Identification of DFS70 Antibodies during ANA screening

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Abstract

Introduction:
Antinuclear Antibody (ANA) testing can be positive in subjects with no clinical evidence of ANA associated rheumatic diseases (AARDA). Dense Fine Speckled 70 (DFS70), also known as lens epithelium-derived growth factor, is a protein expressed in the nucleus of most cells where it functions as transcription coactivator. It has been shown that DFS70 antibodies are more common in healthy subjects than AARDA patients, and that they negatively predict the development of AARDA, especially in absence of other AARDA-specific antibodies. Being able to identify with certainty whether ANA positivity is due to DFS70 would aid diagnosis and avoid unnecessary, repeated testing.

Methods (see right):
1. ANA by immunofluorescence using the Euroimmun platform
2. DFS70 antibodies by ELISA
3. Immunoadsorption of DFS70 antibodies followed by manual ANA

Results:
We evaluated overall 132 samples, out of which 69 were anti-DFS70 Ab positive (**1st group**), 48 had other ANA patterns (**2nd group**) and 15 were ANA negative (**3rd group**) by ANA IF screen utilizing the EuroImmun Sprinter system.
1st group: 57 out of 69 samples (83%) that were identified on IF screen as DFS70 positive, became negative after the immunoadsorption with rDFS70. The remaining 12 sera positive for DFS70 pattern by the IF screen (17%) yielded a new ANA pattern following the immunoadsorption with rDFS70 (**Figure A**). Out of 69 samples with DFS70 pattern on IF screen, 63 were ELISA positive for anti-DFS70 Ab. There was a good agreement between IF results for DFS70 pattern and anti-DFS70 Ab ELISA (Cohen's *kappa*=0.79). **2nd group**: Out of 48 samples, that had a different ANA pattern than DFS70 on the IF screen, 17 (35%) become negative after the immunoadsorption with rDFS70 and 5 (10%) changed ANA pattern (**Figure B**). We found that 8 samples out of 48 were positive by ELISA for anti-DFS70 Ab. **3rd group**: All ANA negative samples as identified on IF screen, were also negative by ELISA for anti-DFS70 Ab and 1 of the 15 (7%) was found to have a speckled pattern after the immunoadsorption with rDFS70 (**Figure C**).

Conclusion:
Our study demonstrated a good agreement between the anti-DFS70 Ab ELISA assay and the DFS70 IF pattern initially viewed on the ANA IF screen. The immunoadsorption technique with rDFS70 was also successful and eliminated the fluorescence seen in the Hep-2 cell substrate. Some samples revealed a new ANA pattern after immunoadsorption and those patients might have a higher risk for development of AARDA and should be farther tested by specific confirmatory tests. In addition, anti-DFS70 Ab positive patients with dsDNA, ENA and other disease related antibodies would not be considered low risk for AARDA.

Materials and Method

1. Initial ANA assay was carried out using HEp-2 IFA slides and a Sprinter automation system (both from Euroimmun, Lübeck, Germany). DFS70 pattern was noted when observed (**Figure 1**).

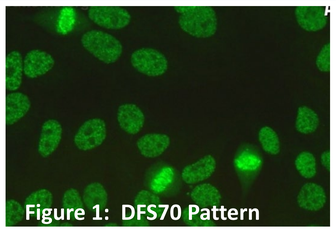


Figure 1: DFS70 Pattern

2. ELISA for DFS70 antibodies was performed using plates coated with recombinant DFS70 (rDFS70) antigen (research use only, Inova Diagnostics, San Diego, CA, USA). Binding of the patient antibodies was quantified as a ratio of optical density (OD) of the patient over OD of a low positive control provided by the manufacturer. Ratios greater than 20 were considered positive.
3.Immunoadsorption of DFS70 antibodies was done by incubating patient sera for 30 minutes at room temperature with a fragment (amino acids 349–435, from Inova Diagnostics) of DFS70, as to deplete them of specific DFS70 reactivity. Sera were then tested for ANA using HEp-2 slides (#708100, Inova Diagnostics) and using a manual, analogic microscope (**Figure 2**).

