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Research Article

Non-Nuclear and Rare Nuclear ANA Patterns in Indirect Immunoflourescence Testing and their Clinical Associations

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Abstract

Objectives: A standardized nomenclature to report Antinuclear antibody(ANA) is given by the International consensus on ANA pattern (ICAP). The cytoplasmic, mitotic and rare nuclear patterns are infrequently reported.The study was done to understand the clinical significance and frequency of these unconventional patterns in our population.

Methods: Retrospective one year blinded study of ANA patterns in serum samples.

Results: Of the 4730 samples, 4568 were included after deleting 162 repeat samples. ANA positivity was seen in 673 cases (14.7%). Cytoplasmic patterns were found in 184 cases (27.3%) and mitotic pattern in 16 (2.4%) cases. Exclusive cytoplasmic patterns were seen in 100 cases (14.3%) and exclusive mitotic pattern in 14 cases (2.08%). Rare nuclear patterns were seen in 30 cases (4.5%).The most common exclusive cytoplasmic pattern was filamentous(n=39), whereas the common cytoplasmic pattern associated with nuclear pattern (mixed pattern) was cytoplasmic homogeneous (AC-19). The rare nuclear patterns included Topo-I (n=9), nuclear envelope (n=5), multiple (n=6) and few (n=8) nuclear dots. While some of the common cytoplasmic patterns like filamentous and homogeneous were more frequent in AIDs the uncommon patterns showed varied clinical associations.

Conclusion: The study demonstrates the clinical significance of reporting exclusive and mixed non nuclear ANA patterns on IIF as many of these have known autoimmune associations.

Keywords: ANA, cytoplasmic patterns, mitotic pattern, rare nuclear pattern, mixed patterns

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Anti nuclear antibody(ANA) screening is a routinely done work up for patients with suspected autoimmune diseases (AIDs).There are many methods available for testing ANA.[1] The American College of Rheumatology ANA task force has recommended IFA using HEp-2 substrate as the "gold standard" for primary screening for ANA in the year 2009.[2] Some clinical laboratories perform solid phase immunoassay (SPIA) to supplement HEp2 IFA screening which adds clinical value to the existing testing algorithm,albeit most laboratories worldwide use HEp 2 IFA as primary screening method.[3]

The International consensus on ANA pattern (ICAP) workshop, which was initiated in the year 2014 was devoted to develop a uniform reporting nomenclature for the various pattern of ANA identified by indirect immunoflourescence (IIF) on HEp-2 cell lines. The identification and reporting of cytoplasmic and mitotic patterns were an important breakthrough and the ICAP committee recommended reporting of these non-nuclear patterns as they carry clinical value in patient diagnosis.[4] Thirty different patterns on HEp-2 were categorized into 4 major groups: negative, nuclear (15 patterns), cytoplasmic (9 patterns) and mitotic (5 patterns).

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Each pattern is denoted by an alphanumeric code and a pattern tree was developed, available in ICAP website for easy reference. The alphanumeric code was abbreviated as "AC" denoting anti-cellular antibodies to encompass all the antigens presents in various components of the cell ie nucleus, cytoplasm and mitotic apparatus. While further refinements in ANA pattern reporting is in progress, the reporting of non-nuclear patterns is not in vogue in many laboratories and requires training.

The prevalence of these patterns and their clinical associations are not well established. These patterns may occur in isolation or along with nuclear patterns as mixed staining patterns. With increasing literature evidence assigning significance to these patterns , it is important to be familiar with these patterns and incorporate them in the routine serology reporting of ANA by IIF.

Although ANA testing is used to screen for autoimmune diseases, a positive ANA screen may be observed in various cancers and infections as well. Although the disease associations of specific autoantibodies are known, overlaps are known to exist and there is also a possibility of ANA positivity in healthy individuals.[5] In this study,we set out to identify the various non-nuclear and rare nuclear patterns in routine ANA reporting and associate its significance in the clinical context.

Methods

The study was conducted in Department of Pathology, St.John's Medical College, a tertiary care referral centre. The study included review of ANA IIF slides for one year period with documentation of ANA pattern with the AC number as per the ICAP consensus statement. The ANA testing by IIF is done with HEp 20-10 cells. The kits used were commercial and procured from EUROIMMUN AG (Germany), with positive and negative control serum provided by the manufacturer. The clinical details, which included age, gender, clinical diagnosis and the clinical department that requested the test, were all collected from the medical records department (MRD) data. The study was approved by the Institutional Ethical committee (Ref No. 95/2019).

