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Dense fine speckled indirect immunofluorescence pattern in an Australian population

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Summary

The dense fine speckled (DFS) pattern is an antinuclear antibody (ANA) pattern that has recently become of interest. This particular pattern has not been associated with systemic autoimmune rheumatic disorders (SARD) but has been associated with other inflammatory conditions such as interstitial nephritis, autoimmune thyroid disease and atopic eczema as well as being found in healthy individuals. We have been reporting this pattern in our laboratory for the past 3 years. The objective of this study was to determine the frequency of the DFS pattern as detected on indirect immunofluorescence (IIF) in an Australian population and to assess association of this pattern with other laboratory autoimmune markers. ANA tests performed by IIF from July 2012 until June 2014 were reviewed and the frequency of DFS pattern was determined. All DFS positive samples that had undergone concurrent testing for antibodies to extractable nuclear antigens (ENA), anti-dsDNA antibodies, rheumatoid factor (RF), anti-citrullinated protein antibodies (CCP) and anti-phospholipid antibodies were compared. Over the 2 year period, 181,819 patients had ANA tests performed and 51,905 were ANA positive. The DFS pattern was found in 5.7% of ANA positive patients. Within this group of patients, only 1.8% were positive for antibodies to ENA and only 0.7% had anti-dsDNA antibodies level greater than 9 IU/mL. RF and anti-CCP antibodies were positive in 6.3% and 4.1% of DFS positive samples, respectively. There were only two samples positive for anti-phospholipid antibodies when the DFS pattern was present. The presence of the DFS pattern as detected by IIF is infrequently associated with autoimmune markers of SARD which is consistent with international studies.

Key words: Dense fine speckled; anti-nuclear antibodies; indirect immunofluorescence; systemic autoimmune rheumatic disease.

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INTRODUCTION

The antinuclear antibody (ANA) indirect immunofluorescence (IIF) assay is a widely used test when autoimmune disease is suspected. ANAs are found in sera from patients with a variety of autoimmune diseases, inflammatory disorders and in healthy individuals. Over 120 patterns have been described but relatively few have clinical significance. A previously described ANA pattern, designated dense fine speckled (DFS), is based on the presence of nuclear distribution of dense fine speckles. This pattern is unique in that unlike other ANA patterns it doesn't appear to be associated with systemic autoimmune rheumatic disorders (SARD). The DFS pattern, produced by autoantibodies to DFS70, was originally described in patients with interstitial nephritis[1] but has been observed in a variety of other inflammatory conditions including atopic eczema, asthma, autoimmune thyroid disease and the rare Vogt–Harada syndrome.[2,3] The pattern is also common in healthy people.[4] Individuals that have the DFS pattern don't appear to progress to SARD. Mariz *et al.*[5] reported that none of 40 healthy individuals with isolated anti-DFS70 reactivity developed a SARD within an average 4-year follow-up. The DFS pattern has been associated with antibodies to a 75 kDa protein also termed lens epithelium derived growth factor (LEDGF)/DNA binding transcription coactivator p75. This protein is thought to be important in cell survival and resistance to cellular stress.[6] The typical IIF DFS staining pattern is described as uniform dense fine speckles of the nucleoplasm and similar speckling of the chromosome plate of metaphase cells (Fig. 1). Other fine speckled patterns do not stain the chromosome plate. The homogenous pattern stains the chromosome plate but in a uniform manner without speckling. Anti-DFS70 antibodies can also be detected by enzyme linked immunoassay, immunoblot and chemiluminescence.

We have been reporting the DFS pattern in our laboratory for 3 years based on IIF HEp 2000 slides. This study was performed to determine the frequency of the DFS pattern as detected by IIF in an Australian clinical population and to assess association, if any, of this pattern with other laboratory autoimmune markers seen in SARD.

