IDIOPATHIC INFLAMMATORY MYOPATHIES (IIM) AND Laboratory Diagnosis

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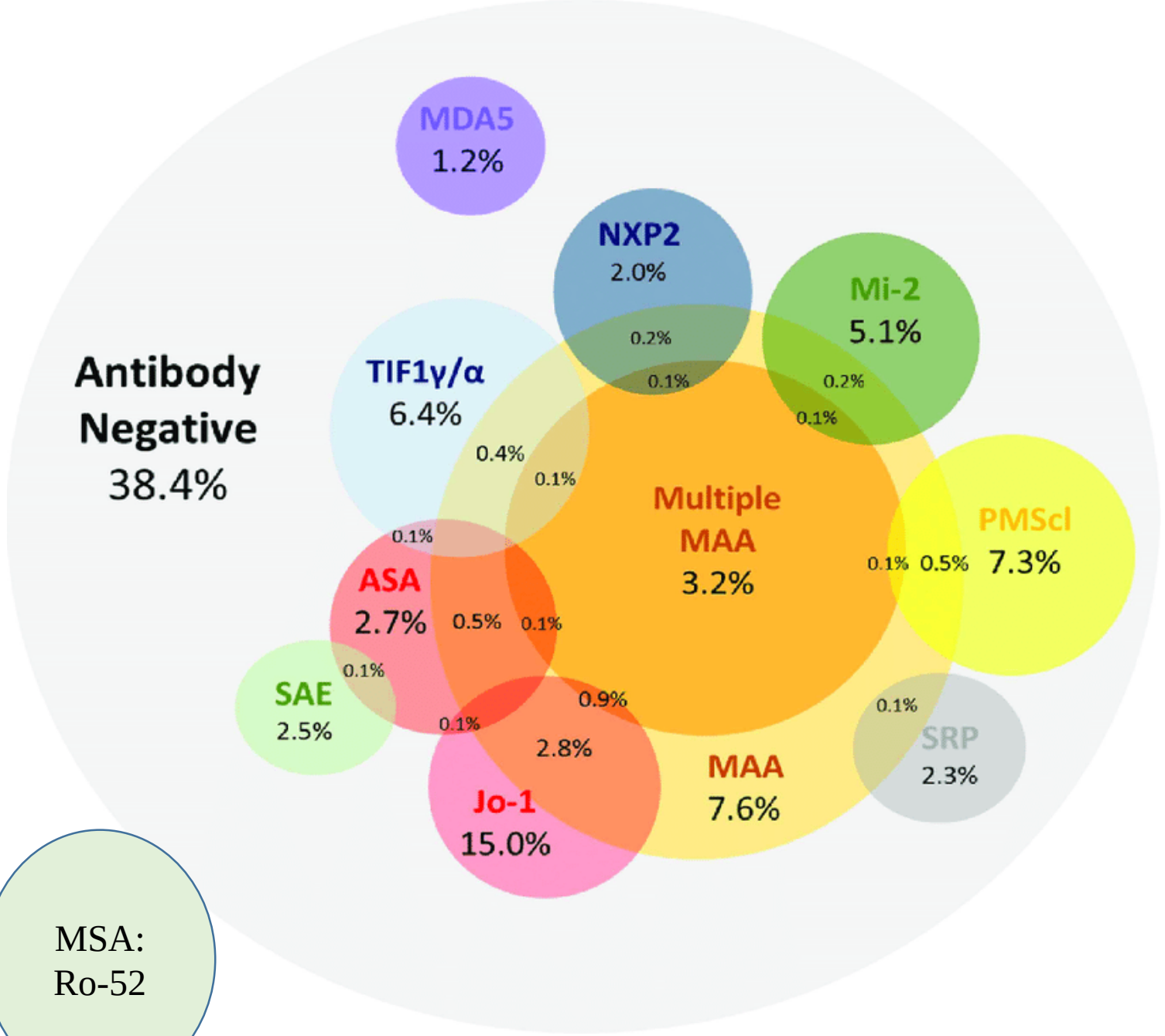
IDIOPATHIC INFLAMMATORY MYOPATHIES (IIM)

- auto-Abs have shown increasing clinical value as biomarkers in the diagnosis of polymyositis (*PM*), dermatomyositis (*DM*), necrotizing myopathies, and inclusion body myositis (*IBM*), collectively referred to as *IIM.*
- **DM** is a major clinical subset of IIM, and both the **typical DM rash** and the presence of perifascicular muscle atrophy have been traditionally regarded as specific findings for this diagnosis.
- More recently, this classification has been challenged and a new one proposed based on **vascular pathology** (myovasculopathy), **immune myopathy** with **perimysial pathology** as 1^{st} subset and the 2^{nd} associated with damage to intermediate-size vessels, capillary loss, membrane attack complex deposition on capillaries, and mitochondrial abnormalities . A 3^{rd} subset of DM is the paradoxic adermatopathic DM or possible DM sine dermatitis

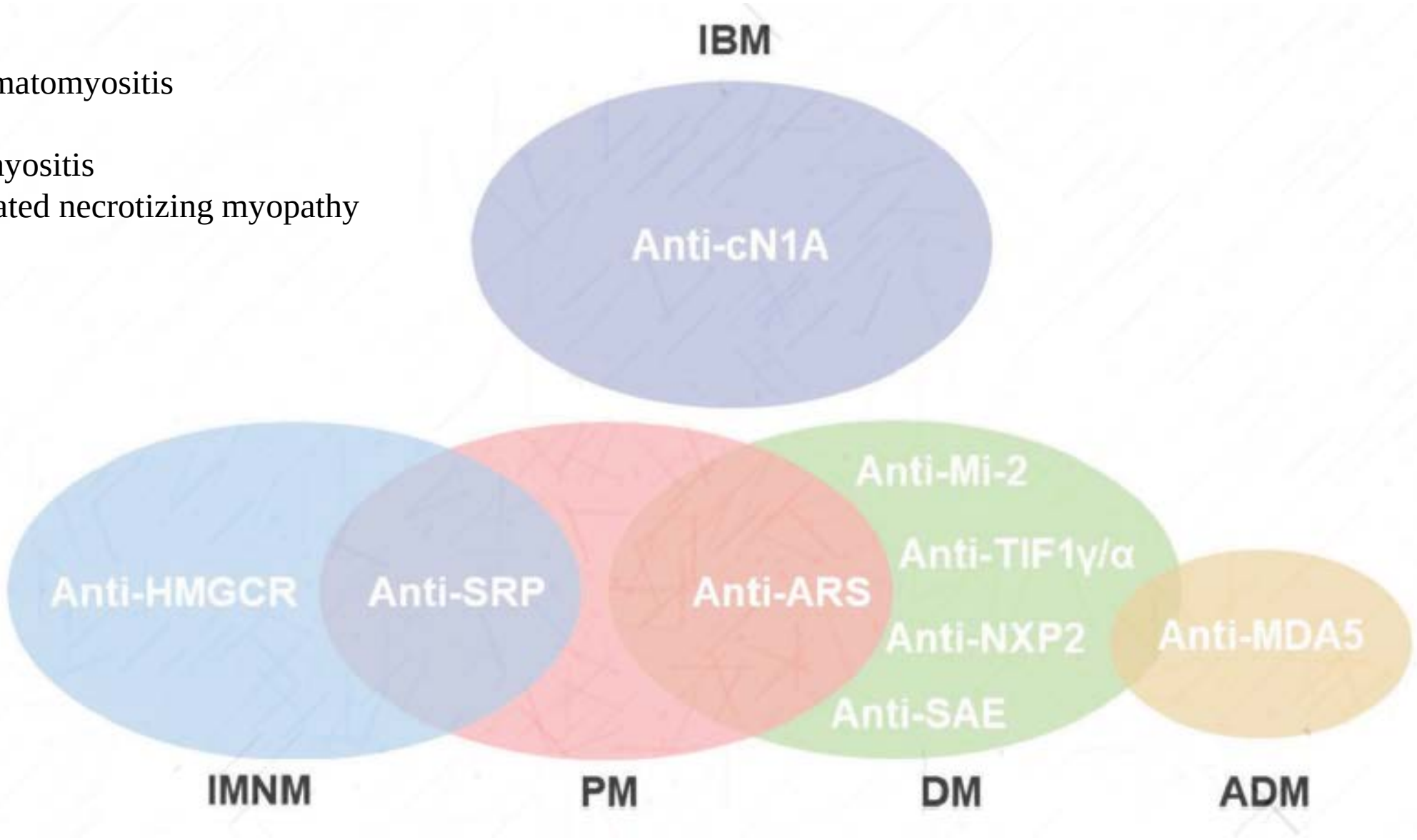
- Most auto-Abs in IIM sera are directed to **intracellular proteins** and, based on their disease specificity, can be grouped into **myositis specific auto-Abs** (MSA) and **myositis associated auto-Abs** (MAA).



MSA:
Ro-52

Subgroups of IIM according to autoantibody phenotype.

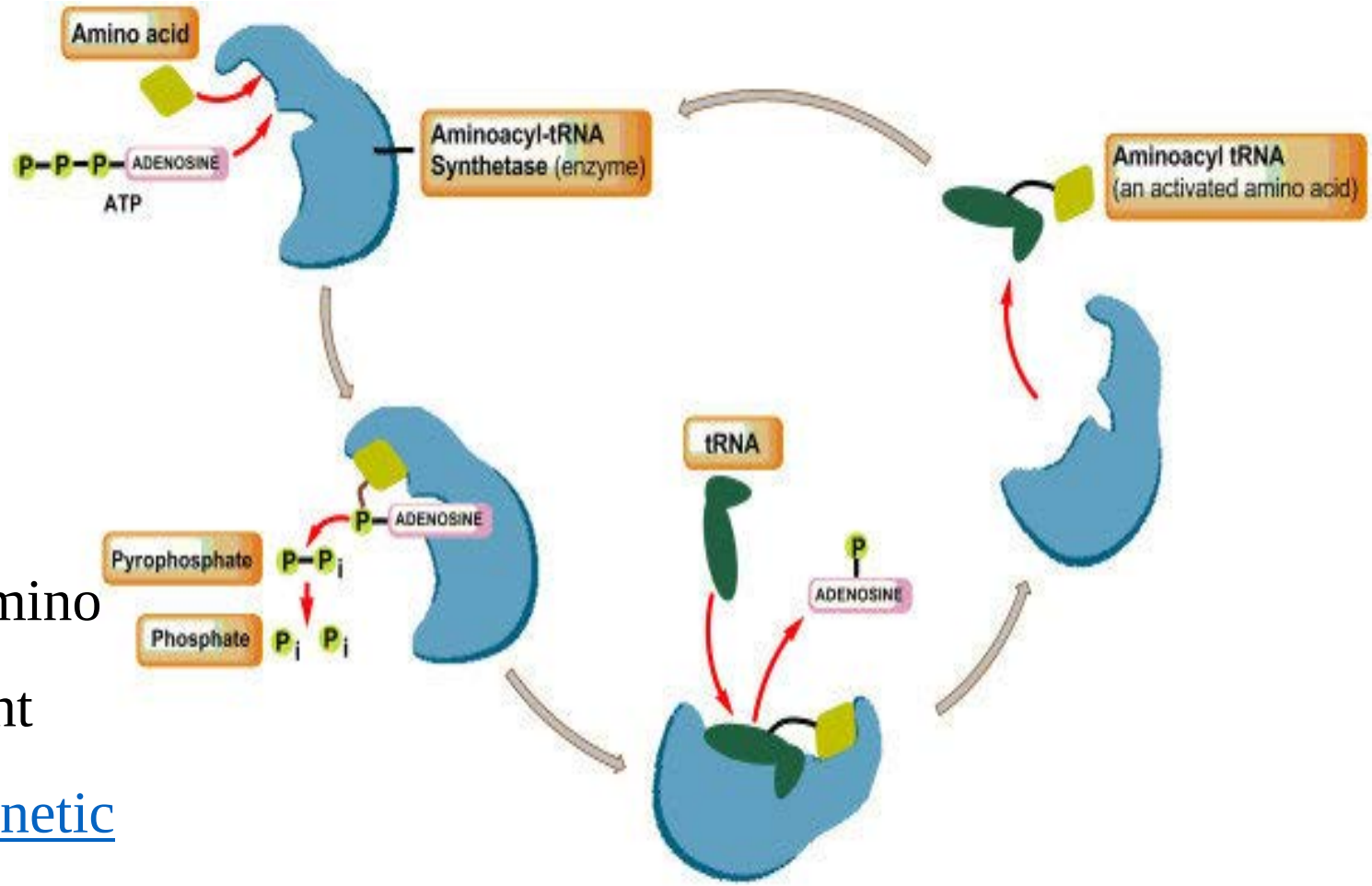
ADM: amyopathic dermatomyositis
DM: Dermatomyositis
IBM: Inclusion body myositis
IMNM: Immune-mediated necrotizing myopathy
PM: polymyositis



Aminoacyl-tRNA synthetase (aaRS or ARS),

- auto-Abs to ARS components include those directed to **Jo-1** (histidyl), **PL-7** (threonyl), PL-12 (alanyl), OJ (isoleucyl), EJ (glycyl), KS (asparaginyl), Zo (phenylalanyl), & Ha (tyrosyl).