The samples were tested at a dilution of 1:100 standardized for our laboratory.[6] For every run a positive and negative control was done.The slides were read using the designated unique identification (ANA) number given for each patient, in a blinded manner independently by all the authors and discrepancies were solved by re-viewing the slide as a team and discussed till consensus was obtained. The immunoflourescence slides were read using Carl Zeiss Flourescent Microscope which uses LED illumination and the slides were read at X400 magnification for the different ANA patterns. The results were then transferred to an excel sheet where the patients MRD numbers were entered. Since multiple testing may be done for patients with clinical suspicion of AIDs, only the index sample finding was included to avoid multiple entry for the same patient and duplication of test results.

Line immunoassay(LIA) findings, wherever available, was noted from the Pathology data base and analyzed. LIA was done by EUROLINE assay (EUROIMMUN, Germany) which are multiparameter line blots. Membrane strips coated with several purified, biochemically characterized antigens as thin parallel lines are used as solid phase. The membranes are fixed as chips at defined positions on plastic foils. Each strip has a control band which indicates whether the individual incubation steps have been performed correctly. An intense dark band at the line of the corresponding antigen appears if the serum sample contains specific antibodies. The LIA used in our lab is EUROLINE ANA profile 3 which has 14 antigens along with PCNA and control band (antigens nRNP / Sm, Sm, SSA, Ro-52, SSB, Scl-70, PM-Scl, PCNA, Jo-1, CENP-B, dsDNA , nucleosomes, histones, ribosomal protein-P, anti-mitochondrial antibodies (AMA-M2). The strips were evaluated using EUROLine scan software.

Results

The total number serum samples received for ANA testing during study period of one year was 4730 with graphical representation of sample details in Figure 1. A shown in Figure 1, the rate of ANA positivity in the population catered by our centre is 14.7%

Baseline Details of the Non-Nuclear and Rare Nuclear ANA Patterns

Of the 673 cases with positive ANA on IIF, cytoplasmic pattern was seen in 184 cases (27.3%) and mitotic pattern in 16 (2.4%) cases. Of the 184 cases, 82 had associated nuclear positivity and 2 cases has associated mitotic pattern. Therefore, exclusive cytoplasmic patterns was seen in 100 cases (14.3%) and exclusive mitotic pattern was seen in 14 cases (2.08%). Rare nuclear patterns was seen in 30 cases

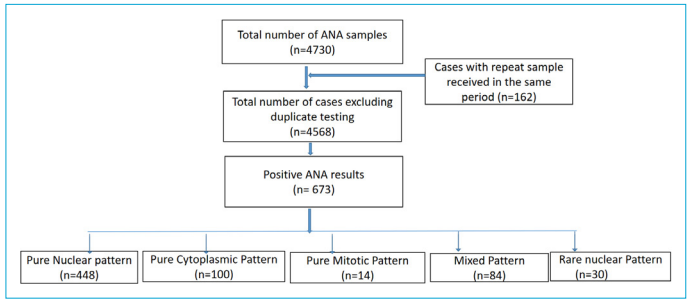

Figure 1. Flow chart representing the ANA sample load and various observed patterns.

(4.5%),with 3 cases having additional cytoplasmic pattern. Clinical diagnosis was available in 200 out of 225 cases with rare nuclear, cytoplasmic and mitotic patterns. The clinical departments from where the samples were received was available in 221 cases, with highest sample load from General medicine department followed by Immunology (Table 1). There were 24.1% males and 75.9% females, with female to male ratio of 3.1:1. Most of the cases were adults (91%), while 8.9% were in pediatric age group. Line immunoblot assay (LIA) was done in 66 cases.

Exclusive Cytoplasmic Pattern on ANA IIF

In 100 cases exclusive cytoplasmic patterns were observed. Nine cases were in pediatric age group and female predominance was seen (n=65). As per the ICAP consensus the cytoplasmic patterns were categorized as: a) fibrillar linear (AC-15, n=1), b) fibrillar filamentous (AC-16, n=39), c) fibrillar segmental (AC-17,n=4), d) discrete dots/GW body like (AC-18,n=17), e) dense fine speckled/homogeneous (AC-19, n=19), f) fine speckled (AC-20, n=4), g) reticular (AC-21,n=9), h) Golgi-like (AC-22,n=6). One case had both discrete dots and Golgi-type staining. The common clinical associations of these various patterns are shown in Table 2. Clinical history was available in 89 cases.

