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# Uncommon patterns of antinuclear antibodies recognizing mitotic spindle apparatus antigens and clinical associations

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**Abstract**

Antinuclear antibodies (ANA) are key biomarkers in the evaluation of rheumatic diseases. The prevalence and clinical significance of uncommon or rare patterns, particularly those directed at the mitotic spindle apparatus (MSA), are not well understood. We aimed to investigate the prevalence and clinical significance of anti-MSA patterns in a Colombian population.

During 2013 and 2014, 113,491 consecutive determinations of ANA were studied for the presence of uncommon patterns. Clinical and laboratory data of anti-MSA positive patients were retrospectively collected and analyzed.

Of the 113,491 patients tested, 60,501 (53%) were positive for ANA, of which 834 (1.3%) were positive for uncommon/rare patterns of ANA (anti-MSA in 592 cases). Of these 592 cases, complete data were available in 329 patients, of whom 116 had an established diagnosis. Anti-MSA antibodies were the only ANA positive test in 81% patients. At least one fine reactivity was identified in 19/116 (16.3%) of ANA-positive patients, of which anti-Ro was the most prevalent (18/116, 15.5%).

The most frequent patterns were nuclear mitotic apparatus (NuMA) (56%) and MSA-2 (25%). The NuMA pattern had the highest ANA titers: mean 320 (range 80–2560) and behaved as monospecific antibodies. The most frequent systemic autoimmune diseases were Sjögren syndrome (SS) (18.1%), rheumatoid arthritis (RA) (13.8%), and systemic lupus erythematosus (SLE) (11%). Undifferentiated connective tissue disease (UCTD) was associated with the centrosome ($P < .001$), NuMA ($P < .02$) and MSA-2 ($P < .45$) patterns. Chronic idiopathic urticaria (CIU) was associated with the NuMA pattern ($P < .02$) and sensorineural hearing loss (SNHL) was associated with the MSA-2 ($P < .001$), centrosome ($P < .68$) and CENP-F ($P < .38$) patterns, previously unreported findings. Malignancies were found in 8 patients (50% were papillary thyroid cancer).

In a large cohort of ANA determinations, uncommon patterns were found in around 1% of cases. The most frequent anti-MSA patterns found were NuMA and MSA-2. More than 50% of patients with anti-MSA had an associated CTD, mainly SS, RA and SLE, and anti-MSA behaved as monospecific antibodies. Other entities of presumed autoimmune origin, like CIU and SNHL, might be associated with these patterns.

**Abbreviations:** ACR = American College of Rheumatology, AIED = autoimmune inner ear disease, ANA = antinuclear antibodies, ANCA = antineutrophil cytoplasmatic antibodies, anti-CCP = anticyclic citrullinated peptide, anti-dsDNA = anti-double-stranded DNA, aPL = antiphospholipid antibodies, APS = antiphospholipid syndrome, CIU = chronic idiopathic urticaria, CTD = connective tissue disease, ENA = antiextractable nuclear antigen antibodies, EULAR = European League Against Rheumatism, ICAP = International Consensus on ANA Pattern, IIF = indirect immunofluorescence, MCTD = mixed connective tissue disease, MD = Meniere's disease, MSA = mitotic spindle apparatus, NuMA = nuclear mitotic apparatus, PCNA = proliferating cell nuclear antigen, RA = rheumatoid arthritis, RF = rheumatoid factor, RNP = ribonucleoprotein, SLE = systemic lupus erythematosus, SNHL = sensorineural hearing loss, SS = Sjögren syndrome, SSc = systemic sclerosis, UCTD = undifferentiated connective tissue disease.

**Keywords:** ANA, atypical patterns, autoantibodies, autoimmunity

## 1. Introduction

Antinuclear antibodies (ANA) are a diverse group of autoantibodies that recognize multiple intracellular antigens, classically consisting of nuclear specificities such as deoxyribonucleic acid or small nuclear ribonucleoproteins. Indirect immunofluorescence (IIF) using HEp-2 cells is the most widely used assay for the detection of ANA.[1]

ANA are key biomarkers in the evaluation of rheumatic diseases[2] such as systemic lupus erythematous (SLE), Sjögren syndrome (SS), systemic sclerosis (SSc), mixed connective tissue disease (MCTD), and polymyositis/dermatomyositis. However, ANA are present in various infectious, inflammatory and neoplastic diseases, and healthy individuals. There are many ANA specificities, and while some antibodies are closely associated with specific diseases, others are non-specific in both patients and healthy individuals. The associations between ANA and some diseases suggest that they may be useful screening and

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diagnostic biomarkers and could provide insights into the understanding of disease mechanisms.[2]

The most common reported antinuclear staining patterns include: homogeneous, speckled, centromere, and nucleolar. Uncommon or rare patterns (occurring in < 1% of ANA-positive patients) have been described and may be divided into 3 groups: cell cycle related (NuMA1, HsEg5, CENP-F, MSA-2, proliferating cell nuclear antigen [PCNA]); nuclear (multiple nuclear dots, centrosome); and cytoplasmatic (Golgi).[3] Of these, the most studied is the mitotic spindle apparatus (MSA) subgroup with at least 5 antigens: NuMA 1/MSA-1, HsEg5/NuMA-2, centrosome, MSA-2 (midbody), and CENP-F.

