Prevalence of anti-dense fine speckled 70 antibodies in healthy individuals and patients with antinuclear antibody-associated autoimmune rheumatic diseases in Japan

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Abstract

Previous studies from various countries have reported anti-dense fine speckled pattern (DFS)70 antibody prevalence but few studies have been from Asia. We investigated the prevalence of anti-DFS70 autoantibodies in a Japanese cohort of healthy individuals (HI) and patients with antinuclear antibody-associated autoimmune rheumatic diseases (AARD).

Enzyme-linked immunosorbent assay and indirect immunofluorescence were performed using samples from 250 HI and 276 AARD patients.

The overall anti-DFS70 antibody prevalence in HI was 16.4%, with 12.8% for males and 20.0% for females (sex difference; P = .12).

In AARD patients, the anti-DFS70 antibody prevalence in systemic lupus erythematosus, mixed connective tissue disease, systemic sclerosis, dermatomyositis and polymyositis (DM/PM), Sjögren syndrome, and rheumatoid arthritis (RA) was 22.1%, 14.3%, 14.3%, 3.0%, 21.3%, and 18.1%, respectively (no significant difference between AARD patients except DM/PM and HI). The prevalence of isolated anti-DFS70 antibody in HI and all AARD patients excluding RA was 14.8% (37/250) and 4.4% (9/204), respectively (P < .01 vs HI). Among anti-DFS70 antibody-positive cases, 63.4% (26/41) were DFS pattern by IIF and 23.5% (8/34) were HI and AARD patients excluding RA, respectively.

The anti-DFS70 antibody prevalence in HI and AARD patients in Japan was similar. Furthermore, the anti-DFS70 antibody prevalence in HI and AARD in Japan is higher than in HI and AARD in regions other than Asia. This makes AARD differential diagnosis by antinuclear antibody screening difficult.

Abbreviations: AARD = antinuclear antibody-associated autoimmune rheumatic diseases, ANA = antinuclear antibody, CIA = chemiluminescence immunoassay, DFS = dense fine speckled pattern, DM/PM = dermatomyositis and polymyositis, ELISA = enzyme-linked immunosorbent assay, EPA = EUROPattern, HI = healthy individuals, IIF = indirect immunofluorescence, MCTD = mixed connective tissue disease, OD = optical density, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SS = Sjögren syndrome, SSc = systemic sclerosis.

Keywords: antinuclear antibodies, autoantibodies, autoimmune disease, dense fine speckles, dense fine speckled 70, healthy individuals, prevalence

1. Introduction

The antinuclear antibody (ANA) test uses indirect immunofluorescence (IIF) for the diagnosis and investigation of the disease state of antinuclear antibody-associated autoimmune rheumatic diseases (AARD) such as connective tissue disease and autoimmune hepatitis.[1] Because HEp-2 cells are used as nuclear substrates,[2] they are currently widely used worldwide as a screening test for ANA.[3] The problem is the high prevalence of
detection in healthy individuals (HI) despite the ability to capture many ANAs.\[4\]

In 2014, the International Consensus on ANA patterns for the staining pattern classification of ANA tests using IIF showed that a dense fine speckled (DFS) pattern was individually determined in the speckled pattern as a competent-level reporting.\[5\] The DFS pattern resulting from anti-DFS70 antibody binding is unique and characterized by dense and heterogeneous fine speckled staining of the nucleoplasm in the interphase, and speckled staining tightly associated with chromatin during mitosis.\[6\] The DFS pattern and autoantibodies were originally described by Ochs et al. in the serum of American patients with interstitial cystitis. These autoantibodies were named anti-DFS70 antibodies based on the DFS staining pattern of HEp-2 cells by IIF\[7\] and the corresponding antigen assessed by SDS-PAGE was a 70-kDa protein.\[8\]

Methods for detecting anti-DFS70 antibodies include enzyme-linked immunosorbent assay (ELISA), immunoblot, line or dot immunooassays, and chemiluminescence immunoassay (CIA).\[9,10\] Although the clinical impact of DFS70 antibodies is not yet clear, it was reported to be useful to help eliminate ANAs.\[11\] The clustering of ANAs.\[12\] The numbers and genders of the patients were as follows: 68 patients with systemic lupus erythematosus (SLE; median age, 35 years; range, 17–72 years; 65 females, 3 males); 14 patients with mixed connective tissue disease (MCTD; median age, 47 years; range, 26–67 years; females only); 42 patients with systemic lupus erythematosus (SLE; median age, 59 years; range, 11–83 years; 37 females, 5 males); 33 patients with dermatomyositis and polymyositis (DM/PM); median age, 54 years; range, 16–79 years; 21 females, 12 males); 47 patients with Sjögren’s syndrome (SS; median age, 55 years; range, 21–71 years; 46 females, 1 male), and 72 patients with rheumatoid arthritis (RA; median age, 51.5 years; range, 19–80 years; 64 females, 8 males).

