Are Anti-DFS70 Autoantibodies Protective?

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The presence of antinuclear antibodies (ANA) against intracellular antigens is a hallmark of ANA-associated autoimmune rheumatic diseases (AARD). Despite advances in methodologies designed to determine ANA, indirect immunofluorescence (IIF) using a human epithelial cell (HEp-2) substrate is still the most commonly used method [1]. However, the diagnostic value of ANA-IIF test results for AARD is affected by limited specificity due to the occurrence of ANA positive results also in subjects not affected by AARD and in healthy individuals. This is the case of antibodies directed to the dense fine speckled (DFS) 70 antigen [2]. These antibodies have recently raised much attention given their high frequency in the sera of patients referred to clinical laboratories for ANA-HEp-2 testing [3-6].

The term DFS70 originated from the IIF pattern and its 70 kDa molecular weight in immunoblotting. The protein was found to be identical to the lens epithelium-derived growth factor (LEDGF) or DNA-binding transcription co-activator. Two main isoforms of the LEDGF protein, 75 kDa (p75) and 52 kDa (p52), are recognized. The C-terminus of the p75 isoform contains a domain that elicits anti-DFS70 autoantibody production. However, in humans, the p52 isoform is deprived of epitopes useful for binding the anti-DFS70 antibodies. DFS70/LEDGFp75 acts as a stress-induced protein, promoting cell survival in response to various environmental factors. This function is exerted by engaging an interaction with specific transcription factors and by activating stress, inflammatory, protective, and antioxidative genes. Autoantibodies targeting the nuclear autoantigen DFS70/LEDGFp75 might function in the removal of protein cleavage fragments from debris generated during cell death and tissue damage. In addition, DFS70/LEDGFp75 has emerged as a highly conserved protein upregulated in several human cancer cells, especially in prostate, breast, colon, liver, thyroid and uterine tumor tissues [7]. Overexpression of DFS70/LEDGFp75 in cancer cells contributes to antagonizing chemotherapeutic stress and to promoting resistance to cell death [8].

The DFS IIF pattern (AC-02 of the ICAP standardized nomenclature) shows a typical fine speckled staining throughout the interphase nucleus and on metaphase chromatin (Figure 1) [9]; however, its proper recognition is challenged by

Figure 1. Dense fine speckled pattern (AC-2) produced by autoantibodies to DFS70/LEDGFp75 in HEp-2 slides: fine speckles in the nucleoplasm of cells in interphase, typically excluding the nucleolus, with increased staining intensity of condensed mitotic chromosomes

DFS70 = dense fine speckled 70 antigen, LEDGFp75 = lens epithelium-derived growth factor, HEp-2 = human epithelial cell

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the low accuracy in the correct interpretation of the DFS pattern (about 50%) [10,11] and by the variability among HEp-2 cells substrates [12]. From these observations, and considering that the AC-02 pattern is a common finding in routine HEp-2 ANA IIF, confirmation of anti-DFS70 antibodies by specific immunoassays is strongly recommended [13].

Despite increased knowledge about the biological role of anti-DFS70 antibodies, their clinical significance is still debated [14]. Over the past years, a heterogeneous array of clinical conditions associated with anti-DFS70 expression, such as Vogt–Harada syndrome, atopic dermatitis, alopecia, interstitial cystitis, and various other inflammatory diseases, as well as cancer, have been reported [15]. Recent studies observed a high frequency of these autoantibodies in apparently healthy individuals (2–8.9%) and a low frequency in AARD (0–5.7%) where they are usually accompanied by AARD-associated autoantibodies.

A recent meta-analysis summarizing the previously published data reported an overall prevalence of anti-DFS70 antibodies in 2.8% of AARD with a frequency of isolated positivity of 0.5% [16]. The high prevalence of anti-DFS70 autoantibodies in ANA-positive healthy individuals suggests that these autoantibodies could play a protective role [17]. The first study that examined the possible protective role of these antibodies was performed by Mariz et al. [18], who reported that none of the 40 healthy individuals with isolated anti-DFS70 antibodies developed AARD within an average 4-year follow-up. Later studies validated this intriguing hypothesis, introducing the suggestion that the presence of isolated anti-DFS70 antibodies (namely, without any other AARD-related antibody) could be used to help to avoid a diagnosis of AARD.