- ARS (also called tRNA-ligase) is an **enzyme** that attaches the appropriate **amino acid** onto its corresponding tRNA during protein translation.
- In humans, the 20 different types of amino acid-tRNA are made by the 20 different **ARSs**, 1 for each amino acid of the genetic code.



- Because the vast **majority** of studies found anti-ARS auto-Abs only in patients with IIM and not in other SARDs, it was concluded that anti-ARS auto-Abs are myositis specific.
- Anti-ARS were present in the **preclinical phase** and predicted **clinical outcomes** of IIM, so this auto-Abs characterize a distinct IIM **clinical phenotype**, which is referred to as the **antisynthetase syndrome (aSS).**
- anti-ARS auto-Abs are the serologic hallmarks of the **aSS**, which is characterized by **inflammatory myopathy** & **multiple organ involvement**, particularly **ILD**.
- aSS is often accompanied by nonerosive arthritis, Raynaud phenomenon, “mechanic’s hands,” skin rashes, sicca syndrome, and constitutional symptoms such as fever.

Anti-Jo-1(histidyl tRNA synthetase)

- **Anti-Jo-1 Ab** is the most prevalent of the ARS auto-Abs, found in 15-30% of patients with **PM,** in 60-70% of patients with interstitial lung disease (**ILD**), but rarely in DM (including juvenile DM) or other systemic autoimmune rheumatic diseases (**SARDs**).
- An association of anti-Jo-1 auto-Abs with **clinical synovitis** was described.
- The other ARS auto-Abs have a prevalence of **<5%** in IIM.

- There are reports that patients with **non-anti-Jo-1 antibodies** have different & uncertain clinical outcomes.

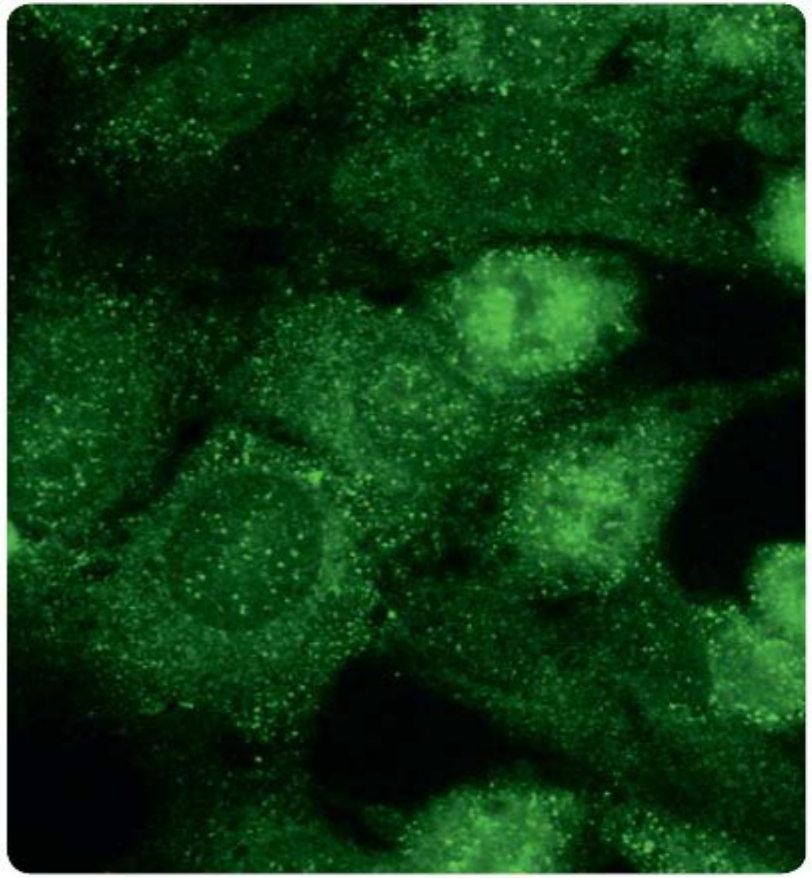
✓ Patients with **anti-PL-7** & **anti-PL-12** auto-Abs frequently develop ILD, gastrointestinal manifestations, and less frequently have myositis compared to anti-Jo-1 positive patients.

- In distinction from inflammatory muscle disease (e.g., myositis), patients with **anti-PL-12** auto-Abs have been reported to develop a noninflammatory necrotizing myopathy, and anti-PL-7 auto-Abs were associated with pericarditis.

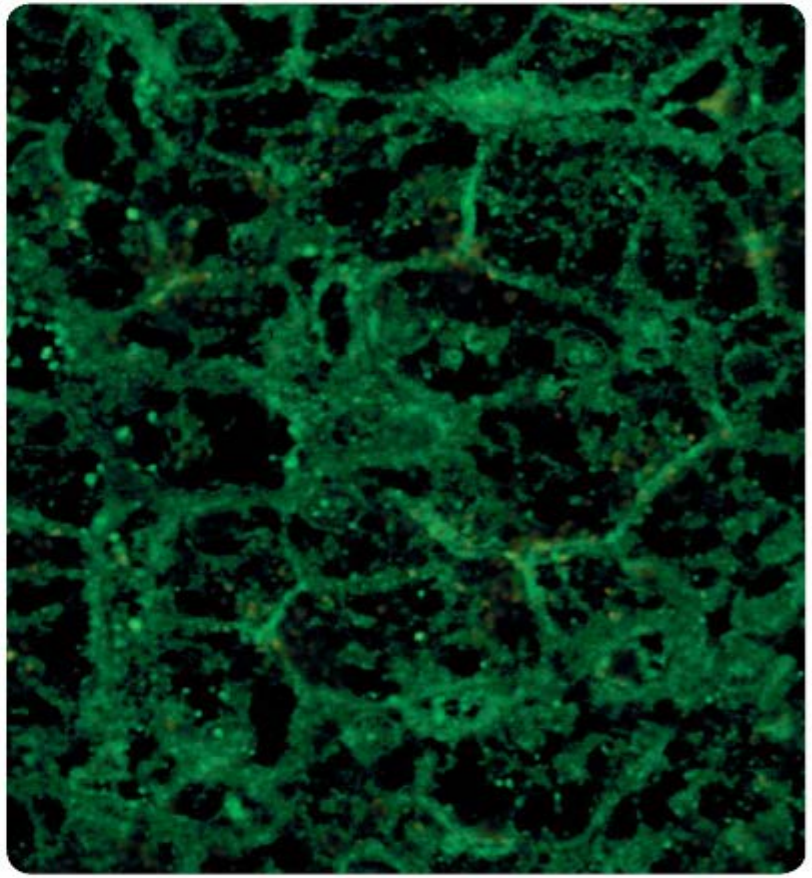
- Serologically, juvenile IIM (JIIM) tends to be rather different from the adult counterpart
- Like adult IIM, JIIM are also characterized by **skeletal muscle weakness**, characteristic rashes, and other systemic features.
- In a large multicenter study, 68% had only 1 myositis auto-Ab and 32% had no identifiable MAA or MSA.
- **Anti-p155/140** auto-Abs were the most frequent serologic subgroup, present in 32% of patients with **juvenile dermatomyositis (JDM)** or **overlap myositis** with **JDM**, followed by anti-MJ auto-Abs, which were seen in 20% of JIIM patients, primarily in JDM. And unlike adult IIM, other MSAs, including anti-synthetase, anti-signal recognition particle, and anti-Mi-2, were present in only 10% of JIIM.
- Hence JIIM is a rather heterogeneous group of illnesses that could be classified on the basis of distinct auto-Ab phenotypes that differ from adult IIM.

- It has been noted that the majority of IIM patients especially those with the aSS also have anti-Ro52/TRIM21 auto-Abs
- Patients with both ARS & Ro52/TRIM21 auto-Abs displayed a different clinical phenotype associated with severe myositis and joint impairment.
- Moreover, the coexistence of anti-Ro52/TRIM21 auto-Abs seems to be associated with an increased risk of cancer & ILD.

Autoantibodies against Jo-1 (AC-20)



