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

Gayatri Ravikumar,1 Judah Pereira,2 Anshika Gupta2

1Department of Pathology, St. John’s Medical College, Sarjapura Road, Bengaluru, India
2Undergraduate MBBS Student, Bengaluru, India

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

Cite This Article: Ravikumar G, Pereira J, Gupta A. Non-Nuclear and Rare Nuclear ANA Patterns in Indirect Immunofluorescence Testing and their Clinical Associations. EJMO 2023;7(2):180–188.
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 present in various components of the cell i.e. 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. 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. 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 immunofluorescence slides were read using Carl Zeiss Fluorescent 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.](image-url)
(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**

<table>
<thead>
<tr>
<th>Clinical departments</th>
<th>Number of cases (n=221)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Medicine</td>
<td>60</td>
<td>27.14</td>
</tr>
<tr>
<td>Immunology</td>
<td>45</td>
<td>20.36</td>
</tr>
<tr>
<td>Dermatology</td>
<td>18</td>
<td>8.14</td>
</tr>
<tr>
<td>Neurology</td>
<td>17</td>
<td>7.69</td>
</tr>
<tr>
<td>Nephrology</td>
<td>15</td>
<td>6.78</td>
</tr>
<tr>
<td>Emergency medicine</td>
<td>12</td>
<td>5.4</td>
</tr>
<tr>
<td>Obstetrics &amp; Gynecology</td>
<td>11</td>
<td>4.97</td>
</tr>
<tr>
<td>Pediatrics &amp; Paediatric haematology</td>
<td>11</td>
<td>4.97</td>
</tr>
<tr>
<td>Pulmonary medicine</td>
<td>7</td>
<td>3.16</td>
</tr>
<tr>
<td>Gastroenterology</td>
<td>6</td>
<td>2.74</td>
</tr>
<tr>
<td>Surgery, Orthopedics, Cardiothoracic surgery (2 each)</td>
<td>6</td>
<td>0.9 (each)</td>
</tr>
<tr>
<td>Ophthalmology, ENT, endocrinology, Medical Oncology, Psychiatry, Urology, Cardiology, Hematology (1 each)</td>
<td>8</td>
<td>0.45 (each)</td>
</tr>
<tr>
<td>Sample received from Referral lab (outside)</td>
<td>5</td>
<td>2.26</td>
</tr>
</tbody>
</table>

**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.
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-
<table>
<thead>
<tr>
<th>Patterns</th>
<th>Autoimmune etiology</th>
<th>Dermatology related</th>
<th>Renal related</th>
<th>Lung related</th>
<th>Neurology related</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. CYTOPLASMIC PATTERNS (n=100)#</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC-16 (n=39)</td>
<td>Rheumatoid arthritis (3) SLE/DLE/APLA(7) JIA(1) AIHA(2) Myasthenia gravis(1)</td>
<td></td>
<td>Urticaria/Spongiotic dermatitis with eosinophils/DRESS syndrome with eosinophils (5)</td>
<td>Nephrotic syndrome/CKD/ HUS with AKI(4)</td>
<td>ILD(1)</td>
<td></td>
</tr>
<tr>
<td>AC-17 (n=4)</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC-18 (n=17)</td>
<td>Adult onset Still's disease(1) Rheumatoid arthritis(1) Aplastic anemia(1)</td>
<td>Erythema nodosum/ urticaria / FDE/ Psoriasis(6)</td>
<td>Nephrotic syndrome/CKD/ HUS with AKI(4)</td>
<td>ILD(1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC-19 (n=19)</td>
<td>Dermatomyositis(1) Sjoegren Syndrome(1) Rheumatoid arthritis(1) SLE/APLA(3) Multiple Sclerosis(1)</td>
<td></td>
<td></td>
<td>Nephrotic syndrome(1)</td>
<td>ILD(3)</td>
<td></td>
</tr>
<tr>
<td>AC-20 (n=4)</td>
<td>Rheumatoid arthritis with ILD(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Viral meningitis(1) Drug induced bone marrow suppression (1) Celiac disease(1)</td>
</tr>
<tr>
<td>AC-21 (n=9)</td>
<td>Suspected AIH/Decompensated Chronic liver disease(2) Sjoegren Syndrome(1) Pure Red cell aplasia(1) Rheumatoid arthritis(1)</td>
<td></td>
<td></td>
<td></td>
<td>Cutaneous vasculitis(1)</td>
<td></td>
</tr>
<tr>
<td>AC-22 (n=6)</td>
<td>AIHA(1) Idiopathic pulmonary fibrosis(1)</td>
<td></td>
<td></td>
<td>Nephrotic syndrome(1)</td>
<td>NIL</td>
<td>Complicated Falciparum with Multiorgan dysfunction syndrome(2) Primary hyperparathyroidism(1)</td>
</tr>
<tr>
<td>II. MITOTIC PATTERNS (n=14)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC-24 (n=4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Skin rash(1)</td>
<td></td>
</tr>
<tr>
<td>AC-25 (n=4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
plasmic discrete dots (AC-18) with mitotic intercellular bridge.