The prevalence of common ANA patterns is relatively well known,[4,5] but less is known about the prevalence and clinical significance of rare ANA patterns. Due to their low frequency, current knowledge of the specificity of rare ANA patterns is based on observational studies or case series with limited numbers of patients. In part, this may be explained by clinical immunology laboratories reporting only "clinically-relevant" ANA patterns, while other "less-important" patterns are ignored. Moreover, most of these patterns are at an expert level according to the International Consensus on ANA Pattern (ICAP) classification because some are difficult to recognize (with frequency playing an important role) and their clinical relevance is unclear.[6,7] Information on atypical patterns is scarce and mostly comes from Caucasian cohorts. Therefore, we evaluated the prevalence of atypical ANA in a general Latin-American population.

## 2. Patients and methods

We included 113,491 consecutive ANA determinations made at Dinámica IPS, a specialized diagnostic center present in 8 main cities across Colombia, in 2013 and 2014. All patients sera submitted for ANA testing were tested by conventional IIF using Hep-2 cells (AESKU DIAGNOSTICS, Wendelsheim, Germany) with serial dilutions commencing at 1:80. Patterns were classified using 3 images for each determination. Antiextractable nuclear antigen antibodies (ENA), including anti-Ro (anti-SSA), anti-La (anti-SSB), anti-RNP, and anti-Sm, were detected using enzyme-linked immunosorbent assay (ELISA) (ALEGRIA ANALYZER, ORGENTEC Diagnostika, Mainz, Germany). Anti-double-stranded DNA (anti-dsDNA) antibodies were detected and quantified by IIF on *Crithidia lucilliae* cells. The presence and titers of antineutrophil cytoplasmatic antibodies (ANCA) were detected and quantified by IIF.

ANA positive patients with anti-MSA antigen patterns (Fig. 1) (NuMA/MSA-1, midbody/MSA-2, CENP-F/MSA-3, and centrosome) were identified via electronic data capture from the electronic patient records database from a private health insurance organization that covers approximately 2.3 million Colombian patients nationwide. Patient characteristics, medical histories, and details of the diagnostic workup, medical treatment, and follow-up were retrieved by chart review. Specific attention was directed at the principal diagnosis, known rheumatic diseases, comorbidities, and the evolution of any clinical progression. Patients were diagnosed with a definite rheumatic disease if they matched the diagnostic/classification criteria.

Diagnoses of connective tissue disease were based on the classification criteria of the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria in the case of rheumatoid arthritis (RA) and SSc.[8,9] SLE was diagnosed according to the SLICC criteria[10] and SS by the classification criteria of the American-European Consensus group.[11] Undifferentiated connective tissue disease (UCTD) was defined according to the criteria of Mosca et al.[12] Antiphospholipid syndrome (APS) was defined according to the Sydney criteria.[13] Vasculitis was diagnosed according to the ACR diagnostic criteria and the Chapel Hill consensus conference.[14,15]

### 2.1. Statistical analysis

Categorical variables were compared using Fisher's exact test or the chi-square test and continuous variables using the *t*-test or Mann–Whitney *U* test when appropriate. A *P*-value of $\leq .05$ was considered statistically significant. All analyses were performed using STATA (version 13.0, Texas).

### 2.2. Ethical considerations

This study was reviewed and approved by the institutional review board of Dinámica IPS. All participants signed written informed consents.

## 3. Results

From 113,491 sera consecutively tested, 60,501 (53%) were positive for ANA. Of these, 834 (1.3%) were positive for rare ANA patterns: Anti-NuMA (MSA-1) (0.46%), antimidbody (MSA-2) (0.32%), centrosome (centriole) (0.17%), cytoplasmatic fibers (0.15%), multiple nuclear dots (0.13%), lysosomal (0.04%), Golgi (0.03%) PCNA (0.03%), anti-CENP-F (MSA-3) (0.013%), and nuclear envelope (0.003%) (Fig. 2). Based on the initial ANA analysis, 592 samples with staining of anti-MSA antigen patterns (NuMA/MSA-1, midbody/MSA-2, CENP-F/MSA-3, and centrosome) were chosen for further analysis (Fig. 2). Among them, 329 patients had a complete medical history and laboratory data (NuMA n = 152, MSA-2 n = 116, centrosome n = 57, CENP-F n = 4), but only 116 patients had a definite diagnosis and comprise the present analysis. The remaining 213 patients were excluded because they did not meet the clinical criteria of autoimmunity or were considered false positives. Their mean ANA titers were lower, most at the low threshold of detection, mean 80 (range 80–320), the majority of the patients had noninflammatory arthralgia in 69 (32%), osteoarthritis in 42 (19%) fibromyalgia in 13 (6%), and in 13 (6%) patients ANA was tested without reason as screening test in asymptomatic patients (Supplemental Table, http://links.lww.com/MD/C394). In the group focus of the study, the median age was $50 \pm 14.6$, and 102 (87.9%) were female (Table 1). Mean ANA titers were 160 (range 80–2560). Anti-MSA antibodies were the only serological marker in 94 (81%) of patients. At least 1 fine reactivity was found in 19 (16.3%) ANA-positive patients: anti-Ro in 18 (15.5%) patients, which was associated with NuMA in 12 (12.9%) patients, MSA-2 in 3 (2.5%) patients, centrosome in 2 (1.7%) and CENP-F in 1 (0.8%) patient. Anti-La was the second most frequent with 6 patients (5.1%), of which 4 (3.4%) corresponded to NuMA and 2 (1.7%) to MSA-2. Anti-RNP were present in 3 (2.5%) patients and were associated with the centrosome in 2 (1.7%) and MSA-2 in 1 (0.8%) patients. Anti-Sm in 1 (0.8%) patient was associated with the MSA-2 pattern, and 1 patient (0.8%) was anti-dsDNA positive and associated with the centrosome pattern. The most commonly associated autoantibodies were rheumatoid factor (RF) in 22 (18.8%) patients, anticyclic citrullinated peptide (anti-CCP) in 7 (6.0%) and antiphospholipid antibodies (aPL) in 9 (2.4%) patients. The most frequent patterns were NuMA (56% of

