivity rates as 0.08%, 0.2% and 0.26%, respectively (50-52). Betancur et al. reported this positivity rate very low as 0.03% (18). The anti-golgi antibody positivity rate among 18985 ANA tests in our study was 0.18% similar to the other studies.

Anti-golgi complex antibodies were first identified in the serum of a patient with Sjögren's syndrome and lymphoma (53). Then it was reported in many different situations such as Sjögren's syndrome, SLE, RA, mixed connective tissue disease, GPA, idiopathic cerebellar ataxia, paraneoplastic cerebellar degeneration, adult Still's disease, and viral infections (14,54,55). Interestingly, Bizzaro et al. reported that high titer anti-golgi antibodies may be an early indicator of systemic autoimmune diseases before significant clinical manifestations appear (56).

Vermeersch et al. identified 20 patients with anti-golgi antibodies of 51586 patients during the 10-year period. Overall, only 3 of the 20 patients had a systemic autoimmune disorder (one Sjögren's, two RA). From the other point of view, only 1 of 164 consecutive patients with Sjögren's syndrome or SLE had anti-golgi autoantibodies (52). Koh et al. reported anti-golgi antibody in 3 patients among 1173 tests, 2 of which were diagnosed as seropositive RA (15).

There are reports/case reports stating that it may be associated with autoimmune hepatitis and/or liver dysfunction (54,57-59). In addition to the aforementioned clinical cases, four women with inflammatory myopathy were reported in different literature that had anti-golgi antibody accompanied by anti-SS-A/Ro antibody (60-63).

Anti-golgi antibody was detected in 6 patients among 66 rheumatology patients. In only one patient no rheumatologic disease was considered, and the remaining patients dispersed into different disease groups. In this

group of 66 patients, there was only one polymyalgia rheumatica patient and this patient had anti-golgi antibody. No laboratory findings suggesting liver dysfunction were found in any of the patients.

Rods & Rings

In ICAP this pattern is coded as AC-23. The rod and ring structures are composed of an enzyme named as inosine monophosphate dehydrogenase type 2. As the presence of this pattern depends on the HEp-2 cell substrate used, the positivity rate in routine ANA tests is unclear. During 5-year period, we found rods and rings pattern in 26 patients which accounts for 0.14%.

When searched in the literature, the first point to notice is the relation of this pattern with HCV positive patients receiving ribavirin/IFN treatment (64,65), but it has also been reported in HCV negative patients (66,67). In addition to ribavirin treatment, patients using mycophenolic acid, azathioprine, methotrexate or acyclovir for diseases other than HCV, patients with autoimmune diseases such as SLE or healthy people can induce rods and rings pattern (17,65,66,68).

Since our study was retrospective, anti-HCV test could not be studied in 13 of 26 patients with this pattern. Anti-HCV was positive in 7 of the remaining 13 patients. This finding supported that many other reasons can trigger the formation of rods and rings pattern. This pattern was detected in four rheumatology patients with negative ANA. Unfortunately, their HCV infection status was unknown.

Anti-tropomyosin-like antibody

The anti-tropomyosin-like pattern, classified under the title AC-16 cytoplasmic fibrillar filamentous in ICAP, is reported to be found in patients with myasthenia gravis (69), ulcerative colitis (70), Crohn's disease (71) and different inflammatory reactions and infections, but exact relationship has not been proven yet. In our study, the positivity rate of anti-tropomyosin antibody was 0.05% (10/18985). This pattern was detected in 3 of 66 rheumatology patients. The diagnoses of these patients were RA, seronegative RA and ankylosing spondylitis. Interestingly, they all had a rheumatologic diagnosis.

Anti-mitotic coat antibody

Mitotic chromosomal, formerly called chromosome coat protein, dividing cell antigen or mitotic chromosome autoantigen, is classified as AC-28 in ICAP. Although this pattern is rare, it has been reported in SLE patients and patients with carcinoma (72,73). Blaschek et al. identified this antibody only in mitotic cells, and reported that it was directed against an antigen called "dividing cell antigen" as known to be histone or histone related protein. In that study, dividing cell antibody was detected in 10 of 183 SLE patients and in one of 39 patients with idiopathic Raynaud's, but not detected in any of the other connective tissue diseases (72).

In our study the positivity rate of mitotic coat pattern was 0.03%. This pattern was detected in a 50-year-old female patient with a complaint of joint pain in the rheumatology group. She was considered as RA and the treatment was initiated but she did not apply for subsequent follow-up.

Anti-lysosome-like antibody

Anti-lysosome–like antibodies are defined as small/medium sized fine-spotted and coarse droplet staining scattered throughout the cytoplasm. It is classified in AC-18 pattern as "cytoplasmic discrete dots/GW body-like" in ICAP. Autoantibodies causing staining as the AC-18 pattern have been reported in distinct systemic autoimmune rheumatologic disorders and in a variety of other diseases; and their prevalence in unselected or specified disease cohorts has not been thoroughly studied (74). While the positivity rate of this pattern was 0.02%, none of these patients were rheumatology patients.

The most important limitation of our study is that we could not identify target antigens monospecifically. Therefore, our comments are made only on IIF images. The second limitation is that the study is retrospective, so we could not reach some data of the patients.

However, our results show that the patterns do not indicate a specific disease and are also detected in the patient group without a rheumatologic disease. Another limitation is that, detailed evaluations were made only in rheumatology patients. Larger studies involving patients from other clinics may provide more valuable information.

Although these patterns constitute a low percentage among all of our ANA tests and some individual patterns are not directly associated with certain diseases; along with the negative ANA report, they should be noted in the description part in order to distinguish significant cytoplasmic patterns and give an idea for some specific conditions. If anti-cell (AC) antibody or HEp-2 indirect immunofluorescence test terms are used in the future, it would be appropriate for all patterns to be reported as positive.

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