HEp-2 cells

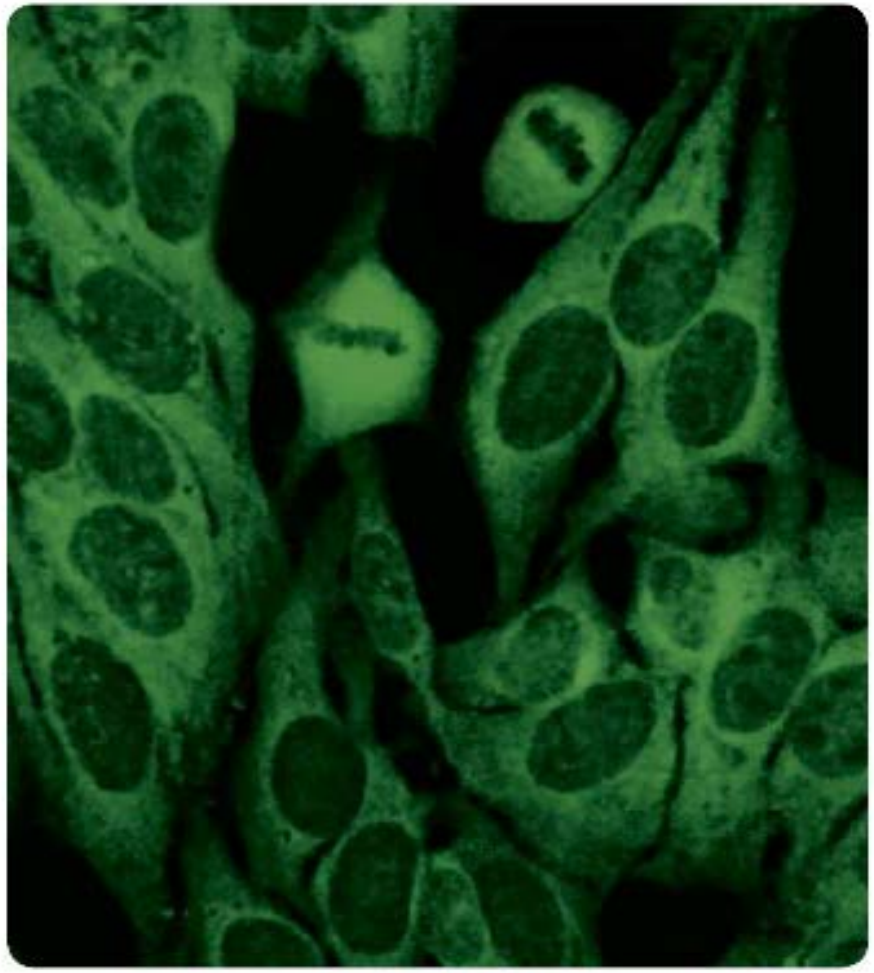


Primate liver

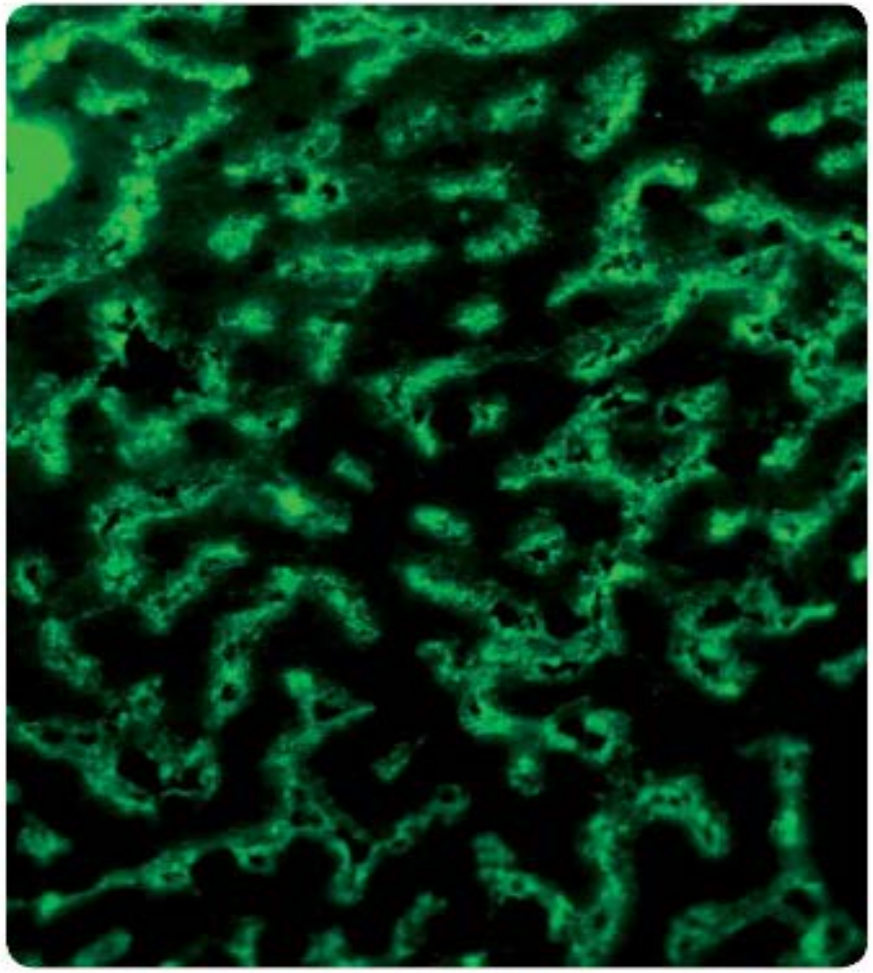
Antibodies against Jo-1 show a fine speckled to homogenous cytoplasmic fluorescence on **HEp-2 cells**. The cell nuclei also show distinct sharp dots in many cases. According to recent findings, Jo-1 target antigens are not solely localised in the cytoplasm, but are also found in the cell nucleus in some species.

On frozen tissue sections of **primate liver** the cytoplasm is only slightly stained. The fluorescence cannot be used for diagnostics.

Autoantibodies against **PL-7 & PL-12** (AC-19)



HEp-2 cells



Primate liver

Autoantibodies against PL-7 and PL-12 show a fine speckled to homogenous cytoplasmic fluorescence with **HEp-2 cells**. The cell nuclei also show distinct clear dots in many cases. According to recent findings, the enzymes PL-7 and PL-12 are not solely localised in the cytoplasm, but are also found in the cell nucleus in some species.

On frozen tissue sections of **primate liver** there is an unspecific fluorescence.

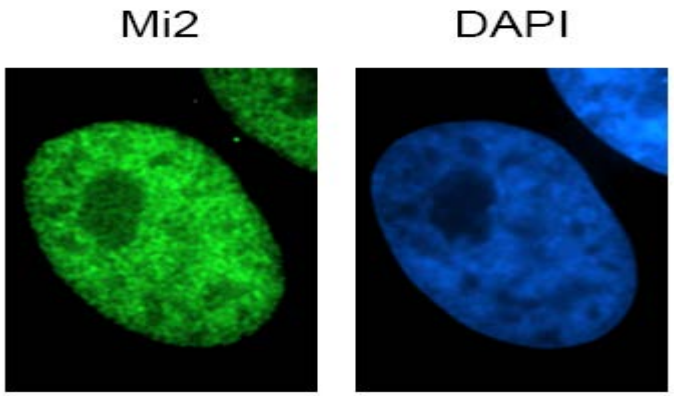
- Anti-ARS auto-Abs can be identified by several methods. Initially, these auto-Abs were defined by IP in which the anti-ARS was found to co-IP both the synthetase and the isoaccepting tRNA .
- Although many patients have cytoplasmic staining on IFA of HEp-2 cells, with the availability of newer methods for the detection of anti-ARS it is recognized that IFA alone lacks sensitivity, making IFA an unreliable screening test for anti-ARS.
- Although anti-Jo-1 auto-Abs can be detected by a variety of ELISA methods, addressable laser bead (ALBIA), and a Chemiluminescent immunoassay (CIA), there is little information about the comparative sensitivity, specificity, and consistency of these assays. Also, auto-Abs to other synthetases are rarely detected by these assays, so protein/RNA IP remains the only assay that identifies all the anti-ARS and other MSA and MAA. Line immunoassays (LIA) (became a popular tool to detect many MSA and MAA. Recently, a screening ELISA (research use only) containing Jo-1, PL-7, PL-12, EJ, and KS has been developed for the detection of anti-ARS auto-Abs.
- Since auto-Abs to ARS are only seen in a minority of patients with IIM, which is by itself a rare condition, the development and application of immunoassays for the detection of these auto-Abs is a challenge.

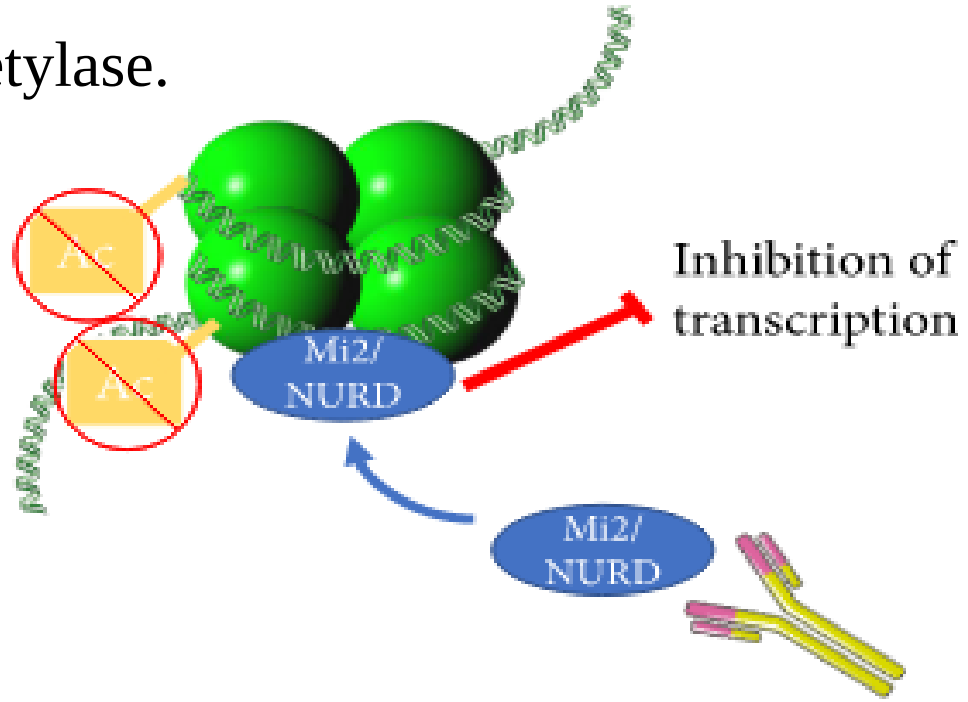
Methods for Detection of Autoantibodies to Nuclear and Intracellular Antigens

Method	Antigen Source	Sensitivity and Use
Immunofluorescence microscopy	Tissue sections; cell lines	Sensitive assay, often used for screening; may be computerized
Double immunodiffusion (Ouchterlony)	Tissue and cell extracts	Requires precipitin reaction, high specificity but not very sensitive
Counterimmunoelectrophoresis	Tissue and cell extracts	Increased sensitivity and speed as compared with immunodiffusion procedure
Immunoblotting, Western blot	Cell extracts	Very sensitive, permits detection of antibodies against soluble and insoluble antigens
Dot blot, linear blot (line immunoassays)	Purified native or recombinant antigens	Qualitative assay, average sensitivity
ELISA	Purified native or recombinant antigens	Very sensitive, quantitative, high throughput, can determine antibody class, low cost
Immunoprecipitation	Radiolabeled native or recombinant proteins	Very sensitive; identity of the actual autoantibody target may require ancillary confirmatory testing
Microsphere multiplexed assay (ALBIA)	Purified native or recombinant antigens	Very sensitive (compares to ELISA), semiquantitative, rapid, expensive proprietary technology
Cell-based assays	Cell line transfected with autoantigen cDNA, followed by IFA	Sensitivity = 45% to >90%; Specificity >95%

MI-2/ NURD COMPLE Anti-Mi-2

- Anti-Mi-2 auto-Abs are found in 10-30% of IIM patients and are more prevalent **DM>PM**.
- **Mi-2** is represented as 2 closely related molecular species, Mi-2α & Mi-2β.
- Anti-Mi-2 auto-Abs bind to a 240 kDa component of a macromolecular enzymatic complex referred to as the **Mi-2/ nucleosome remodeling & deacetylase (NuRD)** complex.
- This moiety is thought to participate in the **regulation** of **gene expression** at the chromatin level via chromatin remodeling ATPase and histone deacetylase.





Clinical Importance of Anti-Mi-2 auto-Ab

- Variations in prevalence of anti-Mi-2 are attributed to ethno geographic variables.

✓ It is the most common MSA in Mesoamerican IIM cohorts , environmental exposure to UV light, & immunoassay variables.

- In both adult and pediatric IIM, anti-Mi-2 associated with characteristic **DM cutaneous** features such as **Gottron sign**, **heliotrope rash**, neck and upper back involvement (aka V & shawl rashes), and cuticular hypertrophy. these patients do not usually develop lung involvement or arthritis &, accordingly, have a good prognosis.
- Anti-Mi-2 Abs are negatively correlated with malignancy-related myositis & have occasionally been reported in amyopathic DM.

frontiers in Immunology

SYSTEMATIC REVIEW
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Geographical Latitude Remains as an Important Factor for the Prevalence of Some Myositis Autoantibodies: A Systematic Review

Andrea Aguilar-Vazquez[1,2†], *Efrain Chavarria-Avila*[2,3,4†], *Oscar Pizano-Martinez*[2,4,5], *Alejandra Ramos-Hernandez*[4], *Lilia Andrade-Ortega*[6], *Edy-David Rubio-Arellano*[7] *and Monica Vazquez-Del Mercado*[2,4,5,8*]

They observed that **anti-Mi-2 prevalence** ↑ near to the **Equator** meanwhile **anti-MJ/NXP2** & **anti-ARS** prevalence had an opposite behavior **increasing** their prevalence in the geographical locations farther to the Equator.

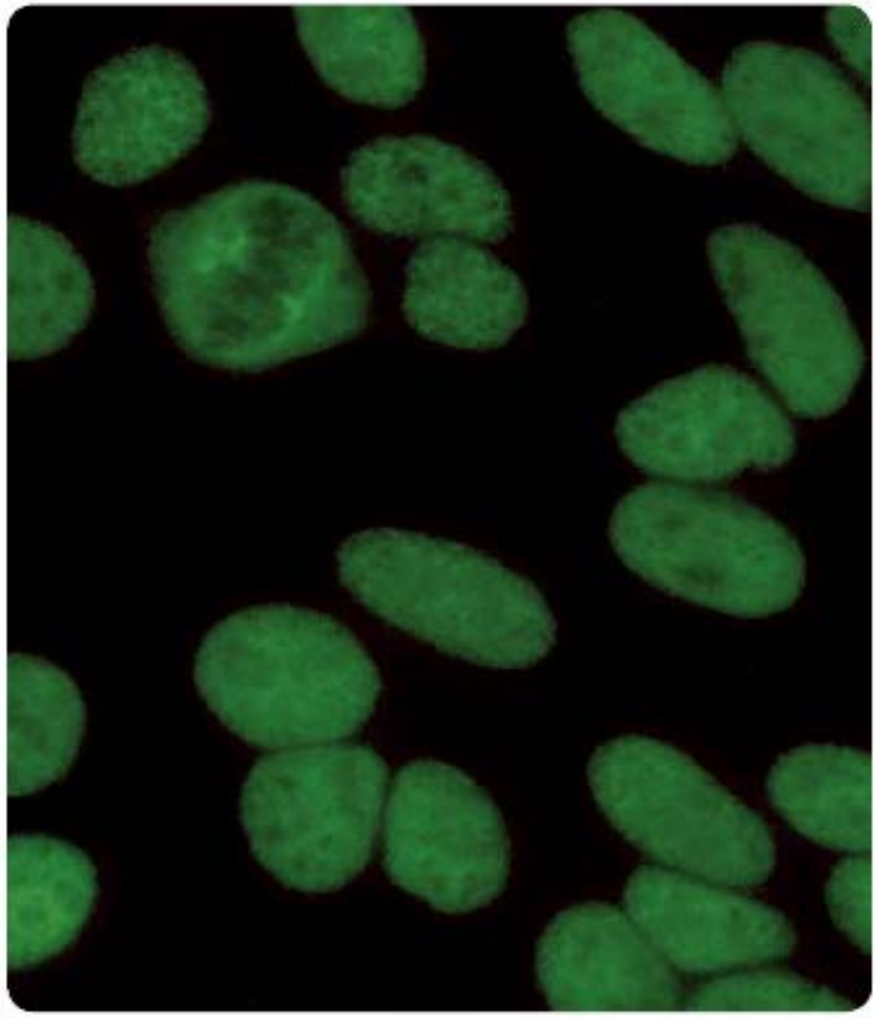
These results **highlighted** the importance to include the **UV radiation** and other environmental factors in IIM studies, in order to clarify its association with MSA and MAA prevalence as well as its possible role in the immunopathogenesis of these diseases.