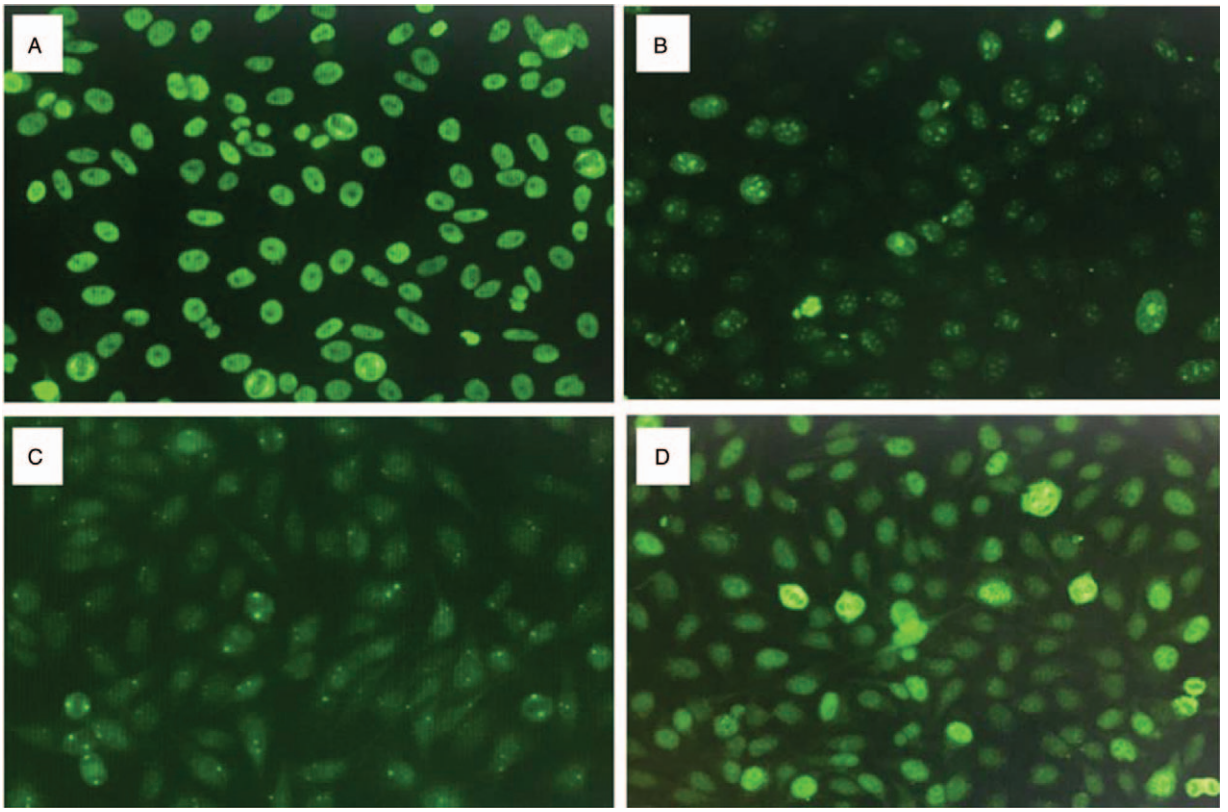


**Figure 1.** Immunofluorescent pattern of sera containing mitotic spindle apparatus antibodies. (A) NuMA (MSA-1), (B) Midbody (MSA-2), (C) centrosome, (D) CENP-F (MSA-3) (images from Dinámica IPS). CENP = CENtromere protein, MSA = mitotic spindle apparatus, NuMA = nuclear mitotic apparatus.

