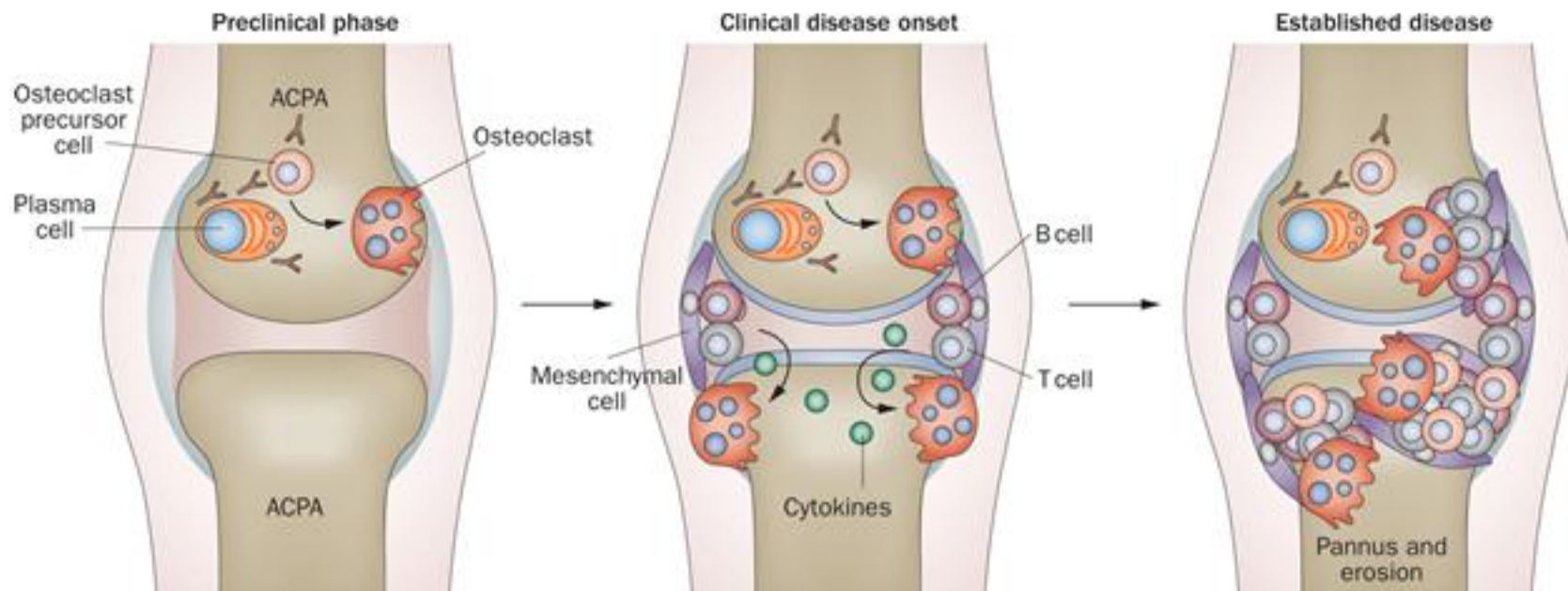


# Biomarkers in Rheumatoid Arthritis



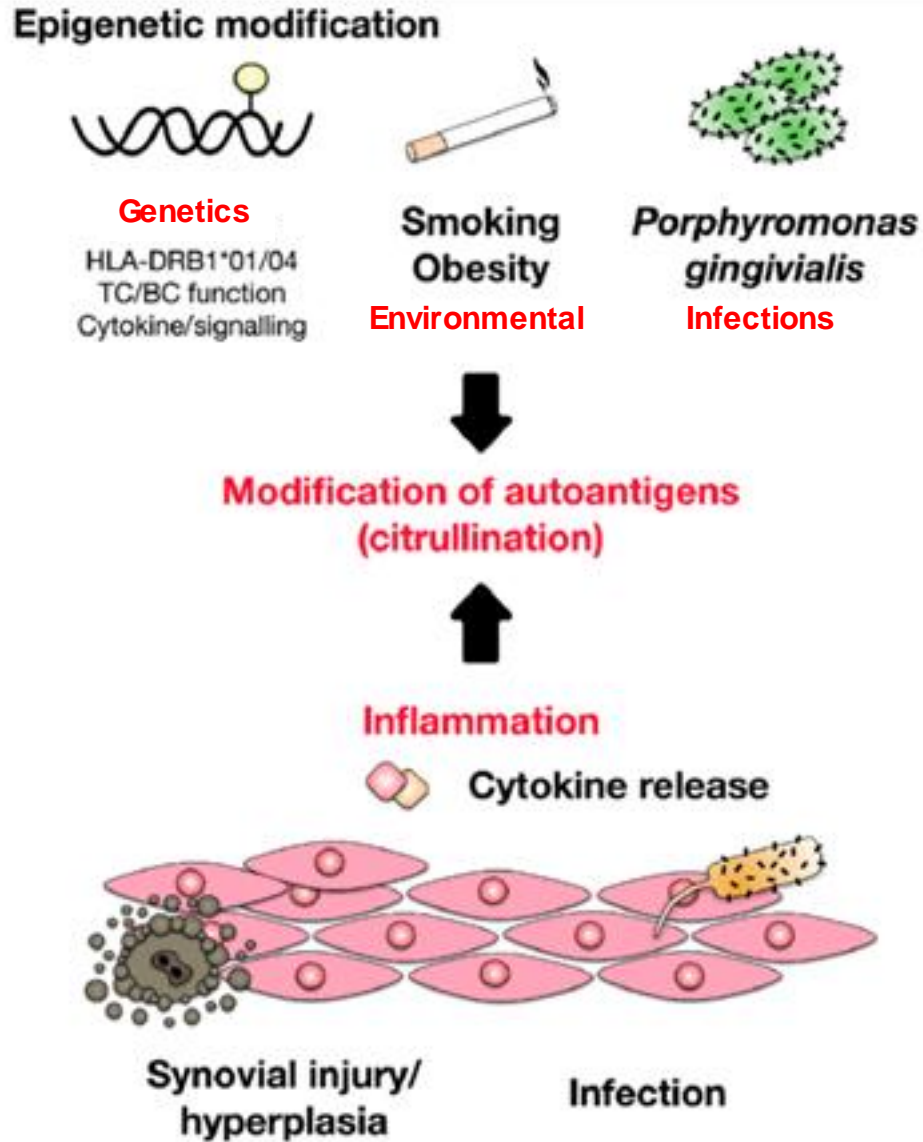
*Schett and Gravalles 2012*

# Rheumatoid Arthritis (RA)

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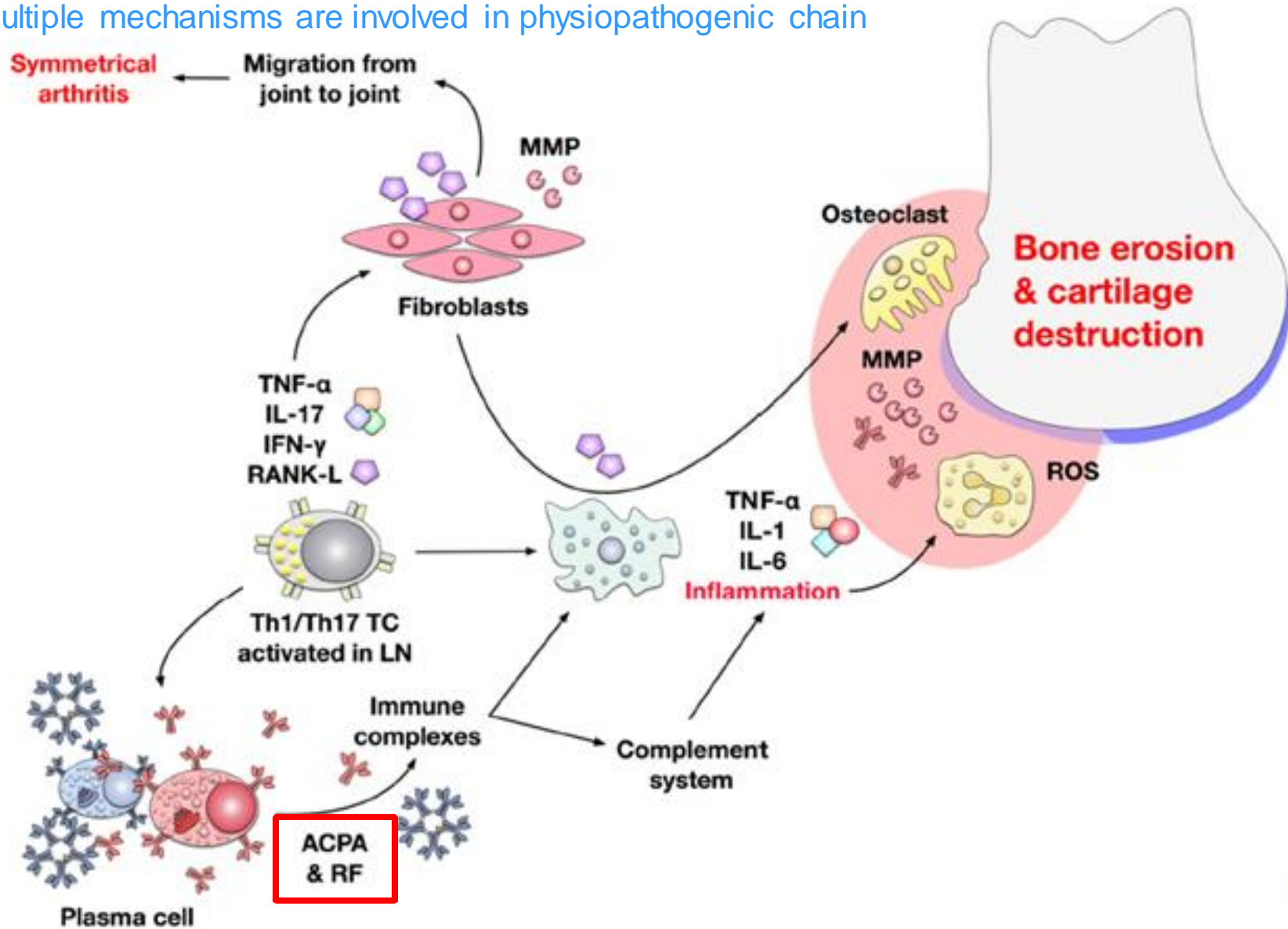
- Chronic inflammatory disease with autoimmune pathogenesis
- It affects multiple small joints (of the hands and feet) resulting in cartilage destruction and bone erosion leading to deforming and destructive arthritis and multiple systemic manifestations.
- Most prevalent chronic inflammatory diseases.
  - Prevalence ranges from 0.4% to 1.3% of the population depending on
    - sex (women are affected two to three times more often than men)
    - age (frequency of new RA diagnoses peaks in the sixth decade of life)