Amongst the cytoplasmic patterns, the most common pattern observed was filamentous (AC-16), with 38% seen in autoimmune conditions. The other unique associations was malignancies and cerebrovascular disease (stroke), seen in 5.4% and 11% respectively. The next common cytoplasmic pattern was dense fine speckled (AC-19) and was seen in 56 % of cases with autoimmune conditions,as shown in Table 2, while 19% were seen in patients with interstitial lung disease (ILD).

Cytoplasmic discrete dots/GW body like pattern (AC-18), which was largely seen in skin related conditions like urticaria and fixed drug eruptions (40%) and lower respiratory infections (LRIs) in 20% cases, and also was found in neurological diseases and AIDs. Cytoplasmic reticular pattern (anti mitochondria {AMA} like, AC-21) was almost always found in patients with autoimmune conditions (83%) including patients with autoimmune liver disease. Golgi like pattern (AC-22) showed a peculiar association with complicated falciparum malaria with multi-organ dysfunction syndrome (33%). Equal percentage of cases were seen in autoimmune conditions. The least common pattern was linear fibrillar pattern (AC-15) seen in a patient with Eale's disease, an idiopathic occlusive vasculitis involving the retina.Figures 2 and 3 illustrates the various cytoplasmic patterns seen in this study

Table 1. Clinical departments from which the samples were received

Clinical departments	Number of cases (n=221)	Percentage (%)
General Medicine	60	27.14
Immunology	45	20.36
Dermatology	18	8.14
Neurology	17	7.69
Nephrology	15	6.78
Emergency medicine	12	5.4
Obstetrics &Gynecology	11	4.97
Pediatrics &Peaditric hematooncology	11	4.97
Pulmonary medicine	7	3.16
Gastroenterology	6	2.74
Surgery,Orthopedics, Cardiothoracic surgery (2 each)	6	0.9 (each)
Opthalmology, ENT, endocrinology, Medical oncology, Psychiatry, urology, cardiology, hematology (1 each)	8	0.45 (each)
Sample received from Referral lab (outside)	5	2.26

Cytoplasmic Pattern Associated with Nuclear Pattern on ANA IIF

We also evaluated the mixed pattern where cytoplasmic patterns were seen along with nuclear patterns and mitotic patterns. There were 82 cases where cytoplasmic pattern was seen along with nuclear patterns and 2 cases where

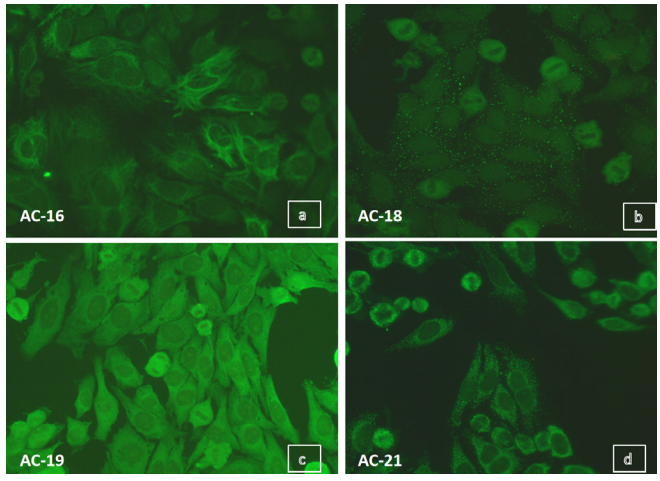

Figure 2. Composite representation of the common cytoplasmic patterns.

Immunoflourescence staining showing: ***(a)*** *cytoplasmic filamentous pattern with staining of intermediate filaments spreading from the nuclear rim,* ***(b)*** *cytoplasmic dots pattern with staining of GW/P bodies in the cytoplasm of interphase cells,* ***(c)*** *cytoplasmic dense fine speckled pattern giving an almost homogeneous cytoplasmic staining and* ***(d)*** *cytoplasmic reticular pattern with coarse granular staining (mitochondria-like) extending throughout the cytoplasm on HEp 20-10 cells..*

