3 Laboratory Interpretation of Rheumatic Diseases

Altaf Abdulkhaliq and Manal Alotaibi

3.1 Introduction

Generally the diagnosis of rheumatic diseases is based on a set of clinical, serological, and radiological measures. The discovery of a novel test that appears to be considerably more disease-specific and preferably sensitive would be of value for the early diagnosis and immediate, effective therapy to prevent joint deterioration, functional disability, and unfavorable disease outcome [1].

However, components of acute phase reaction proteins such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) or rheumatoid factor (RF) lack specificity and sensitivity and could not reach the expectation of earlier diagnosis of specific rheumatic diseases. Therefore, the discovery of immunologic laboratory tests has occupied a valued position in the practice of rheumatology and has helped define the pathophysiology of various rheumatic conditions such as the immunologic basis of rheumatoid arthritis (RA) [2, 3] and explain the contribution of genetic basis to autoimmune disease via the association of ankylosing spondylitis (AS) with HLA-B27 and RA with certain HLA-DR alleles [4, 5].

Hence the salient existence of such immunologic laboratory tests has assisted the more precise diagnosis of diverse rheumatologic conditions that may share some clinical characteristics. In addition, these tests can provide valuable evidence concerning disease manifestation, activity and prognosis, and therapeutic monitoring.

Essential terms concerning the laboratory tests are needed to be defined such as sensitivity, specificity, and positive and negative predictive values. *Sensitivity* refers to the ability of the test to detect the proportion of patients with a disease which usually have a positive test result. However, *specificity* refers to the ability of the test to detect the proportion of patients without the disease which usually have a negative test result. *Predictive value* refers to the likelihood of disease or non-disease based on a positive or negative test result. A high positive predictive value test indicates that the patient with a positive test result most probably has the disease in question. Similarly, a high negative predictive value test indicates that the patient with a negative test result most likely does not have the disease in question.

Unlike with sensitivity and specificity of the test, the predictive value is markedly affected by disease prevalence. For instance, the predictive value of a positive rheumatologic test in patients with polyarthralgia is likely to be higher in a rheumatology clinic than in a family physician's clinic [6].

A. Abdulkhaliq (✉)
College of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia
e-mail: aaabdulkhaliq@uqu.edu.sa

M. Alotaibi
Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Internal Medicine Department, College of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia

© The Author(s) 2021
H. Almoallim, M. Cheikh (eds.), *Skills in Rheumatology*,
https://doi.org/10.1007/978-981-15-8323-0_3

The subsequent sections will discuss the stepwise approach to the diagnostic workup of rheumatic diseases and are presented as follows:

- Inflammatory markers (ESR and CRP)
- Rheumatoid factor (RF)
- Antinuclear antibody (ANA) profile, for instance, anti-double-stranded DNA antibodies (anti-dsDNA) and anti-ribonucleic protein (RNP) antibodies
- Other disease-specific antinuclear antibodies and cytoplasmic antibodies
- Complement deficiencies and decreased complement activity in certain medical conditions
- Components and classification of synovial fluid analysis
- Other biochemical tests: renal function tests and urine analysis (this section is not in the scope of the current chapter but it will be discussed in details in the chapter of "Renal System and Rheumatology")

3.1.1 Objectives

By the end of the current chapter, the candidates should be able to:

- Identify the rule of acute phase reaction proteins in rheumatic diseases.
- Interpret the auto-antibodies' results based on clinical findings.
- Classify various types of joint effusions based on clinical and laboratory analysis of synovial fluid.

3.2 Acute Phase Reactants

Acute phase reactants (APRs) or proteins are defined as those proteins whose serum concentrations increase or decrease by at least 25% during inflammatory states. Changes in levels of APR largely result from the effects of cytokines, including interleukin (IL)-6, IL-1 beta, tumor necrosis factor-alpha (TNF-alpha), and interferon gamma.

Serum APR level measurements are useful because they frequently reflect the presence and intensity of an inflammatory process. The assessment of APR may be most helpful in patients with RA, polymyalgia rheumatica, and giant cell arteritis.

However, APR measurements in clinical use are not specific to any particular disease.

The most widely used indicators of the acute phase response are the ESR and CRP [7].

ESR and CRP definitions, measurements, uses, and other important aspects are addressed in Table 3.1.

3.3 Rheumatoid Factor (RF) and Anti-citrullinated Protein Antibody (ACPA)

3.3.1 Definition

RF is an antibody directed against the Fc fragment of immunoglobulin G (IgG). It may be of any isotype: IgG, IgA, IgE, and IgM. RF-IgM is the only one measured in clinical practice. The origin of RF is incompletely understood [7]. ACPAs are antibodies that are targeted against citrulline which is situated on proteins. Important clinical features of RF including measurement and common issues while dealing with it in clinical practice are all addressed in Table 3.2.

3.4 Antinuclear Antibodies (ANAs)

3.4.1 Definition

ANAs are serologic hallmarks of patients with systemic autoimmune disease. These antibodies should be ordered when the clinical assessment of the patient suggests the presence of an autoimmune or connective tissue diseases [7]. Clinical aspects of ANAs are discussed in Table 3.3.