Indeed, a lower prevalence of anti-DFS70 antibodies, rarely isolated (1.1%), was found in systemic lupus erythematosus (SLE) patients than in healthy controls and, interestingly, these anti-DFS70 positive patients showed lower disease activity than anti-DFS70 negative SLE patients [19]. NZB/WFl female mice at age 14 weeks were treated with affinity purified human anti-DFS70 autoantibodies weekly. At the 35th week, 40% of the mice showed minor lupus nephritis (unpublished data). Another study reported increasing levels of anti-DFS70 antibodies in patients with amyopathic dermatomyositis complicated by interstitial lung disease at disease remission [20]. In these later patients, anti-MDA5 antibodies, a serological marker of dermatomyositis also tested negative after therapy. Conversely, in the same cohort, a decrease for dermatomyositis patients showing concomitant antibody positivity against DFS70 and MDA5 before therapy, revealed unchanged levels of anti-MDA5 during the therapy and disappearance of anti-DFS70 antibodies. In addition, Infantino and co-authors [21,22] found a higher frequency of anti-DFS70 antibodies in long-term undifferentiated connective tissue disease (UCTD) than in CTD patients, concluding that the presence of these autoantibodies could help to identify UCTD patients who will not progress to classical CTD.

Furthermore, a young female with high titer-isolated anti-DFS70 antibodies, suspected of having an underlying autoimmune disease, was eventually diagnosed with glomerulonephritis, showing that the identification of isolated anti-DFS70 antibodies may be useful in excluding an autoimmune pathogenesis [23]. The reason and the immune mechanisms underlying anti-DFS70 antibody production are still unclear but may be related to demographic, racial, genetic, environmental factors or diet or therapeutic interventions. A more recent multicenter study revealed that anti-DFS70 autoantibodies were stable over time and more common in younger individuals, especially females [24]. However, a higher prevalence of anti-DFS70 antibodies in younger individuals and in females, was not confirmed by Mahler et al. [25]. These differences might be explained by the different selections and compositions of the analyzed population, although no distinctive difference regarding anti-DFS70 antibody prevalence were found in 2628 sera from patients of different ethnicity and from different countries.

CONCLUSIONS
This data support the value provided by anti-DFS70 antibodies when added to the diagnostic workflow, because when appropriately recognized, it will help to rule out AARD diagnosis and avoid needless patient referrals. However, further studies are needed to address any questions about the natural or protective nature of these autoantibodies.

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References


**Capsule**

**GM-CSF and CXCR4 define a T helper cell signature in multiple sclerosis**

Cytokine dysregulation is a central driver of chronic inflammatory diseases such as multiple sclerosis (MS). Galli and colleagues sought to determine the characteristic cellular and cytokine polarization profile in patients with relapsing–remitting multiple sclerosis (RRMS) by high-dimensional single-cell mass cytometry (CyTOM). Using a combination of neural network-based representation learning algorithms, the authors identified an expanded T helper cell subset in patients with MS, characterized by the expression of granulocyte–macrophage colony-stimulating factor and the C-X-C chemokine receptor type 4. This cellular signature, which includes expression of very late antigen 4 in peripheral blood, was also enriched in the central nervous system of patients with relapsing–remitting multiple sclerosis. In independent validation cohorts, they confirmed that this cell population is increased in patients with MS compared with other inflammatory and non-inflammatory conditions. Last, they also found the population to be reduced under effective disease-modifying therapy, suggesting that the identified T cell profile represents a specific therapeutic target in MS. Nature Med 2019; 25: 1290

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**Capsule**

**Incidence and time trends of joint surgery in patients with psoriatic arthritis: a register-based time series and cohort study from Denmark**

Guldberg-Møller investigated time-trends and cumulative incidence of joint surgery among patients with psoriatic arthritis (PsA) compared with the general population. In this nationwide register-based cohort study, the Danish National Patient Registry was used to identify incident PsA patients. The 5-year incidence rates and incidence rate ratios (IRR) of joint surgery were calculated in four calendar-period defined cohorts. Each patient was matched with ten non-PsA individuals from the general population cohort (GPC). The cumulative incidences of any joint and joint-sacrificing surgery, respectively, were estimated using the Aalen-Johansen method. From 1996 to 2017, 11 960 PsA patients (mean age 50 years; 57% female) were registered. The IRR of any joint surgery was twice as high for PsA patients compared with GPCs across all calendar periods. Among patients with PsA, 2, 10 and 29% required joint surgery at 5, 10, and 15 years after diagnosis. The risk of surgery in PsA patients diagnosed at 18-40 years was higher (22%) than in GPC 60+ year old (20%) after 15 years of follow-up.

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