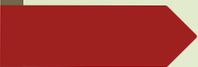




ESTENSIONE PROGETTO PARE: IL VALORE DI CTD SCREEN E DFS70 NELL'INQUADRAMENTO DELLE MALATTIE AUTOIMMUNI



Introduzione

- La diagnosi delle connettiviti sono basate su criteri clinici e test serologici tra cui la ricerca di **anticorpi anti-nucleo** (ANA).
- La metodica in **immunofluorescenza indiretta (IFA) su cellule HEp2** è la metodica gold standard utilizzata per la classificazione e la diagnosi di numerose malattie autoimmuni (Meroni PL, Schur PH. ANA screening: an old test with new recommendations. *Ann Rheum Dis.* 2010 ;69(8):1420-2).
- Sono state sviluppate tecniche che in parte superano i **limiti tecnici** della metodica in IFA nella diagnosi delle patologie reumatiche ANA-associate (AARD).

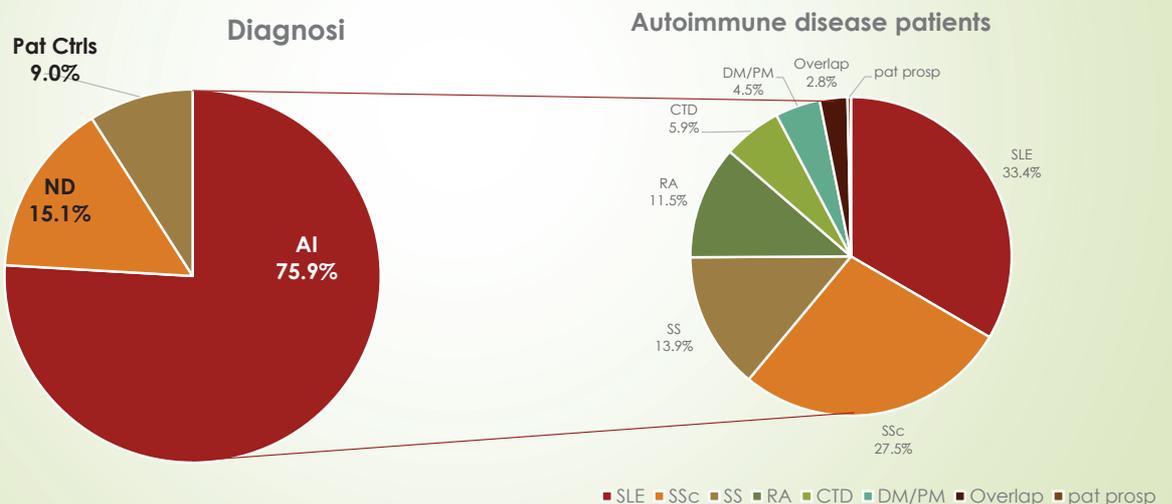
- **Soggettività** dell'interpretazione;
- Mancanza di una completa **standardizzazione**;
- Processo non **automatizzato**.

Scopo dello studio

- ▶ Confrontare due diversi approcci per lo **screening ANA**:
 - ▶ ANA IFA
 - ▶ CTD Screen (Plus per BIO-FLASH e EliA™ per Phadia 250)
- ▶ Valutare l'**associazione** tra i test e la diagnosi clinica
- ▶ Esplorare l'**utilità clinica** dei metodi automatizzati per la ricerca di anticorpi anti-nucleo.

Disegno dello studio

Tutti i campioni sono stati testati con IFA-HEp2 manuale



Metodi



CTD screen EliA™ (Phadia AB)

- ▶ Test fluoroimmunoenzimatico su Phadia 250 (Thermofisher)



