Anti-tRNA Synthetase-specific Immunofluorescence Patterns are Easily Detected in the Suitable Substrate

To the Editor:

We read with interest the article by Aggarwal, *et al*[1]. We agree with the authors when they evoke the importance of cytoplasmic staining for early diagnosis of myositis while refraining from reporting an "antinuclear antibody (ANA)–negative result." However, we disagree with reporting it as a generic concept without its specific description, because as noted by the authors, cytoplasmic staining preserves various patterns, and we should mind the possible diffusion of SS-A/Ro into the cytoplasm during the fixation process of HEp-2 substrate.

Besides the number of pattern types and the diversity of target antigens, the frequency with which they are experienced in laboratories should be considered as another important factor. Under the real circumstance of cytoplasmic staining on HEp-2 cells, we usually encounter anti-Ro, which has intensely higher incidence than anti-tRNA synthetase. We have also frequently observed antimitochondrial antibodies. Moreover, even other patterns, the clinical significance of which is still less known, are observed more often than anti-tRNA synthetases. Therefore, precisely distinguished report type is absolutely required, and the cytoplasmic dense fine speckled pattern and the cytoplasmic fine speckled pattern, which are currently recommended in the International Consensus on ANA Patterns[2,3], can be considered a priority.

Originally, in the beginning of the ANA test, when cryostat sections or touch prints were used as a substrate with animal liver or kidney, there was no disagreement regarding the term. Thereafter, as the ANA substrate was gradually replaced with cultured cells (HEp-2) and the fluorescence pattern in cytoplasm were recognized, the name ANA was called into question. A term "antinucleocytoplasmic antibody" was once suggested. However, because medical terminology customarily maintains a misnomer even if new evidence contradicts it, and some cytoplasmic antigens are derived from the nucleus, the name "ANA" persisted, including its comprehensive meaning. Recently, the term "anticellular antibody" has been proposed[4]. This controversy already existed 20 years ago and we received a great number of questions from many laboratories owing to the unidentified name. Hence, Dr. Think-You Kim, of Hanyang University Medical Center, Seoul, South Korea, presented a lecture on anticytoplasmic antibodies at the inaugural meeting of the Korean Study Group for Diagnostic Immunology on October 8, 1997.

We overcame these problems by establishing the human macrophage cell line, IT-1, in 1989. Instead of the ANA misnomer, we used the test name "autoimmune target (AIT) test" and clarified the confusing concept that the ANA are used either separately from or with cytoplasmic antibodies[5]. With the AIT test, antiribosomal P, which is not well determined in the HEp-2 ANA test, was reported to be easily detected[6], and the anti-Jo1 presents mainly as fine granules with a thick and individual shape in the cytoplasm, so it has been described as "cytoplasmic fine granular pattern" by the AIT test[7]. Further, diffuse cytoplasmic patterns, which are presumed to be other anti-tRNA synthetases, are often observed in patients with myositis.

Since 2002, we have performed the external quality assessment (EQA) program of the autoimmune test authorized by the Korean Society for Laboratory Medicine in South Korea and have involved cytoplasmic patterns in that program[8]. In the early stages of implementation, there were institutions sending out results such as "Negative: cytoplasmic pattern (1:160)," but these participants are diminishing as EQA continues. However, apart from this improved perception of cytoplasmic staining, the assessment of cytoplasmic patterns is still struggling. In the recently performed EQA of ANA/AIT test on anti-Jo1 samples, 38.9% (28/72) of the participating institutes reported the result as negative (27.8%, 20/72) or unrelated to anti-Jo1 (11.1%, 8/72), and the majority rendered the result "cytoplasmic" without a detailed description (Table 1).

Recently, for the automation of the HEp-2 ANA test, we performed a comparative test with 4 main products (Bio-Rad, Euroimmun, INOVA, Zeus). The staining process was done by employees from each company on the same day using the same samples in our laboratory. Even with the same HEp-2 cell, there was a difference in each test, not only in the pattern but also the titer. Regarding anti-tRNA synthetase-specific immunofluorescence patterns, 2 samples of anti-Jo1 were applied and some products showed negative or low titers. After experimentation demonstrated that these discrepancies resulted from substrate differences, we reaffirmed that the EQA results were not merely due to differences in readers' capabilities.

