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Letter to the Editor

Should we look for anti-RNA polymerase III antibodies in Systemic Sclerosis patients with anti-centromere or anti-topoisomerase I antibodies?

Running title: anti-RNA polymerase III in Systemic Sclerosis

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Systemic sclerosis (SSc) is a chronic autoimmune disease characterised by skin and internal organs fibrosis, vascular damage and positive antinuclear autoantibodies (ANAs). The classical antigenic specificities of ANAs are anti-centromere (ACA), anti-topoisomerase I antibodies (anti-topo I) and the more recently described anti-RNA polymerase III antibodies (anti-RNAPIII).

Anti-RNAPIII, firstly described in SSc in 1993[1], are currently admitted as specific SSc-related autoantibodies and have been incorporated into the 2013 ACR/EULAR classification criteria for the disease [2]. The prevalence of anti-RNAPIII partially depends on the geographic origin of patients [3]. In France, the prevalence is low and ranges from 3 to 9% whereas it can reach 14% in North America and 41% in South America [3,4]. The ANA pattern associated with anti-RNAPIII was described as a fine-speckled nuclear stain with additional occasional bright dots, with or without concurrent punctate nucleolar staining but no typical pattern was proposed [5]. Anti-RNAPIII are mainly associated with a diffuse cutaneous subtype, scleroderma renal crisis and a risk of cancer in close temporal relationship to SSc onset [3,4,6,7]. As the specific method for their identification was the radioimmunoprecipitation assay, a cumbersome method not suitable for routine practice, these autoantibodies were not routinely looked for. More recently immunoenzymatic methods have been developed, but, the detection strategy was not clearly established for routine practice [5]. Although the positivity of ACA and anti-topo I is considered to be exclusive, the co-positivity of anti-RNAPIII with these SSc-specific auto-antibodies remains to be clarified. In order to design the best strategy for anti-RNAPIII detection, we aimed to 1/ evaluate the co-positivity of anti-RNAPIII in SSc patients positive for ACA or anti-topo I and to 2/ analyse immunofluorescence patterns and clinical characteristics of anti-RNAPIII positive patients.

Firstly, 76 sera from SSc patients (9 men, 67 women) from Marseilles (South of France) positive for either ACA or anti-topo I antibodies were tested for the presence of anti-RNAPIII. All patients fulfilled the 2013 ACR/EULAR criteria and were further classified as having diffuse or limited cutaneous SSc [2]. All the sera were collected from 2012 to 2016 and were issued from a Biobank (DC 2012-1704) with respect of ethical directives. Antinuclear antibodies (ANAs) were detected by indirect immunofluorescence on HEp-2 cells (Bio-Rad Laboratories, Hercules, CA) at a screening dilution of 1:160. ACA, anti-topo I and anti-RNAPIII were detected by commercially kits (EliA Thermo Fisher). The cutoff value for anti-RNAPIII positivity was 10 Arbitrary Unit/ml (AU).

Secondly, the ANAs immunofluorescence patterns and clinical data of 8 SSc patients positive for anti-RNAPIII were collected from 2012 to 2016 and compared to anti-RNAPIII negative SSc patients (<10 AU/ml). Results were expressed as median +/- interquartile range or as frequencies (fq). Medians were compared using Mann Whitney U Test. Frequencies were compared using Chi 2 Test.

Among the 76 selected sera of SSc patients, 33 patients (43%) were positive for ACA and 43 (57%) for anti-topo I antibodies. Immunofluorescence nuclear patterns were: centromeric (*n*=32), nucleolar homogeneous (*n*=41), speckled-centromeric (*n*=1), speckled-homogeneous (*n*=1) and mixed speckled-nucleolar-centromeric (*n*=1). Anti-RNAPIII were investigated in these SSc patients: only one ACA-positive serum (1.3%) was found also positive for anti-RNAPIII with a titer of 192 AU/ml. This serum corresponded to the mixed speckled-nucleolar-centromeric immunofluorescence nuclear pattern **(Figure 1)**. No other specificities associated with a speckled pattern (anti-Ro/SSA anti-La/SSB, anti-Sm, anti-RNP) were detected. The patient, a 47-year-old female, had a diffuse cutaneous subset, with digital ulcers, joint contractures, a reduced diffuse lung capacity for carbon monoxide and oesophageal reflux disorder. Her medical history was remarkable for an ovarian

adenocarcinoma that was diagnosed 6 years before SSc, and considered in remission after surgery and radio-chemotherapy.