# Factors Contributing to RA Development



# Pathomechanism of RA

Multiple mechanisms are involved in physiopathogenic chain



# Need for Biomarkers

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Early Diagnosis

Starting treatment in early stages prevents osteo-articular destruction

Prognostic Indicators

Identifying patients at high risk for aggressive forms of RA

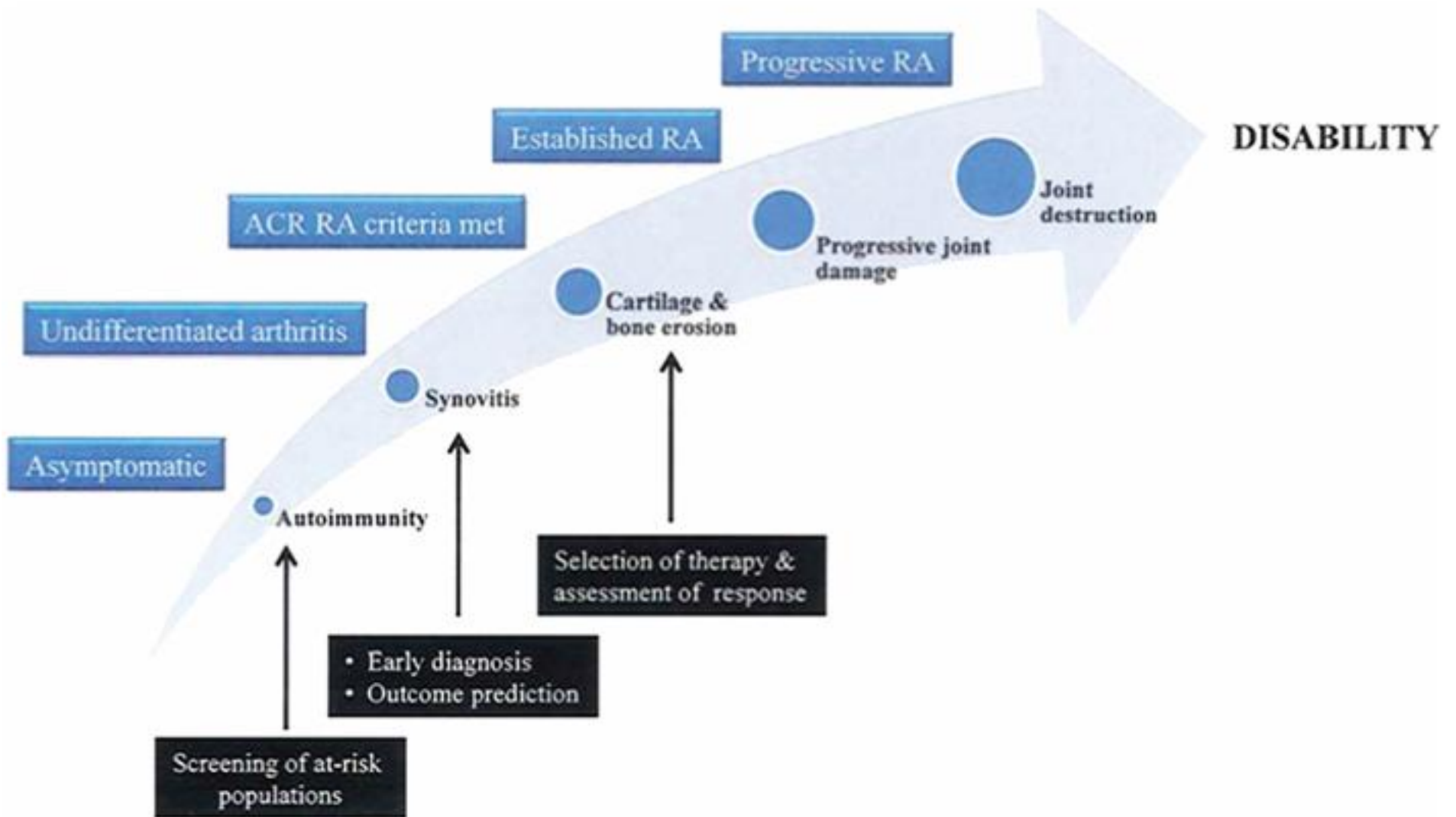
Monitoring Disease Activity

Evaluating treatment efficacy

Selection of Therapy

Predictive biomarkers of the response to treatment

# Schematic Outlining of Development & Progression of RA



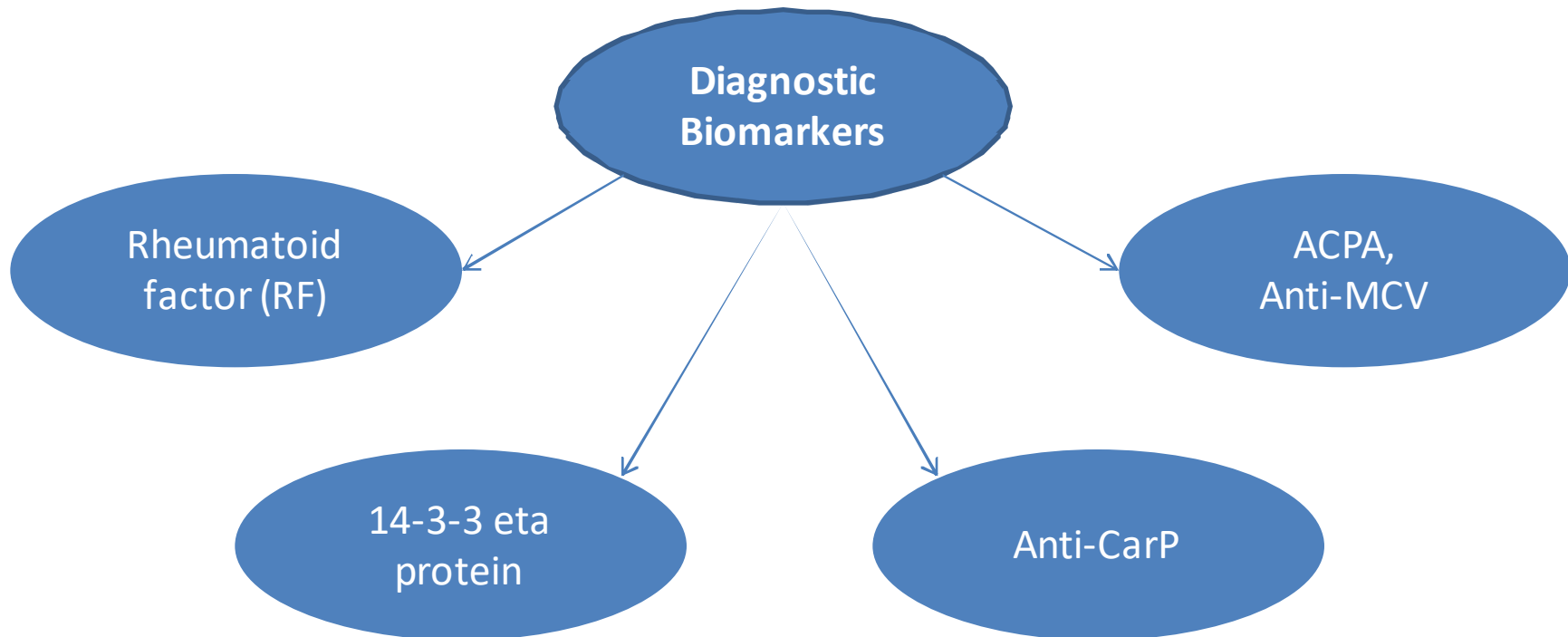
ACR - American College of Rheumatology

Lindstrom TM. Scand J Clin Lab Invest Suppl. 2010;242:79-84

# Diagnostic Biomarkers

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- The concept “window of opportunity” shows that RA identification in early stages is essential to prevent erosion and to stop progression of radiologic changes.



*Anti-CarP – antibodies against carbamylated proteins;  
Anti-MCV – antibodies against mutated citrullinated vimentin*

# ACR 1987 vs. ACR/EULAR 2010 Criteria for Diagnosis of RA

Currently, according to the EULAR 2010 criteria, RF and anti-CCP are used for RA diagnosis

