Clinically pertinent and cost-effective use of laboratory investigations in musculoskeletal diseases

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In diagnosing musculoskeletal (MSK) diseases 80% of information is derived from clinical history, 15% from physical examination and only 5% from laboratory investigations. Therefore, it becomes mandatory to make a provisional diagnosis, a short list of differential diagnoses, followed by a list of minimum investigations for confirming or refuting these diseases before requesting any laboratory investigations. In this approach the 'arthritis panels' or 'arthritis laboratory profiles' that are popular with many practitioners must be forcefully condemned.

Among laboratory investigations for MSK diseases, those differentiating inflammatory (red flag) from non-inflammatory (green flag, biomechanical-structural) MSK diseases are most useful and cost-effective. Erythrocyte sedimentation rate (ESR fasting, Westergren), C-reactive protein (CRP), platelet count and reversal of serum albumin/globulin ratio are used for this purpose. Once inflammatory diseases have been classified, clinical pattern would further classify the patient in inflammatory peripheral arthritis where rheumatoid factor (RF) would then categorize them in seropositive and seronegative groups. In patients with suspected inflammatory spinal disease HLA B27 screening would be useful. In those with suspected collagen vascular diseases anti-nuclear antibody (ANA) using indirect immunofluorescence test (IFT) becomes mandatory before doing ANA subsets including anti-ENA and anti-dsDNA antibodies. Using reliable Crithidia immunofluorescence or Farr radioimmunoassay technique is essential for the latter. Bypassing ANA screening and directly requisitioning anti-DNA antibody may lead to serious difficulties as ELISA-based tests give false positive results. For suspected systemic vasculitides, antineutrophil cytoplasmic antibody (ANCA) screening using indirect immunofluorescence test as the first step is mandatory before the second step of determining the specific reactivity against antigen PR-3 or myeloperoxidase. This review also discusses the misinterpretation or over-interpretation as well as misuse of tests such as ASLO titer and serum uric acid. Finally, the importance of a new test for antibodies against citrullinated cyclic peptides (anti-CCP antibodies) in the diagnosis of rheumatoid arthritis, especially in its very early stage, is discussed.

Key words: musculoskeletal (MSK) diseases, inflammatory markers, inflammatory polyarthritis, laboratory investigations, arthritis

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INTRODUCTION

A recent article in this journal introduced the subject of clinical assessment of musculoskeletal (MSK) diseases.[1] In it the background problem of general lack of training and awareness with regard to MSK diseases was highlighted. The reason for this situation was identified as lack of emphasis on MSK diseases in undergraduate and postgraduate medical curriculums around the world. Therefore, these diseases are neglected by everyone.[2-5] Yet, they are among the commonest conditions seen in any general outpatient clinic, where approximately 15-25% of the patients have complaints related to the musculoskeletal (MSK) system.[6,7]

In clinical medicine there are 3 'tools' used for making a diagnosis, namely clinical history, physical examination and laboratory investigations. Rheumatology (the discipline dealing with MSK diseases) being a strongly clinical

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subject, generally 80% of diagnostic information is provided by accurate clinical history. Physical examination adds only 15% of diagnostic information while laboratory investigations add only 5% towards making a diagnosis.[8] This fact is central to efficient and cost-effective clinical care of the patients with MSK diseases. The discipline of rheumatology is highly clinical, and over-reliance on investigations and their over-interpretation is to be strongly discouraged. This is especially true for the beginners in rheumatology who have yet to hone their history taking skills. They must learn to take an accurate clinical history for making a provisional diagnosis along with a short list of differential diagnoses. This list would usually become even shorter after a careful physical examination. Then, a list of pertinent investigations is drawn up to corroborate the main diagnosis. In this scheme, there is no place for 'arthritis panels' of investigations in which most of the tests would be redundant and may often actually confuse the issue because of false-positive or false-negative results. It is unfortunate that such 'battery of tests' or 'panels' are thriving in most countries (mainly for commercial reasons driven by market forces), playing havoc on patients with MSK diseases. It is not uncommon for these tests to be carried out using substandard techniques and poor quality control, with a high rate of false positive and false negative results causing further problems.

In this write up diagnostically useful investigations commonly used for patients with joint diseases only are discussed. Pertinent investigations, basic principles, 'gold standard' techniques, quality control issues and interpretation of the results are pesented. For more details the reader is referred to standard texts and major reviews.[9-14]

INVESTIGATIONS

1. Acute phase reactants that confirm inflammatory nature of the disease:
   a. Erythrocyte sedimentation rate (ESR)
   b. Platelet count
   c. Albumin – globulin ratio (A/G ratio)
   d. Alkaline phosphatase (ALP)
   e. C-reactive protein, others.
2. Rheumatoid factors (RF)
3. Anti-nuclear antibody (ANA) and its subsets including anti-double stranded DNA antibody (anti-dsDNA), anti-extractable nuclear antigens (anti-ENA) antibodies of different specificities
4. Complement component C3 and C4 levels
5. Antibodies to cyclic citrullinated peptide (anti-CCP)
6. Anti-streptolysin 'O' antibody (ASO) titer
7. Antineutrophil cytoplasmic antibodies (ANCA)
8. Synovial fluid analysis
9. Serum uric acid.