Serum from 250 cases (median age, 24 years; range, 21 to 62 years; 125 females, 125 males) that showed no abnormalities by medical examination by hospital staff were included as HI samples. Each sample was obtained with informed consent. After separation of the serum by centrifugation, the serum samples were stored frozen at −40°C. All samples collected from AARD patients and HI were randomly numbered and blindly examined for ANAs.

This study was approved according to the principles set forth in the Declaration of Helsinki by the Medical Ethics Committee of the Graduate School of Medicine, Kobe University, and samples were collected and analyzed with the consent of the patients (approval number: 1467).

2. Materials and methods

2.1. Subjects

We examined sera from 276 AARD patients who were followed at Kobe University Hospital. Serum samples were collected without conscious bias mostly at patients’ first visits during 1996 to 2010. The patients’ diseases were defined using the established criteria for each disease.\[16–23\] The numbers and genders of the patients were as follows: 68 patients with systemic lupus erythematosus (SLE; median age, 35 years; range, 17–72 years; 65 females, 3 males); 14 patients with mixed connective tissue disease (MCTD; median age, 47 years; range, 26–67 years; females only); 42 patients with systemic lupus erythematosus (SLE; median age, 59 years; range, 11–83 years; 37 females, 5 males); 33 patients with dermatomyositis and polymyositis (DM/PM); median age, 54 years; range, 16–79 years; 21 females, 12 males); 47 patients with Sjögren’s syndrome (SS; median age, 55 years; range, 21–71 years; 46 females, 1 male), and 72 patients with rheumatoid arthritis (RA; median age, 51.5 years; range, 19–80 years; 64 females, 8 males).

2.2. Equipment and reagents

For the anti-DFS70 antibody test, a DFS70 ELISA Kit (MBL, Nagoya, Japan) that uses protein from an insect cell expression system as an antigen was used.\[12,24\] For the measurement of ANAs by IIF, a computer-aided microscope system, the EURO-Pattern (EPA; EUROIMMUN AG, Lübeck, Germany), and IFA and ELISA processor, the Sprinter XL (EUROIMMUN AG) was used, and HEP-20–10 test kit (EUROIMMUN AG) was used as a reagent.\[23,26\] We analyzed 8 disease-specific ANAs, anti-dsDNA antibody, anti-U1RNP antibody, anti-Sm antibody, anti-SSA/Ro antibody, anti-SSB/La antibody, anti-Scl70 antibody, anti-centromere antibody, and anti-Jo-1 antibody using enzyme immunoassay based reagent kits (MESACUP ENA TEST and MESCUP DNA-II TEST, MBL). These were measured according to the manufacturer instructions.

2.3. Methods

ELISA and IIF were performed to compare the prevalence of anti-DFS70 antibodies and the frequency of DFS patterns by IIF in HI and AARD patients by disease type.

2.3.1. ELISA. According to the manufacturer’s instructions, each serum sample was diluted 1:100 into assay diluent, and 100 μL from each pre-diluted sample sera was used for a 1-point calibration (index value=100). Negative controls were assayed at the same time. Then, the plate was incubated for 60 minutes at room temperature. After incubation, the contents of the wells were discarded and the plates were washed 4 times with washing buffer from the kits. A second incubation was conducted by adding 100 μL of enzyme conjugate to each well and the plate was incubated for 60 minutes at room temperature. After washing as in the previous step, 100 μL of substrate was added into each well, and the plate was incubated for 30 min at room temperature. Finally, 100 μL of stop solution was added into each well and the optical density was determined using an ELISA reader at 450 nm wavelength. An index value (recommended by the kit manufacturer) of 15 or more was determined to be positive for anti-DFS70 antibodies.\[24\] Positive cases were used in an immunoabsorption test by ELISA.