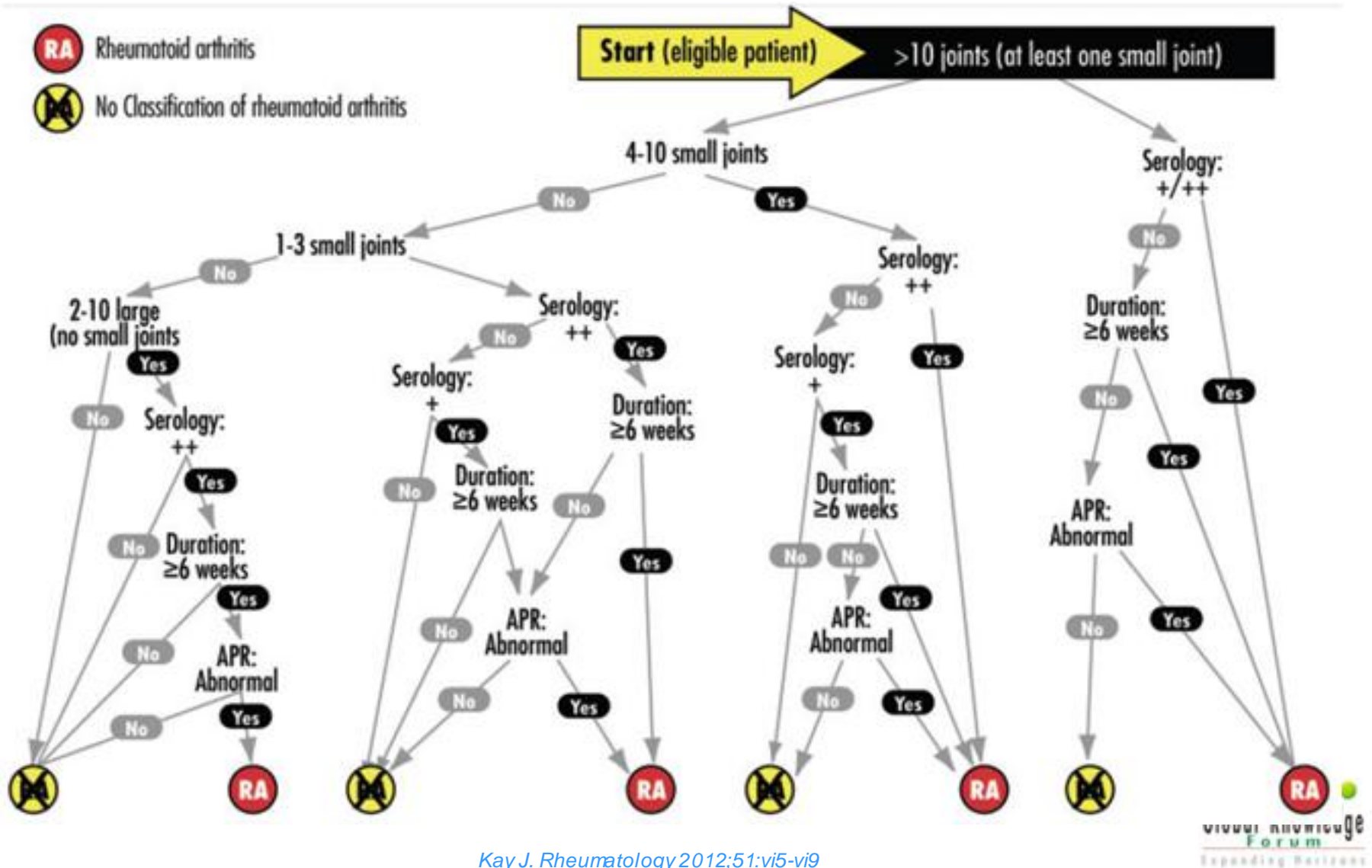
ACR 1987 criteria
○ Morning stiffness (at least 1 h)
○ Arthritis of three or more joint areas
○ Arthritis of hand joints MCF or PIJ
○ Symmetric arthritis (at least 6 weeks)
○ Rheumatoid nodules
○ Serum rheumatoid factor (RF)
○ Radiographic changes (erosions)
<b>4 criteria need to be present for the diagnosis of AR</b>

ACR/EULAR 2010 criteria	
Joint involvement	Score
1 large joint	0
2-10 large joints	1
1-3 small joints	2
4-10 small joints	3
>10 joints (at least one small joint)	5
Serology	