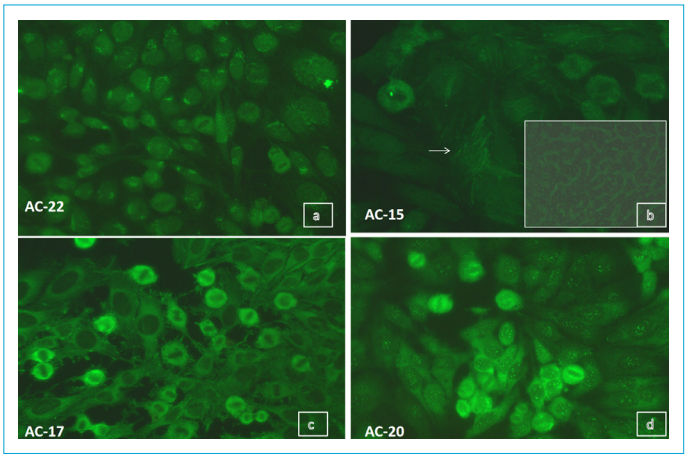

Figure 3. Composite representation of the uncommon cytoplasmic patterns.

*Immunoflourescence staining patterns of **(a)** anti-Golgi apparatus autoantibodies with granular perinuclear staining, **(b)** cytoplasmic linear pattern (actin-like) showing several bunched fibre structures along the long axis of the cells(arrow) with inset showing bile canaliculi staining of primate liver, **(c)** cytoplasmic segmental staining showing short segments along the stress fibres and **(d)** anti-Jo-1 autoantibodies giving a fine speckled cytoplasmic staining with distinct sharp dots in the cell nuclei on HEp 20-10 cells.*

cytoplasmic pattern was seen along with mitotic pattern. Table 3 shows the various cytoplasmic patterns seen with the common nuclear patterns. As seen from the table 3, the most common cytoplasmic pattern associated with both homogeneous and speckled patterns were cytoplasmic dense fine speckled/homogeneous seen in 77% and 79% of these patterns respectively (Fig. 4).

A few cases showed rare mixed patterns. There were 2 cases with nuclear envelope pattern (AC-11,12), one was seen with cytoplasmic discrete dots (AC-18) and other with reticular pattern (AC-21). One case with nuclear dots showed cytoplasmic dense fine speckled staining pattern (AC-19). One cases with centromere pattern had associated cytoplasmic fine speckled (AC-20). Clinical details were available in 75 cases (91.4%), of which 67 cases (87%) had autoimmune diseases.

In 2 cases we found cytoplasmic pattern associated with mitotic patterns. One cases of septic shock where cytoplasmic fibrillar filamentous (AC-16)was seen in association with mitotic spindle fibres (AC-25), while another cases of drug induced liver injury showed a mixed pattern of cyto-