Then, we retrospectively analysed immunofluorescence pattern and clinical characteristics of 8 SSc patients found positive for anti-RNAPIII in our laboratory. The various immunofluorescence aspects were nuclear speckled (*n*=5), nucleolar (*n*=1), nucleolar-speckled (*n*=1) **(Figure 2)** and mixed speckled-nucleolar-centromeric (*n*=1) **(Figure 1)**. The median titer of anti-RNAPIII was 54 [18-298] AU/ml.

Table 1 illustrates the comparison between anti-RNAPIII positive and negative patients**.** Sex ratio did not differ between the two groups. A trend to a higher frequency of the diffuse cutaneous form was observed in anti-RNAPIII positive SSc patients. The two patients with *sine scleroderma* belonged to the group of negative anti-RNAPIII. Scleroderma renal crisis was solely documented in patients with positive anti-RNAPIII. Other variables were not significantly associated with anti-RNAPIII positivity.

The present study extends the previously published data performed in South of France [4] about the anti-RNAPIII screening strategy. This study highlights that anti-RNAPIII are rarely encountered in SSc patients already positive for ACA or anti-topo I antibodies. The case of the patient with coexisting ACA and anti-RNAPIII was interesting regarding two aspects. First, the clinical feature was closer to the one described in patients with anti-RNAPIII with a diffuse cutaneous form and a concomitant cancer [8]. Second, the observed ANAs immunofluorescence pattern was highly remarkable due to the peculiar aspect of immunofluorescence. Therefore, in ACA or anti-topo I positive sera, the search for anti-RNAPIII can be recommended faced to a mixed fluorescence pattern. Conversely, in case of typical fluorescence aspects related to ACA or anti-topo I positivity, the systematic search for anti-RNAPIII is not mandatory. As observed herein, different immunofluorescence pattern

can be associated with anti-RNAPIII [9]. Therefore the search for this auto-antibody should not be restrained to a nucleolar immunofluorescence pattern of ANAs [10].

Regarding the phenotype of anti-RNAPIII positive patients, a higher frequency of scleroderma renal crisis and diffuse cutaneous form was observed. The low number of patients and our geographic location might be a limiting factor to evidence any association with other anti-RNAPIII features such as the frequency of cancer [8].

Although SSc-related autoantibodies are exclusive markers, co-positivity can exist in rare cases. The immunofluorescence reading step appears crucial to focus the screening. In order to improve the benefit cost ratio, anti-RNAPIII should be searched with respect to ANAs fluorescence pattern and clinical characteristics of the patients.

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Table 1: Characteristics of SSc patients with respect to positivity of the anti-RNA Polymerase III (anti-RNAPIII) autoantibodies.

Characteristics	**Anti-RNAPIII + (*n*=8)**	**Anti-RNAPIII- (*n*=75)**	p-value
Sex Ratio (F/M)	6/2	66/9	0.30
Age (years, median) [IQR]	54 [39.5-71.3]	62.4 [49.9-70.6]	0.47
Age at disease onset (years, median) [IQR]	52 [30.5-71.8]	53 [43.8-61.3]	0.77
Age at the apparition of Raynaud phenomenon (years, median) [IQR]	48 [30-70.5]	50 [38-56]	0.97
Diffuse SSc/Limited SSc	5/3	23/50	0.08
Sine Scleroderma SSc	0/8	2/75	0.64
Pulmonary fibrosis (Fq)	2/8	25/70	0.55
FVC (%, median) [IQR]	87.6 [70.2-108.3]	79.5 [68.8-100.5]	0.49
DLCO (%, median) [IQR]	57 [41-65]	51.3 [41.4-63.7]	0.77
DLCO/VA (%, median) [IQR]	67 [48-76]	63.65 [52-73]	0.68
Pulmonary Arterial Hypertension (Fq)	2/8	20/72	0.87
Esophagus Reflux Disorder (Fq)	3/8	24/65	0.97
Intestinal Motility Disorder (Fq)	1/8	26/73	0.26
Medsger Severity Scale (Fq)	3 [3-4]	3 [2-3.37]	0.27
Scleroderma Renal Crisis (Fq)	2/8	0/75	$<10^{-4}$
Cancer occurrence	1/8	9/75	0.96
Anti-Topo 1 antibody (Fq)	0/8	43/75	-
Anti-centromere antibody (Fq)	1/8	32/75	-

Results are expressed as median +/- interquartile range or as frequencies (fq). Medians were compared using Mann Withney U Test. Frequencies were compared using Chi 2 Test.