Table 3.1 ESR versus CRP

Definition	**ESR**		**CRP**
	ESR is an indirect measurement of serum acute phase protein concentrations, defined as the rate (mm/hour) at which erythrocytes suspended in plasma settle when placed in a vertical tube, reflects a variety of factors, most notably the plasma concentration of fibrinogen [7]		CRP is defined as a pentameric protein comprised of five identical, non-covalently linked 23-KD subunits arranged in cyclic symmetry in a single plane. It is a component of the innate immune response and has both pro-inflammatory and anti-inflammatory actions. CRP can activate the complement system and enhance the apoptotic cell clearance [7]
Methods of measurement	Cont. **ESR**		Cont. **CRP**
	The Westergren method	**The Wintrobe method**	It is measured by immunoassay technique or nephelometry [7]
	Uses a 200-mm tube and has a dilution step that correct for the effect of anemia. It is the preferred method and can detect an ESR more than 50–60 mm/h [7, 8]	Uses a 100-mm tube and has no dilution step [7, 8]	
Sensitivity and specificity	An advanced rate does not diagnose a specific disease, but it does indicate that an underlying disease may exist [7, 8]		Although CRP is a sensitive reflector of inflammation, it is not specific for inflammation [9]
	An elevated ESR observed together with a normal CRP is often a false-positive value for the ESR; this may reflect the effects of blood constituents, such as monoclonal immunoglobulins, that are not related to inflammation but that can influence the ESR. However, this conclusion is not always valid. As an example, the ESR may be markedly elevated in patients with active systemic lupus erythematosus (SLE), while the CRP response is muted. These variations may be explained by differences in the production of specific cytokines or their modulators in different diseases [10]		
Normal result	– Normal values for the Westergren method are: **Men** = 0–15 mm/h **Women** = 0–20 mm/h **Children** = 0–10 mm/h – A normal value does not rule out the disease – Non-inflammatory conditions that can elevate ESR include aging, female sex, obesity, pregnancy, and race [7, 8] – The age-adjusted upper limit of normal for ESR is: **Male** = age/2 **Female** = (age+10)/2	– Normal value is less than 0.08 mg/dl – CRP levels vary with age, sex, and race [7] – The age-adjusted upper limit of normal for CRP is: **Male** = age in years/50 **Female** = (age in years/50) + 0.6 [9]	

(continued)

Table 3.1 (continued)

Abnormal results	1-**Causes of marked ESR elevation (more than 100 mm/hr):** 1. Infection (bacterial 33%) 2. Connective tissue diseases (gain cell arteritis, polymyalgia rheumatica, SLE, vasculitides 25%) 3. Malignant neoplasms and renal disease 17% 4. Inflammatory disorders 14% [7, 11] **Causes of marked decreased in ESR (0 mm/h):** 1. Afibrinogenemia/ dysfibrinogenemia 2. Agammaglobulinemia 3. Increased plasma viscosity 4. Extreme polycythemia [7, 11]	**Values between 0.3 and 1 mg/dL may indicate:** 1. Minor degrees of inflammation, e.g., periodontitis 2. Minor degrees of metabolic malfunction (non-inflammatory states), e.g., obesity and insulin resistance [7, 9] **Values greater than 1 mg/dL can indicate:** Clinically significant inflammation [9] **Values greater than 8–10 mg/dL may indicate:** 1. Bacterial infection 2. Systemic vasculitis 3. Metastatic cancer 4. Trauma, burns, and surgery [7, 9]
Advantages and disadvantages	1. Inexpensive, familiar, and easy to perform 2. As a patient's condition worsens or improves, the ESR changes are relatively slow [12] 3. A literature review was conducted for all clinical trials and observational studies of disease-modifying medications and corticosteroids in RA to elaborate on the laboratory results of both ESR and CRP before treatment and 4 weeks to 24 weeks after treatment in the same patients, and it has been concluded that the ESR was more sensitive to change than the CRP at 12 weeks and 24 weeks of treatment [13]	1. It rises more quickly and falls more quickly than ESR [11] 2. Measurements of CRP concentrations are of prognostic value in rheumatoid arthritis and can help guide management [11, 13–15] 3. CRP alone may have been in favor as a simple, validated, reproducible, non-age-dependent test for disease activity assessment [12] 4. CRP had been found to be more sensitive and specific marker for diagnosing bacterial infections in SLE compared to procalcitonin (PCT) [14, 15]. However, further meta-analysis report of studies describing the role of PCT or CRP as a biomarker of infection in autoimmune diseases has determined that PCT test is more specific than sensitive [16]. In addition, a later study has confirmed that PCT test is superior to CRP test in detecting superimposed bacterial infections in active SLE patients, where the PCT levels are correlated with the progression of bacterial infection and used to monitor the response to antibiotic treatment [17]
	The serum protein electrophoresis is the most sensitive test for detecting inflammatory changes. It is the most expensive, directly quantifies the acute phase response [7]. However, there is no single best laboratory test to reflect inflammation The optimal use of acute phase protein measurements may be to obtain several measurements, most commonly ESR and CRP, rather than a single test [9, 14, 18] **Additional tests** suggest systemic inflammation: Low albumin and mild elevation of hepatic alkaline phosphatase [7]	

3.4.2 Methods of Measurement

- Indirect immunofluorescence method using "fluorescence microscope" is the gold standard method to detect ANAs. Currently most laboratories use human epithelial cell tumor line (HEp2 cells) as a substrate to detect antibodies that bind to various nuclear antigens (ANAs) instead of frozen section of rodent organ cells.
- Other methods that can be used for detection of specific ANA include ELISA, immuno-blotting, and Western-blotting methods.

Table 3.2 Characteristics of RF

<table>
<tr><td>Measurement</td><td colspan="3">It is measured by nephelometry, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), and latex agglutination techniques, although there is no single technique that has clear advantage over others. Automated methods, such as nephelometry and ELISA, tend to be more reproducible than manual methods [7]. The most commonly used technique to measure ACPA is the ELISA for antibodies against cyclic citrullinated peptides (CCP).</td></tr>
<tr><td>Sensitivity and specificity</td><td colspan="3">• The sensitivity of RF in RA has ranged from 26% to 90%
• The reported sensitivity of the RF test in RA has been as high as 90%. However, population-based studies, which include patients with mild disease, have found much lower rates of RF-positive RA (26 to 60%) [19]
• The sensitivity of ACPA testing is similar to RF at around 75%. However it provides much higher specificity rates at around 95%.
• The specificity is 85% [19]
• The specificity to a young healthy population is about 96% [19, 23]</td></tr>
<tr><td>Positive results
Cont. Positive results</td><td colspan="3">The common denominator for the production of RF (positive result) is chronic immune stimulation</td></tr>
<tr><td></td><td>Healthy individuals</td><td>Non-rheumatic disorders</td><td>Rheumatic disorders</td></tr>
<tr><td></td><td>• RF is present in some healthy individuals, especially the elderly (3–25%), male and female are affected equally, and only 20% of cases is the RF level significantly positive
• RF has been found in 2–4% of young, healthy individuals [7, 20]</td><td>1. Chronic infection, e.g., AIDS, mononucleosis, parasitic infections, chronic viral infection (hepatitis B or hepatitis C (HCV) 54–76%), chronic bacterial infections (tuberculosis, subacute bacterial endocarditis (SBE))
2. Cryoglobulinemia 40–100% especially with HCV
3. Pulmonary disorders, such as sarcoidosis
4. Malignancy, especially after radiation or chemotherapy and B-cell neoplasms
5. Primary biliary cirrhosis [7, 21]

