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Research paper

Anti-DFS70 antibodies detected by immunoblot methods: A reliable tool to confirm the dense fine speckles ANA pattern

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ABSTRACT

Background: Autoantibodies to the DFS70 (dense fine speckles 70) protein have been identified among the anti-nuclear antibodies (ANA) in patients with various disorders. However, the ANA test in indirect immunofluorescence (IIF) is not a reliable method to identify anti-DFS70 antibodies. We undertook this study to evaluate the diagnostic performance of two new immunoblot methods for the detection of anti-DFS70 antibodies and to investigate whether their different DFS70 antigen composition could affect diagnostic accuracy in detecting anti-DFS70 antibodies.
Methods: 62 samples showing a DFS70 staining pattern by IIF were tested by dot blot (Alphadia) and line blot (Euroimmun) methods. The dot blot method employs a truncated sequence of the DFS70 antigen (residues 349-435), while the line blot uses the full-length protein (aa 1-530). The 62 samples were previously assayed by a chemoluminescent (CLIA) method also using a truncated antigen (aa 349-435): 27 were CLIA positive and 35 were CLIA negative. 120 sera from subjects with infectious diseases were used as controls.
Result: Both immunoblot methods were positive in the 27 IIF/CLIA positive samples; in addition, the Alphadia dot blot identified another seven DFS70 samples and the Euroimmun line blot was positive in five samples that were negative by CLIA. Among the 120 control samples, two false positives were recorded for the CLIA method, six for the Alphadia method and four for the Euroimmun method. Therefore, in this selected series of samples, sensitivity and specificity were 43.5% and 98.3% for the CLIA method, 54.8% and 95% for the dot blot and 51.6% and 96.6% for the line blot, respectively.
Conclusions: Because of great inconsistency in assessing the DFS70 pattern using the ANA-IIF test, specific assays should be used to confirm anti-DFS70 antibodies. The results of this study show that there is no difference in the overall diagnostic accuracy among methods that use the truncated or the full-length DFS70 antigenic sequence and that it is likely that antibodies directed against antigens other than DFS70 may be responsible for producing a DFS70-like ANA-IIF pattern.

1. Introduction

The dense fine speckled 70 (DFS70) antigen (also known as the lens epithelium derived growth factor - LEDGFp75) is a highly conserved DNA-binding protein, contributing to cellular protection against oxidative DNA damage (Singh et al., 1999; Shinohara et al., 2002). The protein is present in cell nuclei of all organs and tissues and may be overexpressed or altered during inflammation, thus stimulating autoantibody responses (Ganapathy et al., 2003; Ochs et al., 2015).

A DFS70 pattern is found at a relatively high frequency during the anti-nuclear antibody (ANA) routine work up by indirect immunofluorescence assay (IIF) on HEp-2 cells. With this method the presence of DFS70 antibodies is suspected when fine speckles uniformly distributed in the nucleus, sparing the nucleoli, are present in interphase cells, and staining of the chromatin region is observed in metaphase cells. Currently, the clinical significance of their finding is not clear as the presence of anti-DFS70 antibodies has been described in patients with diverse chronic inflammatory conditions (Ochs et al., 2000; Muro et al., 2006; Muro et al., 2008; Kuwabara et al., 2009; Mahler and Fritzler,

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2012; Fabris et al., 2014), but also in healthy subjects (Watanabe et al., 2004; Mariz et al., 2011; Mahler et al., 2012a; Schmeling et al., 2015).

The recent availability of analytical methods employing the recombinant DFS70/LEDGFp75 protein gave new vigor to the study of these antibodies and enabled a more accurate definition of their prevalence in different clinical conditions (Mahler et al., 2012a).

However, beside the possible association with specific diseases or clinical conditions, the correct recognition of the DFS pattern and therefore of anti-DFS70 antibodies by methods specifically designed to detect these antibodies, is important to avoid errors in the interpretation of the ANA test. Since ANA positivity, without further specification of the pattern, is one of the classification criteria of systemic lupus erythematosus (SLE), incorrect recognition of the DFS70 pattern may cause an incorrect clinical interpretation of the meaning of ANA positivity, leading to unnecessary analysis, misdiagnosis, possible over-treatment and increased costs for patients and care providers.

Specific chemoluminescence (CLIA) or enzyme immunoassay (ELISA) methods for the detection of anti-DFS70 antibodies are already available (Mariz et al., 2011; Bizzaro et al., 2011; Mahler et al., 2012b; Miyara et al., 2013; Bizzaro et al., 2015). Their diagnostic sensitivity is around 50% in samples that display a presumptive DFS70 pattern in the ANA-IIF test. In this study we evaluated the diagnostic performance of two new immunoblot methods for the identification of anti-DFS70 in samples showing a DFS70-like staining pattern by IIF, and to ascertain whether the use of different antigenic sequences could improve the diagnostic sensitivity of specific tests for anti-DFS70 antibody detection.

2. Methods

2.1. Patients

Sixty two sera displaying a DFS70-like fluorescence pattern at a titer equal to or higher than 1:160 in the ANA test on HEp-2 cells were studied. Sera came from a cohort of samples referred as possible DFS70-positive ANA by expert clinical pathologists, collected consecutively from routine ANA testing.