MATERIALS AND METHODS

Routine diagnostic sera in which ANA was requested from 1 July 2012 until 30 June 2014 were assessed for the DFS pattern. This laboratory receives sera from mainly primary referrers. ANA was detected by IIF using HEp 2000 slides (ImmunoConcepts, USA) according to the instructions of the manufacturer. The screening dilution was 1:80 and read by two experienced technologists. All DFS positive samples that had undergone concurrent testing for extractable nuclear antigen (ENA) antibodies, anti-dsDNA antibodies, anti-cardiolipin antibodies, beta 2 GP1 antibodies, rheumatoid factor (RF) and anti-citrullinated protein antibodies (CCP) antibodies were analysed.

Antibodies to ENA were detected using a commercial addressable laser bead immunoassay (Bioplex 2200; Hercules, USA). Beads are coated with a ligand specific to a particular assay and allow simultaneous detection of multiple analytes from a single sample. As a second method for ENA detection, INNO-LIA (Innogenetics, Belgium) was used. This is a qualitative line blot for identifying the reactivity of serum antibodies to specific antigens coated as discrete lines on a nylon membrane with a plastic

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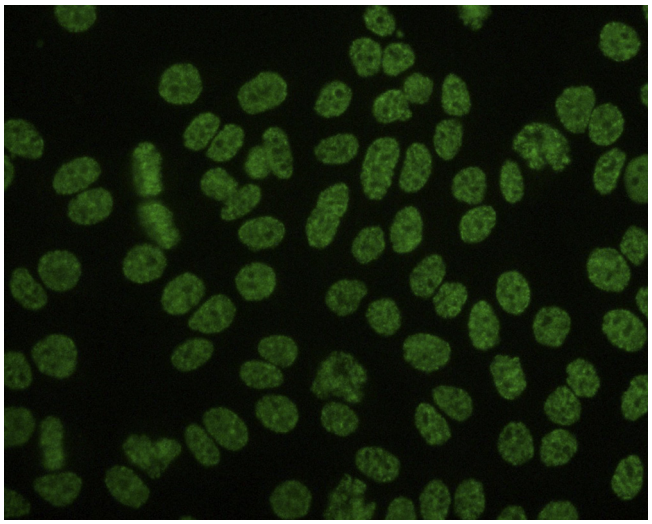

Fig. 1 The DFS pattern on IIF HEp 2000 Slides. Serum dilution 1:80.

backing. The antibodies to ENA that were detected were SSA (Ro 60), SSA (Ro52), SSB, Sm, RNP, topoisomerase I/Scl-70, Jo-1, ribo P and Centromere B.

Anti-dsDNA antibodies were detected with a commercial Farr assay (Amerlex anti-dsDNA radioimmunoassay kit; Trinity Biotech, Ireland) that detects anti-dsDNA antibodies using radio-labelled dsDNA tracer. RF was detected using turbidimetry (Roche Diagnostics, USA) and anti-CCP antibodies were detected by fluorescence enzyme immunoassay (Phadia; Thermo Scientific, Sweden) according to the instructions of the manufacturer. Samples that had concurrent testing for anti-phospholipid antibodies (anti-cardiolipin and beta 2 glycoprotein 1 IgG) were assessed by Bioplex 2200 according to the instructions of the manufacturer. The Fisher exact analysis was used to calculate the significance of the proportion of females who were DFS positive compared to females that were ANA positive and presence of SARD markers in DFS positive group versus speckled positive IIF group.

RESULTS

In the 2 year period, 181,819 unique ANA tests were performed. Of these 51,905 (28.5%) patients were ANA positive. The average age of patients with a positive ANA result was 40.3 years and 78% were female. The most commonly observed patterns were speckled and homogenous. The DFS pattern was observed in 2967 (5.7%) of positive ANA patients (Fig. 2). The distribution of titres of DFS samples is shown in Fig. 3. The majority of DFS positive ANA results were 1 in 320 titre. The average age of all DFS positive patients was 45.4 years (range 2–93) and females made up 82.1% of this group. There was no statistically significant difference between the percentage of females that were DFS positive and percentage of females that were positive for other ANA patterns. DFS positive patients ($n = 2076$) that had undergone testing for antibodies to ENA, dsDNA, RF and/or CCP were compared (Table 1). As can be seen, very few DFS positive samples were positive for other markers of SARD. Rheumatoid factor was found in 6.3% of DFS patients tested but other markers for SARD were less than 5%. Antibodies to ENA and dsDNA were positive in 2.5% of DFS IIF positive patients. In contrast, 12.2% of patients with a speckled pattern had at least one ENA antibody detected or were positive for anti-dsDNA antibodies greater than 9 IU per mL, which is statistically significant ($p < 000001$). Only two patients that

