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Concise report

Anti-pseudo-PCNA type 1 (anti-SG2NA) pattern: Track down Cancer, not SLE

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ABSTRACT

Objective: Describe the clinical significance of anti-SG2NA antibodies also called anti-pseudo-PCNA type 1 (proliferating cell nuclear antigen auto-antibodies) which are rare antinuclear antibodies (ANAs) staining distinctly S/G2 proliferative HEp-2 cells by indirect immunofluorescence. By analogy with anti-PCNA antibodies, they have been suspected to be associated with systemic lupus erythematosus (SLE), cancers or viral diseases.
Methods: From May 2006 to February 2013, 16,827 patients were tested positive for ANAs in the Laboratory of Immunology, Strasbourg, France. We retrospectively analyzed clinical and biological data from 126 patients with anti-pseudo-PCNA type 1 antibodies.
Results: There was a 0.75% prevalence of anti-pseudo-PCNA type 1 Abs among ANAs$^+$ patients. Median age was 56.9 years (standard deviation [SD] 13.4 years) with a sex ratio female/male of 1.9. Compared to ANAs$^+$ patients, many more patients have been hospitalized in the Oncology and Hematology Department (23% vs. 6.3%, $P < 0.05$). Indeed, anti-pseudo-PCNA type 1 Abs were detected in 33 patients suffering from solid and hematological cancers (26%). Another group of patients presented various auto-immune diseases but surprisingly none of our patients was affected with SLE when 5 out of 8 patients in anti-PCNAs$^+$ Abs group ($P < 5.10^{-6}$) were. Finally, the presence of anti-pseudo-PCNA type 1 Abs was associated in 30 cases with other auto-Abs reflecting a more general breakdown of B cell tolerance against other self-antigens.
Conclusion: Considering our results, explorations for tumors should be at least recommended for patients with anti-pseudo-PCNA type 1 Abs. Lupus disease is not associated with these autoAbs.

1. Introduction

Antinuclear antibodies (ANAs) are frequently associated with autoimmunity, tumors, aging, infections or medications [1,2]. Some ANAs specificities are rare and their implications in pathology remain unclear [3].

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Anti-proliferating cell nuclear antigen Abs (anti-PCNA Abs) were described for the first time in 1978 as a rare novel pattern of ANAs on HEp-2 cells [4]. Thus, a speckled pattern of nuclear staining was found with the unique characteristic of being observed on proliferating cells in phase S. PCNA antigen, also called PCNA/cyclin, was then identified as the 34 kDa factor of an auxiliary protein for DNA polymerase δ [5] which is part of a multiprotein complex playing an essential role in DNA repair and replication. Historically described in patients with systemic lupus erythematosus (SLE), anti-PCNA Abs were initially considered as a specific marker [6,7]. Later on, other studies did not confirm a strong association and anti-PCNA Abs were found in patients affected with Sjögren syndrome, lymphoma, various cutaneous diseases, chronic hepatitis B and C virus infections, although not present in healthy controls sera [8–12].

A novel antinuclear antibody staining HEp-2 cells with a PCNA-like pattern by indirect immuno-fluorescence (IIF) was described in 1994 by Landberg et al. in the serum of a patient with bladder and lung cancers [13]. Initially called anti-SG2NA Ab [14], it recognized an antigen related to cell cycle specifically expressed in phases S and G2, and was later also called anti-pseudo-PCNA type 1 Ab because of its PCNA-like pattern [15]. Recently, the target of anti-pseudo-PCNA type 1 Abs was clearly identified as the isoform 2 of the striatin 3 protein, distinct of the PCNA Antigen. This nuclear protein is made of 780 amino-acids with 6 WD40 domains [16,17], plays a scaffolding role and acts as a link between intracellular signal transduction and trafficking in eukaryotic cells.

In this study, we focused on anti-pseudo-PCNA type 1 Abs currently thought to be associated with tumors or lupus by extension with anti-PCNA auto-Abs. Thereafter, we describe for the first time, the clinical and biological features of 126 patients in a French retrospective cohort (Strasbourg University Hospital).

2. Methods

From May 2006 to February 2013, 54,894 sera were tested for ANAs in the Laboratory of Immunology (Strasbourg University Hospital). Patients' sera submitted for ANAs were analyzed by IIF using HEp-2 cells (HEp-2 cells slides and anti-human IgG, IgA, IgM fluorescent conjugate ZEUS Scientific, Raritan, NJ 08869 USA) and titrated when positive (≥1/160). ANAs patterns were defined as classically described [3,18–20]. For high quality images of the different patterns during cell cycle stages, it can be referred to atlases by Bradwell and Hugues [19] and by Humbel [20].

