

**Conundrum of Rare (esoteric)
but
Clinically Important
Autoantibodies
ACR October 2008**

**Marvin J. Fritzler PhD MD
Professor of Medicine
Mitogen Advanced Diagnostics Laboratory
University of Calgary**

©

GOALS

- Understand the importance of the detection and interpretation of “esoteric” autoantibodies.
 - Review recent data on clinical association of esoteric autoantibodies with anti-CENP-F as an example.
- Appreciate what esoteric autoantibodies can teach us about clinical applications of test results.

Autoantibody Snapshot

SLE*	Scleroderma	Sjögren's Syndrome	Myositis
dsDNA chromatin Ribosomal P	CENP-A,-B topoisomerase I (Scl-70)	SS-A/Ro 60 SS-B/La M3 receptor	Jo-1 other tRNA synthetases
snRNPs (Sm, U1-6 RNP) PCNA/proteasome	RNA Pol I/III PM/Scl Exosome	SS-56 Golgins GW Bodies	PM/Scl Ubiquitin-like modifier Exosome
SS-A/Ro60 Histone	Fibrillarin PDGF R	CENP-C ?	Mi-2 MJ/p140
NR 2 Assemblyosome	To/Th B23	NuMA TS-1 RNA	SRP SAE
Cardiolipin-β-2 GP1 Ro52	Myenteric plexus Ro52	α-fodrin ? Ro52	p155 Ro52

*Over 150 autoantibodies identified in SLE. Sherer et al

Some "ESOTERIC" Autoantibodies

- **Golgi complex**
 - golgins-67, -97, 95/gm130
 - golgins-160, 245, giantin
- **Endosomes**
 - Early endosome antigen 1 (EEA1)
 - Cytoplasmic linker protein (CLIP170)
 - Lysobisphosphatidic acid (LBPA)
 - GRASP-1
- **GW Bodies**
 - GW proteins
 - hAgo2, Ge-1/Hedls, RAP55/LSm5
 - LSm4, Dicer, Dcpl, Xrn
- **Centrosome**
 - pericentrin
 - PCM-1, -2
 - Ninein, Mob-1
- **Proteasome**
 - A3-HC9
 - Ki-p28γ
- **Assemblyosome** — SMN complex
 - Sm
 - RNA helicase (Gu)
 - Fibrillarin
 - p80 coilin
- **Intracellular Exosome**
 - PM/Scl-75, -100
 - hCs14
 - hRrp4, 40, 41, 42
- **Extracellular Exosome**
 - Protasomes
 - Microbodies
- **Rods & Rings**
 - Recently described by Dr. E.K.L. Chan, University of Florida

See: Stinton et al. J. Clin. Immunol. 110: 30, 2004

Esoteric Autoantibodies

- Golgi complex SjS 60%
 SLE 20%
 Ataxia/neuro 5%
- GW Bodies SjS 40%
 ataxia/neuro 35%
 PBC 10%
- PCNA SLE 40%
 other 60%
- EEA-1 Ataxia/neuro 30%

Perspectives of the Clinical Laboratory Pet Peeve #1:

- “False Positive” tests:
 - e.g. an (esoteric) autoantibody that has no known clinical associations.
 - Referring physician: “The patient has nothing.” and “What should I tell the patient?”
 - Clinical associations are only as good as the clinical interface. Second & third opinions are often requested.
 - Interpret in context of autoantibodies that antedate diagnosis (predictive antibodies).
 - Chronology of autoantibodies
 - If patient had “nothing” why was ANA ordered.

Perspectives of the Clinical Laboratory Pet Peeve #2:

- “False Negative” tests:
 - “The patient has obvious classical SLE, why is the ANA test negative?”
 - esoteric autoantibodies are often not identified correctly or disregarded by clinical lab
 - growing list of autoantibodies that are missed by IIF staining (i.e. Rib P, PCNA, PM/Scl, CENP, Jo-1)
 - If diagnosis obvious, why was ANA done?

