Clinical Chemistry

Trainee Council

PEARLS OF LAB MEDICINE

Rheumatoid Arthritis

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Rheumatoid arthritis

Systemic autoimmune disease
- 1% in the general population

Inflammation of the synovial membrane of peripheral joints
- Joint swelling
- Stiffness
- Tenderness
- Joint destruction
- Long term disability

Extra-articular manifestations
- Pulmonary fibrosis
- Coronary artery disease
- Osteoporosis
- Vasculitis

Female to male ratio 3:1

Increased mortality in patients with rheumatoid arthritis compared with the general population

Pathogenesis of Rheumatoid arthritis

Inflammation of the synovial membrane of the joint (synovitis)

Early stages of rheumatoid arthritis

- Hyperplastic synovial membrane
- Angiogenesis
- Cellular infiltration
  - $CD4^+$ T cells
  - B cells
  - Neutrophils

Late stages of rheumatoid arthritis

- Pannus formation
- Invasion of cartilage and bone

Immune-mediated mechanisms of Rheumatoid Arthritis

Increased infiltration of **CD4⁺ T cells** in the synovial membrane

- Stimulate macrophages to produce and secrete cytokines
  - TNF-α
  - IL-1
  - IL-6
- Stimulate fibroblasts to secrete matrix metalloproteinases
- Stimulate B cells to produce auto-antibodies
  - Rheumatoid factor
- Stimulate osteoclasts ⟹ bone and cartilage erosion
- Rank-L (receptor activator of NK-κB ligand): regulates osteoclastogenesis

Immune-mediated mechanisms of Rheumatoid Arthritis

Differentiation pathways of T_H17 and Treg from a common progenitor (T_H0)

New cell types in immunopathogenesis of RA

T_H17 cells

- IL-17 (secreted by T_H17 cells) induces osteoclastogenesis which can lead to joint destruction
- Animal models
  - Collagen induced arthritis is suppressed in mice deficient in IL-17

T regulatory cells (Treg)

- Treg in RA patients express reduced levels of FoxP3 – treatment with anti-TNFα reverses Treg function
- Animal models
  - Treg depletion in mice leads to exacerbation of arthritis

Risk factors associated with Rheumatoid Arthritis

Infectious agents

Bacteria, Parvovirus B19, Retroviruses, Epstein-Barr virus

Genetic factors

- HLA-DR4, HLA-DR1

  HLA-DRB1 molecules containing certain conserved amino acids at positions 70 – 74 (“shared epitope” hypothesis)
- Protein tyrosine phosphatase N22 (PTN22)

  Missense single nucleotide polymorphism (R620W) is associated with RA

Environmental factors

Smoking (especially in individuals who carry the shared epitope)

ACPA+ **ACPA-**	different groups of RA patients

Symptoms and signs of Rheumatoid Arthritis

Clinical symptoms of Rheumatoid Arthritis

- Patients present with morning joint pain and stiffness
- Physical examination: symmetric joint swelling

Extra-articular manifestations

- General symptoms
  fever, fatigue, weakness, anorexia, weight loss, anemia
- Pleuritis, interstitial pneumonitis
- Pericarditis, myocarditis
- Nephritis
- Vasculitis
- Osteoporosis

The 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for rheumatoid arthritis*

Joint involvement

- 1 large joint *(score 0)*
- 2 – 10 large joints *(score 1)*
- 1 – 3 small joints *(score 2)*
- 4 – 10 small joints *(score 3)*
- > 10 joints (at least 1 small joint) *(score 5)*

Serology

- Negative RF and negative anti-CCP *(score 0)*
- Low-positive RF or low-positive anti-CCP *(score 2)*
- High-positive RF or high-positive anti-CCP *(score 3)*

Acute-phase reactants

- Normal CRP and ESR *(score 0)*
- Abnormal CRP or ESR *(score 1)*

Duration of symptoms

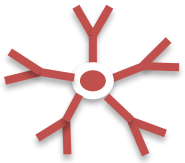
- < 6 weeks *(score 0)*
- ≥ 6 weeks *(score 1)*

A score of **6 and more** is needed to classify a patient as having rheumatoid arthritis

** Aletaha D, Neogi T, Silman AJ et al. Arthritis Rheum. 2010;62(9):2569-81*

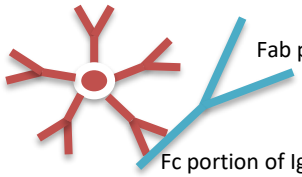
Rheumatoid Factor

IgM molecule



IgG molecule

RF = IgM anti-IgG



Auto-antibody that reacts with the Fc portion of normal polyclonal IgG

IgM is the most common class

65 – 75% sensitive

Not specific for Rheumatoid Arthritis
- Specificity for RA is 75 – 93%

3 – 5% in normal population
- More in elderly individuals

<50% in early Rheumatoid Arthritis

Laboratory Assays for Rheumatoid Factor

Sheep erythrocyte agglutination assay
- Sensitive but has high false positive rates