Quanta Flash® CTD screen Plus

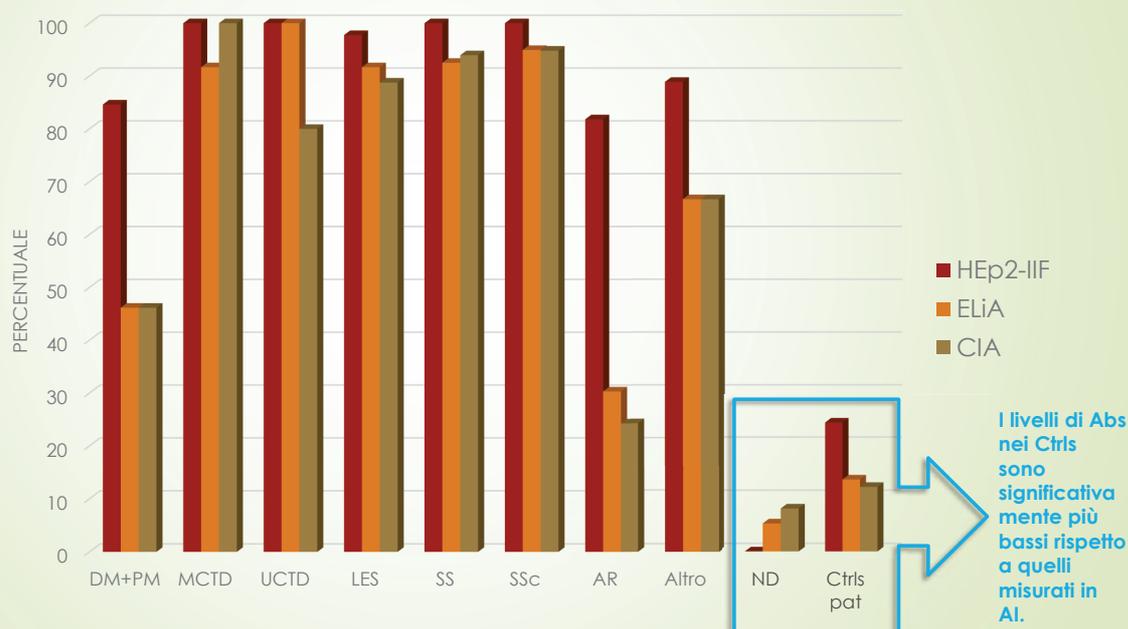
- ▶ Test in chemiluminescenza (CIA) su BIO-FLASH (INOVA)



- ▶ Metodi per lo screening di ANA su sistemi chiusi completamente automatici;
- ▶ Contemporanea rilevazione di autoanticorpi diretti verso i principali antigeni nucleari/citoplasmatici responsabili della positività in IFA su HEp-2.

Antigene	CTD screen EliA™ (Phadia 250)	Quanta Flash® CTD screen Plus	CTD associata
dsDNA	Nativo	Sintetico	LES
Sm	Sintetico	Nativo	LES
Rib-P	Ricombinante	Sintetico	LES
PCNA	Ricombinante	Nativo	LES
U1-snRNP (70kD, A and C)	Ricombinante	Nativo	MCTD, LES
SS-A/Ro (Ro52 and Ro60)	Ricombinante	Nativo	SS, LES lupus neonatale
SS-B/La	Ricombinante	Nativo	SS, LES lupus neonatale
Scl-70 (DNA topoisomerasi I)	Ricombinante	Ricombinante	Scleroderma
CENP	Ricombinante (CENP B)	Nativo (CENP-B + CENP-A)	Scleroderma (CREST)
Fibrillarín	Ricombinante	X	Scleroderma
RNA Polymerase III	Ricombinante	Nativo	Scleroderma
Jo-1 (istidil-tRNA sintetasi)	Ricombinante	Nativo	PM/DM
Mi-2	Sintetico	Nativo	PM/DM
PM-Scl	Ricombinante	Sintetico	PM/Scl overlap, Scl
ThTO	X	Nativo	Scl
Ku	X	Nativo	Scl, LES, MCTD

Distribuzione dei campioni positivi



Concordanza tra CTD Screen e ANA-IFA

	ELiA™ (Thermofisher)		CIA (INOVA)	
	Con AR % (95% CI)	Senza AR % (95% CI)	Con AR % (95% CI)	Senza AR % (95% CI)
Concordanza				
Totale	83.3 (79.6 - 87.1)	87.8 (84.4 - 91.3)	83.1% (76.6-84.9%)	87.7% (84.2-91.2%)
Positiva	81.2 (76.7 - 85.6)	86.4 (82.3 - 90.5)	81.0% (73.4-83.0%)	87.1% (82.9-91.4%)
Negativa	90.7 (84.6 - 96.8)	92.5 (86.7 - 98.3)	88.8% (82.5-95.0%)	89.1% (82.8-95.5%)
K di Cohen*	0.60	0.70	0.62	0.71

(*) Scala di Landis & Koch per l'interpretazione della k di Cohen's:

- $k < 0$ nessuna concordanza
- $0 < k < 0.20$ concordanza lieve
- $0.21 < k < 0.40$ concordanza discreta
- $0.41 < k < 0.60$ concordanza moderata
- $0.61 < k < 0.80$ concordanza sostanziale
- $0.81 < k < 1$ concordanza quasi perfetta

