ANA Screening Methods in the Diagnosis of Connective Tissue Diseases: an Italian Multicenter Study

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INTRODUCTION

Diagnosis of Connective Tissue Diseases (CTD) is based upon clinical criteria and serological testing for detection of autoantibodies such as antinuclear antibodies (ANA). Although indirect immunofluorescence (IIF) on HEp-2 cells is considered the reference technique for ANA testing due to the high sensitivity, the method is burdened with some criticisms.
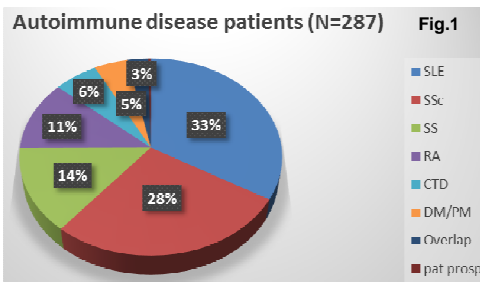
New techniques have been developed to overcome the HEp2-IIF drawbacks. Among the latest generation of "ANA screening assays" the fully automated fluoroenzyme immunoassay EliA[TM] CTD Screen on Phadia 250 (Phadia AB) is reported as a reliable method to help diagnosing ANA-associated rheumatic diseases (AARD).

AIM OF THE STUDY: to evaluate the performance of the EliA[TM] CTD Screen in comparison to HEp2-IIF method for ANA screening.
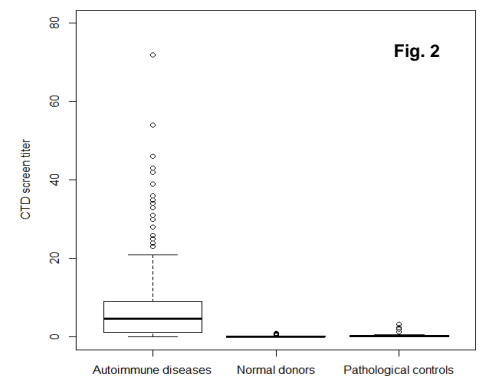
METHODS: results of ANA screening by EliA[TM] CTD Screen, a mix of 14 antigens, the most relevant for AARD (Tab.1) were compared with the HEp2-IIF in 378 subjects (287 autoimmune patients, 34 non-autoimmune pathological controls, 57 healthy donors)(Fig.1).

Marker autoantibodies	Associated CTD
dsDNA	SLE
Sm	SLE
Rib-P	SLE
PCNA	SLE
U1-snRNP (70 kD, A and C)	MCTD, SLE
SS-A/Ro (Ro52 and Ro60)	Sjögren's syndrome, SLE, neonatal lupus
SS-B/La	Sjögren's syndrome, SLE, neonatal lupus
Scl-70	Scleroderma
CENP	Scleroderma (CREST)
Fibrillarin	Scleroderma
RNA Polymerase III	Scleroderma
Jo-1	Polymyositis / dermatomyositis
Mi-2	Polymyositis / dermatomyositis
PM-Scl	Polymyositis-scleroderma overlap, scleroderma

Tab.1

Autoimmune disease patients (N=287)   **Fig.1**
SLE 33%, SSc 28%, SS 14%, RA 11%, CTD 6%, DM/PM 5%, Overlap 3%, pat prosp

RESULTS & DISCUSSION

Fig. 2

Agreement between EliA[TM] CTD Screen & Hep2-IIF

Tab.2

EliA[TM] CTD screen results	Autoimmune diseases	Normal donors	Pathological controls	Tot
Equivocal	18	3	0	21
Negative	50	54	29	133
Positive	219	0	5	224
Total	287	57	34	378

EliA[TM] CTD Screen classifies samples as neg/pos/equivocal, at variance with HEp2-IIF pos/neg results. **Equivocal samples were considered positive in the evaluation of assay agreement & accuracy** (Tab.2)

Tab.3

Agreement	with RA patients	without RA patients
	% (95% CI)	% (95% CI)
Overall	83.3 (79.6 - 87.1)	**87.8 (84.4 – 91.3)**
Positive	81.2 (76.7 - 85.6)	**86.4 (82.3 – 90.5)**
Negative	90.7 (84.6 - 96.8)	**92.5 (86.7 – 98.3)**
Cohen's kappa*	0.6 (0.52 – 0.69)	**0.7 (0.61 – 0.78)**

EliA[TM] CTD Screen in autoimmune disease discrimination

Tab.4

EliA[TM] CTD Screen operative characteristics	with RA patients	without RA patients
	% (95% CI)	% (95% CI)
Accuracy	84.7 (81.0 - 88.3)	**89.9 (86.7 – 93.0)**
Sensitivity	82.6 (78.2 - 87.0)	**89.4 (85.6 – 93.2)**
Specificity	91.2 (85.4 - 97.0)	**91.2 (85.4 – 97.0)**
PPV	96.7 (94.5 – 99.0)	96.6 (94.3 – 98.9)
NPV	62.4 (54.2 – 70.6)	75.5 (67.4 – 83.5)
LR +	9.4 (4.8 -18.2)	**10.2 (5.2 – 19.7)**
LR -	0.2 (0.2 – 0.3)	**0.1 (0.1 – 0.2)**

- The CTD screen levels among groups were significantly different (Kruskal-Wallis chi-squared=150.5, df=2, p-value << 0.001) (Fig.2)
- Autoantibody levels in the positive pathological ctrls were significantly lower than the positive autoimmune samples (W=144.5, p=0.005) (Fig.2)
- Compared to HEp2-IIF, EliA[TM] CTD Screen showed a good overall (83.3%) & negative agreement (90.7%), while the positive one was slightly lower due to the presence in the cohort of 33 RA pts (81.2%)(Tab.3)
- Indeed, the clinical context in which the CTD screen finds the best use is that of diagnosis/confirmation of AARD (ANA Associated Rheumatic Disease, namely SLE, SSc, SjS, AIM and MCTD) rather than SARD (all AARD + RA) because RA is not typically related with ANA or ANA subserology
- Considering diagnosis, EliA[TM] CTD Screen showed a sensitivity of 82.6% & a specificity of 91.2%. As EliA[TM] CTD Screen does not include RA specific antigens, agreement & sensitivity were re-calculated after the exclusion of RA pts (Tab.4).

CONCLUSIONS: The EliA[TM] CTD Screen showed very good agreement with HEp2-IIF and may help in differentiating pts with/without CTD.
Further studies are needed to define its potential position in ANA testing algorithms.

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