**Supplementary materials for ‘Anti-DFS70 antibodies among patient and healthy population cohorts in China: results from a multicenter training program showing spontaneous abortion and pediatric systemic autoimmune rheumatic diseases are common in anti-DFS70 positive patients’**

**Supplementary Tables and Figures**

**Supplementary Table 1.** Characteristics of DFS pattern between routine ANA adult and pediatric patient and healthy population cohorts in Renji Hospital during a 3-month study period.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Routine HEp-2 IFA cohort(RJ-PA cohort) |  | Healthy population cohort(RJ-PE cohort) |
|  | Total | Adult | Pediatric |  |  |
| DFS pattern titer, n (%) |  |  |
| 1/80 | 49 (17.3) | 44 (17.8) | 5 (13.5) |  | 4 (12.1) |
|  1/160 | 65 (22.9) | 62 (25.1) | 3 (8.1) |  | 7 (21.2) |
|  1/320 | 51 (18.0) | 39 (15.8) | 12 (32.4) |  | 9 (27.3) |
|  1/640 | 83 (29.2) | 70 (28.3) | 13 (35.2) |  | 9 (27.3) |
|  1/1280 | 36 (12.7) | 32 (13.0) | 4 (10.8) |  | 4 (12.1) |
| Department distribution of DFS pattern, n (%) |  |  |
| Rheumatology | 121 (42.6) | 110 (44.5) | 11 (29.7) |  |  |
| Gynecology | 96 (33.8) | 96 (38.9) | 0 (0.0) |  |  |
| Pediatrics | 26 (9.2) | 0 (0.0) | 26 (70.3) |  |  |
| Gastroenterology | 12 (4.2) | 12 (4.9) | 0 (0.0) |  |  |
| Hematology | 11 (3.9) | 11 (4.5) | 0 (0.0) |  |  |
| Nephrology | 8 (2.8) | 8 (3.2) | 0 (0.0) |  |  |
| Other departments | 10 (3.5) | 10 (4.0) | 0 (0.0) |  |  |
| Anti-DFS70 antibodies |  |  |
| ELISA | 272 (95.8) | 235 (95.1) | 37 (100) |  | 30 (90.9) |
| LIA | 269 (94.7) | 233 (94.3) | 36 (97.3) |  | 31 (93.9) |
| HEp-2 ELITE | 137 (48.2) | 121 (49.0) | 16 (43.2) |  | 11 (33.3) |

HEp-2 IFA: HEp-2 cell indirect immunofluorescence assay; ELISA: enzyme-linked immunosorbent assay, LIA: line immunoblot assay, HEp-2 ELITE: immunofluorescence assay on HEp-2 ELITE/DFS70-KO substrate.