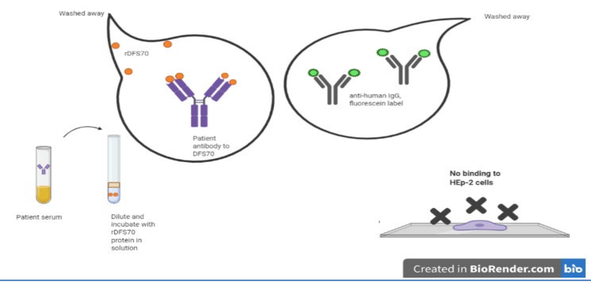


Figure 2: Immunoadsorption technique

Summary

- Good agreement between the anti-DFS70 Ab ELISA assay and the DFS70 IF pattern initially viewed on the ANA IF screen.
- The immunoadsorption technique with rDFS70 eliminated the fluorescence seen in the HEp-2 cell substrate.
- Some samples revealed a new ANA pattern after immunoadsorption and those patients might have a higher risk for development of AARDA and should be farther tested by specific confirmatory tests.
- Immunoadsorption with DFS70 antigen could be used to confirm DFS70 pattern on IF.
- Anti-DFS70 Ab ELISA could be offered as a reflex tool for confirmation of IF DFS70 pattern, avoiding repeated testing of DFS70 positive patients, saving cost and reducing patients' long-term anxiety regarding the ANA positive result.

Future Work

- Consider offering ELISA assay with considerations for the ANA algorithm after discussions with providers
- Investigate the clinical picture of patients with discrepancies
- Perform immunoadsorption technique using other recombinant proteins

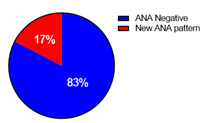
Acknowledgements

Inova Diagnostics (California, USA) has provided these items free of charge for evaluation with our patient population. The DFS70 ELISA and rDFS70 fragment for immunoadsorption are not available commercially in the United States for diagnostic use, and were prepared by the Research and Development team of Inova Diagnostics (Mahler M et al, Autoimmunity reviews 2012; 11: 642-645).
We thank Mary Ann R. Aure (Inova Diagnostics) for her help with this study.

Results

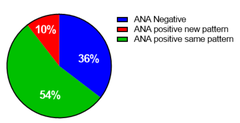
Immunoadsorption with rDFS70

Figure A: Initial DFS IF positive



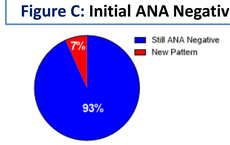
Total initial DFS IF Positive Samples: 69

Figure B: Initial DFS IF Negative



Total initial DFS IF Negative Samples: 48

Figure C: Initial ANA Negative



Total initial ANA negative samples: 15

Figure D: Changes following Immunoadsorption- Initial DFS IF Positive

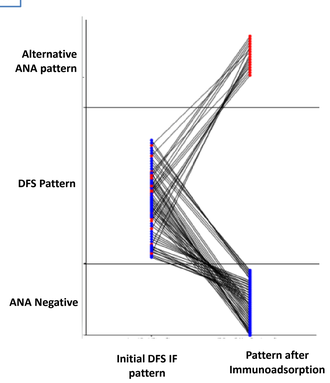


Figure E: Changes following Immunoadsorption- Initial DFS IF Negative

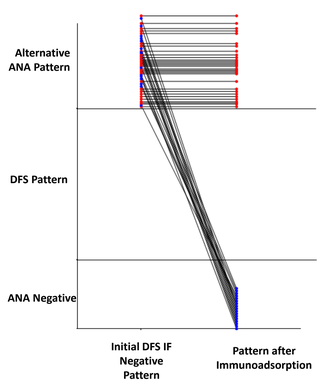


Figure F: Changes following Immunoadsorption- Initial ANA negative

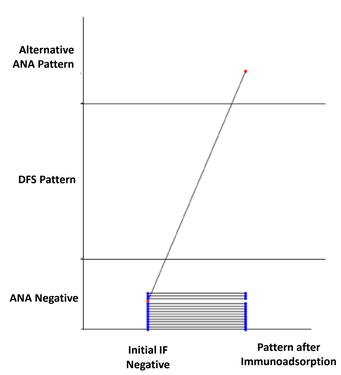


Table1: DFS70 ELISA (RUO) results

	Pos	Neg	Total
Pos	63	8	71
Neg	6	55	61
Total	69	63	132