Should Dense Fine Speckled 70 (DFS70) pattern be reported? Biomnis reference lab experience on 12 619 ANA screening consecutive sera

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SPECIALISED MEDICAL PATHOLOGY

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BACKGROUD

The presence of Anti Nuclear Antibodies (ANA) and antibodies (Ab) to extractable nuclear antigens (ENA) is a hallmark of Systemic Autoimmune Rheumatic Diseases (SARD). The"gold standard" test for ANA screening is the indirect immunofluorescence (IIF) assay on Hep2 cells. The typical DFS70 IIF staining pattern has been described as uniformely distributed fine speckles throughout interphase nuclei and on metaphase chromatin.

The DFS70 pattern has been described in patients with interstitial cystitis, atopic dermatitis even in healthy individuals, but is rarely associated with SARD.

Therefore has the DFS70 pattern to be reported?

The aim of this study is:

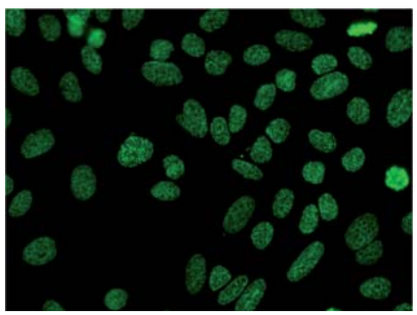
- To determinate the prevalence of DFS70 pattern in a large cohort.
- To study the correlation between IIF and a specific Chemiluminescent Immuno Assay (CIA) for the detection of anti-DFS70 Ab.

METHODS

12 619 consecutive sera collected from 3 to 30 September 2015 were screened for ANA by IIF.

- IIF was performed using Hep 2 cells (NOVAViewTM, Werfen-INOVA), the screening dilution was 1/80, reading and interpretation were done by both technologists and pathologist.
- Among the 12 619 sera, 123 (66 DFS70 IIF pattern and 57 other IIF pattern) were also tested for anti-DFS70 Ab by CIA (QUANTAFlashTM DFS70 assay on BIO-FlashTM, Werfen-INOVA).
- Anti-ENA, chromatin and double stranded DNA (anti-dsDNA) were also determinated in all samples using a multiplexed bead assay (BiopPlexTM 2200, BIO-RAD).

RESULTS AND DISCUSSION



The characteristic Dense Fine Speckled staining pattern of interphase cells and the strong chromatin staining of mitotic cells.

All of the sera with DFS70 pattern presented a titer ≥ 160, and 87% of them a titer ≥ 1/320 (fig. 1).

Among the patients with DFS70 pattern, one (1.2%) presented also low anti-ds DNA Ab at 10 UI OMS (cut off › 9 UI OMS) and was followed for SARD. Another patient presented a low anti-SSA/Ro60 Ab (index: 1.3; cut off › 1.2) but had no SARD diagnosis yet.

Prevalence of DFS70 pattern

- 6 799 sera (53.9%) were IIF non significant ‹ or = 1/80
- ANA were detected in 5 820 sera (46.1%) and among these positive sera, DFS70 pattern was described for 83 sera.
- The global prevalence of DFS70 pattern was 0.66% (83 /12 619) and 1.5% (83/5 820) of ANA positive patterns.

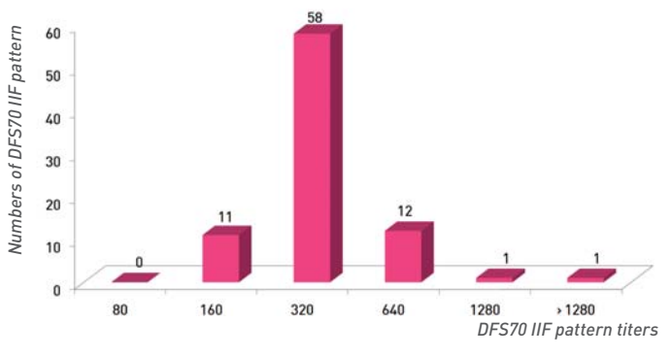


Fig. 1: Repartition of the DFS70 IIF pattern titers

Correlation between IIF and CIA for the detection of anti-DFS70 Ab

		IIF on Hep2 cells		
QUANTAFlashTM Anti-DFS70 Ab		DFS70 pattern	Other pattern	total
	+	61	6	67
	-	5	51	56
	total	66	57	123

Table1: Global agreement between IIF and QUANTAFlashTM for detection of anti DFS70 Ab is 91.06%.

Kappa=0.820 (95% confidence interval: from 0.718 to 0.921).

The strengh of agreement is considered to be very good.

Among the discrepancies, 6 sera were positive for anti-DFS70 Ab with CIA despite the IIF pattern were not evocated of a characteristic DFS70 pattern: 3 sera had a clearly homogeneous speckled pattern and 3 sera had a characteristic DFS70 pattern for interphase cells but not for the mitotic cells.

QUANTAFlashTM DFS70 assay presented an excellent specificity (95%). Among the discrepancies, 5 DFS70 IIF pattern have not been confirmed by CIA; after reviewing, we confirmed the very characteristic pattern for 3 of them but a most doubtful pattern for 2 of them.

CONCLUSION

As previously described, anti-DFS70 Ab seems to be less prevalent in patients with SARD; so even if this Ab can not totally exclude SARD, anti-DFS70 Ab can be proposed as a usefull biomarker in algorithms for ANA testing. The recognition of DFS70 IIF pattern should be reported and anti-DFS70 Ab confirmation should be performed together with other anti-ENA Ab testing.

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