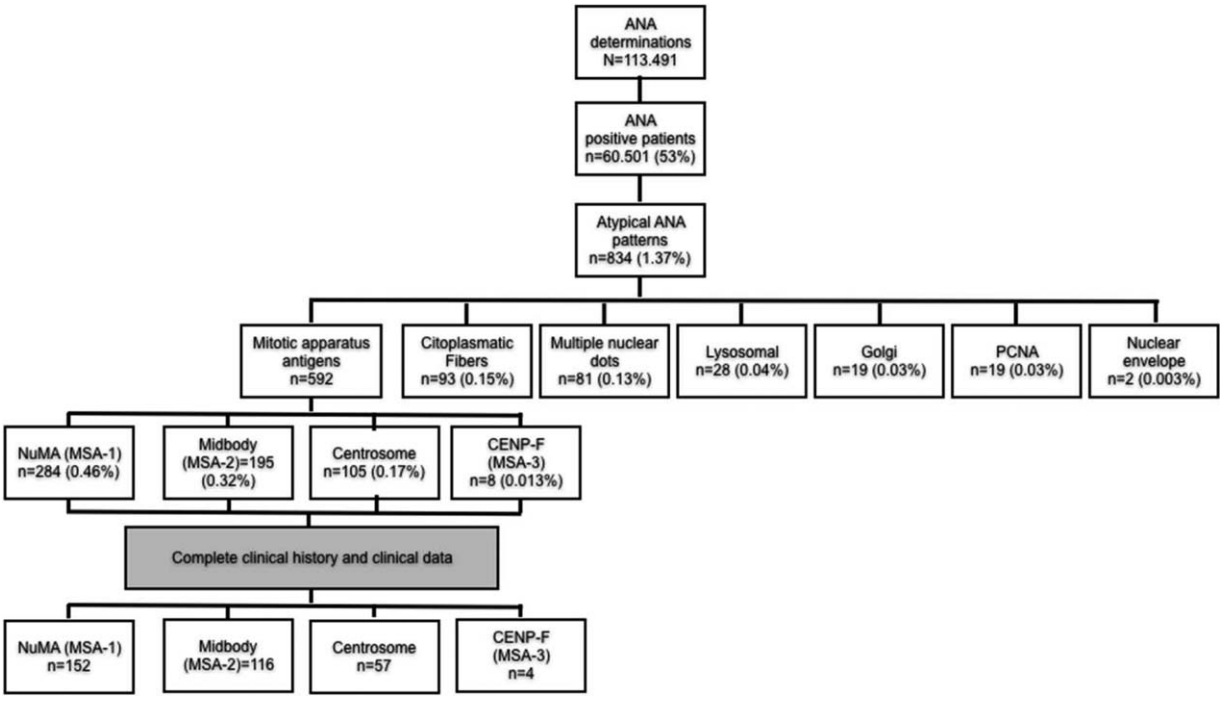


**Figure 2.** Flowchart of ANA determinations and uncommon patterns. ANA = antinuclear antibodies.

**Table 1**

**Laboratory characteristics of anti-MSA antigens.**

| | Total patients | NuMA-1 (MSA-1) | | Midbody (MSA-2) | | Centrosome | | CENP-F (MSA-3) | |
|---|---|---|---|---|---|---|---|---|---|
| Patients | 116 | 65 (56%) | | 30 (25%) | | 17 (14%) | | 4 (3.4%) | |
| Age, years ± SD | 50 ± 14.6 | 49.33 ± 14.9 | | 53.9 ± 12.1 | | 48 ± 18.1 | | 55.7 ± 7.0 | |
| Female | 102 (87.9%) | 55 (84.6%) | | 27 (90%) | | 14 (82.3%) | | 3 (75%) | |
| Median ANA titers (Range) | 160 (80-2560) | 320 (80-2560) | | 80 (80–640) | | 160 (80–1280) | | 160 (80–640) | |
| ENA positive | 19 (16.3%) | 12 (18.4%) | $P<.69$ | 3 (10%) | $P<.27$ | 4 (23.52%) | $P<.49$ | 1 (25%) | $P<.53$ |
| Ro/SSA | 18 (15.5%) | 12 (18.4%) | $P<.32$ | 3 (10%) | $P<.29$ | 2 (11.76%) | $P=1$ | 1 (25%) | $P<.49$ |
| La/SSB | 6 (5.1%) | 4 (6.1%) | $P<.69$ | 2 (6.6%) | $P<.39$ | 0 | $P<.59$ | 0 | $P=1$ |
| Sm | 1 (0.8%) | 0 | $P<.44$ | 1 (3.3%) | $P<.25$ | 0 | $P=1$ | 0 | $P=1$ |
| RNP | 3 (2.5%) | 0 | $P<.08$ | 1 (3.3%) | $P=1$ | 2 (11.7%) | $P<.05$ | 0 | $P=1$ |
| Anti-dsDNA | 1 (0.8%) | 0 | $P<.44$ | 0 | $P=1$ | 1 (5.8%) | $P<.14$ | 0 | $P=1$ |
| Hypocomplementemia | 4 (3.4%) | 4 (6.1%) | $P<.12$ | 0 | $P<.57$ | 0 | $P=1$ | 0 | $P=1$ |
| aPL | 9 (2.4%) | 8 (12.3%) | $P<.005$ | 1 (3.3%) | $P<.11$ | 0 | $P=1$ | 0 | $P=1$ |
| RF positive | 22 (18.9%) | 14 (21.5%) | $P<.42$ | 5 (16.6%) | $P<.79$ | 2 (11.7%) | $P<.52$ | 1 (25%) | $P<.57$ |
| Anti-CCP | 7 (6.0%) | 5 (7.6%) | $P<.46$ | 1 (3.3%) | $P<.67$ | 1 (5.8%) | $P=1$ | 0 (0%) | $P=1$ |
| ANCA positive | 1 (0.8%) | 0 (0%) | $P<.44$ | 0 (0%) | $P=1$ | 0 (0%) | $P=1$ | 1 (25%) | $P<.03$ |