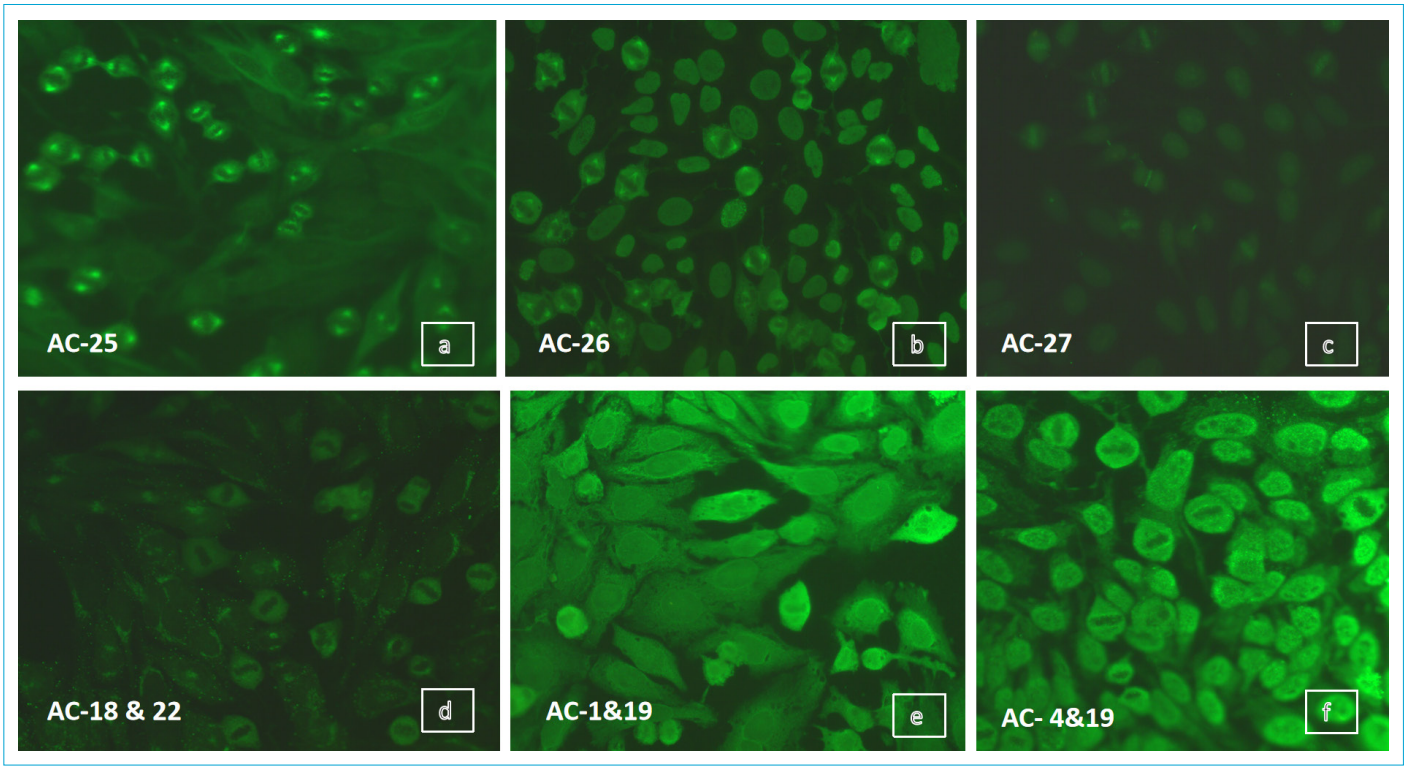

Figure 4. Composite representation of mitotic patterns(top panel) and mixed patterns (bottom panel).

*Top Panel: Immunoflourescence staining patterns of **(a)** spindle fibers stained in mitotic cells, **(b)** nuclear speckled staining with spindle fibers(NuMA-like) and **(c)** staining of intercellular bridges(midbody) on HEp 20-10 cells*

*Bottom Panel: Immunoflourescence staining showing mixed patterns with **(d)** cytoplasmic discrete dots and Golgi apparatus, **(e)** nuclear homogeneous staining with cytoplasmic dense fine speckled pattern and **(f)** nuclear fine speckled staining with cytoplasmic dense fine speckled pattern on HEp 20-10 cells.*

Table 2. Common clinical associations of exclusive cytoplasmic patterns and mitotic patterns

Patterns	Autoimmune etiology	Dermatology related	Renal related	Lung related	Neurology related	Others
I. CYTOPLASMIC PATTERNS (n=100)#						
AC-16 (n=39)	Rheumatoid arthritis (3) SLE/DLE/APLA(7) JIA(1) AIHA(2) Myasthenia gravis(1)	Utricaria/Spongiotic dermatitis with eosinophils/DRESS syndrome with eosinophils (5)	Nephrotic syndrome/CKD/ HUS with AKI(4)	ILD(1)	CVA(acute/old) (4)	Malignancy(2) Takayasu arteritis(1)
AC- 17 (n=4)	NIL	NIL	NIL		Parainfectious cerebellitis(1)	Acute hepatitis / Decompensated chronic liver disease(2) Icerative colitis with arthralgia(1)
AC-18 (n=17)	Adult onset Still's disease(1) Rheumatoid arthritis(1) Aplastic anemia(1)	Erythema nodosum / utricaria / FDE/ Psoriasis(6)	NIL	LRTI (3)	Parainfectious cerebellitis(1) Progressive multifocal leuko encephalopathy (1)	Depression(1)
AC-19 (n=19)	Dermatomyositis(1) Sjogrens Syndrome(1) Rheumatoid arthritis(1) SLE/APLA(3) Multiple Sclerosis(1) ITP(2)	NIL	NIL	ILD(3)		Infections (2)
AC-20 (n=4)	Rheumatoid arthritis with ILD(1)				Viral meningitis(1)	Drug induced bone marrow suppression (1) Celiac disease(1)
AC-21 (n=9)	Suspected AIH/Decompensated Chronic liver disease(2) Sjogren's Syndrome(1) Pure Red cell aplasia(1) Rheumatoid arthriris(1)	Cutaneous vasculitis(1)				
AC-22 (n=6)	AIHA(1) Idiopathic pulmonary fibrosis(1)	NIL	Nephrotic syndrome(1)	NIL		Complicated Falciparum with Multiorgan dysfunction syndrome(2) Primary hyperparathyroidism(1)
II. MITOTIC PATTERNS (n=14)*						
AC-24 (n=4)		Skin rash(1)				Pancytopenia due to infection(2) Plasma cell myeloma(1)
AC-25 (n=4)	ITP(1),AIHA(1),UCTD(2)					

Table 2. CONT.