Positive ACPA can be found in the following non rheumatological diseases:
1. Active tuberculosis (varying rates)
2. Chronic obstructive pulmonary disease (5%)
3. It is important to note that unlike RF, ACPAs are rarely found in patient with hepatitis C virus</td><td>1. Rheumatoid arthritis 26–90%
2. Sjögren's syndrome 75–95%
3. Mixed connective tissue disease 50–60%
4. Mixed cryoglobulinemia (types II and III) 40–100%
5. Systemic lupus erythematosus 15–35%
6. Polymyositis or dermatomyositis 5–10%
7. Sarcoidosis 15% [7, 21]

ACPAs were found to be positive in the following autoimmune diseases:
1. SLE and primary Sjogrens Syndrome (17%)
2. Psoriatic arthritis (8-16%)</td></tr>
<tr><td>Can RF be used as a screening test?</td><td colspan="3">• Measurement of RF is a poor screening test to diagnose or exclude rheumatic disease in either healthy populations or in those with arthralgias but have no other symptoms or signs of rheumatic disease [20]
• In a population study, it has been found that the presence of both RF and anti-citrullinated protein antibody (ACPA) in apparently healthy people substantially increases the probability of having RA. So the presence of the two autoantibodies (RF and ACPA) is associated with a relative risk of approximately 70% [20]
• The RF has a higher positive predictive value (PPV) if ordered more selectively in patients with a modest or higher chance of having an RF-associated rheumatic disease such as RA, Sjögren's syndrome, or the mixed cryoglobulinemia syndrome. Included in this group are patients with prominent morning stiffness, with sicca symptoms, or with arthralgia or arthritis in a rheumatoid distribution (i.e., symmetric polyarthritis involving small joints) [19]
• Higher titers of RF have a higher positive predictive value for RA [19].</td></tr>
</table>

(continued)

Table 3.2 (continued)

Significance of measuring RF and ACPA in known RA cases	• RF-positive patients with RA may experience more aggressive and erosive joint disease and extra-articular manifestations than those who are RF-negative. Similar findings have been observed in juvenile idiopathic arthritis [19] • RF status may be useful in combination with other indicators, including HLA-DRB1, CRP, the ESR, and severity of synovitis on physical exam, to predict progression of radiographic changes in RA patients and to guide treatment [19] • ACPA positivity was found to be associated with more erosive joint disease, especially apparent on radiographs. It was also found to be better at predicting these changes than RF
RF and monitoring of rheumatic diseases	• The change in RF level does not reflect changes in RA disease activity • RF should not be used routinely to monitor RA disease activity in clinical practice • RF titer may fall with effective treatment of RA in patients who are originally RF-positive [19, 22] • In Sjögren's syndrome, the disappearance of a previously positive RF may herald the onset of lymphoma. That is why some clinicians check RF repeatedly in patients with Sjögren's syndrome. The clinical utility of this practice, however, has not been critically assessed [19, 22]
Antibody status (ACPA/RF)	• RF and ACPA have the potential to revert and convert during the early course of disease. Fluctuations in RF and ACPA were not associated with clinical outcomes [23] • Repeated measurement of ACPA or RF during the first year after onset of arthritis does not offer major additional information [24]
RF and the mortality	Patients with RA with positive RF, especially IgA and IgM isotypes, carry a risk of dying earlier than patients without these serological findings [25]

Table 3.3 ANA characteristics

Positive result	• It is defined as the level of ANA that exceeds the level seen in 95% of the normal population • In most laboratories, this level is a titer of 1:40 to 1:80 that are reported positive • Clinically significant titers in laboratories that use HEp-2 cells as substrate are usually more than or equal to 1:160 [7, 26]			
	Systemic autoimmune diseases	**Organ-specific autoimmune diseases**	**Infections**	**Others**
	1. SLE 93% 2. Scleroderma 85% 3. Mixed connective tissue disease 93% 4. Polymyositis/ dermatomyositis 61% 5. Rheumatoid arthritis 41% 6. Rheumatoid vasculitis 33% 7. Sjögren's syndrome 48% 8. Drug-induced lupus 95–100%; (e.g., procainamide, hydralazine, isoniazid, and quinidine) 9. Discoid lupus 15% 10. Pauci-articular juvenile chronic arthritis 71% [7, 26]	1. Hashimoto's thyroiditis 46% 2. Graves' disease 50% 3. Autoimmune hepatitis 63–91% 4. Primary biliary cirrhosis 10–40% 5. Primary autoimmune cholangitis 100% 6. Idiopathic pulmonary arterial hypertension 40% 7. Multiple sclerosis 25% [7, 26]	– Chronic infectious diseases (mononucleosis, hepatitis C infection, SBE, tuberculosis, and HIV) and some lympho-proliferative diseases – Malignancy (rare) with the exception of dermatomyositis [7, 26]	1. Highly relatives of patient 15–25% 2. Normal elderly 20% 3. Patients with silicone breast implant 15–25% [7]
Is ANA used as a screening test?	– No, it cannot be used as a screening test for autoimmune disorders in the general healthy population in the absence of clinical findings as it may be present in very low specificity titer in normal population 5% – It should be used primarily as a confirmatory test when the clinicians strongly suspect SLE or autoimmune disorders – A patient with a negative ANA and strong clinical evidence of SLE or another SS-A-associated disease, antibodies to SS-A should be ordered [7]			

(continued)

Table 3.2 (continued)