The idiopathic inflammatory myopathies (IIM) are characterized by muscular weakness, cutaneous manifestations, muscle damage revealed by increase of muscular enzymes, muscle biopsy, electromyography and changes on magnetic resonance imaging. However, the hallmark of these IIM, is the development of myositis specific antibodies (MSA) or myositis associated antibodies (MAA). The theories about their presence in the serum of IIM is not known. Some studies have suggested that some of these MSA, such as anti-Mi-2 increases according to the intensity of UV radiation. There is scarce information about the environmental factors that might contribute in order to be considered as triggering factors as UV radiation might be. In this review, we analyzed the reported prevalence of MSAs and MAAs regarding to their geographical location and the possible relation with UV radiation. We collected the prevalence data of fifteen MSA and thirteen MAA from 22 countries around the world and we were able to observe a difference in prevalence between countries and continents. We found differences in anti-PL7, anti-Ro52, anti-La and anti-Ku prevalence according to UV radiation level. Otherwise, we observed that anti-Mi-2 prevalence increases near to the Equator meanwhile anti-MJ/NXP2 and anti-ARS prevalence had an opposite behavior increasing their prevalence in the geographical locations farther to the Equator. Our results highlighted the importance to include the UV radiation and other environmental factors in IIM studies, in order to clarify its association with MSA and MAA prevalence as well as its possible role in the immunopathogenesis of these diseases.

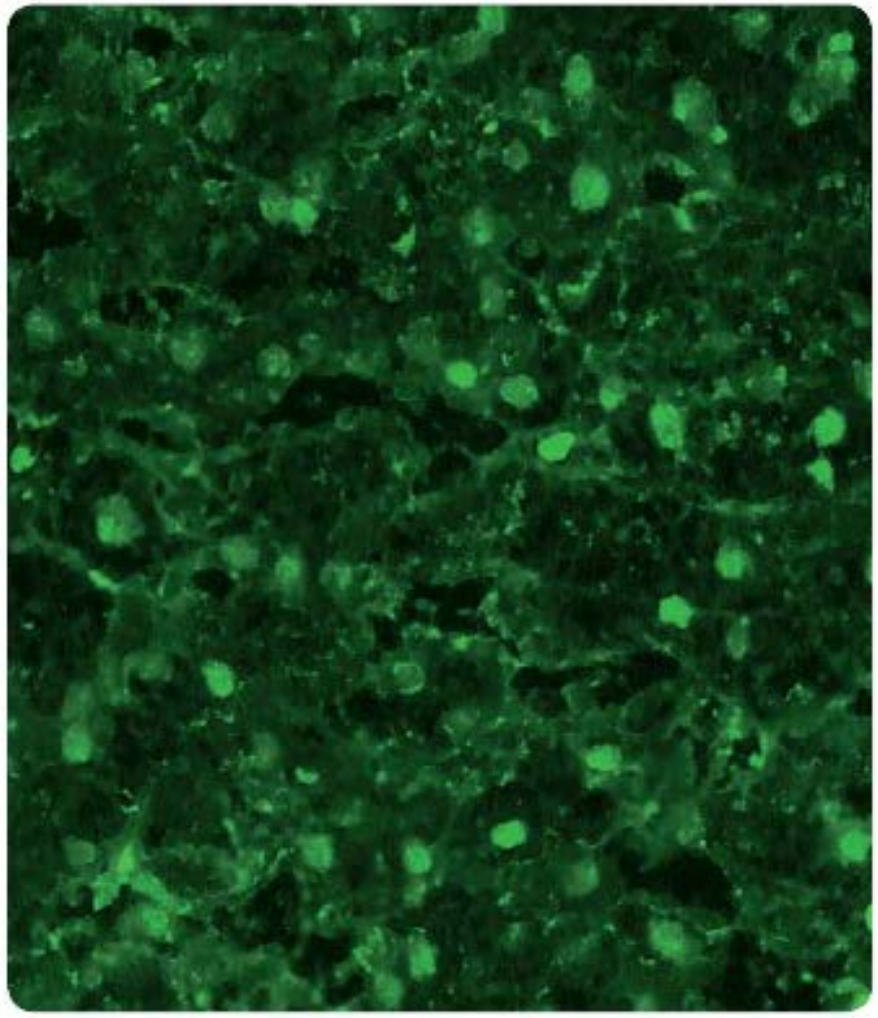
Keywords: idiopathic inflammatory myopathies (IIM), autoantibodies, prevalence, latitude, UV radiation


1 2 3 4 5 6 7 8 9 10 11
Artic Polar Circle (66
Tropic of Cancer (23
Equator
Tropic of Capricorn (-23
Antartic Polar Circle (-6
Canada (3.2%)
United States of America (5.6%)
Mexico (34.1%)
Guatemala (60.0%)
Anti-Mi-2 (%): 6.3 (0.0 – 38.2)
Anti-Mi-2 (%): 38.8 (12.0 – 60.0)
Anti-Mi-2 (%): 6.0 (0.0 – 23.1)
3.9°
5.2°
4.5°
P < 0.001
Czech Republic (6.6%)
Germany (13.6%)
Netherlands (6.0%)
Sweden (NR)
United Kigdom (6.4 %)
Poland (3.7%)
France (NR)
Hungary (7.7%)
Spain (6.1%)
Greece (NR)
Italy (4.6%)
China (4.2%)
Japan (3.7%)
South Korea (11.0%)
India (18.9%)
Chile (23.1%)
Argentina (12.5%)
Australia (3.0%)

Autoantibodies against Mi-2 (AC-4)



HEp-2 cells



Primate liver

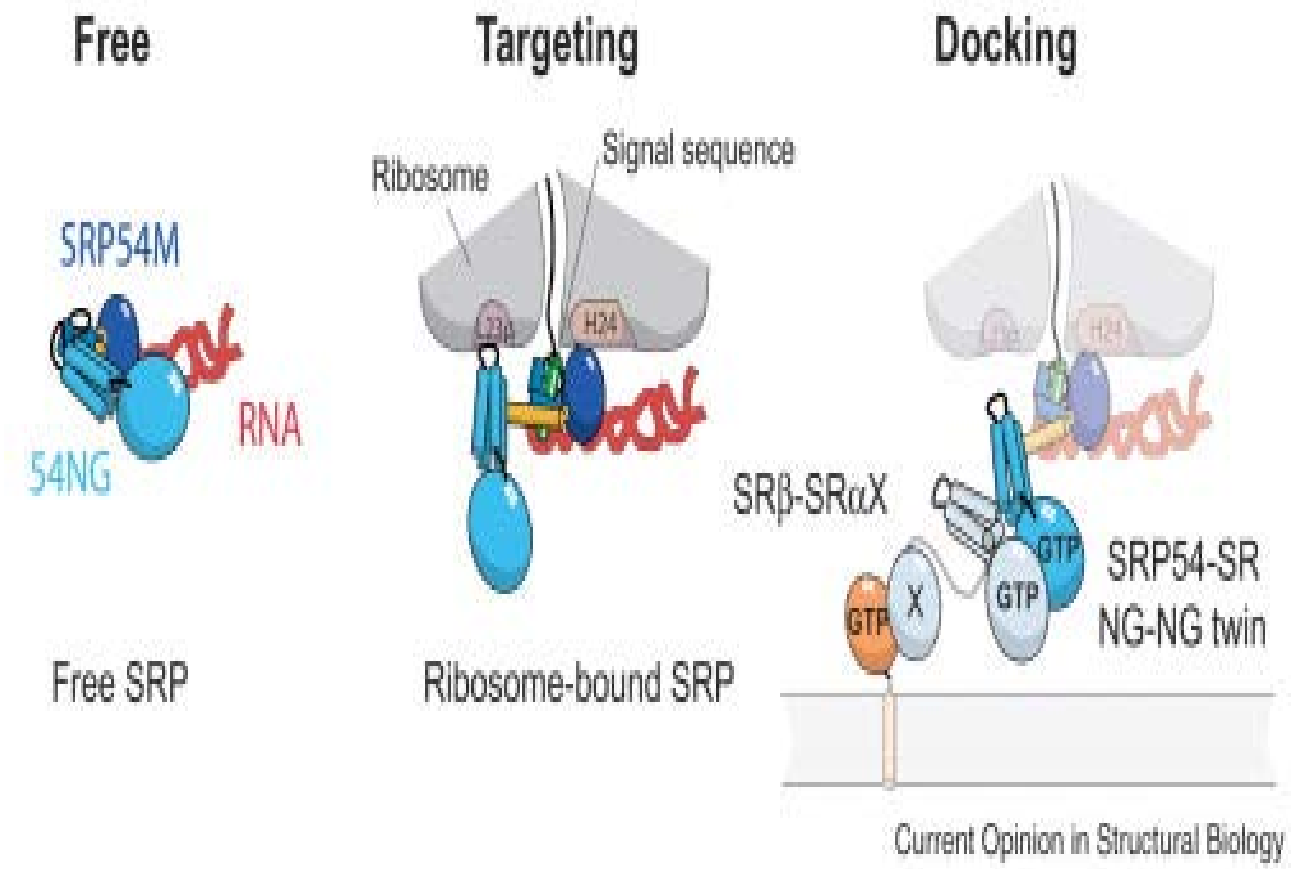
Autoantibodies to Mi-2 show a fine-speckled fluorescence of the cell nuclei in the indirect immunofluorescence test with **HEp-2 cells**. The nucleoli are partly unaffected.

With **primate liver**, autoantibodies against Mi-2 depict a fine speckled fluorescence of the hepatocyte nuclei.