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-I pattern was seen in 9 cases. Nuclear envelope (AC-11 &12), pleomorphic pattern (AC-13), and Topo-I pattern (AC-29) were found in 30 cases. The exclusive 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 in 1 case each. The other clinical associations are detailed in Table 2. Figure 5 illustrates some of the rare nuclear patterns found in this study.

Comparison of ANA Patterns with Line Immoblot 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.

Among the exclusive cytoplasmic patterns, 4 cases had negative LA and 2 cases were NaVA like staining was positive for Ro-52 and weak band at dDNA. The other case of NaVA and MSA band at dDNA. The other case of NaVA and MSA

Table 2. CONT.

<table>
<thead>
<tr>
<th>Patterns</th>
<th>Autoimmune etiology</th>
<th>Dermatology related</th>
<th>Renal related</th>
<th>Lung related</th>
<th>Neurology related</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC-26 (n=4)</td>
<td>Rheumatoid arthritis (1)</td>
<td></td>
<td></td>
<td>LRTI (1)</td>
<td></td>
<td>Carpel tunnel syndrome(1)</td>
</tr>
<tr>
<td>AC-27 (n=2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vasculitic ulcers(1)</td>
</tr>
<tr>
<td>III. RARE NUCLEAR PATTERNS (n=30)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC-6 (n=6)</td>
<td>AIHA(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC-7 (n=9)</td>
<td>DLE(1), Primary Sjogren’s syndrome (1), Bullous pemphigoid(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC-11 &amp;12 (n=5)</td>
<td>JIA(1), Autoimmune hepatitis(2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC-29 (n=9)</td>
<td>Progressive systemic sclerosis(3), Seronegative Rheumatoid arthritis(1), Sjogren’s syndrome(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC-13 (n=1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No clinical details available</td>
</tr>
</tbody>
</table>

# Clinical details available for 89 cases, * clinical details available for 12 cases, † Clinical details available in 23 cases: Systemic lupus erythematosus (SLE), Discoid Lupus erythematosus (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).
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. 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. The Indirect Immunofluorescence (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.

The ANA positivity rate of 14.7% seen in this study is in concordance with that reported from Indian population. Female predominance was noted, similar to other population groups and Indian population. The frequency of cytoplasmic and mitotic patterns is higher than that found in the study by Chhabra et al. 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). Chhabra et al. 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 microtubules.

### Table 3. Cytoplasmic patterns seen in association with nuclear patterns

<table>
<thead>
<tr>
<th>Nuclear patterns</th>
<th>Cytoplasmic fibrillar (AC15-17)</th>
<th>Cytoplasmic discrete dots (AC-18)</th>
<th>Cytoplasmic fine speckled (AC-19,20)</th>
<th>Cytoplasmic reticular AMA -like (AC-21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneous(AC-1) (n=43)</td>
<td>4</td>
<td>1</td>
<td>33</td>
<td>5</td>
</tr>
<tr>
<td>Speckled (AC-4 &amp;5) (n=34)</td>
<td>2</td>
<td>4</td>
<td>27</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 5. Composite representation of the rare nuclear patterns

Immunofluorescence 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 fluorescence of nuclear membrane (arrow) and (d) few nuclear dots with 1-6 nuclear discrete dots in most cells.
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 AIDs. 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. 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. 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 AIDs is available. 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 immunofluorescence pattern observed that anti-Golgi antibodies were not limited to a specific disease and these antibodies were not clinically associated with systemic AIDs. A similar finding was reported by Vermeersch et al. 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. 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 urticaria. 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. 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, 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. 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.