Patterns	Autoimmune etiology	Dermatology related	Renal related	Lung related	Neurology related	Others
AC-26 (n=4)	Rheumatoid arthritis (1)			LRTI (1)		Carpel tunnel syndrome(1)
AC-27 (n=2)						Vasculitic ulcers(1)
III.RARE NUCLEAR PATTERNS (n=30)†						
AC-6 (n=6)	AIHA(1)				Acute CVA(1)	Severe pulmonary hypertension & thrombocytopenia(1)
					Vascular headache(1)	
AC-7 (n=9)	DLE(1), Primary Sjogren's syndrome(1), Bullous pemphigoid(1)	Drug rash(1)			Sensory axonal neuropathy(1)	Severe depressive disorder(2) Microangiopathic hemolytic anemia(1)
AC-11 &12 (n=5)	JIA(1),Autoimmune hepatitis(2)		CKD(1)			Non specific symptoms(1)
AC-29 (n=9)	Progressive systemic sclerosis(3) Seronegative Rheumatoid arthritis(1), Sjogren's syndrome(1)					Adrenal insufficiency(1)
AC-13 (n=1)						No clinical details available

Clinical details available for 89 cases, * clincial details available for 12 cases ,† Clinical details available in 23 cases; Systemic lupus erythematosis (SLE), Discoid Lupus erythematosis (DLE), Antiphospholipid antibody syndrome (APLA), Juvenile Idiopathic arthritis (JIA), Autoimmune hemolytic anemia (AIHA), Drug reaction with eosinophilia (DRESS), Chronic kidney disease (CKD), Hemolytic uremic syndrome (HUS), Acute kidney injury (AKI), Cerebrovascular accident (CKA), Interstitial lung disease (ILD), Lower respiratory tract infection (LRTI), fixed drug eruption (FDE), Immune thrombocytopenic purpura (ITP), Unclassified connective tissue disorder (UCTD).

plasmic discrete dots (AC-18) with mitotic intercellular bridge (AC-27).

Exclusive Mitotic Pattern on ANA IIF

This pattern was very rarely seen in ANA IIF screening. Only 14 cases had exclusive mitotic pattern. There were 4 cases each with centrosome (AC-24), spindle fibre (AC-25) and NuMA (AC-26) pattern and 2 cases showed intercellular bridge pattern. All cases of mitotic spindle fibre pattern (AC-25) had autoimmune associations. Clinical associations detailed in Table 2 and illustrative photographs in Figure 4.

Rare Nuclear Pattern on ANA IIF

Rare nuclear patterns include those that are uncommonly reported on ANA IIF and it included discrete nuclear dots (AC-6&7), nuclear envelope (AC-11 &12), pleomorphic pattern (AC-13) and Topo-I pattern(AC-29).We found these patterns in 30 cases. Topo-I pattern was seen in 9 cases, nuclear envelope in 5 cases, discrete nuclear dots (multiple) in 6 cases and few in 8 cases. One case showed pleomorphic pattern. Topo-1 pattern was more often seen in patients with progressive systemic sclerosis. However, rare association with seronegative rheumatoid arthritis, Sjogren's syndrome and adrenal insufficiency was noted (1 case each). The other clinical associations are detailed in Table 1. Figure 5 illustrates some of the rare nuclear patterns found in this study.

Comparison of ANA Patterns with Line Immunoblot Assay (LIA) :

LIA was available in 66 cases of these 224 cases with non-nuclear and rare nuclear patterns. Of these, 11 cases had exclusive cytoplasmic pattern, 3 cases had exclusive mitotic pattern, 12 cases had rare nuclear pattern and 40 cases had mixed nuclear with cytoplasmic patterns on ANA IIF.