Accuratezza di CTD Screen nel discriminare le patologie autoimmuni

Caratteristiche operative	ELiA™ (Thermofisher)		CIA (INOVA)	
	Con AR % (95% CI)	Senza AR % (95% CI)	Con AR % (95% CI)	Senza AR % (95% CI)
Accuratezza	84.7 (81.0 - 88.3)	89.9 (86.7 - 93.0)	83.3 (79.5 - 87.2)	89.2 (85.9 - 92.5)
Sensibilità	82.6 (78.2 - 87.0)	89.4 (85.6 - 93.2)	80.8 (76.1 - 85.5)	88.7 (84.6 - 92.7)
Specificità	91.2 (85.4 - 97.0)	91.2 (85.4 - 97.0)	90.5 (84.6 - 96.4)	90.5 (84.6 - 96.4)
VPP	96.7 (94.5 - 99.0)	96.6 (94.3 - 98.9)	96.1 (93.5 - 98.6)	95.9 (93.3 - 98.5)
VPN	62.4 (54.2 - 70.6)	75.5 (67.4 - 83.5)	62.3 (54.2 - 70.4)	76.1 (68.2 - 84.0)
LR + / LR -	9.4 / 0.2	10.2 / 0.1	8.53 / 0.21	9.36 / 0.13

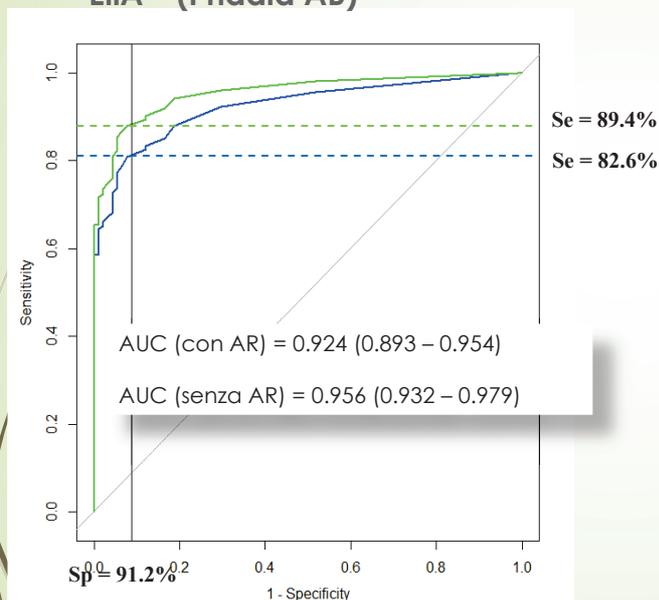
VPP = valore predittivo positivo
 VPN = valore predittivo negativo
 LR = likelihood ratio

Curve ROC

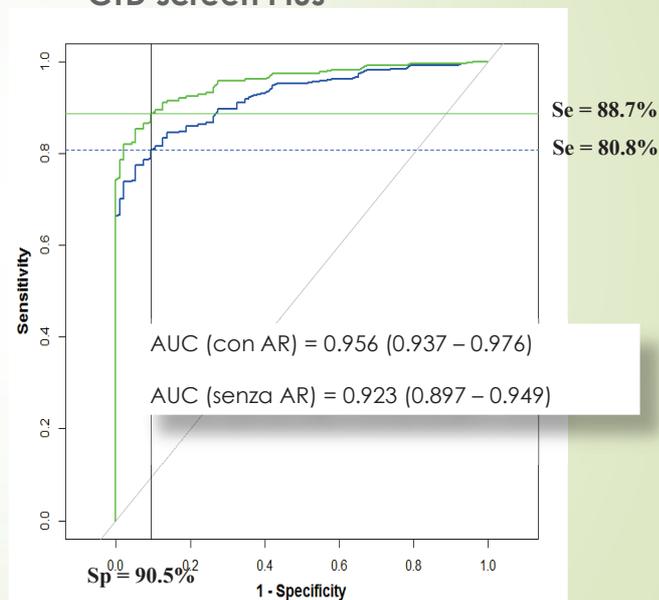
— con AR
 — senza AR



**CTD screen
 ELiA™ (Phadia AB)**



**Quanta Flash®
 CTD screen Plus**



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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 (IF) on HEp-2 cells is considered the reference technique for ANA testing due to the high sensitivity, the method is burdened with some criticism. New techniques have been developed to overcome the HEp-2 IF drawbacks. Among the latest generation of "ANA screening assays" the fully automated Fluorescence Immunoassay EIA™ CTD Screen on ProBea 200 (ProBea 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 EIA™ CTD Screen in comparison to HEp-2 IF method for ANA screening.