Funding: No funds, grants, or other support was received.

Financial Interests: The authors have no relevant financial or non-financial interests to disclose.


References

3. Chan EKL, von Mühlen CA, Fritzler MJ, Damaiseaux J, Infantino
M, Klotz W, et al; ICAP Committee. The International Consen-
sus on ANA patterns (ICAP) in 2021-The 6th workshop and

4. Damoiseaux J, von Mühlen CA, Garcia-De La Torre I, Carballo
OG, de Melo Cruvinel W, et al. International consensus on ANA
patterns (ICAP): the bumpy road towards a consensus on re-

5. Li QZ, Karp DR, Quan J, Branch VK, Zhou J, Lian Y, et al. Risk
factors for ANA positivity in healthy persons. Arthritis Res Ther

6. Rao JS, Shobha V, Thomas T, Kini U. Standardizing initial dilu-
tion titers of antinuclear antibodies for the screening of sys-
temic lupus erythematosus. Indian J Rheumatol 2019;14:211–
7.

7. Bossuyt X, De Langhe E, Borghi MO, Meroni PL. Understand-
ing and interpreting antinuclear antibody tests in systemic

8. Chan EKL, de Melo Cruvinel W, Andrade LEC. The international
consensus on standardized nomenclature of antinuclear anti-
body HEp-2 cell patterns (ICAP) initiative - Current state and
perspectives. In: Conrad K, Chan EKL, Andrade LEC, Steiner G,
Pruijn GJM, Shoenfeld Y, editors. Autoantibody Research to
Standardized Diagnostic Assays in the Management of Hu-
man Diseases series. Report on the 12th Dresden Symposium

9. Tebo AE. Recent approaches to optimize laboratory as-
essment of antinuclear antibodies. Clin Vaccine Immunol

Antinuclear antibody positive autoimmune disorders in North

prevalence of antinuclear antibodies in the general popula-

Prevalence of autoantibodies to cellular cytoplasmic and mi-
totic antigens in routine antinuclear antibody reporting: Im-
plementation of international consensus on antinuclear anti-

T, et al. Anti-mitochondrial autoantibodies in systemic lupus
erythematosus and their association with disease manifesta-

14. Bhanji RA, Eystathioy T, Chan EK, Bloch DB, Fritzler MJ. Cli-
nical and serological features of patients with autoantibodies to

N, Youinou P, et al. Anti-α actinin antibodies as new predictors
of response to treatment in autoimmune hepatitis type 1. Al-

16. Irure-Ventura J, Rodríguez C, Vergara-Prieto E, Vargas ML,
Quirant B, Jurado A, et al; GEAI and EASI groups. Rare immu-
nofluorescence patterns of autoantibodies on HEp-2 cells
defined by ICAP identify different autoimmune diseases in
the absence of associated specificities: a Spanish multicentre

17. Vermeersch P, Van den Bergh K, Blockmans D, Westhovens R,
Bossuyt X. Anti-Golgi autoantibodies are not clinically asso-
ciated with systemic autoimmune diseases. Ann Rheum Dis

18. Betancur JF, Londoño A, Estrada VE, Puerta SL, Osorno SM,
Loaiza A, et al. Uncommon patterns of antinuclear antibodies
recognizing mitotic spindle apparatus antigens and clinical
associations. Medicine (Baltimore) 2018;97:e11727.

19. Sener AG. evaluation of rare antinuclear antibody patterns in

antinuclear antibody patterns as diagnostic indicators. Clin
Biochem 2021;90:28–33.

21. Alpini C, Lotzniker M, Valaperta S, Bottone MG, Malatesta M,
Montanelli A, Merlini G. Characterization for anti-cytoplasmic
antibodies specificity by morphological and molecular tech-