The immunoabsorption test was performed using the following method. Each serum sample was supplemented with the antigen used in the assay system (final concentration; 5 μg/mL, shown by pilot experiments to be an excess amount to ensure complete inhibition). After samples were measured using a predetermined method, and the obtained index values were defined as (B) and (A), respectively. Values of [(A-B)/A] of 50% or more indicated inhibition had occurred.

2.3.2. IIF. Testing and evaluation were carried out according to the manufacturer’s instructions. In brief, microscope slides containing millimeter-sized biochips coated with HEp-20-10 cells were incubated with serially diluted serum (starting with 1:40 in PBS-Tween) for 30 minutes at room temperature, washed with PBS-Tween, and immersed in PBS-Tween for 5 minutes. To detect bound antibodies, fluorescein isothiocyanate-conjugated goat anti-human IgG was applied for 30 minutes at room temperature, followed by washing as described above. Then, samples were mounted using the mounting medium included in the kit. The steps described above were performed automatically.
by sprinter XL. After the slides were cover-slipped in mounting medium, the slides were placed in the EPA. Next, two technicians made a negative/positive determination (cutoff value 1:40) based on an image automatically determined by the EPA, and confirmed the staining pattern and antibody titer. In cases where the automatic verdict was incorrect, the technicians corrected it. EPA could not determine DFS patterns other than the basic pattern; therefore, the technicians observed the image and made a decision with reference to the International Consensus on ANA Patterns (classification algorithm and representative images available at www.ANApatterns.org). Typical images of the classical homogeneous pattern, classical speckled pattern, and the DFS pattern are shown in Figure 1, where fine speckled staining is present throughout the interphase nucleus (specific heterogeneity in size, brightness and density) and similar staining (dense and uneven spots) is present in the chromatic region of the mitotic nucleus. Atypical stained patterns were judged to be “undeterminable,” even though they resembled the DFS pattern.

2.3.3. Anti-DFS70 antibody in isolation. Anti-DFS70 antibody-positive cases assessed by ELISA were tested with 8 types of disease-specific ANAs, and cases that were anti-DFS70 antibody-positive and -negative for all 8 disease-specific ANAs were defined as anti-DFS70 antibody in isolation (monospecific anti-DFS70 antibody).

2.3.4. Correlation between anti-DFS70 antibody level and IIF antibody titer. The correlation between anti-DFS70 antibodies (index value) determined by ELISA and IIF antibody titer (negative, 1:40 to 1:2560, 1:5120 or more) were examined by Spearman rank correlation.

2.4. Statistical analysis

R.3.6.1 (R Core Team 2019, R Foundation for Statistical Computing, Vienna, Austria.) was used for statistical analysis using the chi-square test or Fisher exact test appropriately for categorical variables, and Spearman rank correlation for correlation between anti-DFS70 antibody level and IIF antibody titer. The significance level was set at \( P < .05 \).

3. Results

3.1. Immunoabsorption test by ELISA

An immunoabsorption test performed on 88 cases positive by ELISA showed all cases had inhibition of 50% or more.

3.2. Prevalence of anti-DFS70 antibody in HI

As shown in Table 1, the prevalence of anti-DFS70 antibodies in HI was 20.0% (25/125), 12.8% (16/125), and 16.4% (41/250) in

| HI /Disease | Males | Females | Total | \( P \) value (vs HI in Total) |
|-------------|-------|---------|-------|-----------------------------|
| HI          | 12.8% (16/125) | 20.0% (25/125) | 16.4% (41/250) | \( P \) value (vs HI in Total) |
| AARD        | 10.3% (3/29)    | 17.8% (44/247)   | 17.0% (47/276)  | \( .85 \)            |
| AARD except RA | 14.3% (3/21)    | 16.9% (31/183)   | 16.7% (34/204)  | \( .94 \)            |
| SLE         | 33.3% (1/3)     | 21.5% (14/63)    | 22.1% (15/68)   | \( .28 \)            |
| MCTD        | 14.3% (2/14)    | 14.3% (2/14)     | 14.3% (2/14)    | \( 1.00 \)           |
| SSc         | 10.8% (8/73)    | 10.8% (8/73)     | 10.8% (16/147)  | \( .73 \)            |
| DM/PM       | 4.8% (1/21)     | 4.8% (1/21)      | 4.8% (2/42)     | \( .04 \)            |
| SS          | 21.7% (10/46)   | 21.7% (10/46)    | 21.7% (20/92)   | \( .42 \)            |
| RA          | 20.3% (13/64)   | 20.3% (13/64)    | 20.3% (26/128)  | \( .74 \)            |

AARD = antibody-associated autoimmune rheumatic diseases, DM/PM = dermatomyositis and polymyositis, HI = healthy individuals, MCTD = mixed connective tissue disease, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SS = Sjögren’s syndrome, SSc = systemic sclerosis.
females, males, and both sexes, respectively. No sex difference was found ($P = .12$).

