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**TITLE: Rheumatoid Arthritis**

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**Slide 1:**
Hello, my name is Dr. Evangelos Ntrivalas. I am the Clinical Laboratory Director at the Foundation for Blood Research in Scarborough, ME. Welcome to this Pearl of Laboratory Medicine on "Rheumatoid Arthritis."

**Slide 2:**
Rheumatoid arthritis is a systemic autoimmune disease with a prevalence of about 1% in the general population. It is characterized by inflammation of the synovial membrane of peripheral joints, which can lead to joint swelling, stiffness, and tenderness, and over time, if left untreated, it can lead to joint destruction and long-term disability. Rheumatoid arthritis may also present with extra-articular manifestations, including pulmonary fibrosis, coronary artery disease, osteoporosis, and vasculitis. Rheumatoid arthritis can affect people of all ages, but is most common between the fourth and seventh decades of life, with a female to male ratio of 3:1. Of note, there is increased mortality in patients with rheumatoid arthritis as compared to the mortality in the general population.

**Slide 3:**
The major pathophysiologic characteristic of rheumatoid arthritis is inflammation of the synovial membrane of the joint (synovitis). In a normal joint, the synovial membrane lines the joint capsule and it consists of a thin layer of synovial macrophages and fibroblasts, with an underlying connective tissue, blood vessels and a few immune cells (mostly mast cells and neutrophils). In early rheumatoid arthritis, the synovial membrane becomes hyperplastic and angiogenesis is initiated. There is also an increased cellular infiltration of immune cells (particularly CD4+ T cells, B cells, and neutrophils). In late stages of rheumatoid arthritis, the synovial membrane is transformed into an inflammatory tissue, the pannus, which invades and destroys the adjacent cartilage and bone, through the activation of osteoclasts.

**Slide 4:**
The immunopathogenic mechanisms of the inflammatory response in rheumatoid arthritis are characterized, as stated before, by an increased infiltration of CD4+ T cells in the synovial membrane.

Antigen-activated CD4+ T cells can stimulate the local macrophages to produce and secrete the cytokines TNF-α, IL-1 and IL-6 and fibroblasts to secrete matrix metalloproteinases. These factors further activate local cells to secrete additional inflammatory cytokines and chemokines that can further recruit more immune cells. The end result of this vicious cycle leads to the formation of the inflammatory pannus. Antigen-activated CD4+ T cells can also stimulate the locally recruited B cells to produce auto-antibodies, such as the rheumatoid factor. There is increasing evidence showing that B cells have a major pathogenic role in rheumatoid arthritis, which is further supported by studies showing the efficacy of pharmacologic agents that target B cells in rheumatoid arthritis. Lastly, the antigen-activated CD4+ T cells can stimulate osteoclasts which can lead to bone and cartilage erosion. A new member of the TNF family – the Rank-L – has been identified to have a role in the regulation of osteoclastogenesis in rheumatoid arthritis patients.

**Slide 5:**

Recent studies depicted the role of other T cell types in rheumatoid arthritis. Two of these are the TH17 and the T regulatory cells that derive by the same TH0 progenitor under the influence of distinct cytokines. TH17 cells secrete IL-17, a proinflammatory cytokine that has a pathogenic role in a number of autoimmune diseases such as Lyme disease, multiple sclerosis and systemic lupus erythematosus. Studies have shown that this cytokine has also a pathogenic role in rheumatoid arthritis, inducing osteoclastogenesis which can lead to joint destruction. In animal models, it was shown that collagen induced arthritis is suppressed in IL-17 deficient mice. On the other hand, the T regulatory cells have an immunosuppressive role and participate in immune tolerance. In rheumatoid arthritis patients, these cells express low levels of FoxP3; the molecule which is required for their function. Interestingly, it was shown that treatment with anti-TNF-α agents reverses the Treg function to its normal immunosuppressive role by inducing FoxP3. Also, animal models have shown that depletion of T regulatory cells in mice leads to exacerbation of rheumatoid arthritis, a finding that further supports the useful role of these cells in this disease.

**Slide 6:**

It is clear that rheumatoid arthritis is an autoimmune inflammatory response. What triggers this inflammation is still under investigation. There are not so far clear evidences for infectious agents but efforts have been made to assess their role in the pathogenesis of rheumatoid arthritis. There are some known, though, genetic factors that predispose to rheumatoid arthritis. More specifically, HLA-DRB1 molecules containing certain conserved amino acids at positions 70-74 (the so-called “shared epitope”) have been associated with increased risk of rheumatoid arthritis. It is hypothesized that citrullinated peptides can bind to this shared epitope and be presented to T cells. Another genetic risk factor that has been associated with rheumatoid arthritis is the protein tyrosine phosphatase N22 gene which encodes a phosphatase responsible for T cell activation. A single nucleotide polymorphism at position 620 of this gene has been found in patients with rheumatoid arthritis.