**Supplementary Table 2.** Clinical diagnosis of patients with positive reactivity to DFS70 by both ELISA and LIA assay.

|  |  |  |  |
| --- | --- | --- | --- |
| Disease | Adult  |  | Child |
| No. of patients | Frequency (%) |  | No. of patients | Frequency (%) |
| **Systemic autoimmune disease** |  | 15.5 |  |  | 50.0 |
| Undifferentiated CTD | 28 | 8.3  |  | 8 | 20.0 |
| Rheumatoid arthritis | 13 | 3.9  |  | 0 | 0.0 |
| Systemic lupus erythematosus | 5 | 1.5  |  | 3 | 7.5 |
| Sjogren's syndrome | 5 | 1.5  |  | 0 | 0.0 |
| Juvenile idiopathic arthritis | 0 | 0.0  |  | 9 | 22.5 |
| Antiphospholipid syndrome | 1 | 0.3  |  | 0 | 0.0 |
|  |  |  |  |  |  |
| **Organ-specific autoimmune disease** |  | 0.9 |  |  | 2.5 |
| Autoimmune hepatitis | 2 | 0.6  |  | 0 | 0.0  |
| Autoimmune thyroiditis | 1 | 0.3  |  | 0 | 0.0  |
| Immune thrombocytopenic purpura | 0 | 0.0  |  | 1 | 2.5  |
|  |  |  |  |  |  |
| **Inflammatory diseases** |  | 7.4 |  |  | 20.0 |
| Arthritis | 8 | 2.4  |  | 0 | 0.0 |
| Vasculitis | 4 | 1.2  |  | 2 | 5.0 |
| Urticaria | 3 | 0.9  |  | 0 | 0.0 |
| Anaphylactoid purpura | 1 | 0.3  |  | 3 | 7.5 |
| Iridocyclitis | 0 | 0.0  |  | 2 | 5.0 |
| Alopecia areata | 0 | 0.0  |  | 1 | 2.5 |
| Ulcerative colitis | 1 | 0.3  |  | 0 | 0.0 |
| Crohn's disease | 1 | 0.3  |  | 0 | 0.0 |
| Adult-onset Still disease | 1 | 0.3  |  | 0 | 0.0 |
| Dermatomyositis | 1 | 0.3  |  | 0 | 0.0 |
| Gastroduodenal ulcer | 1 | 0.3  |  | 0 | 0.0 |
| Anterior uveitis | 1 | 0.3  |  | 0 | 0.0 |
| Oral aphthae ulceration | 1 | 0.3  |  | 0 | 0.0 |
| Takayasu arteritis | 1 | 0.3  |  | 0 | 0.0 |
| Fasciitis | 1 | 0.3  |  | 0 | 0.0 |
|  |  |  |  |  |  |
| **Gynecologic syndromes** |  | 35.1 |  |  | 0.0 |
| Spontaneous abortion | 94 | 28.0  |  | 0 | 0.0  |
| Female infertility | 16 | 4.8  |  | 0 | 0.0  |
| Pregnancy | 5 | 1.5  |  | 0 | 0.0  |
| Pregnancy with other diseases a | 3 | 0.9  |  | 0 | 0.0  |
|  |  |  |  |  |  |
| **Neoplasms** |  | 4.5 |  |  | 0.0 |
| Multiple myeloma | 9 | 2.7  |  | 0 | 0.0  |
| Lymphoma | 2 | 0.6 |  | 0 | 0.0 |
| Hysteromyoma | 2 | 0.6 |  | 0 | 0.0 |
| Duodenum cancer | 1 | 0.3  |  | 0 | 0.0  |
| Prostate cancer | 1 | 0.3  |  | 0 | 0.0  |
|  |  |  |  |  |  |
| **Infectious diseases** |  | 3.6 |  |  | 5.0 |
| Hepatitis B | 6 | 1.8  |  | 0 | 0.0  |
| Pneumonia | 3 | 0.9  |  | 1 | 2.5  |
| Urinary tract infection | 2 | 0.6  |  | 0 | 0.0  |
| Tubercle bacilli | 1 | 0.3  |  | 0 | 0.0  |
| Epstein-Barr virus | 0 | 0.0  |  | 1 | 2.5  |
|  |  |  |  |  |  |
| **Miscellaneous** |  | 33.0 |  |  | 22.5 |
| Checkup | 42 | 12.5  |  | 6 | 15.0  |
| Joint pain | 39 | 11.6  |  | 0 | 0.0  |
| Proteinuria | 6 | 1.8  |  | 1 | 2.5  |
| Chronic kidney disease | 6 | 1.8  |  | 0 | 0.0  |
| Baldness | 5 | 1.5  |  | 0 | 0.0  |
| Coronary atherosclerosis | 3 | 0.9  |  | 0 | 0.0  |
| Hypertension | 3 | 0.9  |  | 0 | 0.0  |
| Diabetes mellitus | 2 | 0.6  |  | 0 | 0.0  |
| Rheumatic heart diseases | 1 | 0.3  |  | 0 | 0.0  |
| Allergic contact dermatitis | 1 | 0.3  |  | 0 | 0.0  |
| Chronic obstructive pulmonary disease | 1 | 0.3  |  | 0 | 0.0  |
| Nephrotic syndrome | 1 | 0.3  |  | 1 | 2.5  |
| Gout | 1 | 0.3  |  | 0 | 0.0  |
| Hyperuricemia | 0 | 0.0  |  | 1 | 2.5  |

a Pregnancy with cervical insufficiency, Hashimoto's thyroiditis, and hyperthyroidism respectively.