3.3. Prevalence of anti-DFS70 antibodies in AARD patients

Most disease-specific autoantibodies in systemic autoimmune diseases are antinuclear antibodies which can be detected by IF-ANA. In contrast, disease-specific autoantibodies in RA, anticyclic citrullinated peptide antibody and rheumatoid factor, are not antinuclear antibodies, and the prevalence of antinuclear antibodies in RA are much lower than that in other AARDs. Thus, we analyzed “AARD excluding RA” as a separate group.

As shown in Table 1, the prevalence of anti-DFS70 antibodies in patients with SLE, MCTD, SSc, DM/PM, SS, and RA was 22.1%, 14.3%, 14.3%, 3.0%, 21.3%, and 18.1%, respectively. In addition, the prevalence of anti-DFS70 antibodies was 17.0% for all AARD patients and 16.7% for AARD patients excluding RA. When comparing all AARD patients, there were no significant differences between different diseases, except for DM/PM compared with HI. The prevalence of patients with DM/PM was 3.0%, which was significantly lower than that of HI ($P = .04$).

3.4. Frequency of monospecific anti-DFS70 antibody

As shown in Table 2, the number of monospecific anti-DFS70 antibody-positive cases in the HI group, all AARD patients, and AARD patients excluding RA was 37, 18, and 9 cases, respectively; and the prevalence of monospecific anti-DFS70 antibody-positive cases was 90.2% (37/41), 38.3% (18/47), and 26.5% (9/34), respectively. The prevalence in all cases was 14.8% (37/250), 6.5% (18/276), and 4.4% (9/204), and the prevalence of monospecific anti-DFS70 antibody-positive cases was higher in the HI group compared with all AARD patients (AARD patients: $P < .01$ vs HI, AARD patients excluding RA: $P < .01$ vs HI).

3.5. Frequency of DFS pattern by IIF

Among anti-DFS70 antibody-positive cases, the number of cases determined to have a DFS pattern in HI, all AARD patients, and AARD patients excluding RA was 26, 15, and 8 cases, respectively (Table 3); and the frequency of the DFS pattern was 63.4% (26/41 cases), 31.9% (15/47 cases) and 23.5% (8/34 cases), respectively. Of note, the number of DFS pattern-undeterminable cases in the HI and all AARD groups was 2 and 6 cases, respectively, and anti-DFS70 antibodies were positive in all cases.

3.6. Correlation between anti-DFS70 antibody level and IIF antibody titer

As shown in Figure 2A, a significant correlation between anti-DFS70 antibody level and IIF antibody titer was observed in HI

### Table 2

Comparison of the prevalence in the presence of an isolated anti-DFS70 antibody by enzyme-linked immunosorbent assay in healthy individuals and antinuclear antibody-associated autoimmune rheumatic disease patients.

| HI /Disease | Prevalence of anti-DFS70 antibody-positive population | $P$ value (vs HI) | Prevalence of the whole population | $P$ value (vs HI) |
|-------------|-----------------------------------------------------|-----------------|-----------------------------------|-----------------|
| HI          | 90.2% (37/41)                                       | (reference)     | 14.8% (37/250)                    | (reference)     |
| AARD        | 38.3% (18/47)                                       | .01             | 6.5% (18/276)                     | < .01           |
| AARD except RA | 26.5% (9/34)                                   | .01             | 4.4% (9/204)                      | < .01           |
| SLE         | 20.0% (3/15)                                        | .01             | 4.3% (3/68)                       | .02             |
| MCTD        | 0.0% (0/2)                                          |                 | 0.0% (0/14)                       | .23             |
| SSc         | 16.7% (1/6)                                         | .01             | 2.4% (1/42)                       | .03             |
| DM/PM       | 100.0% (1/1)                                        | 1.00            | 3.0% (1/33)                       | .10             |
| SS          | 40.0% (4/10)                                        | .01             | 6.5% (4/67)                       | .25             |
| RA          | 69.2% (9/13)                                        |                 | 12.5% (9/72)                      | .02             |

The cases that were anti-DFS70 antibody-positive and negative for all 8 disease-specific ANAs (anti-dsDNA antibody, anti-U1RNP antibody, anti-Sm antibody, anti-SSA/Ro antibody, anti-SSB/La antibody, anti-Scl70 antibody, anti-centromere antibody, and anti-Jo-1 antibody) were defined as anti-DFS70 antibody in isolation. AARD = antibody-associated autoimmune rheumatic diseases, DM/PM = dermatomyositis and polymyositis, HI = healthy individuals, MCTD = mixed connective tissue disease, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SS = Sjögren’s syndrome, SSc = systemic sclerosis.