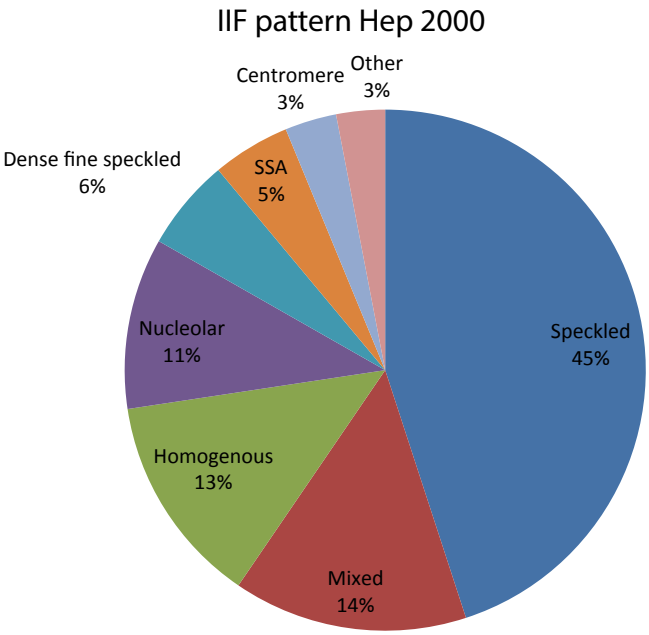

Fig. 2 ANA patterns as detected by IIF in 51,905 patient samples. Mixed patterns were predominantly made up of speckled/homogenous, nucleolar/speckled and nucleolar/homogenous patterns. Other patterns were predominantly multiple nuclear dot and nuclear membrane patterns.

were DFS positive were positive for antiphospholipid antibodies. The ENA results that were positive in 30 DFS IIF positive patients showed a predominance of SSA and SSB with none of the samples positive for Jo-1 or Scl-70 antibodies (Fig. 4).

DISCUSSION

We assessed the frequency of the DFS pattern in a large clinical population of patients in a routine diagnostic laboratory using IIF on HEp 2000 cells. The DFS pattern was found in 5.7% of ANA samples tested by IIF in our laboratory. This is the first reported frequency of this IIF pattern in an Australian population and suggests that this pattern is reasonably frequent. The DFS pattern has been reported in other populations with differing frequencies. In Brazil Dellavance *et al.*[7] reported a frequency of 37% of ANA positive samples having DFS pattern. Another study in a Brazilian population[8] reported that 7.2% of samples were DFS positive by HEp 2 IIF. Watanabe *et al.*[4] reported a frequency of 11% in a healthy population of Japanese hospital workers. In this group DFS made up 54% of all positive ANA. Using an enzyme linked immunoassay, Ayaki *et al.*[9] determined the frequency of anti-LEDGF autoantibodies in a group of 650 sera obtained from the Chicago Blood Bank to be 5.4%. In a Korean population the DFS pattern was present in 28.7% of samples.[10] However, some studies have shown a lower frequency. One study reported that only 172 of 21,512 (0.8%) samples showed the typical DFS pattern by IIF.[11] Another study[12] found 1.62% of 3263 samples were positive for DFS by IIF. In summary, the DFS pattern has been described at a frequency range of 0.8–37% of ANA samples. The frequency of DFS samples in our population is within this and consistent with other studies.

The DFS pattern has been reported to be more common in females.[4,6,7] While some have reported this to be statistically significant we found no significant difference between the