Is ANA used for monitoring diseases?	– No, there is no evidence about use of ANA titer as a monitor to follow disease activity in patients with SLE and autoimmune diseases [7]				
ANA patterns	The pattern type has been found to have relatively low sensitivity and specificity for different autoimmune disorders, and thus tests for specific antibodies have largely replaced the use of patterns				
	The homogeneous or diffuse pattern Represents antibodies to the DNA-histone complex (anti-DNP (LE cell) and anti-histone)	**The peripheral or rim pattern** It is produced by antibodies to DNA (anti-dsDNA) and antibodies to nuclear envelope antigens (anti-laminin)	**The speckled pattern** It is produced by antibodies to SM, RNP, Ro/SSA, La/SSB, Scl-70, centromere, proliferating cell nuclear antigen (PCNA), and other antigens	**The nucleolar pattern** It is produced by antibodies to RNA polymerase I, proteins of the small nucleolar RNP complex (fibrillarin, Mpp10, and hU3–55 K), Th/to, B23, PM-Scl, and NOR-90, and other antigens	**The centromeric pattern** It is produced by antibodies to proteins that are associated with the site of chromosomal constriction. Proteins designated, CENP-A, CENP-B, CENP-C, etc., are only present on active centromeres (i.e., during meiosis and mitosis) [7, 26]
ANA titer	– The presence of very high concentrations of antibody (titer >1:640) should arouse suspicion of an autoimmune disorder. However, its presence alone is not diagnostic of disease – If no initial diagnosis can be made, it is our practice to watch the patient carefully over time and to exclude ANA-associated diseases – An accurate ANA with titer, in combination with a full history and physical examination, can be extremely useful in the diagnosis and exclusion of connective tissue disease [26]				
	– 1–2% of patients who have active and untreated SLE will have a negative ANA, and this is because the substrate used in ANA test did not contain a sufficient antigen to detect SS-A antibodies – 10–15% of SLE patients will become ANA-negative with treatment or inactive disease – 40–50% of SLE patients with end-stage renal disease on dialysis will become an ANA-negative [7]				

3.5 ANA Profile

3.5.1 Definition

An ANA profile consists of many antibodies to measure specific ANAs for certain nuclear antigens. It should be performed when the screening test for ANA is positive and when further information is needed regarding the type of autoimmune disorder [7].

ANA profile antibodies and their specific uses are elaborated on Table 3.4.

3.6 Other Disease-Specific Antinuclear Antibodies and Cytoplasmic Antibodies

These antibodies have to be ordered individually according to the set-up diagnosis based on patient's symptoms and clinical presentations, and they include:

1. **Anti-histone antibodies**: sensitive (70%) for drug-induced lupus but nonspecific and have limited diagnostic utility because they may also be present in patients with SLE. The best test to conduct in patient with suspected drug-induced lupus is antichromatin antibody test, not anti-histone antibody test [7]. However, anti-histone antibody test might be of value in patients having a positive ANA test with history of exposure to medications-induced lupus, such as procainamide (Pronestyl) and isoniazid (INH) [27].
2. **Anti-Th/To antibodies**: crest syndrome 20% [7].
3. **Anti-SCL-70 antibodies** (topoisomerase1): diffuse **systemic** sclerosis (scleroderma) 22–40% [7]. They are highly specific but not sensitive for scleroderma [29].
4. **Anti-tRNA synthetase antibodies (anti-Jo-1, other)**: polymyositis 20–30% [7].
5. **Anti-neutrophil cytoplasmic antibodies (ANCAs)**:

Table 3.4 The standard ANA profile

Measured antibodies	Associated diseases	Characteristics
Anti-dsDNA antibodies (directed against double-stranded DNA)	SLE 60%	– It is very specific for SLE – It is the one that used to follow SLE disease activity; high titers are associated with lupus nephritis or a flare of lupus activity [27]
Anti-U1 RNP antibodies (ribonuclear protein)	SLE 30%, progressive systemic sclerosis (low titer), and mixed connective tissue disease (MCTD)	– A very high level of this antibody is highly suggestive of MCTD [28]
Anti-SM (smith) antibodies	SLE 30%	– It is very specific for SLE – The sensitivity of anti-dsDNA and anti-Sm for the diagnosis of SLE is relatively low – Anti-Sm antibodies generally remain positive, even when a patient has entered remission; therefore it may be especially useful diagnostically when a SLE patient's disease is relatively inactive [28]
Anti-SS-A (RO) antibodies	SLE 30%, primary Sjögren's syndrome 70%, neonatal lupus, sub-acute cutaneous lupus (SCLE), secondary Sjögren's syndrome (rare) [28]	
Anti-SS-B (LA) antibodies	SLE 15%, Sjögren's syndrome 60% [28]	
Anti-centromere antibodies	Crest syndrome 98%, diffuse scleroderma 22–36% [28]	

- **Cytoplasmic anti-neutrophil cytoplasmic antibodies (C-ANCA)**, the most common c-ANCA target is serine proteinase-3: granulomatous polyangiitis (GPA) (Wegener granulomatosis) 90%, microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (EGPA) (rare). Its titer can correlate with GPA disease activity [30].
- **Perinuclear anti-neutrophil cytoplasmic antibodies (P-ANCA)**, the most common p-ANCA target is myeloperoxidase: MPA 70%, pauci-immune glomerulonephritis, and EGPA, or myeloperoxidase (−)—ulcerative colitis, chronic infection, and neoplasm (rare) [30].

6. **Anti-mitochondrial antibodies (AMAs)**: primary biliary cirrhosis 80% [7].
7. **Antibodies to the gp210 and p62 proteins of the nuclear pore complex**: primary biliary cirrhosis 10–40% [7].

3.7 Circulatory Complement Components

Complement is an important effector pathway of innate immunity and has a role in the pathogenesis of some of rheumatic conditions, namely, SLE.