ELISA methods
- Can measure IgM, IgG, or IgA
- Quantitative results (IU/ml)

Nephelometric methods
- Rate nephelometry

Great precision
Can measure low positive values

Assay interferences
- C1q
- Fibrin/fibrinogen in plasma
- Heterophile antibodies

Rheumatoid Factor

Clinical significance of Rheumatoid Factor

Only serologic test that is included in both 1987 and 2010 classification criteria for RA

Specificity is increased when > 50 IU/ml

High levels correlate with radiographic joint destruction and extra-articular manifestations

RF levels may correlate with the degree of inflammation and disease activity (used to monitor disease activity in combination with other inflammatory markers)

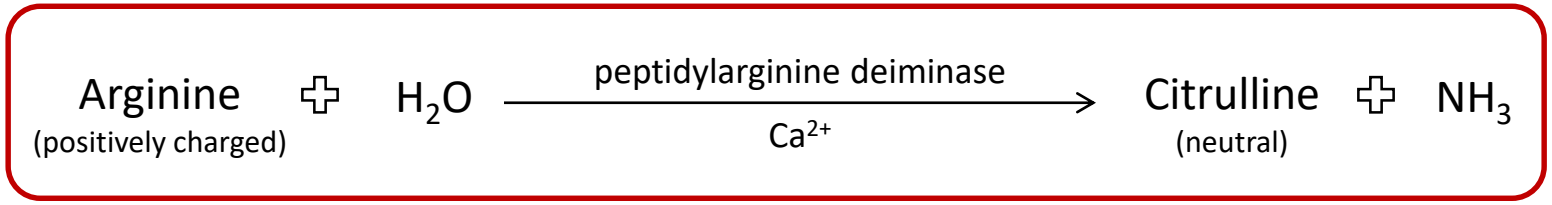
Anti-filaggrin antibodies

Filaggrin (filament aggregating protein) is involved in the organization of cytoskeletal structures of epithelial cells

- **Anti-perinuclear factor (APF) antibody**
  Binds to filaggrin present in cytoplasmic granules of buccal mucosal cells

- **Anti-keratin (AKA) antibody**
  Binds to filaggrin in epithelial cells or rat esophagus

Filaggrin has arginine residues that can be enzymatically deimidated to citrulline residues (citrullination)



This post-translational modification is essential for the auto-antigenicity of filaggrin

Anti-cyclic citrullinated peptide antibodies

Cyclic citrullinated peptides

- Synthetic peptides in which the amino- and carboxy-terminal of a linear polypeptide chain are linked together to form a circular chain
- Citrulline residue easily available to antibodies

Anti-cyclic citrullinated protein assays (anti-CCP)

	Sensitivity	Specificity
anti-CCP1	44 – 56%	90 – 97%
anti-CCP2	64 – 89%	88 – 99%
anti-CCP3	61 – 83%	93 – 98%

Anti-cyclic citrullinated peptide antibodies

Comparison of sensitivity and specificity between RF and anti-CCP

	Sensitivity	Specificity
anti-CCP3	61 – 83%	93 – 98%
Rheumatoid Factor	65 –75%	75 – 93%

Addition of anti-CCP assay improves the diagnosis of RA

Anti-CCP antibodies

- Appear earlier in RA
- Can identify patients who will benefit from early aggressive therapy

Treatment options for Rheumatoid Arthritis

Disease-modifying anti-rheumatic drugs (DMARDs)

Synthetic DMARDs

- High efficacy – first line treatment: steroids, sulfasalazine, methotrexate, leflunomide
- Low efficacy (high toxicity): gold salts, azathioprine, chloroquine, cyclophasphamide, 6-mercaptopurine, D-penicillamine, minocycline, chlorambucille

Biologic DMARDs

Anti-TNF-α agents, CD28/B7 T cell co-stimulation target, $CD20^+$ B cell target, IL-6 receptor target, IL-1 receptor target

References

1. Aletaha D *et al*. 2010 Rheumatoid arthritis cassification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010;69:1580-1588
2. Choy E and Panayi G. Cytokine pathways and joint inflammation in rheumatoid arthritis. *N Engl J Med* 2001;344(12):907-915
3. Anderson A *et al*. Recent developments in the immunobiology or rheumatoid arthritis. *Arthritis Research & Therapy* 2008;10:204-212
4. Bax M *et al*. Genetics of rheumatoid arthritis; what have we learned? *Immunogenetics* 2011;63:459-466
5. van Boekel M *et al*. Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. *Arthritis Res* 2002;4:87-93
6. Nielwood T *et al*. Anti-CCP antibody testing as a diagnostic and prognostic tool in rheumatoid arthritis. *Q J Med* 2007;100:193-201
7. Taylor P *et al*. A systematic review of serum biomarkers anti-cyclic citrullinated peptide and rheumatoid factor as tests for rheumatoid arthritis. *Autoimmune Diseases* 2011; 2011:1-18
8. Agarwal S. Biologic agents in Rheumatoid arthritis: an update of managed care professionals. *JMCP* 2011;17:S14-S18