The diagnoses of these 62 selected patients (52 females and 10 males, mean age 53 years, range 14–90) were very heterogeneous: 21 had a connective tissue disease (SLE, rheumatoid arthritis, Sjögren's syndrome, scleroderma, undifferentiated connective tissue disease), six undifferentiated arthritis, seven a non-autoimmune rheumatic disease (polymyalgia rheumatica, osteoarthrosis, ankylosing spondylits, Horton arteritis), 11 an autoimmune organ-specific disease (Hashimoto's disease, Graves' disease, celiac disease, autoimmune gastritis, multiple sclerosis, Crohn's disease), and 17 other inflammatory diseases. As a control group we studied 120 ANA-negative patients (101 females and 19 males, mean age 38 years, range 3–82) with active infectious disease (CMV, EBV, HBV, HCV, toxoplasma, syphilis).

All patients and controls gave their informed consent to this retrospective study according to the Declaration of Helsinki and to the Italian legislation (Authorization of the Privacy Guarantor No. 9, December 12th, 2013).

2.2. Immunoblot tests

Immunoblot analysis was conducted using two commercial methods that include the DFS70 antigen in the test panel: the dot blot ANA + DFS70 IgG manufactured by Alphadia (Wavre, Belgium) and the lineblot Euroline ANA Profile 3 + DFS70 IgG manufactured by Euroimmun (Luebeck, Germany). The DFS70 antigen used in the Alphadia method is a 349-435 aa recombinant protein expressed in *Escherichia coli,* while that of the Euroimmun method is a full-length (aa 1-530) recombinant (*E. coli*) protein. Assays were performed according to the manufacturers' instructions and the intensity of the positive dots or lines was read using the Dr. Dot system (Alphadia) (cutoff 10 units) and the Euroline Scan system (Euroimmun) (cutoff 18 units).

Sera from both patients and controls had been previously tested for anti-DFS70 antibody by a chemoluminescent immunometric method (CLIA-QUANTA-Flash DFS70, Inova Diagnostics, San Diego, CA) (Bizzaro et al., 2015). The antigen used in this test consists of a recombinant DFS70 fragment expressed in *E. coli*, spanning from aminoacid 349 to 435, coated onto paramagnetic beads.

We performed immunoblot tests specifically to investigate whether a different DFS70 antigen composition than that used for the CLIA assay could increase the sensitivity in the detection of anti-DFS70 antibodies maintaining the same diagnostic specificity. To this end, we randomly selected 27 sera that were IIF-positive/CLIA-positive and 35 sera that were IIF-positive but CLIA-negative.

The ANA-IIF pattern was carefully checked in all samples to verify whether differences in pattern morphology could explain the different results obtained with the CLIA method. A typical IIF-DFS70 pattern was confirmed in all samples (e.g., either CLIA-positive or CLIA-negative), thus eliminating any possible bias in sample selection.

3. Results

Both the immunoblot methods were DFS70-positive in the 27 CLIA-positive sera; in addition, the Alphadia dot blot method recognized as DFS70-positive seven samples and the Euroimmun lineblot method identified five samples that were negative by CLIA. Among the 120 control samples, two false positives were recorded for the CLIA method, six for the dot blot method and four for the line blot method (Fig. 1 and Table 1). Since the choice of a control group is a critical issue when dealing with anti-DFS70 antibodies, as they can be found in any disease and even in healthy individuals, the fact that serum samples with positive results in the control group differed among methods (data not shown), argues in favor of the non-specificity of these findings.

Comparing the results obtained by the CLIA method and by the two immunoblot methods, in this selected series samples, sensitivity (measured as agreement with the IIF method) and specificity were 43.5% and 98.3% for the Inova CLIA method, 54.8% and 95% for the Alphadia dot blot and 51.6% and 96.6% for the Euroimmun line blot, respectively. The ROC curve analysis showed that the area under the curve was quite comparable for the three methods investigated: 0.712 for CLIA, 0759 for dot blot and 0.750 for line blot (Fig. 2).

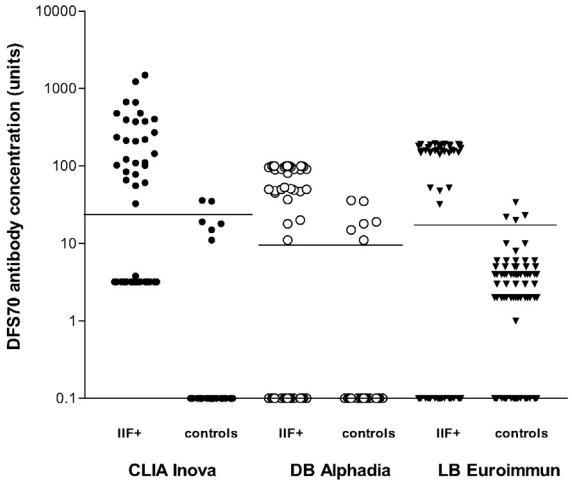

Fig. 1. Anti-DFS70 antibody concentration measured by a chemoluminescent (CLIA), a dot blot (DB) and a line blot (LB) method in samples displaying the DFS70 immunofluorescence (IIF) pattern and in 120 controls (lines show the cutoffs for each method).

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