Legends of the figures

Figure 1: The mixed fluorescence pattern (speckled-nucleolar-centromeric) of the ACA and Anti-RNAPIII positive serum.

A: x 400 magnification.

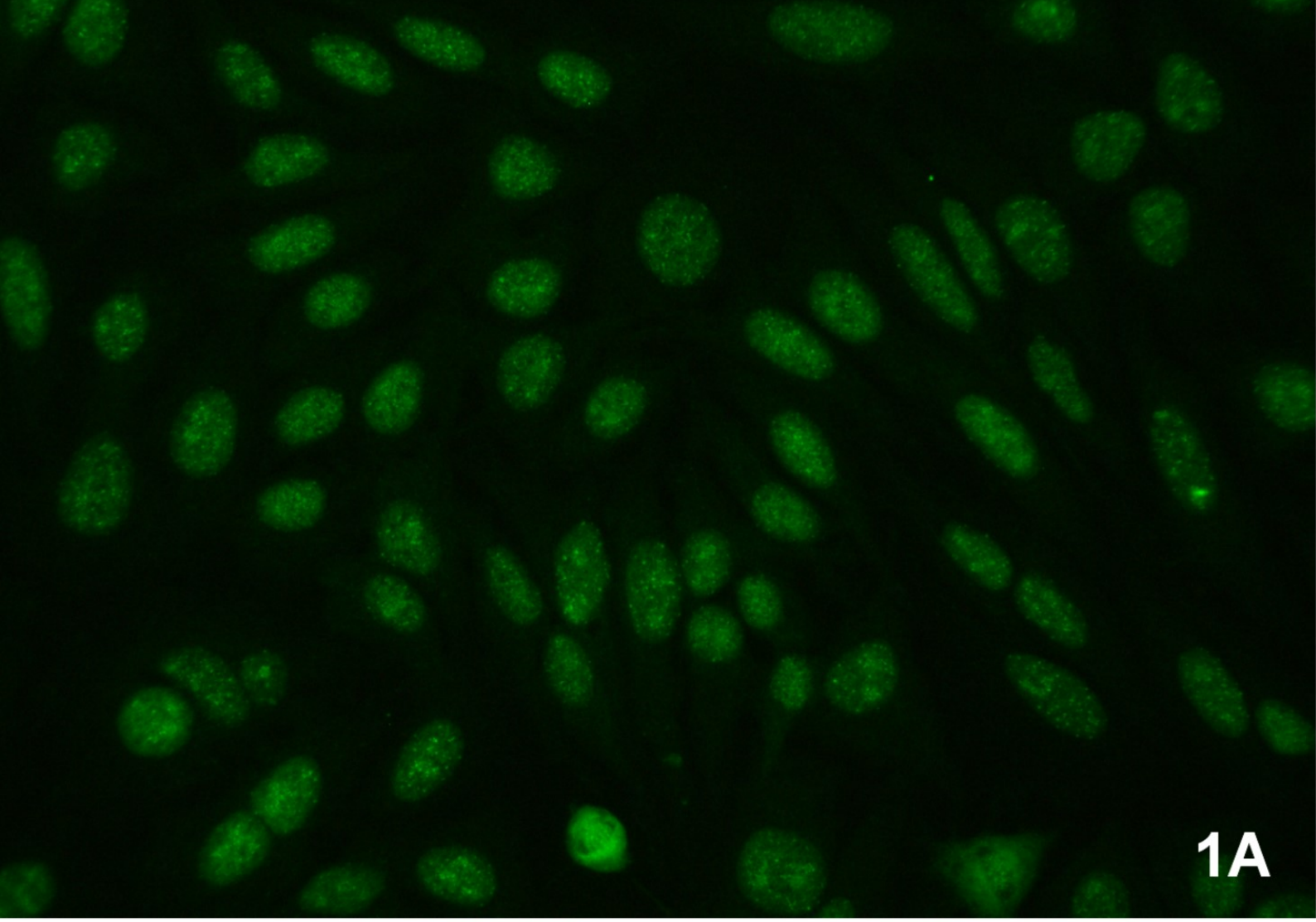
B: Typical features of the centromeric pattern are highlighted with characteristic dots at the interphase mitotic stage (left arrow) and a “block” of condensed dots at the metaphase stage (right arrow).