Additionally, environmental factors, such as smoking, have been linked to rheumatoid arthritis, especially in individuals who carry the shared epitope. It has to be noted that with the discovery of the anti-citrullinated protein antibodies (ACPA), two groups of rheumatoid arthritis patients are recognized, the ACPA+ and the ACPA-. These two groups have different pathogenic and clinical characteristics and also differ in prognosis.

**Slide 7:**
The diagnosis of rheumatoid arthritis is primarily clinical. Patients present with a history of morning joint pain and stiffness and the physical examination reveals symmetric joint swelling. Patients can also present with a number of extra-articular manifestations, including fever, fatigue, unexplained weakness and weight loss and anemia. Symptoms can also be related to specific organs such as lungs (pleuritis and interstitial pneumonitis), heart (pericarditis and myocarditis) and kidneys (nephritis). Patients also can present with symptoms of vasculitis and osteoporosis. The clinical findings can be further supported by radiographic and immunologic tests.

**Slide 8:**
In 2010, the American College of Rheumatology and the European League Against Rheumatism published revised classification criteria for rheumatoid arthritis, to improve the already adopted 1987 criteria. These new criteria are based on a scoring system of four domains that include (1) the joints that are involved; (2) the serologic tests of the auto-antibodies that may be present in rheumatoid arthritis (rheumatoid factor and anti-cyclic citrullinated peptide antibodies); (3) the inflammatory markers C-reactive protein and erythrocyte sedimentation rate; and (4) the duration of the symptoms. The target population includes patients who present with at least 1 joint with definitive clinical synovitis that cannot be better explained by another disease. According to the scoring system of these criteria, patients are considered to have rheumatoid arthritis if they have a score of at least 6 or more. It should be pointed out that these new criteria were developed to help identify patients presenting with early symptoms of rheumatoid arthritis, who will benefit from treatment.

**Slide 9:**
It is obvious from the published criteria that the immunology laboratory has a significant role in identifying patients with rheumatoid arthritis. One laboratory marker that appears in both 1987 and 2010 criteria is the Rheumatoid Factor. Rheumatoid factor is an auto-antibody that reacts with the Fc portion of normal polyclonal IgG. Rheumatoid factor can be IgM, IgG, or IgA, with the most common being the IgM anti-IgG. Its sensitivity for rheumatoid arthritis is around 65-75%. Rheumatoid factor, though, has a limited specificity (75-93%) for rheumatoid arthritis, since it can be found in patients with other auto-immune diseases (such as Sjögren's syndrome, systemic lupus erythematosus, and mixed connective tissue disease) and also infectious diseases (hepatitis C and tuberculosis). Additionally, 3-5% of the normal healthy population may have rheumatoid factor and this percentage increases with age (10-30% of healthy elderly individuals may have rheumatoid factor as a laboratory finding). Another disadvantage of the rheumatoid factor is that it is present in less than 50% of patients with early rheumatoid arthritis.

**Slide 10:**
There are different immunological assays to measure rheumatoid factor in serum. The first assay that was described was the sheep erythrocyte agglutination assay. This assay relies on the agglutinating properties of the IgM rheumatoid factor. Sheep erythrocytes that are coated with rabbit IgG are incubated with patient's serum. If the serum contains IgM rheumatoid factor, this can bind to the IgG on the erythrocytes causing them to agglutinate. Newer agglutination methods use particles other that sheep erythrocytes, such as latex and charcoal. The latex agglutination test is sensitive, but it has a high

false positive rate. ELISA methods can also measure IgM rheumatoid factor. An advantage of ELISAs is that they can measure also other classes of rheumatoid factor, such as IgG and IgA. The results of the ELISA are quantitative and are expressed as IU/ml. Lastly, newer methods using the concepts of rate nephelometry have been used to measure rheumatoid factor. In this method, when antigen and antibody are mixed, under antibody excess conditions, antigen-antibody complexes are formed and their concentration can be determined by light scatter. In general, ELISA and nephelometric techniques show a greater precision and can be used to measure low positive results. Caution should be taken with factors that may interfere with rheumatoid factor measurement. It is known that high levels of C1q, which is an acute phase reactant that is present in inflammatory disorders, can give false positive results for rheumatoid factor in agglutination and nephelometric assays. Likewise, the presence of fibrin or fibrinogen in plasma or incompletely clotted serum can yield false positive or false negative results for rheumatoid factor in agglutination and nephelometric assays. Lastly, the presence of heterophile antibodies may yield false results in ELISA methods for rheumatoid factor.