ANA = antinuclear antibodies, ANCA = antineutrophil cytoplasmic antibodies, aPL = antiphospholipid antibodies, CENP = CENtromere protein, CCP = citrullinated cyclic peptide, CIU = chronic idiopathic urticaria, ENA = extractable nuclear antigens, La/SSB = Anti-Sjögren's-syndrome-related antigen B, MSA = mitotic spindle apparatus, NuMA = nuclear mitotic apparatus, RF = rheumatoid factor, Ro/SSA = Anti-Sjögren's-syndrome-related antigen A.

patients) and midbody (25% of patients). The NuMA pattern had the highest titers of ANA: mean 320 (range 80–2560). RF and anti-CCP were also more prevalent in this pattern (21.5% and 7.6%, respectively). Eight out of 9 patients with positive aPL (12.3%) ($P<.00$) and all patients with hypocomplementemia were NuMA positive. The MSA-2 pattern had the lowest titers of ANA (mean 80, range 80–640) and ENA positivity of all anti-MSA patterns (3/30, 10%), and RF was the most common associated autoantibody (5/30, 16.6%). The centrosome pattern had the second highest frequency of ENA positivity (4/17, 23.5%) with 2 patients (11.7%) being ribonucleoprotein (RNP) positive ($P<.05$). The CENP-F pattern was the least frequent (4/116, 3.4%), and only 1 patient was ENA positive; this was the only ANCA-positive patient (1/116, 0.8%) ($P<.03$).

### 3.1. Relationship with systemic autoimmune diseases

Most anti-MSA-positive patients (75/116, 64%) had a diagnosis of an underlying autoimmune disease. Sixty patients (56%) had a connective tissue disease (CTD) (Table 2). The most frequent systemic autoimmune diseases were SS (21, 18.1%), RA (16, 13.8%), and SLE (13, 11%). All were more frequent in the NuMA pattern. Of note, in comparison with the rest of patterns, 10 patients had UCTD associated with the centrosome pattern in 7 patients ($P<.00$) followed by 2 patients in the NuMA ($P<.02$) and 1 with the MSA-2 ($P<.45$) pattern.

The association with UCTD was stronger for the centrosome pattern with a relative risk (RR) of 13.58 (95% CI: 3.88–47.46), whereas the presence of NuMA seems to be less likely to have UCTD (RR: 0.19; 95% CI: 0.43–0.88) (Table 3).

**Table 2**

**Clinical associations with mitotic apparatus antigens.**

| | All patients | NuMA-1 (MSA-1) n = 65 | | Midbody (MSA-2) n = 30 | | Centrosome pattern n = 17 | | CENP-F (MSA-3) n = 4 | |
|---|---|---|---|---|---|---|---|---|---|
| Connective tissue disease | 65 (56%) | 39 (60%) | $P<.94$ | 13 (43.3%) | $P<.10$ | 11 (64.7%) | $P<.60$ | 2 (50%) | $P=1$ |
| SLE | 13 (11.2%) | 9 (13.8%) | $P<.38$ | 2 (6.6%) | $P<.51$ | 2 (11.7%) | $P=1$ | 0 | $P=1$ |
| Sjögren syndrome | 21 (18.1%) | 14 (21.5%) | $P<.27$ | 4 (13.3%) | $P<.58$ | 2 (11.7%) | $P<.73$ | 1 (25%) | $P<.55$ |
| RA | 16 (13.7%) | 11 (16.9%) | $P<.29$ | 4 (13.3%) | $P=1$ | 1 (5.8%) | $P<.46$ | 0 | $P=1$ |
| UCTD | 10 (8.6%) | 2 (3.0%) | $P<.02$ | 1 (3.3%) | $P<.45$ | 7 (41.1%) | $P<.005$ | 0 | $P=1$ |
| APS | 7 (6.0%) | 6 (9.2%) | $P<.13$ | 1 (3.3%) | $P<.67$ | 0 | $P<.59$ | 0 | $P=1$ |
| Spondyloarthropaties | 4 (3.45%) | 3 (4.6%) | $P<.63$ | 1 (3.3%) | $P=1$ | 0 | $P=1$ | 0 | $P=1$ |
| Vasculitis | 2 (1.7%) | 1 (1.5%) | $P=1$ | 0 | – | 1 | $P=1$ | 1 (25%) | $P<.06$ |
| SSc | 1 (0.8%) | 1 | $P=1$ | 0 | $P=1$ | 0 | $P=1$ | 0 | $P=1$ |
| Autoimmune Hypothyroidism | 3 (2.5%) | 2 (3.0%) | $P=1$ | 1 (3.3%) | $P=1$ | 0 | $P=1$ | 0 | $P=1$ |
| Vitiligo | 5 (4.3%) | 2 (3.0%) | $P<.65$ | 3 (10%) | $P<.10$ | 0 | $P=1$ | 0 | $P=1$ |
| Glomerulopathy | 4 (3.4%) | 4 (6.1%) | $P<.12$ | 0 | – | $P<.57$ | – | 0 | $P=1$ |
| Demyelinating inflammatory disease | 2 (1.7%) | 2 (3.0%) | $P<.50$ | 0 | $P=1$ | 0 | $P=1$ | 0 | $P=1$ |
| CIU | 10 (8.6%) | 10 (15.3%) | $P<.02$ | 0 | $P<.06$ | 0 | $P<.35$ | 0 | $P=1$ |
| SNHL | 13 (11.2%) | 0 | $P<.005$ | 11 (36.6%) | $P<.005$ | 1 (5.8%) | $P<.68$ | 1 (25%) | $P<.38$ |
| Cancer | 8 (6.9%) | 4 (6.1%) | $P<.72$ | 2 (6.6%) | $P=1$ | 1 (5.8%) | $P=1$ | 1 (25%) | $P<.25$ |
| Miscellaneous* | 4 (3.4%) | 2 (3.0%) | $P=1$ | 1 (3.3%) | $P=1$ | 1 (5.8%) | $P<.47$ | 0 | $P=1$ |