CTD, connective tissue disease.

**Supplementary Table 3.** Monospecific anti-DFS70 antibodies are primarily detected in non-SARD patients.

|  |  |
| --- | --- |
| Anti-DFS70 positive casesn=376 | Total |
| Monospecific anti-DFS7092.0% | Anti-DFS70 plus other anti-ENAs8.0% | 100% |
| Non-SARD76.8% | SARD15.2% | Non-SARD4.0% | SARD4.0% | 100% |

DFS70: dense fine speckled 70 kDa antigen; ENA: extractable nuclear antigen; SARD: systemic autoimmune rheumatic disease.

**Supplementary Table 4.** Additional ENAs in adult and pediatric patients with anti-DFS70 in different clinical diagnosis groups.\*

|  |  |  |  |
| --- | --- | --- | --- |
|  | Adult |  | Child |
|  | Additional ENAFrequency (%) | ENA (n) |  | Additional ENAFrequency (%) | ENA (n) |
| Systemic autoimmune disease | 12/52 (23.1) | nRNP/Sm (2), SSA/Ro60 (5), Ro-52/TRIM 21 (7), SSB/La (1), Rib-P (3) |  | 3/20 (15.0) | nRNP/Sm (1), histones (2) |
| Organ-specific autoimmune disease | 0/3 (0.0) | - |  | 0/1 (0.0) | - |
| Non-Systemic autoimmune disease |  |  |  |  |  |
| Inflammatory diseases | 2/25 (8.0) | SSA/Ro60 (1), Ro-52/TRIM 21 (2) |  | 2/8 (25.0) | histones (2) |
| Gynecologic syndromes | 4/118 (3.4) | SSA/Ro60 (2), Ro-52/TRIM 21 (3) |  | 0/0 (0.0) | - |
| Neoplasms | 0/15 (0.0) | - |  | 0/0 (0.0) | - |
| Infectious diseases | 0/12 (0.0) | - |  | 0/2 (0.0) | - |
| Miscellaneous | 7/111 (6.3) | SSA/Ro60 (2), Ro-52/TRIM 21 (6), Rib-P (1) |  | 0/9 (0.0) | - |
| Total | 25/336 (7.4) |  |  | 5/40 (12.5) |  |

\* Anti-DFS70 defined by both ELISA and LIA assays.