Negative RF and negative ACPA	0
Low-positive RF or low-positive ACPA	2
High-positive RF or high positive ACPA	3
Acute-phase reactants	
Normal CRP and normal ESR	0
Abnormal CRP or abnormal ESR	1
Duration of symptoms	
< 6 weeks	0
≥ 6 weeks	1
<b>A total score ≥ 6 is necessary for AR</b>	

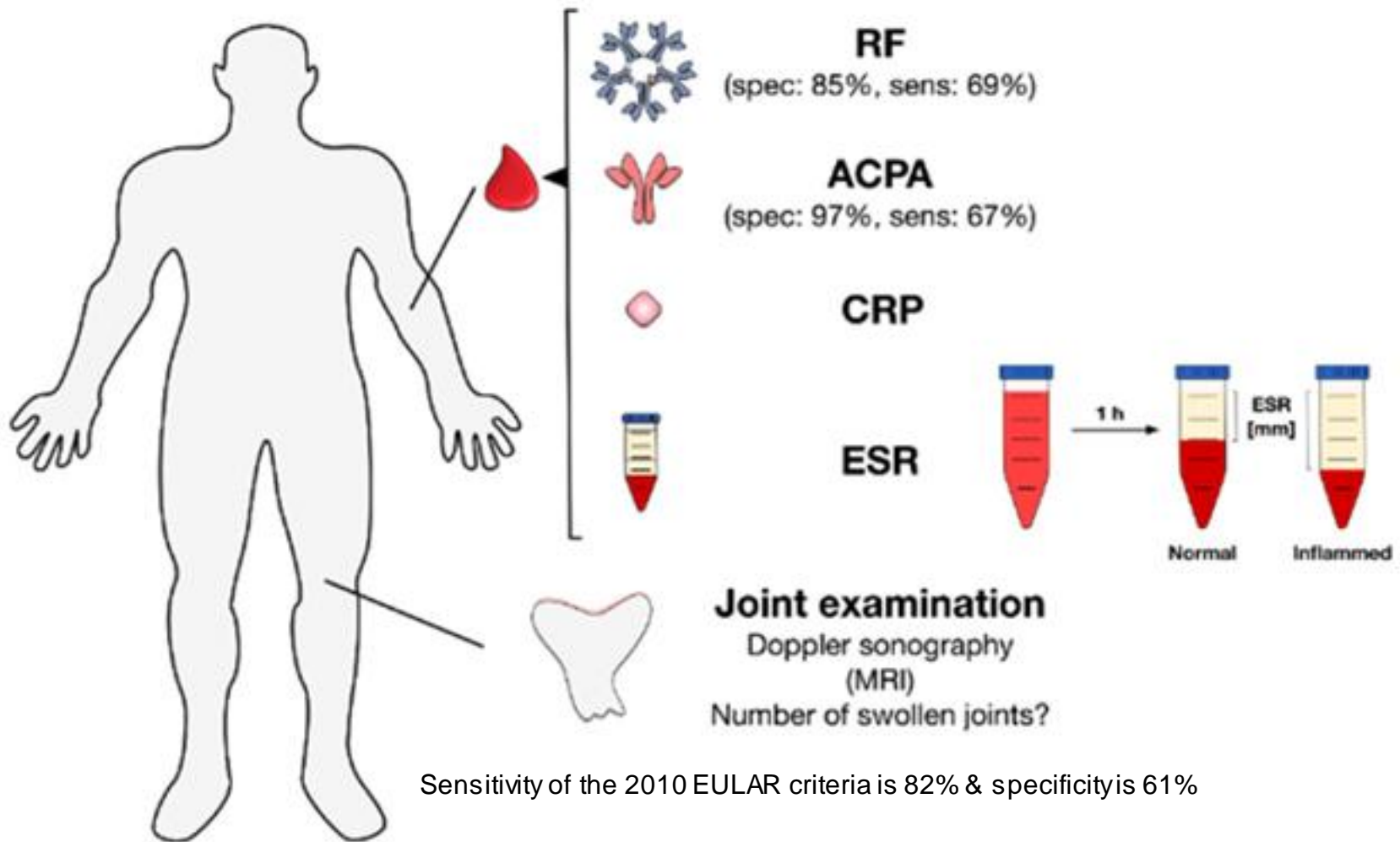
ACR - American College of Rheumatology;  
 EULAR - European League Against Rheumatism; ACPA - Anti-citrullinated protein antibodies; CRP - C-reactive protein; ESR - Erythrocyte sedimentation rate