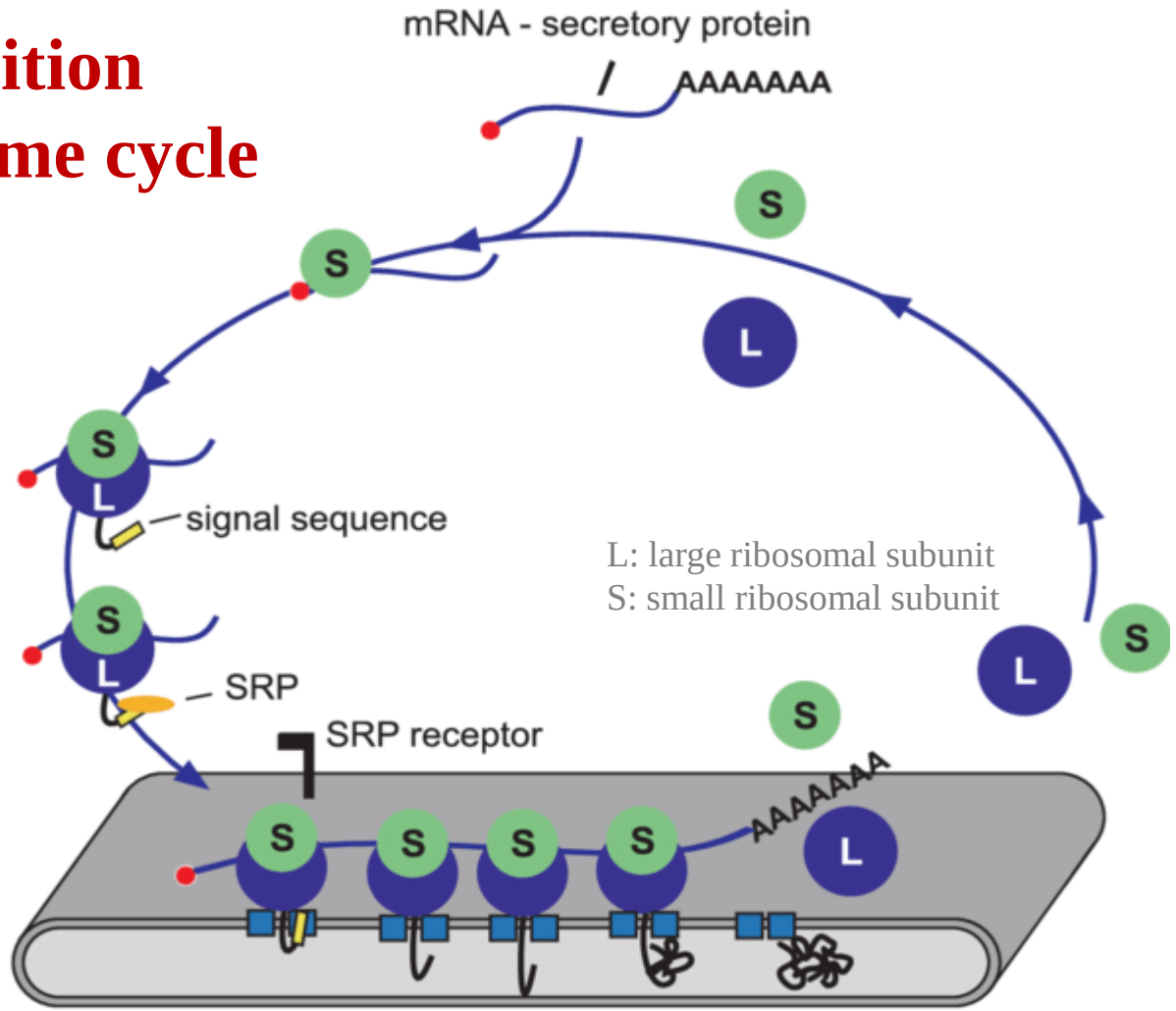
SIGNAL RECOGNITION PARTICLE

- The prevalence of auto-Abs to a cytoplasmic RNP called SRP ranges from 4-8% in IIM cohorts.
- SRP is a **highly conserved** cytoplasmic macromolecular RNP complex, consisting of 6 polypeptides & 7SL RNA in a macromolecular complex.

- Anti-SRP Abs selectively IP the 7SL RNA & bind to a 54 kDa protein moiety.
- It is widely acknowledged that anti-SRP Abs are highly specific for IIM and thus are thought to be useful in differentiating IIM from genetic or sporadic degenerative myopathies, such as **muscular dystrophies**.



The signal-recognition particle (SRP)-ribosome cycle

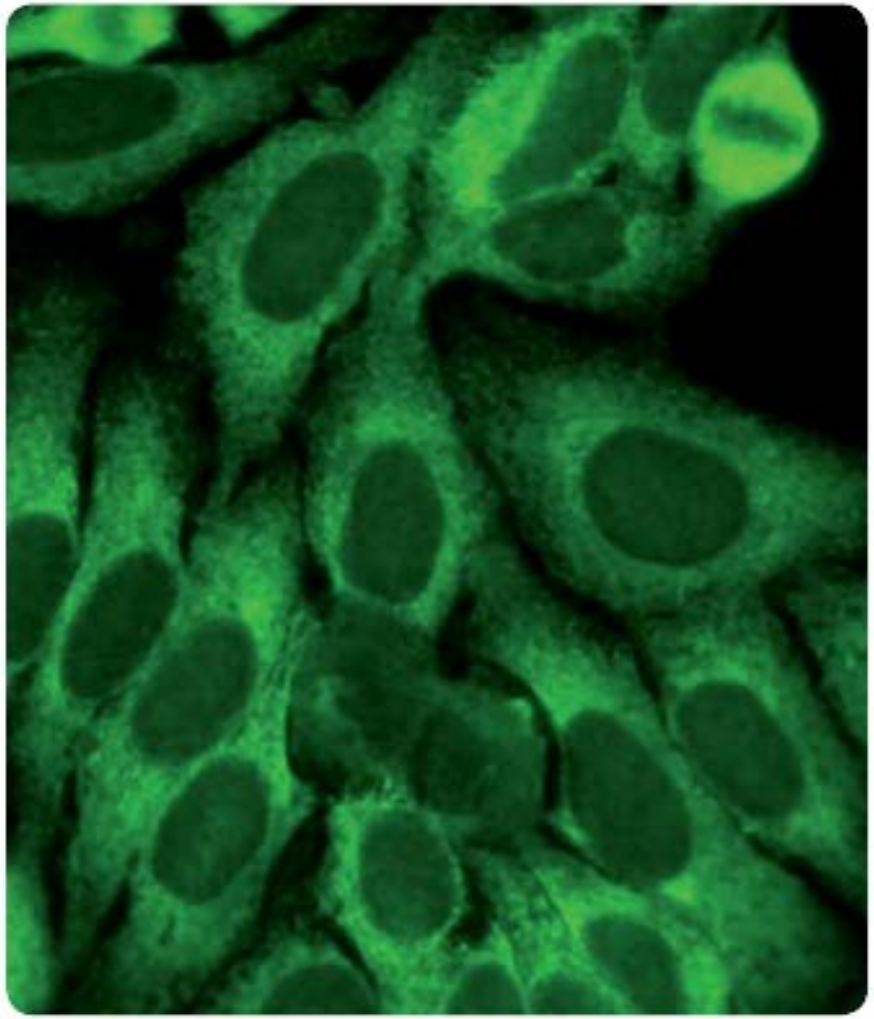


A pathway for mRNA partitioning to the ER. Cytosolic ribosomes engaged in the translation of mRNAs encoding secretory or membrane proteins are targeted through the SRP pathway to the ER membrane. At the ER, the signal sequence engages the protein-conducting channel & protein translocation ensues. The termination of protein synthesis leads to the release of ribosomal subunits from the ER membrane to the cytosol.

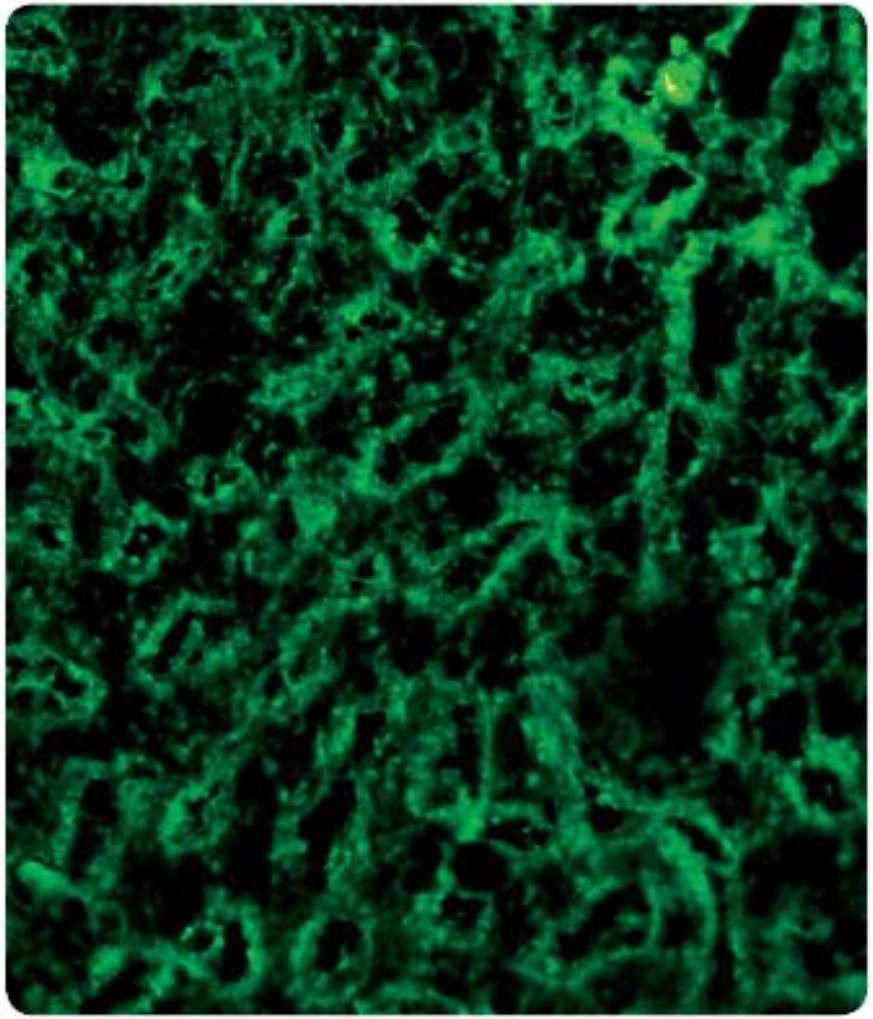
Clinical Importance of Anti-SRP auto-Ab

- Akin to the ARS, anti-SRP have been the **key** auto-Ab marker in what is referred to as "anti-SRP syndrome".
- Characteristics of this syndrome include a severe, rapidly progressing and necrotizing myopathy that is relatively unresponsive to conventional therapies.
- Characteristic histopathologic features consist in predominant muscle fiber necrosis and/or regeneration associated with nonspecific myopathic signs & scarce inflammatory cell infiltration.
- The clinical course of anti-SRP myopathy appears clinically similar to PM, although patients who did not develop overt myositis after some years of follow-up have been reported

Autoantibodies against SRP (AC-19)



HEp-2 cells



Primate liver

Autoantibodies against SRP produce a mainly cytoplasmic, smooth to fine speckled fluorescence on **HEp-2 cells**. In mitotic cells the fluorescence is peri-chromosomally intensified, the chromosomes are unaffected.

Hepatocytes of the **primate liver** generally show a fine speckled fluorescence distributed over the whole organ.

MDA5 (CADM-140) Anti-MDA5

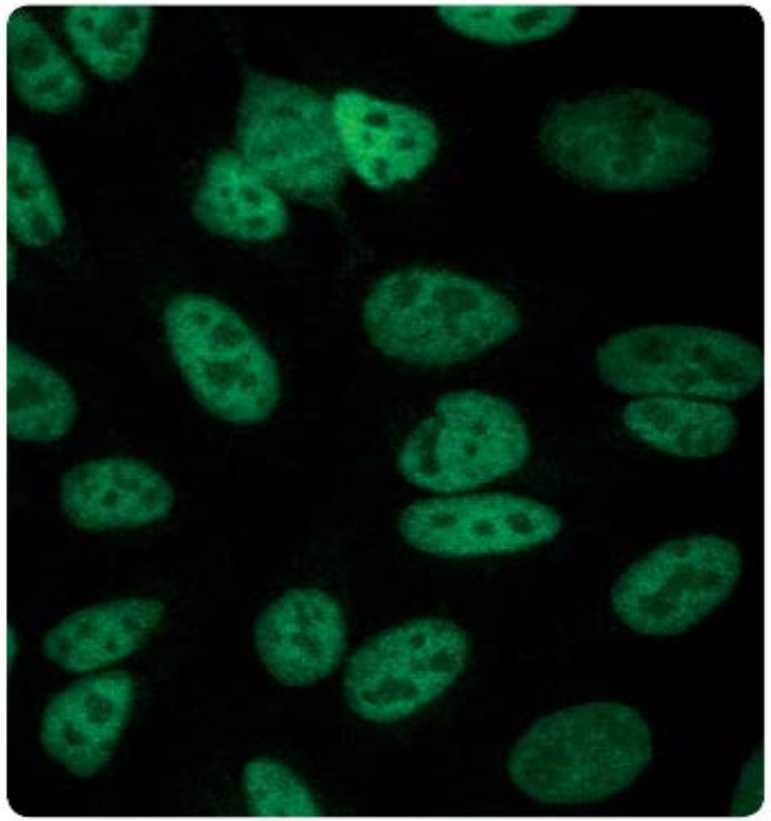
- Anti-MDA5 auto-Abs have been primarily reported in 20-30% of Asian (Japanese, Korean, Chinese) cohorts.
- These auto-Abs were associated with a DM subset characterized by **clinically amyopathic DM (CADM)** accompanied by acute, rapidly progressive ILD.
- Suggested that Asian IIM patients that are anti-MDA5 positive are at **higher risk** of developing rapid progressive ILD than white patients (serve as a prognostic biomarker for ILD).