METHODS: results of ANA screening by EIA™ CTD Screen, a mix of 14 antigens, the most relevant for AARD (Tab. 1) were compared with the HEp-2 IF in 378 subjects (287 autoimmune patients, 91 non-immunologic controls, 87 healthy donors) (Fig. 1).

RESULTS & DISCUSSION

Agreement between EIA™ CTD Screen & HEp-2 IF

Agreement	with RA patients	with RA patients
Agreement	9 (2.9%)	9 (9.0%)
Disagreement	31 (9.7%)	31 (31.0%)
Indeterminate	21 (6.6%)	21 (21.0%)
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EIA™ CTD Screen in autoimmune disease distribution

ANA screening method	RA patients	RA patients
Agreement	9 (2.9%)	9 (9.0%)
Disagreement	31 (9.7%)	31 (31.0%)
Indeterminate	21 (6.6%)	21 (21.0%)
Indeterminate	21 (6.6%)	21 (21.0%)
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CONCLUSIONS: The EIA™ CTD Screen showed very good agreement with HEp-2 IF and may help in differentiating pts with/without CTD. Further studies are needed to define its potential position in ANA testing algorithms.

INVESTIGATION OF ANA SCREENING METHODS IN THE DIAGNOSIS OF AUTOIMMUNE DISEASES

Laura Cervera¹, Maria Elena Magari², Francesca Pignatelli¹, Nadia Bernardi¹, Chiara Sestini¹, Michael Mader¹, Paul-Louis Merckel^{1,3,4}

KEY MESSAGES
• HEp-2 IF is still recommended for ANA screening
• Limitations of the assay are acknowledged
• Good performance was found for QUANTA Flash CTD Screen, ANA ez-IF

INTRODUCTION
Antinuclear antibodies (ANA) detection is important in the diagnosis of several autoimmune diseases and related rheumatological (RI) or rheumatoid (RH) diseases. HEp-2 cells are the most commonly used substrate for ANA screening. However, HEp-2 IF is not recommended for ANA screening in the diagnosis of AARD. The aim of this study was to evaluate ANA screening in patients with a diagnosis of systemic autoimmune disease and regular follow up by a novel ANA screening method.

METHODS
174 with different autoimmune diseases were enrolled (Fig. 1). Clinical parameters, including rheumatoid factor (RF) and anti-CCP antibodies, were also measured.

RESULTS
ANA screening was performed using the standardised HEp-2 IF and the QUANTA Flash CTD Screen. The QUANTA Flash CTD Screen showed a significantly higher sensitivity (82.8%) and specificity (91.2%) compared to HEp-2 IF (68.4% and 88.1%, respectively) (Fig. 2). The QUANTA Flash CTD Screen showed a significantly higher sensitivity (82.8%) and specificity (91.2%) compared to HEp-2 IF (68.4% and 88.1%, respectively) (Fig. 2).

CONCLUSIONS
The QUANTA Flash CTD Screen showed a significantly higher sensitivity (82.8%) and specificity (91.2%) compared to HEp-2 IF (68.4% and 88.1%, respectively) (Fig. 2).

Conclusioni

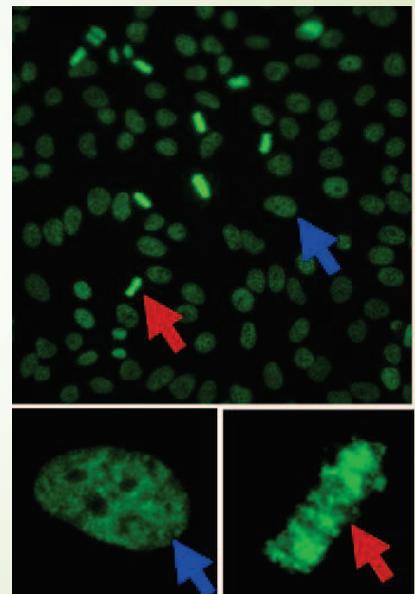
- ▶ Entrambe le metodiche CTD screen mostrano un ottima concordanza con la metodica ANA - IFA, in particolare quando si escludono dalla valutazione i campioni da pazienti affetti da AR (~ 88%);
- ▶ Dal punto di vista clinico, le metodiche CTD screen mostrano una performance migliore nella diagnosi/conferma delle AARD (ANA Associated Rheumatic Disease, quali SLE, SSc, SS, AIM and MCTD) piuttosto che le SARD (AARD + AR) dal momento che non sono compresi Ag specifici per AR;
- ▶ Sono necessari ulteriori studi per definire la posizione di queste metodiche nel processo diagnostico delle patologie autoimmuni;
- ▶ Non sostituisce ANA-IFA ma se eseguito in parallelo può dare un valore aggiunto:
 - campioni negativi per i 2 metodi: si può escludere connettivite AI
 - campioni positivi per i 2 metodi: prosecuzione del percorso diagnostico >>>> test di 2° livello per la specificità antigenica.



