

Anti-DFS70 Antibodies: A Useful Biomarker in a Pediatric Case With Suspected Autoimmune Disease

abstract

Antidense fine speckles 70 (anti-DFS70) antibodies, a peculiar antinuclear antibody (ANA) pattern by indirect immunofluorescence, is frequently observed in ANA-positive individuals with no evidence of systemic autoimmune rheumatic disease. They may be found in many different inflammatory conditions and in healthy individuals. We herein report a case of an 8-year-old girl presenting with generalized edema, hypertension, hepatomegaly, and a history of pharyngitis, which occurred 3 weeks earlier. Laboratory analysis revealed low complement C3 (6 mg/dL), microhematuria, and proteinuria. A diagnosis of acute glomerulonephritis was made. Anti-dsDNA, antiextractable nuclear antigens, and antineutrophil cytoplasmic antibodies were negative. However, a highly positive (1:640) ANA immunofluorescence test with dense fine speckles pattern was found. The presence of anti-DFS70 immunoglobulin G antibodies was confirmed by a specific immunoassay. In conclusion, the presence of isolated anti-DFS70 antibodies may be useful to exclude an autoimmune pathogenesis in those children with a positive ANA test and a clinical picture possibly attributable to systemic autoimmune rheumatic disease. This will avoid further unnecessary investigation with the potential for incorrect diagnosis and possibly harmful treatment. *Pediatrics* 2014;134:e1706–e1708

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KEY WORDS

anti-DFS70 antibodies, anti-nuclear antibodies, systemic autoimmune disease, differential diagnosis

ABBREVIATIONS

ANA—antinuclear antibody

anti-DFS70—antidense fine speckles 70

IIF—indirect immunofluorescence

SARD—systemic autoimmune rheumatic disease

Dr Fabris did a fundamental contribution to conception of the study and acquisition, analysis, and interpretation of laboratory data; she made the first draft of the article and collected all the co-authors' revisions to make the final version, then re-sent it to co-authors for their final approval before submission; Dr Zago did a substantial contribution to acquisition of laboratory data and to draft the first version of the article (introduction and laboratory data); Dr Tosolini did a substantial contribution to acquisition and interpretation of clinical data and to draft the first version of the article (clinical case description); Dr Melli, as clinical supervisor of Dr Tosolini, helped him during the period of hospitalization of the patient and revised clinical data to draft the case description; Dr Bizzaro did a substantial contribution to acquisition of laboratory data (anti-DFS70 IgG antibody detection by the QUANTA Flash DFS70 method) and critically revised the first draft thanks to his important knowledge in the field of autoimmunity and, in particular, in DFS70 analysis and interpretation; Dr Tonutti did a fundamental contribution to conception of the study and acquisition, analysis, and interpretation of data; he revised the draft critically for important intellectual content. All authors approved the final version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Detection of antinuclear antibodies (ANAs) by indirect immunofluorescence (IIF) assay on HEp-2 cells is the test of choice when suspecting a systemic autoimmune rheumatic disease (SARD).^{1,2} Antidense fine speckles 70 (anti-DFS70) antibodies were initially identified as an ANA IIF pattern from a patient with interstitial cystitis,³ but were later associated with a variety of chronic inflammatory conditions^{4,5} with no evidence of SARD (atopic dermatitis, alopecia areata, autoimmune thyroiditis, cancer, etc) and in healthy individuals.⁶ Anti-DFS70 antibodies were found to be frequently associated with anti-p80 coilin antibodies in patients with allergic diseases but rarely in patients with SARD.⁷ These findings suggested that the presence of isolated anti-DFS70 antibodies (ie, the unique antigenic specificity responsible for ANA-positive results) could be taken as strong evidence against a diagnosis of SARD, such as systemic lupus erythematosus, making it a possible key biomarker to discriminate SARD from other clinical conditions in ANA-positive individuals.^{8–10}

The typical DFS70 IIF staining pattern appears as a particular combination of a dense fine speckles fluorescence uniformly distributed throughout the interphase nucleus and a characteristic staining of the chromatin area in mitotic cells (Fig 1A).³ According to this peculiar IIF pattern, the antigen

was initially termed DFS70, due to the apparent molecular weight in immunoblot assays. Later, the main target autoantigen was first identified as the lens epithelium-derived growth factor,¹¹ and more recently, as the DNA-binding transcription coactivator p75.¹²