- The target Ag of MDA5 auto-Abs is a **cytoplasmic** 140 kDa protein initially referred to as clinically amyopathic DM-140 (CADM-140).
- More recently the target has been more precisely identified as the IFN-induced melanoma differentiation-associated gene 5 (MDA5).
- Curiously, in the context of a possible etiology of this serologic subset of IIM, MDA5 functions as a **viral sensor** by recognizing cytoplasmic viral RNA and triggers an innate immune response.

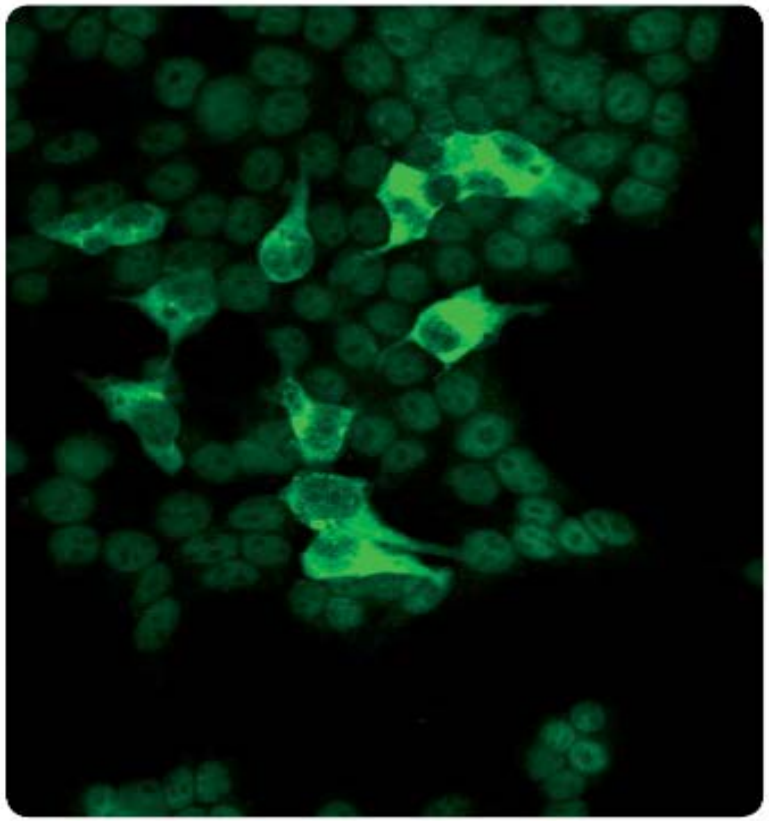
TIF1-Γ/TIF1-α (P155/140)

- An auto-Ab that bound a 155/140 kDa doublet protein was reported in 20-30% of adult & juvenile DM.
- p155/140 was identified as proteins related to the human transcription intermediary factor 1 (TIF-1) family of proteins that include **TIF1-α**, **TIF1-γ**, & **TIF1-β**.
- The main target recognized by anti-p155/140 Abs is the 155 kDa TIF1-γ auto-Ag, while the 140 kDa Ag is the TIF1-α protein.
- In addition, some anti-p155/140-positive sera also recognize the TIF1-β protein.
- TIF1-γ is the most commonly targeted protein of anti-p155/140 auto-Abs and the most common DM-specific auto-Abs in IIM.
- Clinically they have been associated with life-threatening complications, including **malignancy** in adult DM.
- A diagnostic algorithm for the diagnosis of occult malignancy in adult DM that includes testing for anti-p155 Abs has been proposed.
- Prospective studies and reliable, standardized immunoassays are needed to confirm the predictive value of anti-p155 as a risk factor for **cancer-associated DM**.

Autoantibodies against **TIF-1 gamma** (AC-4)



HEp-2 cells



TIF1-gamma-transfected cells

Autoantibodies against TIF1-gamma cause a fine speckled fluorescence on **HEp-2 cells**, which is distributed over the whole cell nucleus but leaves the nucleoli free. Mitotic cells also exhibit a fine speckled fluorescence, but the chromosomes are spared.

Antibodies against TIF1-gamma react with the **transfected cells** of the test substrate. They produce a smooth to fine speckled fluorescence in the cytoplasm. The cell nuclei are generally only slightly stained.

Clinical association: Antibodies against TIF1-gamma can be detected with a prevalence of 5% in patients with dermatomyositis. In particular, they are specific for cancer-associated (paraneoplastic) (dermato)myositis (CAM).

MJ/NXP2

- Anti-MJ Ab was initially found in approximately 25% of juvenile DM & associated with **calcinosis**, severe disease, and muscle contractures.
- Anti-MJ auto-Abs bind a nuclear matrix protein, NXP2/MORC3.
- The association of anti-MJ auto-Abs and cancer was not confirmed in other studies, but a more recent report indicated that IgG2 anti-NXP2 Abs are distinctively associated with cancer in IIM.

SMALL UBIQUITIN-LIKE MODIFIER ACTIVATING ENZYME (SAE)

- Auto-Abs to SAE were identified in less than 10% of adult DM patients who presented with severe skin disease & mild myopathic involvement, although over the course of the disease more profound myositis & systemic features became manifest.
- The target auto-Ag has been identified as the SUMO-1 activating enzyme heterodimer composed of 40 kDa SAE1 & 90 kDa SAE2 subunit.
- SUMO is localized to the nucleus and is structurally similar to proteins responsible for a posttranslational modification of proteins referred to as sumoylation.

3-HYDROXY- 3-METHYLGLUTARYL-COENZYME A REDUCTASE (HMGCR)

- One of the more intriguing MSA is directed to 200/100 kDa proteins identified as 3-hydroxy-3-methylglutaryl-coenzyme A reductase, an enzyme inhibited by statins and involved in cholesterol biosynthesis.
- Although anti-HMGCR was initially described as a biomarker of **statin-associated necrotizing myopathy,** subsequent studies have not confirmed a direct or causal association.
- Nevertheless, a link to patient's statin use is still a relatively common clinical correlation.

- **Immunogenetics** play a key role in the B-cell response to HMGCR.
- Clinical parameters include markedly elevated CK levels, myopathic features on electromyography, & a better prognosis because of a favorable response to immunosuppressive therapy.
- Anti-HMGCR Abs are considered **highly specific** for a **necrotizing myopathy**.
- Anti-HMGCR sera are generally negative for all other auto-Abs tested, although one series from China showed some patients with **concomitant anti-MDA5 Abs**.
- A number of commercially available immunoassays, including **ELISA, CIA**, and other solid-phase immunoassays, are increasingly available.

CYTOSOLIC 5’-NUCLEOTIDASE 1A (NT5c1A)

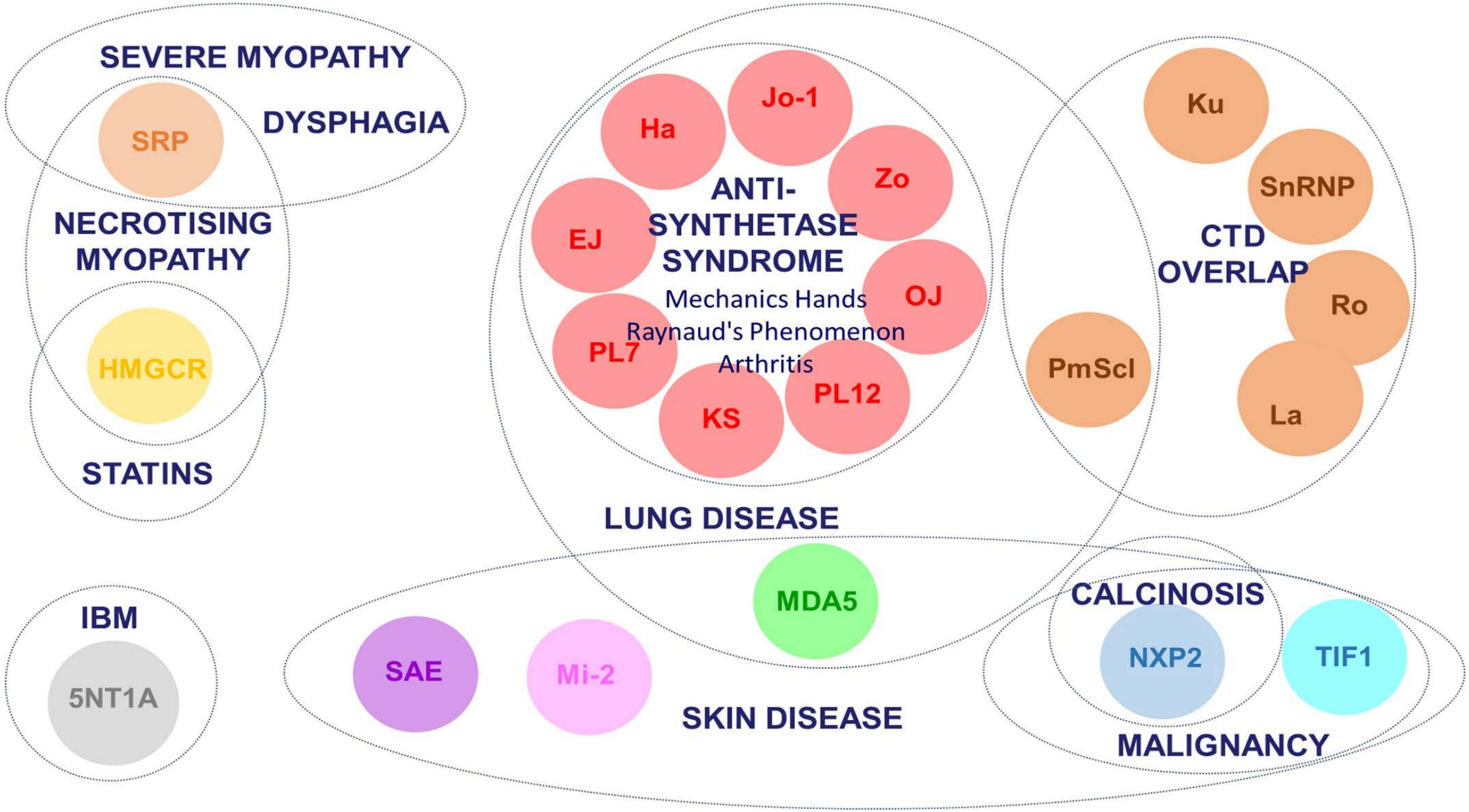
- **Sporadic inclusion body myositis (sIBM)** is a subset of IIM characterized by asymmetric muscle involvement, predominantly affecting the **long finger flexors**, quadriceps muscles, & posterior oropharynx.
- Highest diagnostic sensitivity and specificity including a combination of clinical, electrodiagnostic, and pathologic assessment.
- Although the precise pathogenesis of sIBM remains **unclear** and the disease is not responsive to conventional immunosuppression, the immune system is thought to play a significant role in its pathogenesis.
- A biomarker with high sensitivity and specificity for sIBM was not established until 2011, when Salajegheh et al. (2011) described a serum auto-Ab detected by immunoblot of skeletal muscle lysates that targeted a ~44 kDa human **mu**scle **p**rotein (Mup44) in 52% of sIBM sera with 100% specificity.
- Subsequent studies confirmed that **anti-Mup44**, also known as cytosolic NT5c1A, was relatively sensitive (ranging from 33-80%) but not specific for sIBM.

- One study indicated that anti-NT5c1A was associated with a 6-9 fold increase in the likelihood of having sIBM as compared to other IIM and proposed possible advantages of avoiding unnecessary steroid treatment and/or invasive muscle biopsies.
- Hence interpretation of a positive anti-NT5c1A result needs to be taken in the context of clinical and pathologic findings; therefore caution should be taken when applying this biomarker to patients with IIM. The value of anti-NT5c1A auto-Abs will become clearer when validated and reliable immunoassays are developed.

- **IFA** on commercial HEp-2 substrates had no utility in screening for anti-NT5c1A antibodies.
- In a most recent study using addressable laser bead immunoassay and purified recombinant NT5c1A, anti-NT5c1A auto-Abs had a sensitivity of 48.8% and a specificity of 91.8% for sIBM, establishing a relationship between anti-NT5c1A and **higher disease severity**.