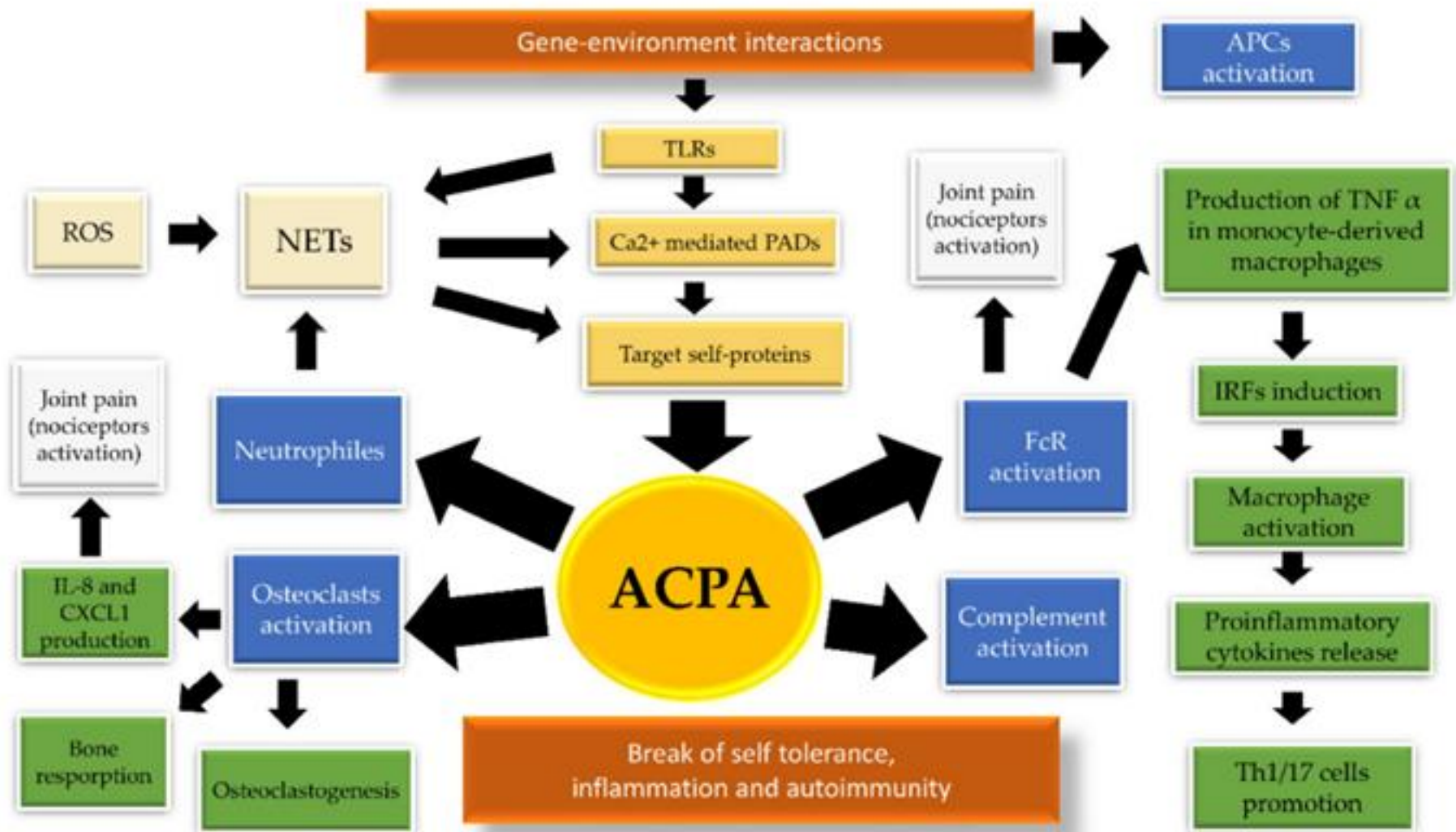
# Algorithm for Classifying Definite RA (red circles) or for Excluding its Current Presence (yellow circles)