### Table 3

Comparison of the prevalence presented as a DFS pattern by indirect immunofluorescence among positive sera of anti-DFS70 antibody by enzyme-linked immunosorbent assay in healthy individuals and antinuclear antibody-associated autoimmune rheumatic disease patients.

| HI /Disease | Prevalence of anti-DFS70 antibody-positive population | Prevalence of the whole population | The number of DFS pattern-undeterminable cases by IIF |
|-------------|-----------------------------------------------------|-----------------------------------|---------------------------------------------------|
| HI          | 63.4% (26/41)                                       | 10.4% (26/250)                    | 2                                                 |
| AARD        | 31.9% (15/47)                                       | 5.4% (15/276)                     | 6                                                 |
| AARD except RA | 23.5% (8/34)                                   | 3.9% (8/204)                      | 6                                                 |
| SLE         | 13.3% (2/15)                                        | 2.9% (2/68)                       | 3                                                 |
| MCTD        | 0.0% (0/2)                                          | 0.0% (0/14)                       | 0                                                 |
| SSc         | 16.7% (1/6)                                         | 2.4% (1/42)                       | 1                                                 |
| DM/PM       | 0.0% (0/1)                                          | 0.0% (0/33)                       | 0                                                 |
| SS          | 50.0% (5/10)                                        | 10.6% (5/47)                      | 2                                                 |
| RA          | 53.8% (7/13)                                        | 9.7% (7/72)                       | 0                                                 |

AARD = antibody-associated autoimmune rheumatic diseases, DM/PM = dermatomyositis and polymyositis, HI = healthy individuals, MCTD = mixed connective tissue disease, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SS = Sjögren’s syndrome, SSc = systemic sclerosis.
Figure 2. A: Correlations between anti-DFS70 antibody levels obtained by enzyme-linked immunosorbent assay and titers of antinuclear antibodies by indirect immunofluorescence in healthy individuals. Open circles show HI sera of monospecific anti-DFS70 antibodies and cases negative for 8 disease-specific ANAs, filled squares show HI sera of anti-DFS70 antibody positive and positive for at least one of 8 disease-specific ANAs, including anti-U1RNP antibody, anti-Sm antibody, anti-SSA/Ro antibody, anti-SSB/La antibody, anti-Scl-70 antibody, anti-Jo-1 antibody, anti-centromere antibody, and anti-dsDNA antibody individuals negative for all of these ANAs. Spearman rank correlation coefficient; $r = 0.53, P < 0.01$. ANAs = antinuclear antibodies, HI = healthy individuals. B: Correlations between anti-DFS70 antibody levels obtained by enzyme-linked immunosorbent assay and titers of antinuclear antibodies by indirect immunofluorescence in antinuclear antibody-associated autoimmune rheumatic patients. Open circles show AARD sera of monospecific anti-DFS70 antibodies and cases negative for all 8 disease-specific ANAs, filled squares show AARD sera of anti-DFS70 antibody positive and positive for at least one of 8 disease-specific ANAs, including anti-U1RNP antibody, anti-Sm antibody, anti-SSA/Ro antibody, anti-SSB/La antibody, anti-Scl-70 antibody, anti-Jo-1 antibody, anti-centromere antibody, and anti-dsDNA antibody individuals negative for all of these ANAs. Spearman rank correlation coefficient; $r = 0.11, P = 0.07$. AARD = antinuclear antibody-associated autoimmune rheumatic, ANAs = antinuclear antibodies.
(Spearman rank correlation; \( r = 0.53, P < .01 \), but no significant correlation was observed in AARD patients (Fig. 2B, \( r = 0.11, P = .07 \)). Compared with AARD patients, most IIF positive cases in HI were positive for monospecific anti-DFS70 antibody.