Amongst the exclusive cytoplasmic patterns, 4 cases with filamentous pattern (AC-16) had negative LIA (n=2) and Ro-52 bands (n=2) . Three cases with dense fine speckled (AC-19) showed Ribosomal -P Protein (n=1) and no extractable nuclear antigen {ENA} in 2 cases. Two cases with reticular pattern (AC-21) had AMA-M2 on LIA. One case of Golgi-like (AC-22) and discrete do t(AC-18) showed as weak dsDNA band and histone band respectively. Amongst the mitotic patterns, 2 cases were NuMA like (AC-26) and one case of mitotic spindle apparatus (AC-25). One case with NuMA like staining was positive for Ro-52 and weak band at dsDNA. The other case of NuMA and MSA

Table 3. Cytoplasmic patterns seen in association with nuclear patterns

Nuclear patterns	Cytoplasmic fibrillar (AC15-17)	Cytoplasmic discrete dots (AC-18)	Cytoplasmic fine speckled (AC-19,20)	Cytoplasmic reticular AMA -like (AC-21)
Homogeneous(AC-1) (n=43)	4	1	33	5
Speckled (AC-4 &5) (n=34)	2	4	27	1

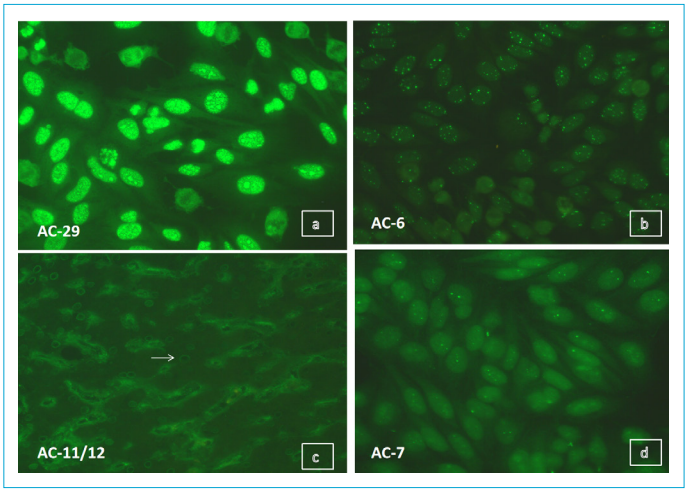

Figure 5. Composite representation of the rare nuclear patterns

*Immunoflourescence staining showing **(a)** Topo-I like pattern with prominent fine speckled nuclear staining along with nucleolar staining and a delicate weak cytoplasmic web like staining, **(b)** multiple nuclear dots, **(c)** nuclear envelope staining on primate liver with characteristic linear flourescence of nuclear membrane (arrow)and **(d)** few nuclear dots with 1-6 nuclear discrete dots in most cells.*

were negative for ENAs.

Of the 12 cases with rare nuclear patterns, majority were Topo-I like(AC-29) (n=7) of which 6 cases showed positive band for Scl-70, some of which also has PM-Scl 100 along with weak bands for other antigens. There were 2 cases each of multiple and few discrete nuclear dots (AC-6 &7) and all were negative on LIA. One case with nuclear envelope pattern (AC-11,12) showed weak band for many ENAs including cytoplasmic antigens like ribosomal P protein and AMA-M2.

Amongst the 40 cases with mixed nuclear and cytoplasmic patterns, 37 cases showed corresponding ENAs ,concordant with the staining pattern on IIFT, while 3 cases were negative.

Discussion

Detection of ANA play a major role in the serological diagnosis and classification of various autoimmune diseases. [7] Classification systems have been created to correlate specific profiles of ANA tests (i.e. relative concentrations, specificity, and sensitivity, immunofluorescence staining patterns) with specific diseases. We attempt to extend the scope of this classification by discovering novel associations between observed ANA staining pattern and corresponding clinical features in this study.

The robustness of HEp 2 cell lines in terms of stability and easy visibility of various subcellular structures along with technical feasibility has made IIF the most viable technique for ANA screening.[8] The Indirect Immunoflourescence (IIF), detects the binding of specific autoantibodies in the patient's serum with intracellular components, resulting in various patterns detected by fluorescence technique with intensity of staining dependant on the titre/ concentration of the antibody present in the serum.[9]

The ANA positivity rate of 14.7% seen in this study is in concordance with that reported from Indian population.[10] Female predominance was noted, similar to other population groups and Indian population.[10,11] The frequency of cytoplasmic and mitotic patterns is higher than that found in the study by Chhabra et al.[12] The occurrence of multiple autoantibodies in autoimmune disorders is a known phenomenon. These autoantibodies are easily recognized on LIA, while the predominant fluorescence pattern is usually recognized on IIF. In this study, we have reported that 12 % of nuclear patterns can have an associated cytoplasmic pattern. It is important to identify and report the mixed pattern, as these patterns are frequent occurrence in AID. The most common cytoplasmic pattern seen in association with nuclear pattern was dense fine speckled(AC-19). Autoantibodies against ribosomal-P-protein, PL-7, PL-12 and SRP produce this pattern of fluorescence and they are commonly seen in autoimmune diseases. This pattern was also the second common pure cytoplasmic pattern where it was seen predominantly in AIDs and few cases of interstitial lung disease(ILDs). Chabbra et al.[12] reported AC-19 as the most common cytoplasmic pattern found in their population. The importance of reporting this pattern is that a significant number of cases of suspected AID, only cytoplasmic dense fine speckled pattern may be present and this will help in further workup of patients with suspected AIDs.