Causes of Decreased Circulatory Complement Components

- Hereditary complement deficiencies (decreased production)
- Secondary complement deficiencies (acquired) [31]

3.7.1 Mechanism of Acquired Complement Deficiencies

1. Increased level of circulatory immune complexes (increased consumption of complements) due to:
   - Infectious causes
   - Glomerulonephritis
   - Rheumatic diseases:
     (a) **SLE**: Low C4 and C3 levels occur in about 50% of patients with SLE. Levels of C3 and C4 are decreased with increased severity of SLE, especially renal disease. A return to normal levels with treatment is a good prognostic sign. Serial observations reveal decreased levels preceding clinical exacerbation.
     (b) **Cryoglobulinemia**: The complement profile shows decreased levels of C4 and C2 with normal or slightly lowered C3.
     (c) **Systemic vasculitis** especially polyarteritis nodosa, urticarial vasculitis: 50% of patients with polyarteritis may have decreased serum complement levels. Its values can be helpful in assessing the clinical course, especially the response to therapy.
     (d) **RA** with extra-articular manifestation (rare) [7, 32].
2. Reduced hepatic synthesis (uncommon)
3. Loss of complement components in the urine (rare) [30]

3.8 Synovial Fluid Analysis

The presentation of one or more hot, swollen joints is a common medical emergency, and synovial fluid aspiration, the so-called arthrocentesis, is the single most important test helping in the diagnosis of different types of arthropathies [33].

Therefore, specialized laboratories analyze synovial fluid to either confirm the diagnosis of crystal-associated arthropathies, support the diagnosis of septic arthritis, or establish other rheumatologic diagnoses such as mono-arthritis or joint effusion [34].

The complete analysis of synovial fluid includes macroscopic (gross appearance), microscopic, and specific stain tests to provide detailed information about the joint's condition and helps in establishing the diagnosis and treatment [35]. Description of macroscopic analysis of synovial fluid includes color, clearance, volume, and viscosity. However, the microscopic analysis can differentiate between inflammatory and infectious processes by measuring a complete leukocyte count. In addition, a differential of the synovial WBC count should be ordered, so that if the results came positive for infectious process, the performance of Gram-stain and culture tests will provide guidance to diagnosis and/or antibiotic therapy [36].

Microscopic examination specifically can also allow the detection and identification of various types of crystal by using polarized light microscope. Refer to Table 3.5 for an overview on important issues as regards arthrocentesis and synovial fluid analysis. However, Table 3.6 shows the classification of joint effusions into normal, inflammatory, non-inflammatory, and septic effusion based on clinical and laboratory analysis of synovial fluid with the causes of each type [37, 38]. Indications, contraindications, complications, and specimen analysis of synovial fluid are presented in Table 3.5. Classification and causes of joint effusions based on laboratory analysis of synovial fluid are presented in Table 3.6. Fig. 3.1 is the clinical diagnostic approach for painful peripheral joint.

3.9 Key Notes

- The likelihood diagnosis of septic arthritis is markedly increased with higher synovial WBC counts. It has been illustrated that for synovial WBC count the likelihood ratio (LR) of having septic arthritis is as follows [34]:
  – WBC count <25,000/μL, the LR is 0.32 at 95% CI.
  – WBC count ≥25,000/μL the LR is 2.9 at 95% CI.
  – WBC count >50,000/μL, the LR is 7.7 at 95% CI.

Table 3.5 Overview on arthrocentesis and synovial fluid analysis

Indications	Contraindications	Complications	Specimen handling	Synovial fluid analysis
1. According to the American College of Rheumatology (ACR), synovial fluid analysis should be performed in the febrile patient with an acute flare of established arthritis to rule out superimposed septic arthritis 2. Unexplained joint, bursa, or tendon sheath swelling 3. Suspected crystal-induced arthritis 4. Repeated aspiration and analysis may be indicated to follow up the response of septic arthritis to treatment and may also be valuable for diagnosis of some cases of gout in which the initial aspirate does not have detectable crystals [34]	1. There is no absolute contraindication 2. Bleeding diatheses and cellulites are considered as relative contraindication; it could make the approach to the joint space difficult due to the overlying swelling [37]	1. Infection 2. Hemarthrosis 3. Pain 4. Cartilage injury 5. Vasovagal syncope [37]	1. Aspiration is performed under aseptic technique with quick transfer of specimen for culture to the sterile tubes and plated as soon as possible 2. If the transfer is delayed more than 6 hours, many changes would occur, for example, decrease in leukocyte count or decrease in crystal numbers [36]	The WBC count and the percentage of PMN cells can help to differentiate between non-inflammatory, inflammatory, and septic joint conditions. These tests are the best diagnostic tool available for detecting bacterial arthritis [36] 1. Gram stain and cultures should be ordered even with relatively low suspicion of infection 2. Crystal search using polarized light microscopy 3. Chemistry analysis should not be routinely ordered [37]

Table 3.6 Classification and causes of joint effusions based on laboratory analysis of synovial fluid

Fluid features	Normal	Non-inflammatory	Inflammatory	Pyarthrosis or septic arthrosis
Appearance	Clear, highly viscous, colorless	Clear to slightly turbid	Slightly turbid, yellow or yellow-green	Turbid to very turbid, yellow or yellow-green
Total WBC count/MM3	0–200	200–2000	2000–50,000	50,000–150,000
Polymorphonuclear cell (PMN)%	<10%	<20%	20–70%	≥75%
Causes		– Osteoarthritis – Joint trauma – Hypertrophic osteoarthropathy – Neuropathic arthropathy – Avascular necrosis [37, 38]	– RA – Gout – Pseudogout – Psoriatic arthritis – AS – SLE – Reiter syndrome – Sarcoidosis – Rheumatic fever – Wegener granulomatosis – Infectious arthritis – SBE [37, 38]	1. It is a septic arthritis until proven otherwise by the fluid culture 2. Pseudosepsis include reactions to intra-articular injections, gout, Reiter's syndrome, leukemic infiltration, and RA [37, 38]