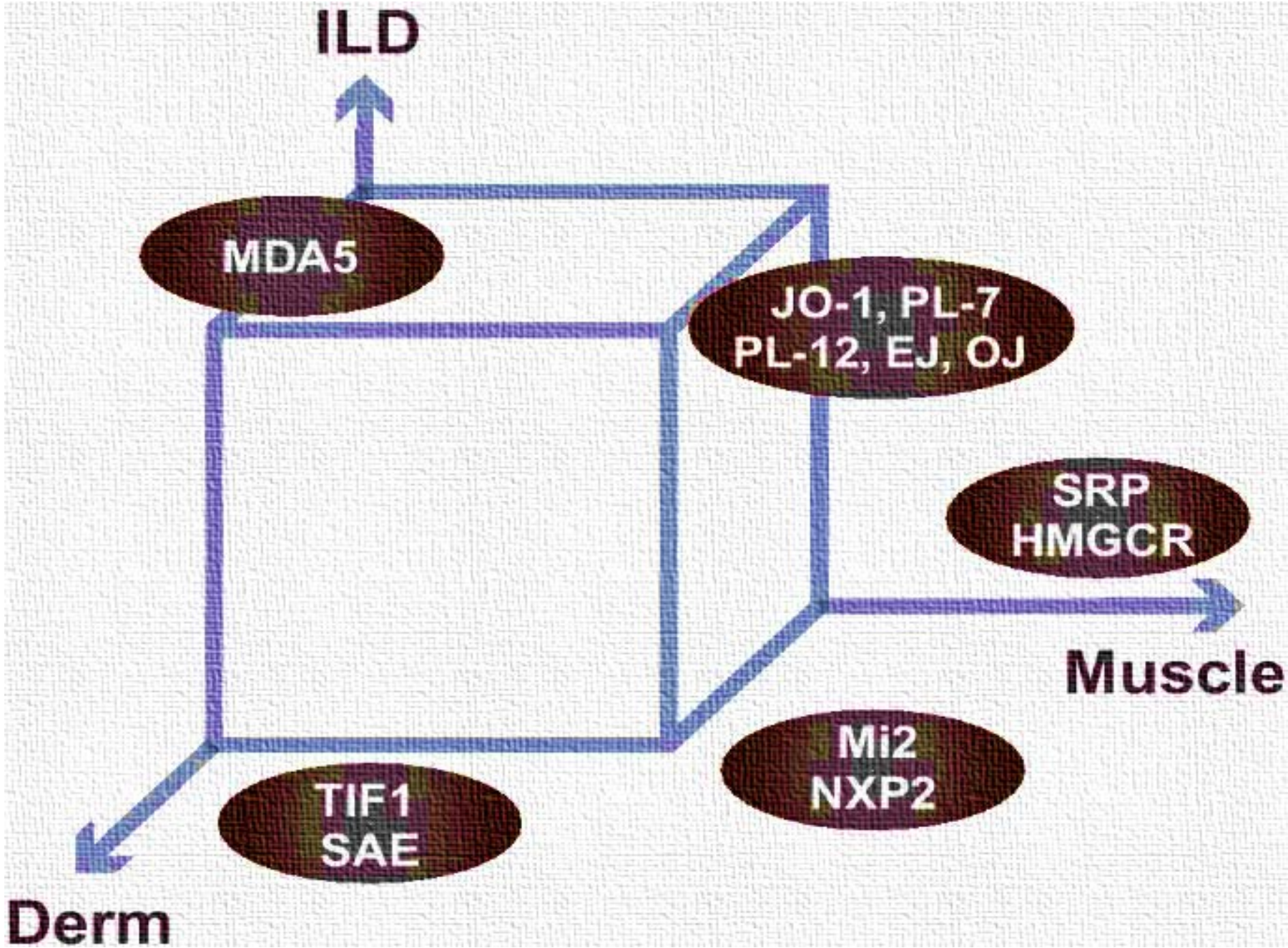
Auto-Ags and Autoantibodies in Idiopathic Inflammatory Myopathies

Autoantigen	Molecular Structure	Antibody Frequency
Jo-1	Histidyl tRNA synthetase protein, 52 kD	23–36% in PM; 60–70% in ILD subset
PL-7	Threonyl tRNA synthetase protein, 80 kD	4% in PM
PL-12	Alanyl tRNA synthetase protein, 110 kD	3% in PM
Zo	Phenylalanyl-tRNA synthetase	<3% in PM
YRS	Tyrosyl-tRNA synthetase	<3% in PM
KS	Asparaginyl-tRNA synthetase	<3% in PM
EJ	Glycyl-tRNA synthetase	<3% in PM
OJ	Isoleucyl-tRNA synthetase	<3% in PM
MDA5/CADM-140	IFN-induced melanoma differentiation-associated gene 5	20–30% in Asian amyopathic DM patients and ILD
Mi-2	Nuclear protein complex, proteins 53 and 61 kD	15–35% in PM; 5–9% in DM
Signal recognition particle (SRP)	54 kD protein complexed with 7 SL RNA	4–8% in PM; does not occur in DM
PM-Scl	Complex of 11 proteins,110–120 kD	8–12% in PM; 25% in PM/scleroderma overlap
U1nRNP	Spliceosome complex	4–17% in PM/DM
SAE	Small ubiquitin-like modifier activating enzyme	4% in myositis patients, 8% in DM
p155/p140 (TIF-1 α/γ)	p155-TIF-1γ (involved in nuclear transcription and cellular differentiation)	21% of myositis patients, 75% in adult DM associated with cancer
HMGCR	200/100 kDa proteins: 3-hydroxy-3-methylglutaryl-coenzyme A reductase	Statin-induced myopathy
MJ/NXP2	Nuclear matrix protein, NXP2/MORC3	25% in juvenile DM
NT5c1A/Mup44	44 kDa human muscle protein	33–80% in inclusion body myositis; specificity >90%


SEVERE MYOPATHY
DYSPHAGIA
SRP
NECROTISING MYOPATHY
HMGCR
STATINS
IBM
5NT1A
Ha
Jo-1
Zo
EJ
ANTI-SYNTHETASE SYNDROME
Mechanics Hands
Raynaud's Phenomenon
Arthritis
OJ
PL7
KS
PL12
LUNG DISEASE
Ku
SnRNP
CTD OVERLAP
Ro
PmScl
La
MDA5
SAE
Mi-2
SKIN DISEASE
CALCINOSIS
NXP2
TIF1
MALIGNANCY

MYOSITIS-ASSOCIATED auto-Abs (MAA)

- **MAAs** are not specific for IIM & are commonly found in the setting of overlap syndromes with myositis.
- The more common MAA bind to Ro60/SSA, Ro52/TRIM21, 75 & 100 kDa PM/Scl auto-Ags, Ku (p70/80) nuclear heterodimer, and components of U1RNP.


ILD
MDA5
JO-1, PL-7
PL-12, EJ, OJ
SRP
HMGCR
Muscle
Mi2
NXP2
TIF1
SAE
Derm

Autoimmune disease	ANA prevalence (%)
Systemic lupus erythematosus (SLE, active)	95–100
Drug-induced lupus erythematosus	100
Mixed connective tissue disease (MCTD, Sharp syndr.)	100
Rheumatoid arthritis	20–40
Other rheumatic diseases	20–50
Progressive systemic sclerosis	85–95
Polymyositis/dermatomyositis	30–50
Sjögren's syndrome	70–80
Autoimmune hepatitis (AIH)	30–40
Ulcerative colitis	26

Polymyositis / dermatomyositis

Autoantibodies against PM-Scl occur in polymyositis and dermatomyositis. Other anti-nuclear antibodies (Mi-1, Mi-2 and Ku) and antibodies against Jo-1 can also be found in these diseases.

Autoantibodies in polymyositis and dermatomyositis

Antigen	Prevalence (%)
PM-Scl (PM-1), incl. overlap syndrome with PSS	24–55
Jo-1 (histidyl-tRNA synthetase)	25–35
Mi-1	10
Mi-2	5–30
Ku, incl. overlap syndrome with PSS	25–50
Single-stranded DNA	40–50
SRP	5
TIF1-gamma	5
PL-7, PL-12 (aminoacyl-tRNA synthetases)	3–4

Anti-nuclear autoantibodies: The most important associated diseases

Antigen	Disease	Prevalence (%)
Histones	Drug-induced SLE	95
	SLE	50–80
	RA	15–50
U1-nRNP	MCTD (Sharp syndrome)	95–100
	SLE	15–40
	RA	3
Sm	SLE	5–40
SS-A (Ro)	Sjögren's syndrome	40–95
	SLE	20–60
	Neonatal lupus syndrome	100
SS-B (La)	Sjögren's syndrome	40–95
	SLE	10–20
Fibrillarin	PSS, diffuse	5–10
RNA polymerase I	PSS, diffuse	4
RNA helicase A	SLE	6
PM-Scl (PM-1)	Poly-/dermatomyositis/overlap syndr.	24–55
	PSS, diffuse	13
Centromeres	PSS, limited	80–95
Topoisomerase I	PSS, diffuse	25–75
PCNA-like	SLE	3
Ku	SLE	10
	Poly-/dermatomyositis, PSS	25–50
Mi-1, Mi-2	Dermatomyositis	5–30

CONCEPT OF OVERLAP SYNDROMES

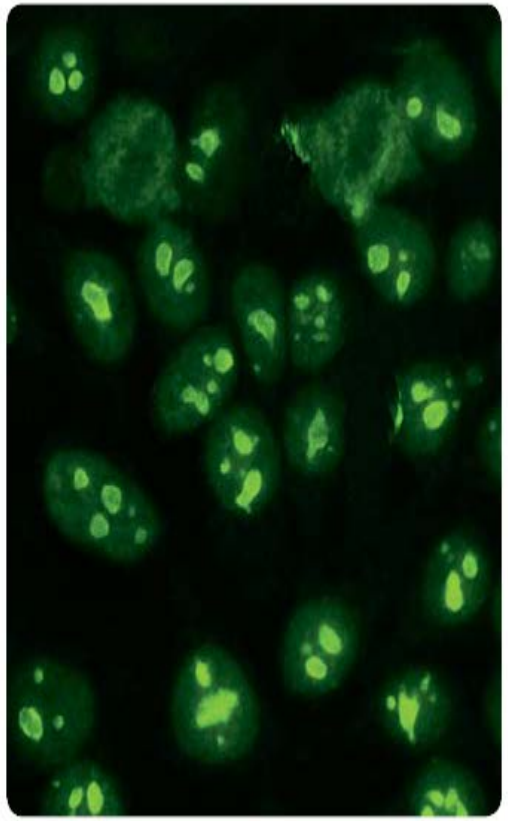
- Overlap syndrome is a term used when patients exhibit symptoms of more than one disease.

✓ For example, patients who meet the diagnostic criteria for SLE and have typical manifestations suggestive of a second diagnosis, such as RA.

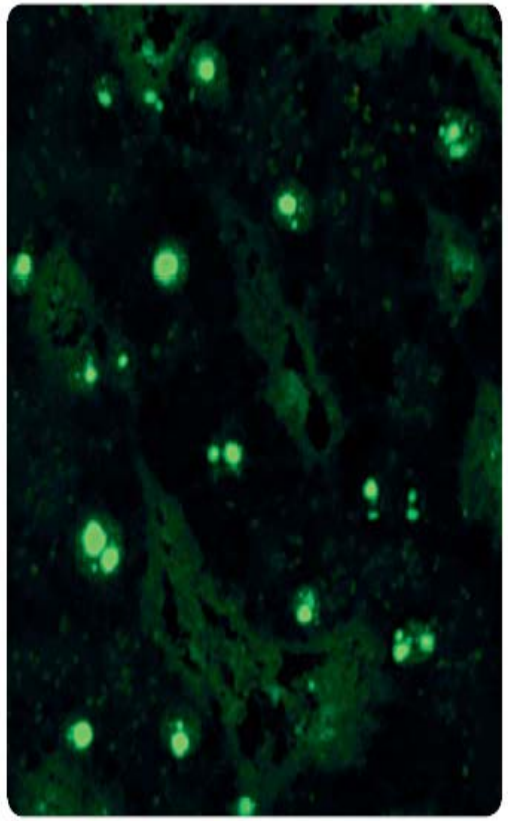
✓ It is uncertain whether overlap syndromes may represent the coexistence of 2 or more different diseases or the syndrome is a distinct entity.