APS = antiphospholipid syndrome, CENP = CENtromere protein, CIU = chronic idiopathic urticaria, MSA = mitotic spindle apparatus, NuMA = nuclear mitotic apparatus, RA = rheumatoid arthritis, SLE = systemic lupus erythematous, SNHL = sensorineural hearing loss, SSc = systemic sclerosis, UCTD = undifferentiated connective tissue disease.

* *Miscellaneous*: autoimmune hepatitis, Cröhn disease, chronic lichen simplex, chronic Raynaud's phenomenon.

**Table 3**

**Association between atypical patterns and autoimmune diseases.**

| | NuMA-1 (MSA-1) RR | Midbody (MSA-2) RR | Centrosome pattern RR | CENP-F (MSA-3) RR |
|---|---|---|---|---|
| SLE | 1.76 (0.57–5.40) | 0.06 (CI 0.12–2.21) | 1.05 (CI 0.25–4.36) | 0 |
| Sjögren syndrome | 1.56 (0.68–3.59) | 0.67 (CI 0.24–1.84) | 0.61 (0.15–2.39) | 1.4 (0.24–8.0) |
| RA | 1.72 (0.64–4.65) | 0.95 (0.33–2.73) | 0.38 (0.54–2.75) | 0 |
| UCTD | 0.19 (0.43–0.88) | 0.31 (0.04–2.41) | 13.58 (3.88–47.46) | 0 |
| APS | 4.70 (0.58–37.87) | 0.47 (0.05–3.80) | 0 | 0 |
| CIU | 0 | 0 | 0 | 0 |
| SNHL | 0 | 15.76 (3.70–67.09) | 0.48 (0.67–3.49) | 2.33 (0.39–13.83) |
| Cancer | 0.78 (0.20–2.98) | 0.95 (0.20–4.48) | 0.83 (0.10–6.34) | 4 (0.63–25.2) |
| Hypothyroidism | 1.56 (0.14–16.82) | 1.43 (0.13–15.24) | 0 | 0 |
| Vitiligo | 0.52 (0.90–3.01) | 4.3 (0.75–24.5) | 0 | 0 |
| Glomerulopathy | — | 0 | 0 | 0 |
| Spondyloarthropathies | 2.35 (0.25–21.96) | 0.95 (0.10–8.83) | 0 | 0 |
| Vasculitis | 0.78 (0.05–12.24) | 0 | 0 | 28 (2.10–371.8) |
| SSc | — | 0 | 0 | 0 |
| Demyelinating inflammatory disease | — | 0 | 0 | 0 |

APS = antiphospholipid syndrome, CENP = CENtromere protein, CIU = chronic idiopathic urticaria, MSA = mitotic spindle apparatus, RA = rheumatoid arthritis, RR = relative risk, SLE = systemic lupus erythematous, SNHL = sensorineural hearing loss, SSc = systemic sclerosis, UCTD = undifferentiated connective tissue disease.

### 3.2. Relationship with organ-specific autoimmune disease

Ten of the 116 patients had an organ-specific autoimmune disease, including vitiligo (5), autoimmune hypothyroidism (3), and demyelinating inflammatory disease (2). Four patients were classified as miscellaneous: autoimmune hepatitis, Cröhn disease, chronic lichen simplex, and Raynaud's phenomena.