4. Discussion

The prevalence of anti-DFS70 antibodies in HI in our study was 16.4% and was higher in females (20.0%) than in males (12.8%) although there was no significant sex difference. A large cohort of blood donors from 7 countries (\( n = 2628 \), not including Japan) tested all samples (as did our study) for anti-DFS70 antibodies by CIA.[15] According to a detailed study in this cohort, the prevalence of anti-DFS70 antibodies varied between 7 countries and was significantly different among the different sites (ANOVA \( P < .05 \)).[15] Albesa et al conducted an analysis of the potential influence of ethnicity in the United States (181 Caucasian subjects, 56 African American subjects, 48 Hispanic subjects, 6 Asian subjects) and reported no statistical difference in the prevalence of anti-DFS70 antibodies.[15] Because a small number of Asians were used in this evaluation, it is necessary to conduct research in multiple countries, including other Asian countries. The prevalence of anti-DFS70 antibodies in this large cohort was between 1.2% to 8.5%.[15] Compared with the highest prevalence in the United States (8.5%, 42/497), the prevalence of anti-DFS70 antibodies in HI (16.4%) in our study was high (\( P = .0011 \)). Watanabe et al reported a high prevalence of anti-DFS70 antibodies in Japanese.[22] In hospital staff (\( n = 597 \)), anti-DFS70 antibodies (tested by IIF and immunoblotting assays) were positive in 64 subjects (10.7%), and the positive rate was significantly higher in individuals younger than 35 years compared with those 35 years and older (\( P < .003 \)).[22] Albesa et al also reported the prevalence of anti-DFS70 was significantly higher in younger individuals than in older individuals (<35 years vs >35 years) for the whole cohort (4.9% vs 2.7%, \( P = .0017 \)) and for females (6.1% vs 2.3%, \( P = .0027 \)). The prevalence of anti-DFS70 antibodies in Japanese HI in our study was higher than apparently healthy blood donors in other nationalities and higher even than in the hospital staff reported by Watanabe (\( P = .02 \)), although, our study included some bias due to our population (79% were under 35 years old), which might have higher numbers of young people than the study by Watanabe et al.

Anti-DFS70 antibodies in apparently HI have been widely reported to be in the range 0.0%–21.6% (including reports from Japan).[128] One limitation of these reports is that most studies were performed as a secondary test of IIF-positive cases or DFS pattern-positive cases to determine the prevalence, and IIF-negative cases with anti-DFS70 antibodies were ignored.

According to reports from several institutions, the prevalence of anti-DFS70 antibodies in AARD patients with SLE, MCTD, SSc, DM/PM, SS, and RA was 0.0% to 5.7%, 0.0% to 7.7%, 0.0% to 6.4%, 0.0% to 28.6%, and 0.0% to 2.6%, respectively.[24] In comparison, the prevalence rates in our study were 22.1%, 14.3%, 14.3%, 3.0%, 21.3%, and 18.1%, respectively, which were high overall. This might be explained by many of the reports already conducting anti-DFS70 antibody tests on IIF-positive samples. The prevalence of anti-DFS70 antibodies may have been underestimated because of differences in IIF reagent kits (differences in cell fixation methods) and cutoff values. A recent paper suggested this limitation.[129] In a cohort of multinational SLE patients, CIA of all samples showed the prevalence of anti-DFS70 in SLE patients was higher than previously published ranges (7.1% vs 0.0%–2.8%).[29] In addition, Infantino et al reported no significant difference in the prevalence of anti-DFS70 antibodies in patients with AARD, non-AARD, and undifferentiated connective tissue disease (UCTD) (2.1% [7/333] vs 2.3% [9/384] vs 5.9% [3/51], respectively; \( P = .188 \)).[13] In our study, there was no significant difference in the prevalence of anti-DFS70 antibodies between HI and each type of AARD (excluding DM/PM). Although bias due to age should be considered, anti-DFS70 antibodies might occur at a certain rate in HI and AARD patients. As Mahler et al reported, anti-DFS70 antibodies may be expressed at a certain level in HI and patients with other diseases, reflecting the B cell autoantibody repertoire.[6] Therefore, HI will only have a certain level of anti-DFS70 antibodies whereas AARD patients will have disease-specific ANAs in addition to this certain level of anti-DFS70 antibodies.