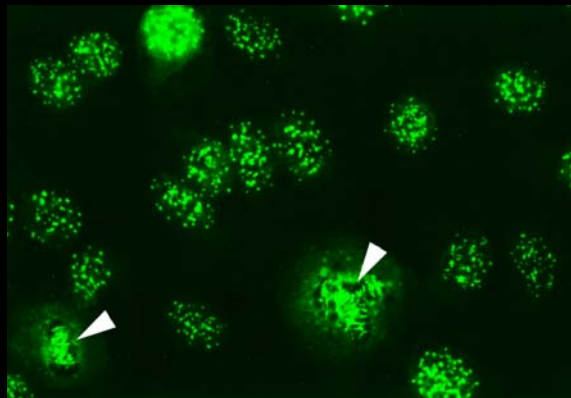
Anti-Centromere Antibodies

- 1980: Described as an autoantibody that bound the primary constriction of metaphase chromosomes. Seen in >85% of CREST. (Am. J. Med. 69:520; Arthritis Rheum 23: 617)
- Numerous subsequent studies confirmed clinical association with CREST/lcSSc and that anti-CENP are predictive.
- Target antigens are centromere proteins (CENPs). 84 kDa CENP-B the “universal” antigen.
- CENP-C associated with Sjögren’s syndrome. (J. Rheum. 31: 121, 2004)

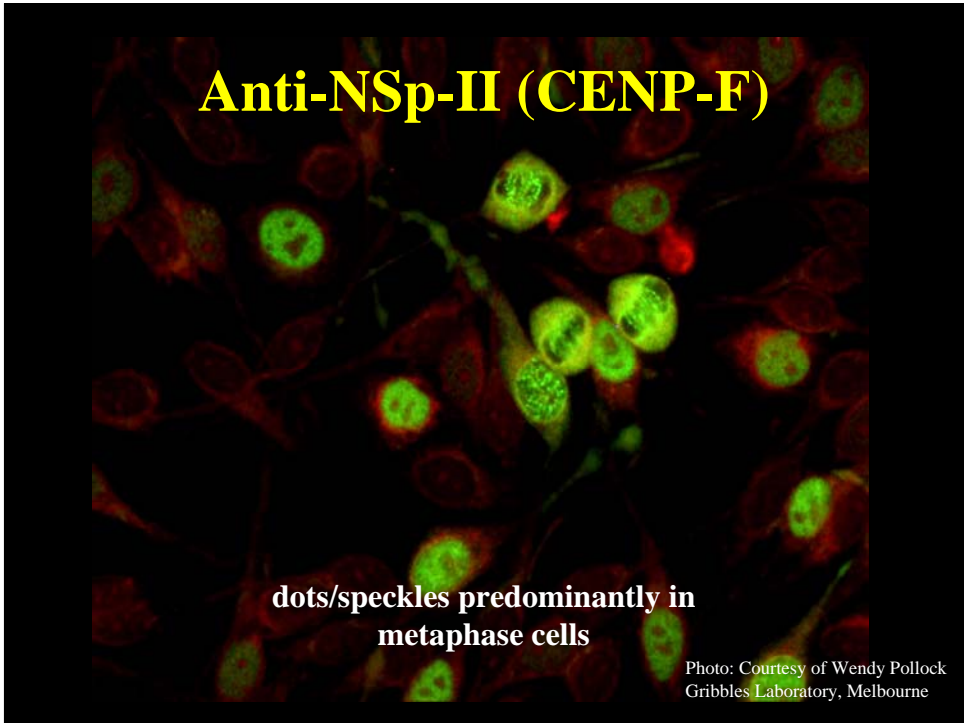
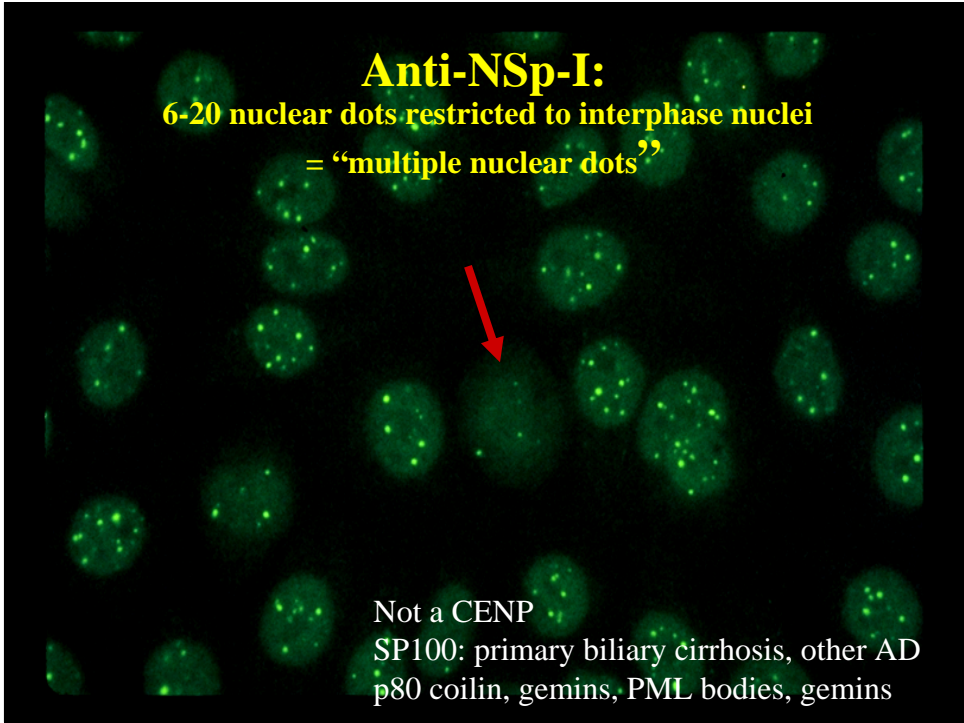
Antibodies to CENPs