Sera displaying a pleiomorphic pattern with a variety of nucleoplasmic speckles from fine to very coarse in 40%–60% of the non mitotic cells (S phase) as well a nucleolar staining in some cells were considered positive for anti-PCNA Abs (Fig. 1A) and tested by Ouchterlony's immunodiffusion (ENA extract, Immunoconcept, Sacramento, USA) and immunodot (D-Tek, Mons, Belgium) in order to confirm their anti-PCNA specificity.

Sera displaying a pleiomorphic nuclear pattern like PCNA pattern but clearly distinguishable by the size, distribution and number of speckles–very fine, uniform and dense speckles with negative nucleoli (Fig. 1B)–were considered positive for anti-SG2NA Abs as defined by Landberg et al. [13] or anti-pseudo-PCNA type 1 Abs by Humbel [15,20].

The individualization of anti-pseudo-PCNA type 1 from anti-PCNA antibodies patterns was confirm by random testing for cyclin reactivity in 25 sera out of 126: the immunodiffusion or cyclin immunodot test was constantly negative in sera from anti-pseudo-PCNA type 1 pattern and positive in sera from anti-PCNA pattern.

If there were several positive sera from a single patient, only the first serum was included, to avoid duplicates. Positive patients for anti-pseudo-PCNA type 1 Abs at the titer of 1/160 or more (≥1/160) where selected for further clinical and biological analysis, after being approved by the Institutional Review Board.

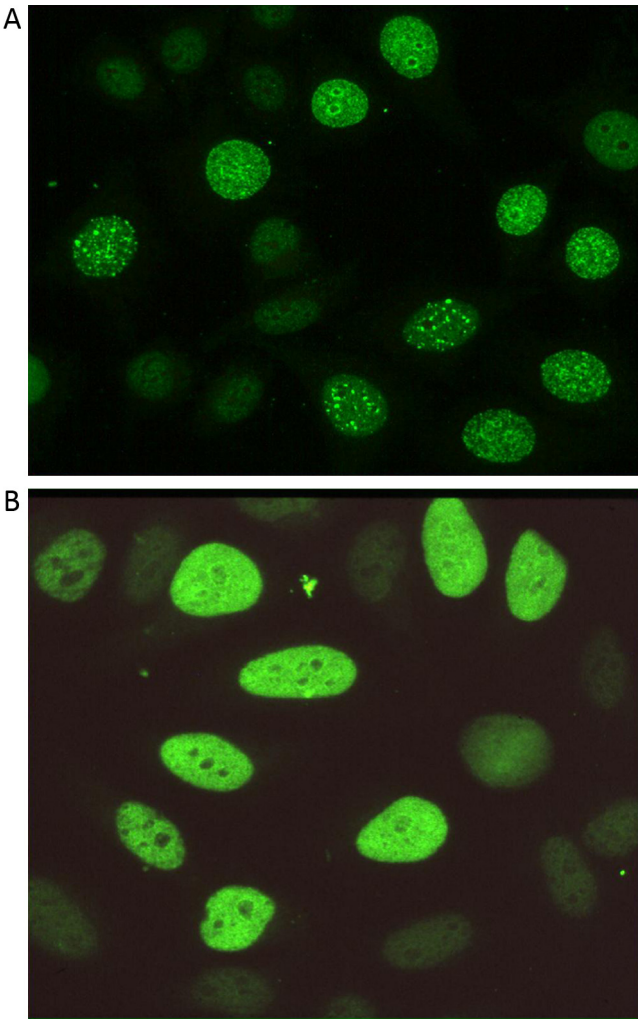

Fig. 1. Characteristic aspects of anti-PCNA and anti-pseudo-PCNA type 1 Abs by indirect immunofluorescence (IIF) on interphase HEp-2 cells. A. Anti-PCNA pattern: fine to coarse, numerous and polymorphic nuclear staining of 30% to 60% of S phase HEp-2 cells. In some cells the nucleoli are stained as well. By immunodiffusion or immunodot, this IIF pattern is characteristic of cyclin positivity. B. Anti-pseudo-PCNA type 1 pattern: dense, uniform and fine speckles in S/G2 phase cells with negative staining of the nucleoli.

Meanwhile, sera positive for anti-PCNA Abs and anti-dsDNA (Kallestad Anti-dsDNA EIA microplate, ref 31008, Bio-Rad Laboratories, Hercules, CA, USA and Varelisa dsDNA Antibodies, ref 141 96, Phadia GmbH, Freiburg, Germany) were distinguished among ANAs positive sera.

The presence of autoimmune diseases or tumors was checked for patients with anti-PCNA Abs.

Autoimmune diseases were diagnosed considering clinical and biological datas, and according to international criteria of diagnosis (American College Rheumatology [ACR] criteria). Tumors were identified according to histological proofs or medical reports.

Fisher's exact test was used for statistical analysis. Statistical significance was defined at $P < 0.05$.

3. Results

During the seven-year period, 54,894 sera were tested for the presence of ANAs and 16,827 patients were positive (excluding duplicates). We identified 126 patients with anti-pseudo-PCNA