We found that the prevalence of anti-DFS70 antibody in patients with DM/PM was significantly lower than that in HI (3.0%; 1/33, \( P = .04 \) vs HI). This finding is consistent with a previous report by Muro et al showing the low prevalence of anti-DFS70 antibody in patients with dermatomyositis (6.0%; 7/116, \( P = .69 \) vs our data).[130] Unfortunately, however, the clinical significance of this finding is unclear.

Next, the difference in reactivity related to the use of different antigens in the measurement system to detect anti-DFS70 antibodies will be described. ELISA and line or dot immunoassays systems manufactured by EUROIMMUN and others use full-length DFS70 antigens to detect anti-DFS70 antibodies.[6,31] The ELISA system produced by MBL used in this study uses antigen from an insect cell expression system, into which cDNA (encoding the 323 to 530 amino acid region) encompassing the DFS70 autoimmune epitope has been inserted.[6,24] The QUANTA Flash DFS70 (CIA) and NOVA Lite HEp-2 Select system (Inova Diagnostics) use an 86-amino acid C-terminal region that has been further fine-tuned.[6] However, in a comparative study between ELISA (MBL) and CIA (Inova Diagnostics), it was reported that the 2 methods had an equivalent detection frequency and excellent correlation despite differences in antigens used (Spearman rank correlation; \( r = 0.91; P < .0001 \)).[12] therefore, it is unlikely that this was due to differences in the measurement system. Regarding the specificity of the ELISA system manufactured by MBL, in 88 cases that were positive by ELISA, all samples showed more than 50% inhibition in an immunobassorption test demonstrating good specificity.

In our study, there was a difference in the effect of anti-DFS antibodies on the staining pattern of IIF between HI and AARD patients coexisting with several types of disease-specific ANAs. Antibody levels of anti-DFS70 antibodies and IIF ANAs were significantly correlated in HI but not in AARD patients. Furthermore, the frequency of monospecific anti-DFS70 antibody-positive cases was 90.2% (37/41) in HI, and 26.5% (9/34) in AARD patients excluding RA; therefore, we considered the presence of anti-DFS70 antibodies in HI was the cause of IIF positivity, although there was a bias because the definition of monospecific anti-DFS70 antibody-positive cases only included 8 types of disease-specific ANAs, which was a study limitation. A previous study reported that HI with anti-DFS70 antibodies mostly had a typical DFS pattern by IIF,[6,12,27] and that the DFS pattern, which is rarely seen in AARD patients, was commonly detectable in HI.[11,12,27]
Among anti-DFS70 antibody-positive cases, 63.4% (26/41) and 23.5% (8/34) were determined to be DFS pattern in HI and AARD patients excluding RA, respectively. Therefore, coexisting disease-specific ANAs reduce the capture rate of anti-DFS antibodies by DFS pattern in AARD patients. When the anti-DFS70 antibodies and the disease-specific ANAs coexisted, there was a tendency to show a staining pattern derived from the disease-specific ANAs. Presumably, the higher antibody titer and affinity of the disease-specific ANAs compared with the anti-DFS70 antibodies inhibited their reactivity to each component of the nuclear material; therefore, the DFS pattern may have been masked.

Finally, the frequency of monospecific anti-DFS70 antibody positive cases was higher in HI than in AARD patients, and the anti-DFS70 antibodies caused IIF to be positive for ANAs in HI. As a result, there is an over-diagnosis of AARD by clinicians based on ANA positive findings, which increases anxiety in patients and leads to unnecessary examinations. The high prevalence of anti-DFS70 antibodies in Japan has led to confusion in clinician triages for patients with possible AARD and non-AARD, making AARD differential diagnosis in ANAs screening more difficult.

4.1. Limitations
Several limitations of this study should be acknowledged. First, HI in our study had relatively higher numbers of young people. Thus there might be a slight selection bias in this study. Second, there might be a bias because the definition of monospecific anti-DFS70 antibody-positive cases only included 8 types of disease-specific ANAs. Third, there might be a difference in reactivity related to the use of different antigens in the measurement system to detect anti-DFS70 antibodies might be included.

4.2. Future directions
The Identification of the DFS pattern by IIF remains challenging and specific assays for the detection of anti-DFS70 antibodies are needed. In addition, the finding that the prevalence of anti-DFS70 antibody in patients with DM/PM was lower than that in HI should be explored by future studies.

5. Conclusion
The anti-DFS70 antibody prevalence in HI and AARD patients in Japan was similar. Furthermore, we found that the anti-DFS70 antibody prevalence in HI and AARD in Japan is higher than in HI and AARD in regions other than Asia.

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