Estensione dello studio per la valutazione di QUANTA Flash® DFS70

ANA-IFA: Pattern dense fine speckled (DSF70)

- ▶ **Antigene** = Lens epithelium-derived growth factor (LEDGF)
- ▶ **Caratteristiche del pattern**
 - ▶ Punteggiatura fine e fitta delle cellule in interfase
 - ▶ Colorazione della cromatina nelle cellule in metafase



Anticorpi anti-DFS70

Watanabe A et al. Anti-DFS70 antibodies in 597 healthy hospital workers. *Arthritis Rheum* 2004; 50: 892-900

Muro Y et al. High concomitance of disease marker autoantibodies in anti-DFS70/LEDGF autoantibody-positive patients with autoimmune rheumatic disease. *Lupus* 2008; 17: 171-176

Mariz HA et al. Pattern on the antinuclear antibody-HEp-2 test is a critical parameter for discriminating antinuclear antibody-positive healthy individuals and patients with autoimmune rheumatic diseases. *Arthritis Rheum* 2011, 63:191-200.

Mahler M et al. Anti-DFS70/LEDGF antibodies are more prevalent in healthy individuals compared to patients with systemic autoimmune rheumatic diseases. *J Rheumatol* 2012; 39: 2104-2110.

Lee H et al. Application of anti-DFS70 antibody and specific autoantibody test algorithms to patients with the dense fine speckled pattern on Hep-2 cells. *Scand J Rheumatol* 2016; 45:122-128.



Marker di esclusione per SARD

Recognizing the dense fine speckled/lens epithelium-derived growth factor/p75 pattern on HEp-2 cells: not an easy task! Comment on the article by Mariz et al

Bizzaro N. et al. *Arthritis Rheum* 2011;63:4036-7

The dense fine speckled antinuclear antibody pattern in autoimmune rheumatic disease: comment on the article by Mariz et al

Craig WY & Ledue TB. *Arthritis Rheum* 2011;63:1759

Towards a better understanding of the clinical association of anti-DFS70 autoantibodies

Mahler et al. *Autoimmunity Reviews* 2016;15:198-201

Disegno dello studio

► **Obiettivo:**

valutazione della prevalenza di sieri DFS70-positivi

► **Casistica:**

368 tra i sieri del progetto PARE

► **Metodo:**

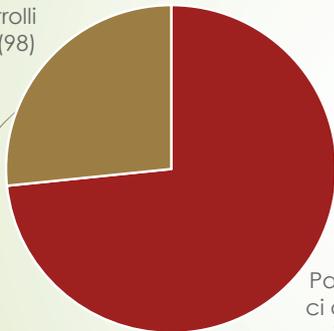
CLIA con kit QUANTA Flash® DFS70 e analizzatore BIO-FLASH



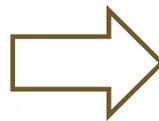
Risultati

Diagnosi (N=368)

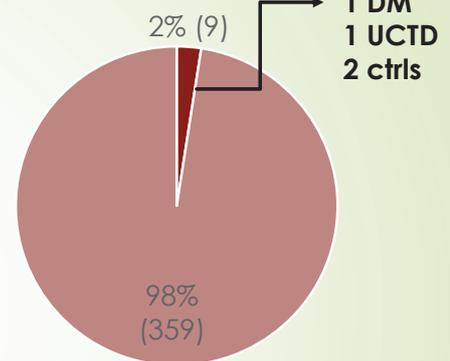
Controlli
27% (98)



Patologici
definiti
73%
(270)



DFS70



■ POS ■ NEG

Profilo anti-DFS70 positivi

- Pattern ANA:
 - 4 omogenei + 1 speckled
 - 3 centromerico
 - 1 nucleolare
- 6/7 ENA e/o CTD screen positivi in AI



Conclusioni

- ▶ **Anticorpi anti-DFS70 presenti sia in pazienti con connettiviti autoimmuni che in controlli sani anche se nella maggior parte delle AARDs sono presenti anche altri Abs specifici;**
- ▶ **Necessità di test di conferma per accertare la reattività vs DFS70**
- ▶ **Necessità di ulteriori studi, ben definiti dal punto di vista epidemiologico**

per determinare se

- a) gli anti-DFS70 possano essere usati per indirizzare la diagnosi vs una patologia specifica o escludere altre patologie o
- b) siano anticorpi senza alcun particolare significato diagnostico e clinico