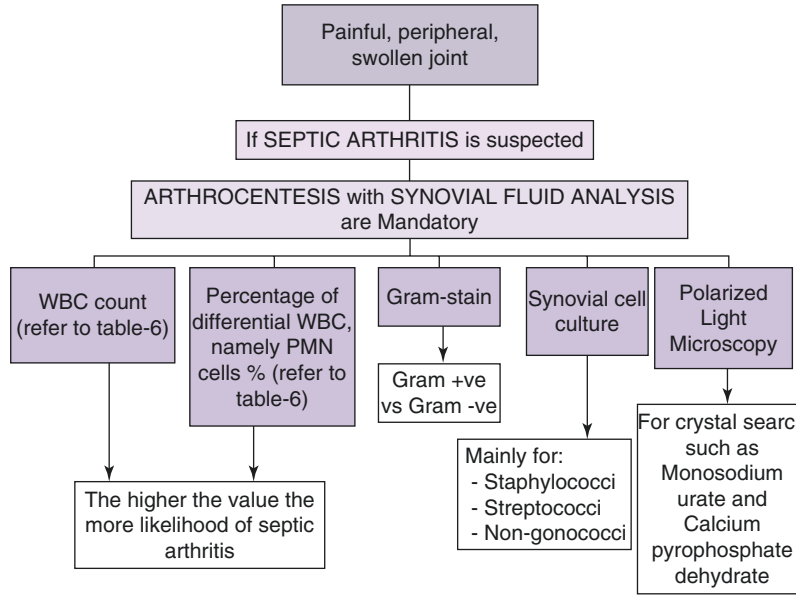

Fig. 3.1 Clinical approach for painful peripheral joint

  - WBC count >100,000/μL, the LR is 28.0 at 95% CI.
- Polymorphonuclear (PMN) cells of 90% are associated with increasing likelihood of septic arthritis of 3.4, while if the percentage of PMN cells is less than 90%, the likelihood decreases down to 0.34 (95% CI) that supports the clinician's suspicion of bacterial arthritis [38, 39].
- Eosinophilic cells in the synovial fluid suggest parasitic infection, allergy, Lyme disease, or neoplasm [40].
- If there is a suspicion of joint involvement by a neoplasm or hematologic malignancy, formal cytological examination should be ordered [38].
- Hemorrhagic effusions may be caused by hemophilia, anticoagulation or other bleeding diathesis, scurvy, trauma, neuropathic arthropathy, and tumors [38].

3.9.1 Gram Stain

- It is used to identify common bacterial organisms (Gram-positive versus Gram-negative coverage) for the diagnosis and treatment of septic arthritis.
- It may be the only evidence of infection with fastidious organisms that are not able to grow in culture [41].
- The *sensitivity* is not known precisely.
  - In non-gonococcal bacterial arthritis, it is in range from 50% to 70%.
  - In gonococcal arthritis, it is <10% [41].
- The *specificity* is high when performed and interpreted by an experienced clinician or technician [41].

3.9.2 Synovial Fluid Culture

- The synovial fluid samples should be routinely sent for culture for staphylococci followed by streptococci and Gram-negative bacteria (non-gonococcal causes).
- Antibiotics should generally not be given prior to joint aspiration [42, 43].
- **The specificity**: Positive synovial culture should be indicative of septic arthritis in 100% of cases with exclusive of contamination and laboratory error [42, 43].
- **The sensitivity**: It is not known precisely because of the lack of an alternative gold standard. The joint aspirate should be cultured for

Table 3.7 Gout versus pseudo-gout

	Crystal	Shape	Birefringence	Color of crystals parallel to axis of red-plate compensator
Gout	Monosodium urate (MSU)	Needle	Negative	Yellow
Pseudogout	Calcium pyrophosphate dehydrate (CPPD)	Rhomboid or rectangular	Positive	Blue [48]

N. gonorrhoeae or unusual organisms (TB, Lyme disease, or fungal infections) when the history is suggestive [42, 43].

3.9.3 Diagnostic Approach

- It should be noted that the absence of organisms on Gram stain or a negative subsequent synovial fluid culture does not rule out the diagnosis of septic arthritis especially if clinical suspicion is high. In such condition, an empirical treatment of the case as septic arthritis should be implemented [44–46].
- Moreover, it has been suggested that the "gold standard" for the diagnosis of septic arthritis is the level of clinical suspicion by an expert physician in the management of patients with musculoskeletal disease [35, 45].
- Similarly, another study had concluded that combining Gram stain and culture of synovial fluid with clinical follow-up is the best approach used to detect patients missed by Gram stain and culture alone [36].

3.9.4 Crystal Search Using Polarized Light Microscopy

Polarized light microscope (PLM) is a fundamental tool for detection and identification of various types of crystals present in synovial fluid depending on their shape (needle, rhomboid, cigar-shaped, etc.) birefringence, location (intracellular or extracellular), and quantity (scarce or plentiful). The obtained results of PLM help the clinicians in diagnosing and managing a case of monoarthritis. However, the presence of artifacts in microscopic analysis can confuse the inexperienced observer; therefore, a suitable interpretation of the synovial fluid analysis using PLM requires at least two experienced observers [47]. The microscopic features of common types of crystals that can differentiate between clinical cases of gout and pseudogout are illustrated in Table 3.7.

3.10 Summary

Due to the fact that musculoskeletal symptoms are exceedingly common compared with the prevalence of systemic rheumatic disease, the pretest probability of systemic rheumatic disease in the population is rather low compared with musculoskeletal symptoms that are nearly ubiquitous. Therefore, establishing the diagnosis of a rheumatic disease may require exclusion of other differential diagnoses that present in a similar fashion. Even the disease established-guidelines, which are often used by clinicians, perform poorly during the assessment of a patient presenting with new polyarthritis [49]. As a consequence, widely used laboratory tests can be very specific and permit rapid diagnosis and appropriate management. However, clinicians should be aware of the false-positive tests that may result in inappropriate management and unnecessary concern.

Generally, serum rheumatologic tests are most helpful for confirming a clinically suspected diagnosis. For instance, testing for RF is appropriate when suspecting RA, Sjögren's syndrome, or cryoglobulinemia, whereas ANA testing is highly sensitive for SLE and drug-induced lupus. Although an elevated ESR is a sensitive test for polymyalgia rheumatica and temporal arteritis, its specificity is quite low. In addition, ESR levels are frequently linked to the disease activity in rheumatoid arthritis and may found to be of value for monitoring therapeutic response. However, anti-double-stranded

DNA antibodies are usually associated with lupus nephritis, and their titer often correlates with disease activity in SLE. On the other hand, cytoplasmic anti-neutrophil cytoplasmic antibody test is highly sensitive and specific for GPA.