### 3.3. Relationship with other diseases

Chronic idiopathic urticaria (CIU) was associated with the NuMA pattern (10/10 patients) ($P < .02$) and sensorineural hearing loss (SNHL) with MSA-2 positivity (11/13) ($P < .001$), centrosome (1/13) and CENP-F (1/13). The association was stronger for the MSA-2 pattern with an RR of 15.76 (95% CI 3.70–67) for SNHL (Table 3).

Four patients had glomerulopathy: 2 with IgA nephropathy, 1 with focal, and segmental glomerulosclerosis, and 1 with proliferative membranous glomerulonephritis. Three of these patients had the NuMA pattern, and 1 patient with IgA nephropathy had the MSA-2 pattern.

Malignancy was detected in 8 patients: 4 had a NuMA pattern (2 papillary thyroid cancer, 1 breast cancer, and 1 leukemia), 2 had an MSA-2 pattern (papillary thyroid cancer and breast cancer, respectively) and 1 each had a centrosome (papillary thyroid cancer) and CENP-F (ovarian cancer) pattern. Given the small sample of patients with cancer, associations with specific ANA patterns were not analyzed.

## 4. Discussion

In a substantial cohort of ANA determinations, we found an uncommon pattern in around 1% of samples. Anti-MSA antigens were the most frequent atypical pattern.

ANA constitute a significant tool for the diagnosis of systemic autoimmune diseases and represent about 30% of overall autoantibody determinations.[16] The prevalence of positive ANA in population-based samples is 23% to 27%.[4,17] To date, our ANA positive population (53%) doubles the highest reported prevalence of consecutive positive ANA consecutive determinations. This might be, in part, because our laboratory is a diagnostic reference center in Colombia, with a wide range of specialties including internal medicine, rheumatology, dermatology, and nephrology, among others.

A rare ANA pattern is defined as a pattern that occurs in < 1% of patients testing positive on IIF.[3] We found a slightly higher prevalence of 1.3%, although this could be an overestimate as our laboratory is a national reference center for autoimmune testing.

To our knowledge, this is the largest reported cohort of rare ANA patterns and anti-MSA antibodies. Vermeersch and Bossuyt[3] and Bonaci-Nikolic et al[18] reported 2 cohorts of 236 and 56 patients, respectively. Most of our anti-MSA positive patients (87.9%) were female, consistent with the known higher prevalence of autoimmune disease in females.

The MSA is composed of centrosomes, spindle poles, spindle microtubes, chromosomes, and intercellular bridge/midbody. Many autoantibodies recognize MSA antigens (at least 5): Nuclear MSA (NuMA-1/MSA-1 and NuMA-2) centrosome, midbody (MSA-2), and centromere-F.[18–20]

Anti-NuMA autoantibodies were first described by McCarty et al[21] in 1981. Later, in 1996, 2 groups simultaneously described 2 autoantibody systems that reacted to mitotic poles and spindles; with nuclear mitotic apparatus protein NuMA-1, also known as centrophilin, SPN, SP-H, and the kinesin-like protein HsEg5 as potential antigens. The latter can be distinguished from NuMA-1 by indirect immunofluorescence (NuMA-1 antibodies stain interphase cells whereas HsEg5 antibodies do not) and Western blotting.[20,22,23]

We found a prevalence of 0.46% for the NuMA pattern compared with 0.047% in a Chinese study[24] and 0.77% in a European cohort,[3] indicating evident geographical and racial/ethnic variations, as shown for other ANA patterns.[25,26]

NuMA-1 antibodies were more frequent than HsEg5 antibodies in sera.

The prevalence ranged from 0.26% to 0.71% for NuMA-1 to 0.06% to 0.12% for NuMA-2. We did not confirm anti-NuMA specificity (NuMA-1 or anti-HsEg5) by immune blotting of sera. The NuMA patients in our cohort had the highest titers of ANA and was the only positive marker in 81.5% of patients, similar to previous reports.[27] The specificity against antigens is singular, with behavior that resembles a monospecific antibody. In some cases it can be accompanied by specific antibodies, this could be

explained by the apparent multiplicity of ANA occurring in autoimmune diseases related to extensive cross-reactive proteins, whose structural basis is not known. Anti-NuMA has a close relationship with autoimmunity, as previously reported: 76% for NuMA1 and 64% for HsEg5.[27] Of reported consolidated NuMA patients, 40% (108/268) had a CTD. The most common diseases were SS in 28% (31/108), SLE: 27% (29/108), RA: 19% (21/108), UCTD: 13% (15/108), and MCTD in 4.6% (5/108). Our results showed that 60% (39/65) of NuMA positive patients had a CTD: SS in 21.5%, RA: 16% and SLE 14% (9/65). We found APS in 9.2% and spondyloarthropathies in 4.6% (3/65) patients, which has not been reported previously for this pattern.

Taken together, more accurate information defines a NuMA positive patient phenotype: a monospecific antibody patient, with high titers of ANA, associated with CTD in up to 40% of cases.