MIXED CONNECTIVE TISSUE DISEASE (MCTD)

- The concept of **MCTD** was initially proposed by Sharp et al. (1972). The 20 patients described in the initial report had a combination of features usually associated with SLE, systemic sclerosis, and polymyositis.
- Characteristically, a high titer of autoantibody to a nuclear ribonucleoproteins (U1nRNP) was found in all the patients by hemagglutination
- The lack of renal and neurologic abnormalities and excellent response of these patients to small doses of oral corticosteroids initially justified the classification apart from SLE and systemic sclerosis. However, the concept of MCTD as a separate group has changed with time.
- Many feel that MCTD represents an overlap of systemic sclerosis, SLE, and polymyositis: A group of patients originally diagnosed as MCTD was restudied 8 years later and showed a general evolution out of the overlap pattern to one of single disease, and scleroderma was the most prevalent diagnosis.
- There appears to be a large overlap that becomes apparent when the abovementioned criteria are applied to a certain population of patients, but recent studies support the existence of MCTD as an individual clinical entity.



HEp-2 cells



Primate liver

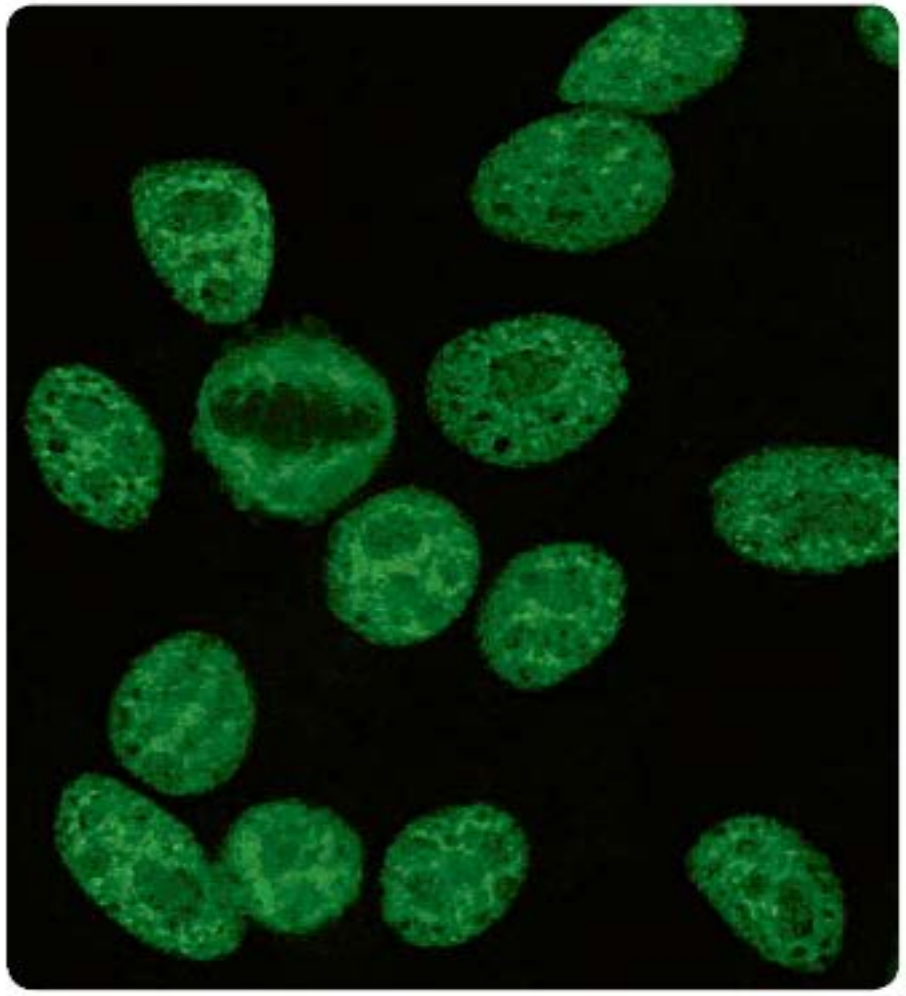
In the immunofluorescence test with **HEp-2 cells**, autoantibodies against PM-Scl exhibit a homogeneous fluorescence of the nucleoli with a simultaneous weaker, fine-speckled reaction of the nucleoplasm. The condensed chromosomes of the mitotic cells are unaffected; a fine, speckled fluorescence is shown outside of the chromosomes.

A homogeneous fluorescence of the nucleoli also appears on frozen sections of **primate liver**, as well as a very weak, fine-speckled to reticular staining of the cell nucleus.

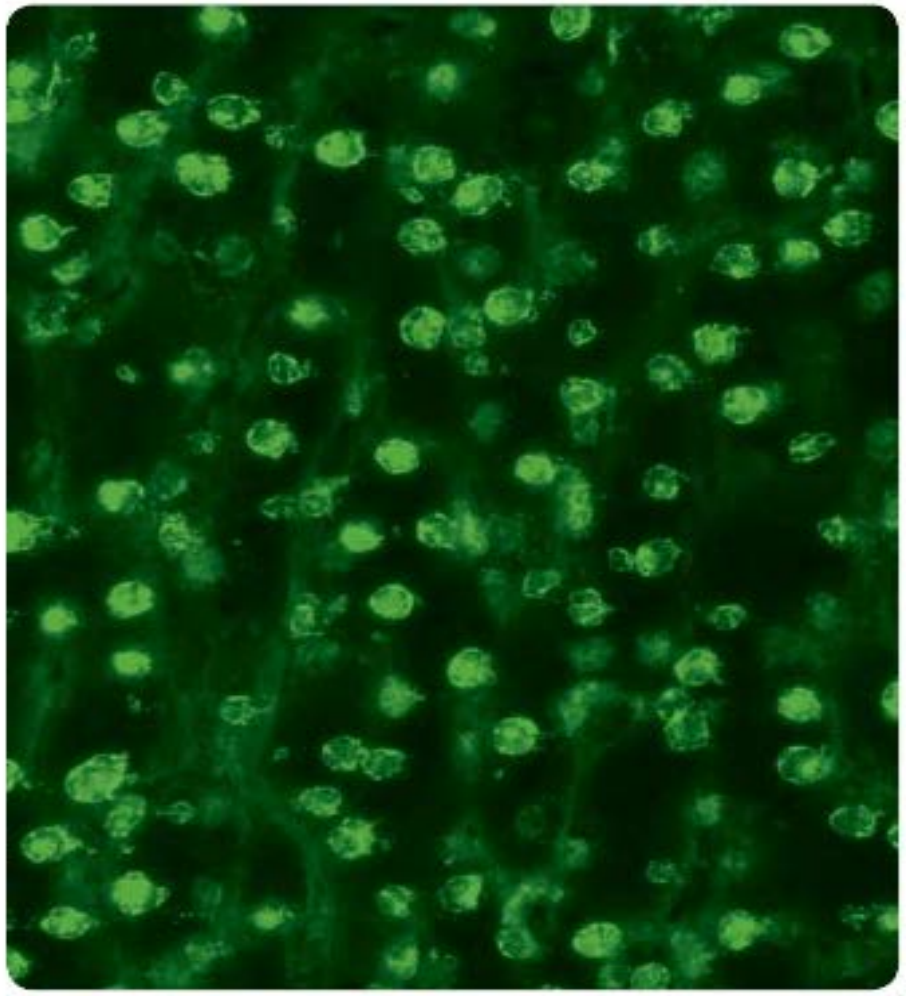
Clinical association: PM-Scl antibodies can be detected in 24–55% of patients with polymyositis/systemic sclerosis overlap syndrome. Here, the autoantibodies are usually directed against both main antigens: PM-Scl75 and PM-Scl100. If progressive systemic sclerosis is exclusively present, antibodies to PM-Scl75 show a prevalence of 10%, and antibodies to PM-Scl100 a prevalence of 7%. Using test systems which detect only anti-PM-Scl100, some patients with progressive systemic sclerosis remain unidentified.

Autoantibodies against **PM-Scl** (AC-8)

Autoantibodies against **Ku** (AC-4)



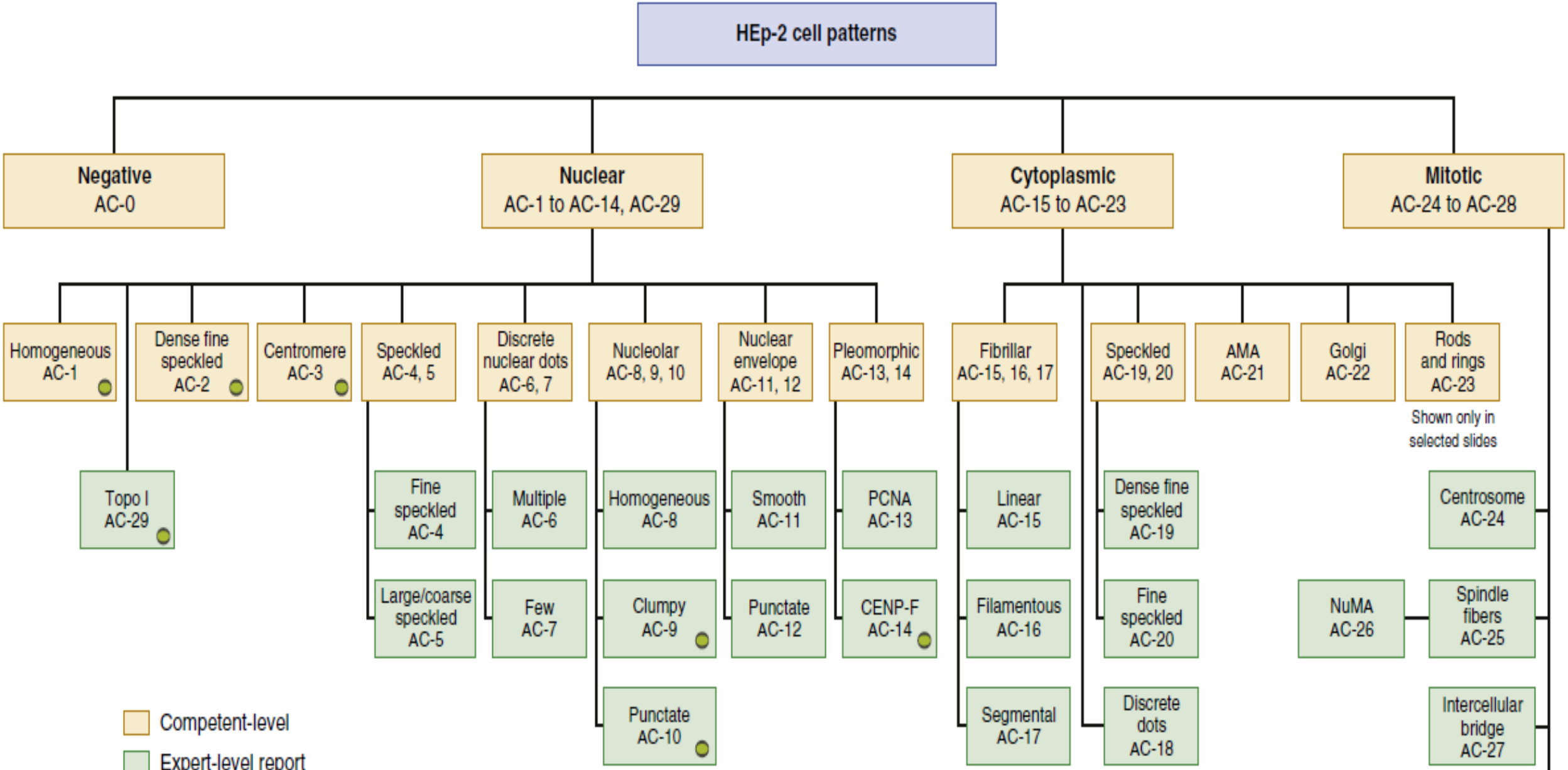
HEp-2 cells



Primate liver

In the indirect immunofluorescence test with **HEp-2 cells**, antibodies against Ku exhibit a fine speckled fluorescence of the cell nuclei and the nucleoli are positive in parts. There is hardly any difference noticeable to antibodies against SS-A, SS-B, Sm and RNP.

However, if **primate liver** sections are incubated in parallel, possibly in the same field, a typical clumpy-speckled staining of the cell nuclei is found, which is an almost certain proof of antibodies to Ku.



Nomenclature & classification trees for IFA patterns on HEp-2 cells. Each of these patterns has an alphanumeric code for anti cell Ab from **AC0-AC29** designating cell organelle that is recognized by each auto-Ab.