In order to increase the utility and decrease the cost-effectiveness of the laboratory testing of rheumatic disease, these tests should be used more selectively and avoid absolute overreliance on lab results. However, a logic combination of the clinical background and the testing results would provide the appropriate diagnosis of the rheumatic conditions. Finally, as Shmerling RH has stated, "the passage of time is one of most useful diagnostic tests as many patients with musculoskeletal symptoms improve over time without a clear diagnosis" [50].

Acknowledgments The authors would like to thank Dr. Waleed Hafiz for his assistance in the development of this chapter.

Abbreviations

(IL)-6	Interleukin-6
AMAs	Anti-mitochondrial antibodies
ANA	Antinuclear antibody profile
ANCAs	Anti-neutrophil cytoplasmic antibodies
anti-dsDNA	Anti-double-stranded DNA antibodies
anti-gp210	Anti-glycoprotein-210 antibodies
anti-p62	Anti-protien-62 antibodies
anti-SCL-70	Anti-topoisomerase1 antibodies
anti-Th/To	Antibodies to Th/To ribonucleoprotein
APRs	Acute phase reactants or proteins
AS	Ankylosing spondylitis
C4 and C3	Complements
C-ANCA	Cytoplasmic anti-neutrophil cytoplasmic antibodies
CI	Confidence interval
CRP	C-reactive protein
EGPA	Eosinophilic granulomatosis with polyangiitis
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
GPA	Granulomatous polyangiitis
HEp2 cells	Human epithelial cell tumor line
HLA-B27	Human leukocyte antigen B27
HLA-DR	Human leukocyte antigen MHC class II
IgG	Immunoglobulin G
IL-1	Interleukin-1
INH	Isoniazid
LR	Likelihood ratio
MPA	Microscopic polyangiitis
MPA	Myeloperoxidase
P-ANCA	Perinuclear anti-neutrophil cytoplasmic antibodies
PLM	Polarized light microscope
PMN	Polymorphonuclear cells
Pronestyl	Procainamide
RF	Rheumatoid factor
RNP	Anti-ribonucleic protein antibodies
SLE	Systemic lupus erythematosus
TNF-alpha	Tumor necrosis factor-alpha
WBC	White blood cell count

References

1. Szodoray P, Szabo Z, Kapitany A, Gyetvai A, Lakos G, Szanto S, et al. Anti-citrullinated protein/peptide autoantibodies in association with genetic and environmental factors as indicators of disease outcome in rheumatoid arthritis. Autoimmun Rev. 2010;9(3):140–3.
2. Rose HM, Ragan C, et al. Differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with rheumatoid arthritis. Proc Soc Exp Biol Med. 1948;68(1):1–6.
3. Ragan C. The history of the rheumatoid factor. Arthritis Rheum. 1961;4:571–3.
4. Schlosstein L, Terasaki PI, Bluestone R, Pearson CM. High association of an HL-A antigen, W27, with ankylosing spondylitis. N Engl J Med. 1973;288(14):704–6.
5. Stastny P. Mixed lymphocyte cultures in rheumatoid arthritis. J Clin Invest. 1976;57(5):1148–57.
6. Lane SK, Gravel JW Jr. Clinical utility of common serum rheumatologic tests. Am Fam Physician. 2002;65(6):1073–80.
7. Sterling G. Rheumatology secrets. 3rd Edition. Philadelphia, PA; 2002. ISBN: 9780323641869.
8. Simple rule for calculating normal erythrocyte sedimentation rate. Br Med J. 1983;286(6364):557–8.

9. Kushner I, Rzewnicki D, Samols D. What does minor elevation of C-reactive protein signify? Am J Med. 2006;119(2):166. e17-28
10. Gaitonde S, Samols D, Kushner I. C-reactive protein and systemic lupus erythematosus. Arthritis Rheum. 2008;59(12):1814–20.
11. Fincher RM, Page MI. Clinical significance of extreme elevation of the erythrocyte sedimentation rate. Arch Intern Med. 1986;146(8):1581–3.
12. Crowson CS, Rahman MU, Matteson EL. Which measure of inflammation to use? A comparison of erythrocyte sedimentation rate and C-reactive protein measurements from randomized clinical trials of golimumab in rheumatoid arthritis. J Rheumatol. 2009;36(8):1606–10.
13. Ward MM. Relative sensitivity to change of the erythrocyte sedimentation rate and serum C-reactive protein concentration in rheumatoid arthritis. J Rheumatol. 2004;31(5):884–95.
14. van Leeuwen MA, van der Heijde DM, van Rijswijk MH, Houtman PM, van Riel PL, van de Putte LB, et al. Interrelationship of outcome measures and process variables in early rheumatoid arthritis. A comparison of radiologic damage, physical disability, joint counts, and acute phase reactants. J Rheumatol. 1994;21(3):425–9.
15. Kim HA, Jeon JY, An JM, Koh BR, Suh CH. C-reactive protein is a more sensitive and specific marker for diagnosing bacterial infections in systemic lupus erythematosus compared to S100A8/A9 and procalcitonin. J Rheumatol. 2012;39(4):728–34.
16. Wu JY, Lee SH, Shen CJ, Hsieh YC, Yo PH, Cheng HY, et al. Use of serum procalcitonin to detect bacterial infection in patients with autoimmune diseases: a systematic review and meta-analysis. Arthritis Rheum. 2012;64(9):3034–42.
17. Yu J, Xu B, Huang Y, Zhao J, Wang S, Wang H, et al. Serum procalcitonin and C-reactive protein for differentiating bacterial infection from disease activity in patients with systemic lupus erythematosus. Mod Rheumatol. 2014;24(3):457–63. https://doi.org/10.3109/14397595.2013.844391. Epub 2013 Oct 21. PMID: 24252006.
18. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999;340(6):448–54.
19. Shmerling RH, Delbanco TL. How useful is the rheumatoid factor? An analysis of sensitivity, specificity, and predictive value. Arch Intern Med. 1992;152(12):2417–20.
20. Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi H, Deyo RA, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. Arthritis Rheum. 2008;58(1):26–35.
21. Shmerling RH, Delbanco TL. The rheumatoid factor: an analysis of clinical utility. Am J Med. 1991;91(5):528–34.
22. van der Heijde DM, van Riel PL, van Rijswijk MH, van de Putte LB. Influence of prognostic features on the final outcome in rheumatoid arthritis: a review of the literature. Semin Arthritis Rheum. 1988;17(4):284–92.
23. Nishimura K, Sugiyama D, Kogata Y, Tsuji G, Nakazawa T, Kawano S, et al. Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. Ann Intern Med. 2007;146(11):797–808. https://doi.org/10.7326/0003-4819-146-11-200706050-00008. PMID: 17548411.
24. Mjaavatten MD, van der Heijde DM, Uhlig T, Haugen AJ, Nygaard H, Bjorneboe O, et al. Should anti-citrullinated protein antibody and rheumatoid factor status be reassessed during the first year of followup in recent-onset arthritis? A longitudinal study. J Rheumatol. 2011;38(11):2336–41.
25. Sihvonen S, Korpela M, Mustila A, Mustonen J. The predictive value of rheumatoid factor isotypes, anti-cyclic citrullinated peptide antibodies, and anti-neutrophil cytoplasmic antibodies for mortality in patients with rheumatoid arthritis. J Rheumatol. 2005;32(11):2089–94.
26. Solomon DH, Kavanaugh AJ, Schur PH, American College of Rheumatology Ad Hoc Committee on Immunologic Testing G. Evidence-based guidelines for the use of immunologic tests: antinuclear antibody testing. Arthritis Rheum. 2002;47(4):434–44.
27. Smeenk R, Brinkman K, van den Brink H, Termaat RM, Berden J, Nossent H, et al. Antibodies to DNA in patients with systemic lupus erythematosus. Their role in the diagnosis, the follow-up and the pathogenesis of the disease. Clin Rheumatol. 1990;9(1 Suppl 1):100–10.
28. Tokano Y, Yasuma M, Harada S, Takasaki Y, Hashimoto H, Okumura K, et al. Clinical significance of IgG subclasses of Anti-Sm and U1 ribonucleoprotein antibodies in patients with systemic lupus erythematosus and mixed connective tissue disease. J Clin Immunol. 1991;11(6):317–25.
29. Moder KG. Use and interpretation of rheumatologic tests: a guide for clinicians. Mayo Clin Proc. 1996;71:391–6.
30. Seo P, Stone JH. The antineutrophil cytoplasmic antibody-associated vasculitides. Am J Med. 2004;117(1):39–50.
31. Saeki T, Ito T, Yamazaki H, Imai N, Nishi S. Hypocomplementemia of unknown etiology: an opportunity to find cases of IgG4-positive multiorgan lymphoproliferative syndrome. Rheumatol Int. 2009;30(1):99–103.
32. Guidelines for the initial evaluation of the adult patient with acute musculoskeletal symptoms. American College of Rheumatology Ad Hoc Committee on Clinical Guidelines. Arthritis Rheum. 1996;39(1):1–8.