Of patients with a NuMA pattern, 15.3% (10/65) had CIU, a novel association not reported previously. Autoimmune chronic urticaria is a principal cause of CIU, potentially explaining 30% to 50% of previously idiopathic cases.[28] Approximately 29% of CIU patients are ANA positive, but the pattern is not reported in these studies.[28–30] ANA positivity in CIU is associated with a higher prevalence of thyroid autoimmunity, a low basophil count, a worse response to therapy and a significantly higher incidence of CTD like RA, SS, and SLE, mostly diagnosed during the 10 years after the diagnosis of CIU, indicating that it may be the prelude to multiple autoimmunity.[28–30]

The midbody is a transient "organelle-like" remnant of cell division just before cellular cleavage. It is formed in the final stages of cell division and is critically involved in cytokinesis. The midbody contains microtubules derived from the spindle midzone and some associated proteins. The target antigens recognized by these autoantibodies have not been completely identified, but Aurora kinase B, an enzyme involved in the attachment of the mitotic spindle to centromeres, is a possible candidate.[3] The midbody (MSA-2) pattern is one of the rarest reported. The largest reported series had only 12 patients.[3] MSA-2 are principally associated with cancer, SSc, Raynaud's phenomena, autoimmune thyroiditis, and migraine, among others.[31,32] In our cohort, the midbody pattern was the second most common (0.32%), contrary to other reports. Patients with a midbody pattern had lower titers of ANA, and these were the only positive ANA in 90% of patients, with RF as the most common associated antibody. Unlike previous reports, we found a high frequency of CTD of 43% (mostly SS, RA, and SLE) and a very low rate of cancer (6%). Interestingly, we found SNHL in 36.6% of patients. In general, an autoimmune etiology of bilateral SNHL, particularly Meniere's disease (MD), is presumed, based on studies that have shown a higher prevalence of elevated positive ANA compared with controls. In these studies, ANA titers ranged from 21% to 38% of patients with MD.[33,34] Similarly, there is an increased prevalence of CTD, particularly RA, SLE, and ankylosing spondylitis, in patients with MD.[35] Alternatively, SNHL could be explained by autoimmune inner ear disease (AIED) a syndrome of rapidly progressive bilateral SNHL with coexisting autoimmune disease in 15% to 30% of patients.[36]

The centrosome is the principal microtubule-organizing center during interphase and mitosis and is composed of 2 centrioles surrounded by a matrix. During the M phase, the 2 centrosomes form the poles of the mitotic spindle.[3]

Anti-centrosome antibodies (also known inappropriately as anticentriole, as their reactivity is rarely restricted to the centriole) are rare, with a prevalence of $< 0.1\%$.[3] Different centrosome proteins are the target of autoantibodies: pericentrin, ninein, Cep250, pericentriolar material 1, α, and γ enolase.[32] At high titers (>1:320), anticentrosome antibodies are associated with CTD, including SS, SLE, scleroderma, UCTD, and Raynaud phenomena.[3] They are also described in patients with viral or mycoplasmal infections.[32] In our retrospective cohort, the centrosome pattern was the third more frequent and was present in 0.17% of patients, in contrast to previous reports.[3] Unfortunately, we only had access to complete medical histories in half the patients, leaving a very small amount of patients for the analysis. This pattern was more closely associated with CTD, specifically UCTD in 41% (7/17) SS and SLE with 11.7% (2/17), in both diseases, respectively.

Anticentromere/kinetochore antibodies were first described in patients with CREST. More than 10 proteins associated with the centromere are described as autoantigens. These proteins are divided into 4 groups: CENPs (CENtromere proteins), chromodomain proteins, topoisomerase II, and other less-well characterized proteins. Antibodies to CENP-F show unique features: unlike other CENPs, they do not tend to occur in concert with other centromere autoantibodies, and they identify a subset of patients with a high frequency of malignancies (50%–80%), commonly lung, breast, and prostate cancers, and non-Hodgkin's lymphoma, and might aid the early diagnosis of cancer.[32] Although paradoxically, anti-CENP-F antibodies are uncommon in unselected cancer cohorts (<10%).[37] Anti-CENP-F antibodies have infrequently been reported in SSc or SS patients.[37] In our cohort, the anti-CENP-F pattern was the least frequent anti-MSA, with only 4 patients with complete data: 2 patients had a CTD, one with SLE, and another with RA. Only one patient had cancer.

Our study has several limitations and strengths. Given the retrospective design used, we did not have access to all clinical data in medical records. Therefore, it is possible that some patients were not recorded as having a CTD. In addition, there is a possibility of misclassification, because not all diagnoses were recorded by a specialist. Furthermore, our study did not confirm anti-NuMA specificity (NuMA-1 or anti-HsEg5) by immune blotting of sera.

However, our study analyzed the largest reported sample of atypical ANA patterns. Besides, a single investigator (JFB), blinded to ANA patterns, reviewed medical records, providing a more homogeneous definition for disease classification.

In conclusion, there are geographical and racial differences in the prevalence of rare ANA patterns. The most frequent anti-MSA patterns found were NuMA and midbody. In more than half the patients, anti-MSA were associated with CTD, mainly SS, RA and SLE, and behave as a monospecific antibody. Other entities of presumed autoimmune origin, such as CIU and SNHL might be associated with these patterns. Further research is needed to replicate our novel findings of uncommon ANA patterns in patients with CIU and SNHL.

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