# Clinical Parameters Frequently Used in Diagnosis of RA and their Quantification using 2010 ACR-EULAR



# Biological Effects of ACPA



Antigen presenting cells (APCs), toll-like receptors (TLRs), peptidyl-arginine-deiminase (PAD), neutrophil extracellular traps (NETs), reactive oxygen species (ROS).

# Distinct Characteristics of ACPA Compared to RF

	ACPA	RF
Isotypes	mainly IgG and IgA	IgM > IgG > IgA
Clinical association	specific for RA	common in various autoimmune diseases
N-glycosylation	extensive	limited
Germinal center reactions	repeated	limited
Somatic hypermutations	extensive	limited
Class switching	extensive	limited
B cell activation	T cell dependent	T cell dependent and/or T cell independent
Producing plasma cells	long lived plasma cells	short lived plasma cells and/or plasmablasts

## ACPA:

- Sensitivity is comparable to RF (50-75%)
- High Specificity (>95%) for RA, (Present in 70% of RA patients vs <5% of controls)
- Predictive of development of RA in undifferentiated arthritis
- May be detected in healthy individuals years before onset of clinical RA
- Marker of erosive disease

# Acute Phase Reactants/ Inflammatory Markers

- **C-reactive Protein (CRP)**

- Unaffected by factors like age, gender, and abnormalities in erythrocytes and serum proteins.
- CRP levels positively correlate both disease activity, histological changes in the synovium, and radiological progression and clinical parameters such as morning stiffness, pain, fatigue, grip strength, articular index, and disability.
- Useful marker in RA diagnosis, as well as the monitoring of disease progression and prognosis of joint damage.

- **Erythrocyte Sedimentation Rate (ESR)**

- Found in presence of inflammatory processes, infections, and autoimmune disorders (e.g., RA).
- Also seen in pregnancy, anemia, certain kidney diseases, and some cancers (e.g., lymphoma and multiple myeloma).

# Disease Activity Score (DAS28) Calculator (CRP/ESR)

## DAS 28-CRP Calculator / DAS-28 ESR Calculator

<https://www.rheumatology.org/Portals/0/Files/DAS28%20CRP%20Calculator.xls>

<https://www.rheumatology.org/Portals/0/Files/DAS28%20ESR%20Calculator.xls>

Clinical Variable	Value
Tender Joint Count (0-28)	0
Swollen Joint Count (0-28)	0
CRP (mg/L)	0
Patient Global Activity (0-100 mm)	0
Tool	Result
DAS28-CRP	0.96

Clinical Variable	Value
Tender Joint Count (0-28)	0
Swollen Joint Count (0-28)	0
ESR (mm/hr)	1
Patient Global Activity (0-100 mm)	0
Tool	Result
DAS28-ESR	0.00

	Remission	Low	Moderate	High
	< 2.6	≥ 2.6 - < 3.2	≥ 3.2 - ≤ 5.1	> 5.1

## Other Diagnostic Biomarkers

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- **Mutated Citrullinated Vimentin Antibodies (Anti-MCV)**

- There is no difference between anti-MCV and anti-CCP for RA diagnosis. Thus, the anti-MCV may be second line of test, used in patients suspected of RA, but with anti-CCP and RF negative.

- **14-3-3 eta Protein**

- 14-3-3 eta protein sensitivity and specificity for RA was 77% and 93% respectively.
- In early stages of the disease, determination of protein 14-3-3 eta along with RF and anti-CCP increases the diagnostic rate from 72% (RF + anti-CCP) to 78% (RF + anti-CCP + 14-3-3eta).

- **Antibodies Against Carbamylated Proteins (Anti-CarP)**

- Anti-CarP has sensitivity of 44% and a specificity of 89% compared with 54% and 96% for anti-CCP and 59% and 91% for RF respectively.

# Prognostic Biomarkers

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- **RF** presence and high titers correlates with –
  - Increased risk of developing RA
  - Develop more aggressive forms of the disease
  - More severe functional impairment
    - The risk may increase even 26 times if the initial titers of RF are > 100 IU/ml.
    - The presence of the IgA isotype is associated with extra-articular manifestations.
- **Anti-CCP** presence at diagnosis is associated with
  - More important radiological progression
  - Severe forms of disease
- Presence of other biomarkers (**anti-MCV, 14-3-3 eta protein**) is associated with more severe forms of RA but these tests are not commonly used.