The most commonly identified pure cytoplasmic pattern in this study was fibrillar filamentous(AC-16). The autoantibodies are against intermediate filaments and microtu-

bules in the cell cytoplasm and detected antigens include cytokeratins, vimentin and tropomyosin.The clinical significance of these patterns are not well established. We found 33% positivity in patients with AID. There are no specific immunoassays available for confirmation, but the staining pattern on IIF is distinct for identification of these antigens.

Cytoplasmic reticular pattern AMA-like (AC-21), has well known association with autoimmune liver disease especially primary biliary cholangitis. We also found positivity in other autoimmune diseases.[13] We did not find this pattern in non specific conditions, thereby signifying its association with AIDs. AC-18 pattern was also commonly found in our study. This pattern was found in AIDs, dermatological conditions, lower respiratory tract infections(LRIs) and neurological conditions. Bhanji et al.[14] 2007 had reported similar associations.

The other cytoplasmic patterns has no specific disease associations. The cytoplasmic segmental pattern, with antibodies against alpha actinin and vinculin was seen only in 4 cases in our study and was seen in patients with liver disorders and ulcerative colitis. Rare reports on its association with AID is available.[15] The Golgi pattern was seen in 2 cases of complicated falciparum malaria, whose significance is unknown. Irure-Ventura et al. in their Spanish multicentric study on rare immunoflourescence pattern observed that anti-Golgi antibodies were not limited to a specific disease and these antibodies were not clinically associated with systemic AIDs.[16] A similar finding was reported by Vermeersch et al.[17] Irure-Ventura's study found higher association of cytoplasmic autoantibodies with Systemic sclerosis and inflammatory myopathies, while nuclear patterns were more frequent in SLE and Sjogren's syndrome.[16] In the present study, no such specific disease associations were found for cytoplasmic autoantibodies and they were widely distributed across various autoimmune and non-autoimmune conditions. The mitotic patterns were rare and few. Autoantibodies against spindle fibres showed clinical association with AIDs and those against centrosomes were seen in infections. Betancur et al. report a significant association of autoantibodies against mitotic spindle apparatus with connective tissue disorder and conditions with presumed autoimmune origin like chronic urtricaria.[18] Amongst the rare nuclear patterns, Topo-I was more frequent and was seen in progressive systemic sclerosis. However, this pattern was also found in a single case each of seronegative Rheumatoid arthritis and Sjogren's syndrome.

The significance of non nuclear and rare nuclear ANA patterns are less well studied in literature. Senez studied rare ANA patterns in Turkey population over a period of 6 years and report no clinical importance of these patterns. They also found association of these patterns with non autoimmune conditions.[19] A recent study from central India by Nanda et al report a frequency of 6.39% for these uncommon ANA patterns.The prevalence of uncommon patterns range from 0.6% -3.3% in their study,[20] similar to what we found in our population group.

When we emphasize the importance of reporting these patterns, we are also aware that the clinical significance of the rare cytoplasmic patterns could not be derived from this study. Although there are commercial assays for identification of the some of the autoantibodies against cytoplasmic organelles, the IIF technique guides in easy recognition of these autoantibodies due to their subcellular localization and distinct staining patterns.[21] The IIF technique has stood the test of time for diagnosing these rare and infrequent patterns and it is good clinical practise to report these patterns an not restrict to only standard nuclear patterns, while reporting ANA serology.

In conclusion, this study is the first of its kind analyzing the associated cytoplasmic pattern with nuclear patterns,exclusive cytoplasmic and mitotic patterns and rare nuclear patterns with clinical diagnosis in a significant number of cases. The study followed the ICAP guidelines for identifying and reporting ANA pattern and the gold standard HEp-2 IIF method for testing serum samples, making it useful for comparison across the globe.

Disclosures

Ethics Committee Approval: The study has been approved by the Institutional Ethical committee of St. John's Medical College, Bengaluru, India: Ref No. 95/2019.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

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