33. Al-Shakarchi ICG. The treatment and care of hot, swollen joints. Rheumatol Pract. 2009;7(7):9.
34. Swan A, Amer H, Dieppe P. The value of synovial fluid assays in the diagnosis of joint disease: a literature survey. Ann Rheum Dis. 2002;61(6): 493–8.
35. Dieppe PAH, Swan A. Synovial fluid analysis. Annu Meet Int Soc Technol Assess Health Care Meet 2000; 16 (112).
36. Mathews CJ, Kingsley G, Field M, Jones A, Weston VC, Phillips M, et al. Management of septic arthritis: a systematic review. Ann Rheum Dis. 2007;66(4):440–5.
37. Margaretten ME, Kohlwes J, Moore D, Bent S. Does this adult patient have septic arthritis? JAMA. 2007;297(13):1478–88.
38. Kerolus G, Clayburne G, Schumacher HR Jr. Is it mandatory to examine synovial fluids promptly after arthrocentesis? Arthritis Rheum. 1989;32(3):271–8.
39. Krey PR, Bailen DA. Synovial fluid leukocytosis. A study of extremes. Am J Med. 1979;67(3):436–42.
40. Kortekangas P, Aro HT, Tuominen J, Toivanen A. Synovial fluid leukocytosis in bacterial arthritis vs. reactive arthritis and rheumatoid arthritis in the adult knee. Scand J Rheumatol. 1992;21(6):283–8.
41. Kay J, Eichenfield AH, Athreya BH, Doughty RA, Schumacher HR Jr. Synovial fluid eosinophilia in Lyme disease. Arthritis Rheum. 1988;31(11):1384–9.
42. Dougados M. Synovial fluid cell analysis. Baillieres Clin Rheumatol. 1996;10(3):519–34.
43. Shmerling RH. Synovial fluid analysis. A critical reappraisal. Rheum Dis Clin N Am. 1994;20(2):503–12.
44. Sorlin P, Mansoor I, Dagyaran C, Struelens MJ. Comparison of resin-containing BACTEC plus aerobic/F* medium with conventional methods for culture of normally sterile body fluids. J Med Microbiol. 2000;49(9):787–91.
45. Sharp JT, Lidsky MD, Duffy J, Duncan MW. Infectious arthritis. Arch Intern Med. 1979;139(10):1125–30.
46. Coakley G, Mathews C, Field M, Jones A, Kingsley G, Walker D, et al. BSR & BHPR, BOA, RCGP and BSAC guidelines for management of the hot swollen joint in adults. Rheumatology. 2006;45(8):1039–41.
47. Kaandorp CJ, Van Schaardenburg D, Krijnen P, Habbema JD, van de Laar MA. Risk factors for septic arthritis in patients with joint disease. A prospective study. Arthritis Rheum. 1995;38(12):1819–25.
48. Martinez-Castillo A, Nunez C, Cabiedes J. Synovial fluid analysis. Reumatol Clin. 2010;6(6):316–21.
49. Harrison BJ, Symmons DP, Barrett EM, Silman AJ. The performance of the 1987 ARA classification criteria for rheumatoid arthritis in a population based cohort of patients with early inflammatory polyarthritis. American Rheumatism Association. J Rheumatol. 1998;25(12):2324–30.
50. Shmerling RH. Diagnostic tests for rheumatic disease: clinical utility revisited. South Med J. 2005;98(7):704–11; quiz 12-3, 28

Open Access This chapter is licensed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.