# Biomarkers for Monitoring Disease Activity

- In the “treat to target” recommendations, 3 composite scores for monitoring of the disease evolution are used –
  - Disease activity score (DAS 28)
  - Simple disease activity index (SDAI)
  - Clinical disease activity index (CDAI)
- **Multi-biomarkers Disease Activity (MBDA) Test Parameters** –
  - Disease activity can be classified into mild (score 1-28), moderate (score 29-43) or severe (score >44)

Parameters	DAS 28	CDAI	SDAI	MBDA
Tender Joints	Yes	Yes	Yes	Acute phase reactants: <b>CRP, SAA</b>
Swollen Joints	Yes	Yes	Yes	Adhesion molecules: <b>VCAM-1</b>
Patient Global Assessment of Disease Activity	Yes	Yes	Yes	Cytokines and related proteins: <b>IL-6 &amp; TNF-RI</b>
Clinician Global Assessment of Disease Activity		Yes	Yes	Matrix metalloproteinases (MMP): <b>MMP-1 &amp; MMP-2</b>
ESR or CRP	Yes		Yes	<b>Human cartilage glycoprotein 39</b>

SAA - serum amyloid A, VCAM-1 - vascular cell adhesion molecule-1, IL-6 - interleukin-6, TNF-R1 - tumor necrosis factor receptor 1, EGF - epidermal growth factor, VEGF-A - vascular endothelial growth factor A

Growth factors: **EGF & VEGF-A**  
Hormones: **leptin & resistin**



# Predictive Biomarkers of Response to Biologic Therapy

Biomarker	Presence(P)/absence(A)	Predictive role	Therapy
FR, Anti-CCP	Neither P or A	-	Anti-TNF
FR, Anti-CCP	P	++	RTX
Anti-MCV	Neither P or A	-	Anti-TNF
Anti-MCV	P	+	RTX
14-3-3eta	A or low levels	+	TCZ, anti-TNF
COMP	A or low levels	+	ADA
Calprotectin	P	+	ADA, IFX, RTX
Survivin	A or low levels	+	IFX

Rheumatoid factor (FR), Antibodies directed to cyclic citrullinated peptides (Anti-CCP), Antibodies against mutated citrullinated vimentin (Anti-MCV), Cartilage oligomeric matrix protein (COMP) failed to predict (-), Confirmed by small studies, require testing on larger groups (+) confirmed by several studies (++) Anti tumor necrosis factor (Anti-TNF), Adalimumab (ADA), Infliximab (IFX) Rituximab (RTX), Tocilizumab (TCZ)

# Biomarkers in RA-associated Interstitial Lung Disease (ILD)

Name	Source	Evidence	Utility Within RA-ILD
RF	Serum, sputum, BALF	Low specificity, higher titers associated with pulmonary involvement of RA	Diagnosis
ACPA	Serum, sputum, BALF	High specificity for RA diagnosis, higher titers associated with pulmonary involvement of RA	Diagnosis
PAD	Synovial tissue	Possible amplification of disease severity	Severity
HSP90/70	Serum and BALF	Elevated in patients with RA-ILD, not patients with RA	Diagnosis
MMP7	Serum	Elevated in patients with RA-ILD and not in patients who had RA without ILD; suggestive of fibrotic ILD	Diagnosis
CXCL10	Serum	Elevated in patients with RA-ILD and not in patients who had RA without ILD	Diagnosis
KL-6/MUC1	Serum	Correlation with severity of RA-ILD on computed tomography	Severity
LOXL2	Serum	Higher with shorter disease duration; does not differentiate between RA patients with and without ILD	Specificity for diagnosis
Anti-CEP-1	Serum	Marker of RA-ILD in patients with synovial disease	Diagnosis
MUC5B	Genetic sequencing	Marker suggesting RA-ILD, specifically in those who have RA and UIP pattern on HRCT	Diagnosis

*PAD, peptidylarginine deiminases; HSP90/70, citrullinated heat shock protein 90/70; MMP7, matrix metalloproteinase-7; CXCL 10, C-X-C motif chemokine 10; KL-6/MUC1, Krebs von den Lungen 6; LOXL2, lysyl oxidase-like 2; anti-CEP-1, anti-citrullinated alpha enolase peptide-1; MUC5B, mucin 5B; BALF, bronchoalveolar lavage fluid; UIP, usual interstitial pneumonia; HRCT, high-resolution computed tomography.*

# Tests Done in SRL

TEST	METHOD	CODE
Rheumatoid Arthritis Panel (ANA, RF, Anti-CCP)	Nephelometry	5008
Rheumatoid Factor (RF) Antibodies		1540D
Anti-Cyclic Citrullinated Peptide Antibodies (Anti-CCP)	Fluoroenzyme Immunoassay	3301
RA Total (Anti-CCP, ANA, CRP, RF)	Nephelometry/CMIA/EIA	4635M
Arthritis Panel-1 (CBC, ESR, Protein electrophoresis, CRP, Uric Acid, ANA, ASO, RF & Urine routine)	Automated Cell Counter/ Automated Photometrical Capillary Stopped Flow Kinetic Analysis/ Manual Modified Westergren/ SPE/ LPA/ Spectrophotometry/ Immunofluorescent Antibody/ Dipstick/ Microscopy/ Nephelometry/ Electrophoresis/ IFA)	1576
Arthritis Panel-2 (CBC, ESR, CRP, Uric Acid, ANA, ASO, RF & Urine routine)		1582



Thank You