**Supplementary Table 5.** Prevalence of DFS pattern and anti-DFS70 antibodies in routine ANA referral cohorts.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Continent | Country | Total patient analyzed (n) | ANA positive n (%) | IFA cutoff | DFS pattern n (%)a | DFS pattern in ANA positive serum (%) | Anti-DFS70 in DFS patternn (%)/method | References |
| **Europe** |  |  |  |  |  |  |  |  |
|  | Italy | 21,516 | ND | 1:40 | 172 (0.8) | ND | ND | (1) |
|  | Italy | 3,175 | 1,191 (37.5) | 1:160 | 120 (3.8) | 10.1 | 55 (45.8)/IB | (2) |
|  | Italy | 1,005 | ND | 1:80 | 22 (2.2) | ND | 19 (86.4)/HEp-2 Elite | (3) |
| **North America** |  |  |  |  |  |  |  |
|  | Canada | 3,263 | ND | 1:80 | 53 (1.6) | ND | 52 (98.1)/CIA53 (100)/ELISA | (4) |
|  | USA | 6,511 | 5,339 (82.0) | 1:40 | 1,758 (27.0) | 32.9 | 720 (41.0)/LIA | (5) |
| **South America** |  |  |  |  |  |  |  |
|  | Brazil | 7,733 | 2,168 (28.0) | 1:80 | 650 (8.4) | 30.0 | ND | (6) |
|  | Brazil | 2,788 | 790 (28.3) | 1:40 | 57 (2.0) | 7.2 | ND | (7) |
| **Asia** |  |  |  |  |  |  |  |  |
|  | South Korea | 10,528 | ND | 1:100 | 181 (1.7) | ND | 109 (60.2)/ELISA | (8) |
|  | South Korea | 2,654 | 352 (13.3) | 1:40 | 101 (3.8) | 28.7 | ND | (9) |
|  | South Korea | 5,509 | 639 (11.6) | 1:80 | 125 (2.3) | 19.6 | 75 (60.0)/ELISA | (10) |
|  | Turkey | 5,800 | 1,302 (22.4) | 1:100 | 16 (0.3) | 1.2 | 0 (0.0)/LIA | (11) |
|  | Turkey | 3,432 | 1,968 (57.3) | 1:100 | 277 (8.1) | 14.1 | 187 (67.5)/LIA | (12) |
|  | China | 5,961 | 2,507 (42.1) | 1:100 | 58 (1.0) | 2.3 | ND | (13) |
|  | China | 95,131 | 34,417 (36.2) | 1:80 | 513 (0.5) | 1.5 | 473 (92.2)/ELISA459 (89.5)/LIA244 (47.6)/HEp-2 Elite | Present studyb |
|  |  |  |  | 1:100 | 552 (0.6) | 1.6 | 488 (88.4)/ELISA461 (83.5)/LIA239 (43.3)/HEp-2 Elite | Present studyc |
| **Australia** |  |  |  |  |  |  |  |  |
|  | Australia | 181,819 | 51,905 (28.5) | 1:80 | 2,967 (1.6) | 5.7 | ND | (14) |

ND: no data; IB: immunoblot; CIA: chemiluminescence assay; ELISA: Enzyme-Linked Immunosorbent Assay; LIA: line immunoassay.

a Ratio of DFS pattern in all routine ANA referral cohort.

b HEp-2 IFA based on Inova slides.

c HEp-2 IFA based on Euroimmun slides.

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**Supplementary Figure 1.** Three illustrative cases showing fine differences in IFA results by Inova and Euroimmun HEp-2 slides.



Case 1 presented DFS pattern (AC-2) in both slides and was identified as anti-DFS70 positive. Case 2 was anti-DFS70 positive and presented homogeneous pattern (AC-1) by Inova slide versus DFS pattern by Euroimmun slide. Case 3 was anti-DFS70 negative and exhibited homogeneous pattern (AC-1) and weak spindle fibers pattern (AC-25) by Inova slide versus DFS pattern by Euroimmun slide. All images were at the magnification of 400x. Sera were diluted 1:80 for Inova and 1:100 for Euroimmun slides.

**Supplementary Figure 2.** HEp-2 IFA patterns of the anti-DFS70 reference material for ICAP pattern AC-2 using different commercial slides.



(A) Anti-DFS70 reference material for ICAP pattern AC-2 (CAT#: IS2726) analyzed by Inova slide at a dilution of 1:100. (B) Anti-DFS70 reference material tested by Euroimmun slide at a dilution of 1:80. Both images show the typical DFS pattern with characteristic dense fine speckled staining of interphase nuclei and strong coarse speckled staining of the metaphase plate. (C) Anti-DFS70 reference material performed by HEp-2 ELITE/DFS70-KO substrate (magnification of 200x). Conventional HEp-2 cell nuclei indicated by arrows showed DFS pattern and the DFS70 KO cells were not stained. (D) Anti-DFS70 reference material performed by HEp-2 ELITE/DFS70-KO substrate (magnification of 400x).

**Supplementary Figure 3.** Quantitative correlation between HEp-2 IFA and ELISA.



(A) Regression analysis between HEp-2 IFA nuclear intensities (NI) read by NOVA View and anti-DFS70 antibodies concentration by ELISA (O.D. value). (B) Correlation between the HEp-2 IFA titers and the anti-DFS70 antibodies ELISA O.D. value.