Therefore, we believe that selecting a suitable substrate is the most important factor for accurately assessing and reporting cytoplasmic staining.

LA-HE JEARN, MD, Professor, Department of Diagnostic Immunology/Laboratory Medicine, Hanyang University Medical Center; THINK-YOU KIM, MD, Professor, Department of Diagnostic Immunology/Laboratory Medicine, Hanyang University Medical Center, Seoul, Republic of Korea. Dr. Kim holds patents relating to the IT-1 cell line. Address correspondence to Dr. T.Y. Kim, Department of Diagnostic Immunology/Laboratory Medicine, Hanyang University Medical Center, 222-1 Wangsimni-ro, Seongdong-gu, Seoul, 04763, Republic of Korea. E-mail: tykim@hanyang.ac.kr

REFERENCES

1. Aggarwal R, Dhillon N, Fertig N, Koontz D, Qi Z, Oddis CV. A negative antinuclear antibody does not indicate autoantibody negativity in myositis: role of anticytoplasmic antibody as a screening test for antisynthetase syndrome. J Rheumatol 2017;44:223-9.

Table 1. EQA results of ANA/AIT test on anti-Jo1 according to participants' description (n = 72). Products of the following manufacturers were used by participant institutes: Bio-Rad Laboratories, Euroimmun, ImmunoThink, INOVA Diagnostics, MBL, and Zeus Laboratories.

Described Patterns	n	Described Patterns	n
Cytoplasmic	27	Cytoplasmic + speckled	4
Cytoplasmic fine granular	2	Cytoplasmic + nuclear dots	2
Cytoplasmic (fine speckled)	1	Cytoplasmic + fine nuclear dots	1
Cytoplasmic fine speckled (anti-Jo1)	1	Cytoplasmic (granular) + homogeneous	1
Cytoplasmic coarse granular	1	Cytoplasmic discrete + speckled	1
Cytoplasmic (mitochondrial)	1	Speckled	2
Cytoplasmic (weak-positive)	1	Peripheral	1
Antimitochondrial	2	Nuclear membranous	1
Anti-Jo1	1	Weakly positive	1
Lysosomal	2	Negative	19

EQA: external quality assessment; ANA: antinuclear antibody; AIT: autoimmune target.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2017. All rights reserved.

Downloaded on April 9, 2024 from www.jrheum.org

2. Chan EK, Damoiseaux J, Carballo OG, Conrad K, de Melo Cruvinel W, Francescantonio PL, et al. Report of the first international consensus on standardized nomenclature of antinuclear antibody HEp-2 cell patterns 2014-2015. Front Immunol 2015;6:412.
3. Damoiseaux J, von Mühlen CA, Garcia-De La Torre I, Carballo OG, de Melo Cruvinel W, Francescantonio PL, et al. International consensus on ANA patterns (ICAP): the bumpy road towards a consensus on reporting ANA results. Auto Immun Highlights 2016;7:1.
4. Dellavance A, de Melo Cruvinel W, Francescantonio PL, Andrade LE. Antinuclear antibody tests. In: Detrick B, Schmitz JL, Hamilton RG, editors. Manual of molecular and clinical laboratory immunology, 8th ed. Washington: ASM Press; 2016:843-58.
5. Jearn LH, Kim DA, Kim TY. Limitations of antinuclear antibody tests (HEp-2) are overcome with the autoimmune target test (IT-1) in systemic lupus erythematosus. J Rheumatol 2009;36:1833-4.
6. Jearn LH, Kim TY. AIT test has no problem in the detection of anti-ribosomal P. Arthritis Res Ther 2009;11:407.
7. Jearn LH, Kim TY. Anti-Jo1 antibody in polymyositis/dermatomyositis is still closely associated with lung rather than joints. J Rheumatol 2015;42:1060.
8. Jearn LH, Kim TY. Should the external quality assessment sample of antinuclear antibodies be of a typical monospecific pattern? Clin Chem Lab Med 2010;48:1189-90.

J Rheumatol 2017;44:10; doi:10.3899/jrheum.170414

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2017. All rights reserved.

Downloaded on April 9, 2024 from www.jrheum.org