**Slide 11:**

As mentioned before, rheumatoid factor, although non-specific for rheumatoid arthritis, is the only serologic test that was included in the 1987 criteria and still included in the 2010 criteria for rheumatoid arthritis classification. Its specificity for rheumatoid arthritis is increased when it is measured in high titers (more than 50 IU/ml). Its specificity can be enhanced further if it is the IgA isotype. High levels of rheumatoid factor (higher than 3 times the upper limit of normal for the laboratory and assay) have been shown to correlate with radiographic joint destruction and extra-articular manifestations. Also, rheumatoid factor has been used to monitor disease activity (in combination with other inflammatory markers of rheumatoid arthritis).

**Slide 12:**

The relatively low specificity of rheumatoid factor created the need for identification of new laboratory markers for rheumatoid arthritis. This was further facilitated by the prior identification of two different antibodies that were found to recognize the same antigen. This antigen is filaggrin (or filament aggregating protein) which is a protein involved in the organization of cytoskeletal structures of epithelial cells. The first antibody was originally identified in 1964 and it was named anti-perinuclear factor antibody, since it reacted with a protein component present in cytoplasmic granules of differentiated buccal mucosal cells. In 1979, another antibody was described that reacted with keratin-like structures of rat esophagus; hence it was named anti-keratin antibody. Both of these antibodies had acceptable sensitivity and higher specificity than rheumatoid factor, and, as stated before, both of them target filaggrin. An interesting feature about filaggrin is that it has arginine residues that can be enzymatically deimidated to citrulline residues by the enzyme peptidylarginine deimidase (PAD). It was found that this post-translational modification is essential for the auto-antigenicity of filaggrin.

**Slide 13:**

The epitope recognition of these antibodies was improved by the development of cyclic citrullinated peptides that made the citrulline residue more easily accessible to them. These assays are referred to as anti-cyclic citrullinated protein assays (anti-CCP). The initial assays (anti-CCP1) were based on a synthetic circular peptide that detected IgG antibodies with a specificity for rheumatoid arthritis that ranged from

90% to 97%, but only moderate sensitivity (44-56%). Subsequently, second generation anti-CCP2 assays were developed using better epitopes. Sensitivity was improved to 64-89% and the specificity ranged from 88% to 99%. The 3rd generation anti-CCP assays used a refining epitope and incorporated a dual conjugate that detects both IgG and IgA antibodies. These improved the test performance by increasing the clinical sensitivity by 5% (and by additional 10% in patients with early onset rheumatoid arthritis) while maintained a specificity of 98%. All of these assays are ELISA based.

**Slide 14:**

The implementation of anti-CCP assays improved the diagnosis of rheumatoid arthritis; the new 2010 criteria include anti-CCP assays – in addition to RF – as a serologic marker for the disease. Anti-CCP assays show better specificity compared to rheumatoid factor. Another important feature of the anti-CCP assays lies on the fact that these auto-antibodies may appear early in pre-clinical stages of rheumatoid arthritis. In older patients, anti-CCP antibodies can be detected before the development of clinical symptoms, whereas in younger patients, these auto-antibodies can be detected close to the onset of the disease. In both cases, though, anti-CCP antibodies can serve as a prognostic indicator of the development of rheumatoid arthritis. Of note, these auto-antibodies can also identify patients who will benefit from early aggressive therapy, since their presence has been correlated to an increased risk for erosive disease.

**Slide 15:**

It is clear that identifying and treating early these patients will improve the clinical outcome. The therapeutic approach for rheumatoid arthritis includes the disease-modifying anti-rheumatic drugs which can be either synthetic or biologic. The first line of treatment includes steroids, sulfasalazine, methotrexate, and leflunomide. There are also various biologic agents, with the most prominent being the anti-TNF-α agents, that are used in the treatment of rheumatoid arthritis. There is also ongoing research in identifying new biomarkers that can be used in early diagnosis and can also predict response to therapy.

**Slide 16: References**

**Slide 17:**

Thank you for joining me on this Pearl of Laboratory Medicine on “Rheumatoid Arthritis.” I am Evangelos Ntrivalas.