- Shortly after publication of first anti-CENP reports of anti-CENP in a number of other diseases.
 - Fritzler, Valencia, McCarty. Speckled pattern antinuclear antibodies resembling anti-centromere antibodies. *Arthritis Rheum.* 27: 92-96, 1984.
- Three main IIF patterns to consider:

Classical Anti-CENP Targets: CENP-A, -B, -C



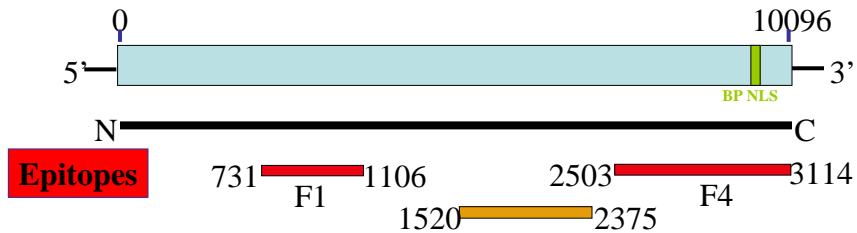
limited cutaneous scleroderma



Brief History of CENP-F

- ✓ 1993: NSp-II first identified as CENP-F: 367 kDa protein
 - Rattner, Rao, Fritzler et al. Cell Motil Cytoskel 26:214, 1993.
- ✓ 1995: Anti-CENP-F associated with cancer
 - Casiano et al J Autoimmun 8: 575, 1995.
- ✓ 1997: 50% of anti-CENP-F cohort have a malignancy
 - Rattner et al. Clin Invest Med 20:308, 1997.
- ✓ 2005: ~7% of NHL have anti-CENP-F by RIA
 - Bencimon et al. Ann NY Acad Sci 1050: 319, 2005.

CENP-F/mitosin 367 kDa



CENP-F ALBIA (Luminex)

Based on 2 recombinant F1 & F4 epitopes

- 98% of CENP-F cohort +ve positive by TnT IP +ve by ALBIA
- 2008 Fox Chase (T. Yen) ~300 sera from various malignancies
 - 20% positive (not associated with any one malignancy or stage of disease)
 - 50% of positive ALBIA had IIF pattern compatible with CENP-F (retrospective)
- 190 Breast Cancer (Calgary): 7 (3.7%) positive
- 150 Prostate Cancer (C. Casiano, Loma Linda) 6 (3.3%) positive
- 50 children with non-Hodgkins Lymphoma (Calgary) (2%)
- 100 Normal Controls: negative
- **CONCLUSION: Anti-CENP-F uncommon in cancer cohorts**

CENP-F ALBIA (Luminex)

▪ BUT the PARADOX and CONUNDRUM:

In 2007/08 18 sera referred to Mitogen Advanced Diagnostics with NSp-II IIF pattern

- 15 (83%) positive CENP-F by ALBIA
- 13/15 (87%) had a malignancy
- **CONCLUSION: Cancer is a common diagnosis of patients with CENP-F antibodies**

SUMMARY

The Autoantibody Conundrum

✓ Study of **disease cohorts** indicates that the frequency of certain autoantibodies is uncommon or even rare.

- <5%: CENP-F, PCNA, NuMA, HsEg5, GWB, Golgi, EEA1.

BUT

✓ Study of **serological cohorts** indicate that the frequency of certain diseases (e.g. SLE) is high.

- >30% of sera with anti-PCNA, -NuMA, -HsEg5, -GWB, -Golgi, EEA1 have SLE and/or Sjögren's syndrome
- >50% CENP-F have malignancy