Figure 2: Various immunofluorescence patterns obtained from sera positive for anti-RNAPIII (x 400 magnification).

A: nuclear-speckled pattern

B: nucleolar-speckled pattern

C: nucleolar pattern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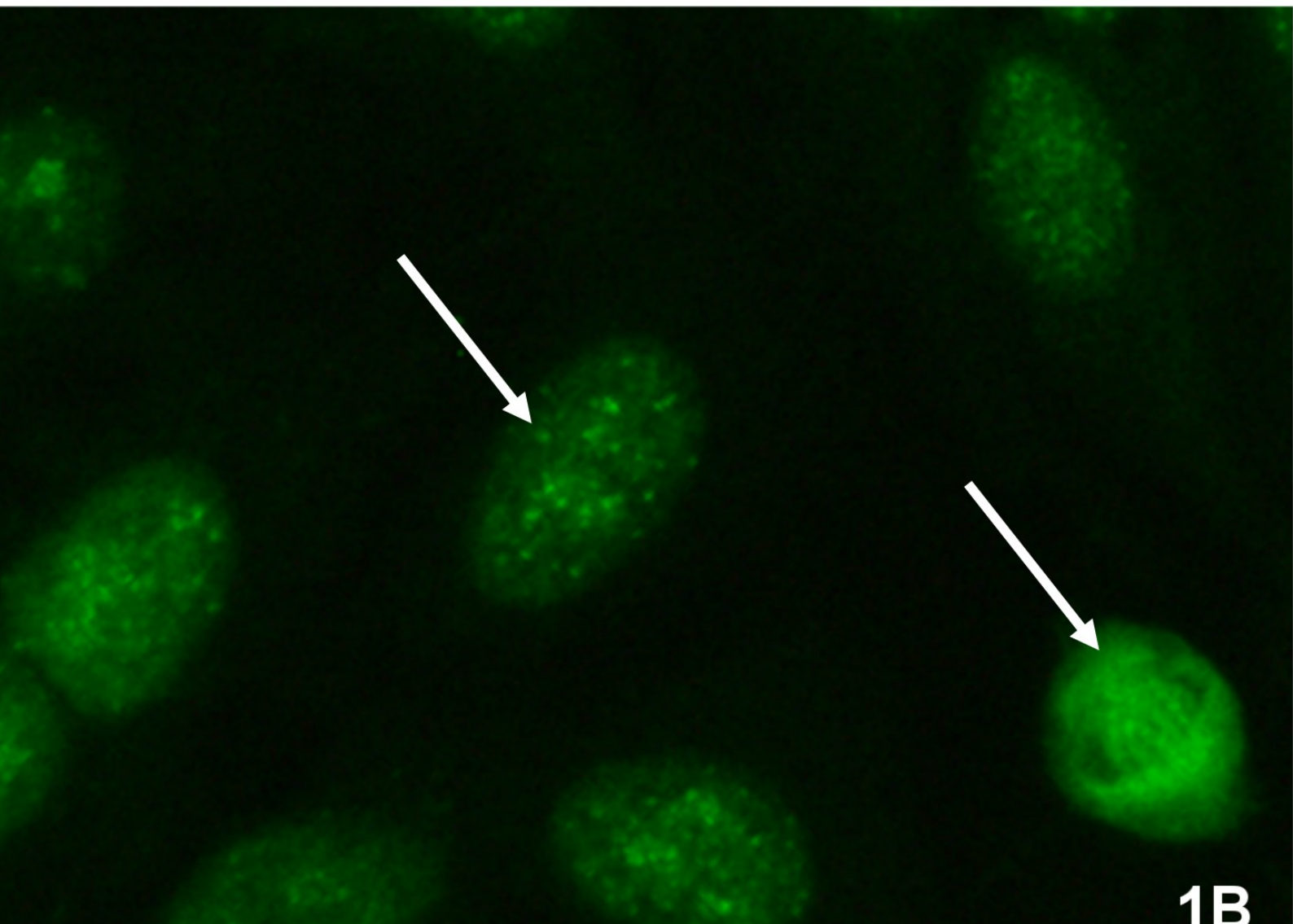
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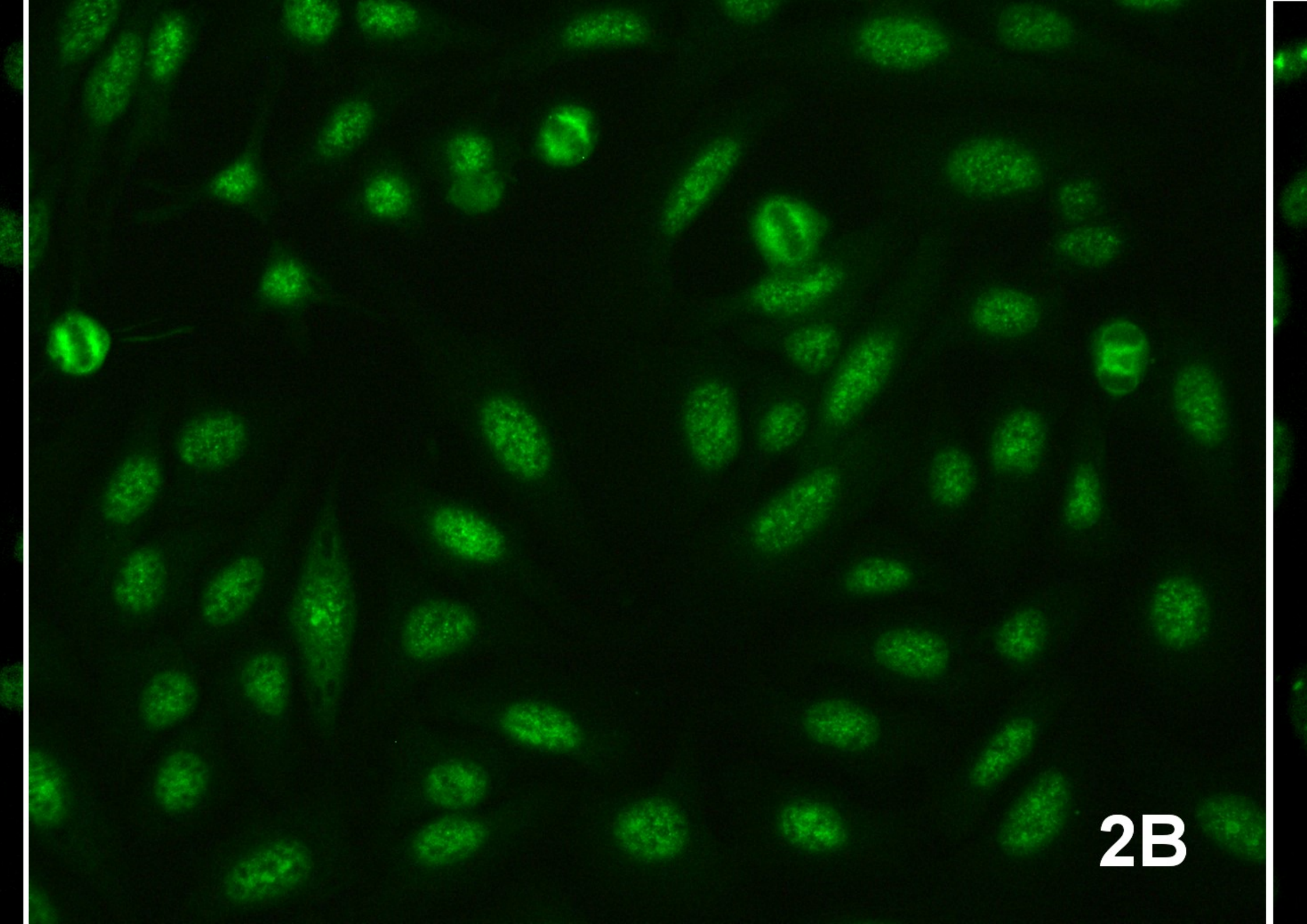
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