CASE PRESENTATION

An 8-year-old girl was referred to the emergency department with a 5-day history of progressive periorbital and lower limb edema, ascites, and increased weight. She complained of respiratory distress in the preceding 2 days. On physical examination, the child presented generalized edema, hypertension (140/100 mm Hg), and hepatomegaly. Parents noted a history of pharyngitis 3 weeks earlier. Blood analysis revealed white blood cells $9300 \times 10^6/L$, with 55% neutrophils and 31% lymphocytes, hemoglobin 9.6 g/dL, platelet count $229 \times 10^9/L$, total protein 60.8 g/L, albumin 33.5 g/L, complement C3 6 mg/dL and C4 19 mg/dL, serum urea nitrogen 35 mg/dL, creatinine 0.72 mg/dL, and electrolytes were in the normal range. Urine analysis revealed microhematuria and proteinuria. After a few hours of observation, the girl developed progressive dyspnea and a chest radiograph confirmed pulmonary edema. An echocardiogram revealed normal cardiac function. She was treated with a continuous infusion of furosemide

and nifedipine with prompt recovery. No extrarenal symptoms or signs potentially associated to systemic lupus erythematosus were noted, and the clinical presentation appeared characteristic of poststreptococcal glomerulonephritis. Accordingly, the antistreptolysin O test presented an elevated titer (1110 UI/mL; range, 0–200), the measure of total proteins renal excretion in a 24-hour urine collection test revealed a significant proteinuria (1236 mg/24 hours), urinary casts and numerous red blood cells, and the throat swab demonstrated the presence of a *Streptococcus pyogenes* infection. Antibiotic therapy was administered; proteinuria and hematuria resolved completely within a few days with loss of weight and normal values of blood pressure without any further therapy.

In the meantime, to exclude the presence of an occult SARD, a large panel of autoantibodies was ordered. Anti-dsDNA antibodies, antiextractable nuclear antigens, and antineutrophil cytoplasmic antibodies were negative, while ANA by IIF on HEp-2000 (ImmunoConcepts, Sacramento, CA) were detected at high titer (1:640) and were characterized by a dense fine speckles pattern distributed also in the chromatin region of metaphase nuclei. In addition, highly fluorescent 1–2 large grains per cell were also observed, which could be attributed to the presence of anti-p80 coilin (Fig 1B). Based on these observations, anti-DFS70 immunoglobulin G antibodies were measured by the QUANTA Flash DFS70 method (INOVA Diagnostics, San Diego, CA) that uses a partial length human recombinant DFS70/lens epithelium-derived growth factor protein. The assay was performed on the fully automated random access BIOFLASH chemiluminescence analyzer (Biokit SA, Barcelona, Spain). The sample resulted positive at high titer for anti-DFS70 antibodies (143.3 U; reference interval <20 U).

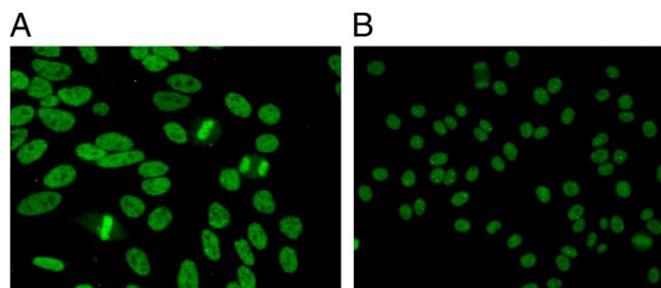


FIGURE 1

A, A typical anti-DFS70 IIF ANA pattern on HEp-2000 (400 \times); B, The ANA pattern observed in the reported case: dense fine speckles distributed also in metaphase nuclei plus 1–2 highly fluorescent large grains per cell (200 \times).

CONCLUSIONS

In clinical practice, ANA assays, often with subsequent automatic cascade profile tests, are often inappropriately ordered. This case represents such a frequent scenario, where the typical clinical and laboratory findings of acute glomerulonephritis might be misinterpreted in favor of an autoimmune pathogenesis, due to the occurrence of a significantly positive ANA test. Anti-DFS70 antibodies are rarely observed in autoimmune diseases¹⁵

and whenever observed, additional autoantibodies related to systemic autoimmune diseases are frequently present as well.

Therefore, the finding of an ANA-positive test caused by anti-DFS70 antibodies as the sole antibody specificity represents a useful tool to exclude an autoimmune disorder and thus avoids further unnecessary autoantibody cascade testing, which may be misleading and lead to an incorrect diagnosis and possible treatment with immunosuppressive

therapeutic agents. However, due to the subjectivity of interpretation and differences in substrate performance,¹⁴ a DFS70-like ANA pattern identified by IIF is not sufficient to indicate the presence of this autoantibody, but it should always be confirmed by a specific immunoassay for the identification of DFS70 specificity.

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