ACUTE PHASE REACTANTS THAT CONFIRM INFLAMMATORY NATURE OF THE DISEASE

Erythrocyte Sedimentation Rate (ESR)

Overproduction of asymmetric plasma proteins (e.g. fibrinogen, immunoglobulins) occurs as part of acute phase reaction during inflammation, irrespective of whether it is due to infection or autoimmune or neoplastic process. These plasma proteins react with red blood cell (RBC) membrane causing them to stick together in stacks (rouleaux).[15] Because these stacks of RBC are heavier than individual RBC, they rapidly fall to the bottom of a column of anticoagulated (citrate anticoagulant) blood. ESR measures the rate of fall of RBC in a vertical column of anticoagulated blood over a period of 1 hour. It is among the cheapest, sturdiest, easiest to perform and read, and possibly one of the most useful investigations for distinguishing inflammatory from non-inflammatory musculoskeletal diseases. However, there are certain caveats. Firstly, there are normal variations of ESR values related to age, fasting or non-fasting state, and menstrual cycle that need to be taken in account. Secondly, there is a small proportion of normal individuals who may always have an abnormally high ESR without any obvious reason. Moreover, ESR is rather non-specific in the sense that it does not distinguish between different forms of inflammation (i.e. sepsis, autoimmune reaction, malignancy etc.). Lastly but not the least, among musculoskeletal diseases there are well known conditions where ESR may not rise even when the disease is in clinical relapse or may not drop

down to normal levels even during remission. This problem is seen most frequently in systemic lupus erythematosus (SLE), ankylosing spondylitis-spondyloarthritides (AS-SpA) group and scleroderma.[14] If these points are taken care of, ESR is useful both in distinguishing inflammatory from non-inflammatory MSK conditions as well as in assessing the response to treatment. The American College of Rheumatology (ACR) criteria for clinical remission of RA[16] also recommends ESR (Westergren technique) for confirming clinical remission. The test should be done using a fasting blood sample. In most laboratories, the upper limit of normal values is 15 mm/hr for women and 10 mm/hr for men. However, this does not take into account the increase in ESR values with aging. Therefore, for men the upper limit of ESR is his age divided by 2. For women, it is 10 added to her age and then divided by 2.[17] ACR criteria for clinical remission in rheumatoid arthritis (RA) has suggested ESR of < 30 mm/hr.[16] Therefore, rheumatologists traditionally consider 30 mm/hr as the upper limit of normal for patients with inflammatory rheumatic diseases. The value also increases in certain non-inflammatory conditions like anemia, renal failure and pregnancy, and decreases in congestive heart failure, cryoglobulinemia, hypofibrinogenemia, certain diseases with changes in RBC morphology, and in conditions causing polycythemia.[18] Wintrobe technique is not the recommended method for ESR measurement.

Platelet Count

Rise in platelets above normal levels as part of acute phase reaction is called reactive thrombocytosis.[14,19] Recent studies have shown that increased IL-6 synthesis in inflammation stimulates megakaryocytopoiesis in the bone marrow causing reactive thrombocytosis.[20] In rheumatoid arthritis, as also in several other inflammatory diseases, the platelet count rises during flares of disease activity and falls with remission.[21] Therefore, this is a useful indicator for monitoring disease activity in routine practice. The caveat is that SLE and antiphospholipid syndrome are two diseases where platelet counts may be low as part of the disease activity.

Albumin/Globulin Ratio

Albumin is among a small group of proteins (e.g. transferrin, and a few other rare ones) the concentration of which decreases in the blood during inflammation.[9] On the other hand, inflammation is associated with a rise in the levels of a large number of acute phase proteins in the blood (including fibrinogen, haptoglobin, cerulopasmin, serum amyloid-A, C-reactive proteins, complement components, polyclonal increase in immunoglobulins, and several others).[9] This leads to the reversal of albumin to globulin ratio to <1. Normally serum albumin level being more than the level of all the other serum proteins combined, normal albumin to globulin ratio (A/G ratio) is 1 or > 1. Therefore, reversal of A/G ratio is a good indicator of the presence of inflammation in the body.

Alkaline Phosphatase

This enzyme is also one of the acute phase reactants and, therefore, rises non-specifically in many inflammatory conditions including inflammatory MSK diseases.[9] Therefore, mild to moderate isolated rise in serum alkaline phosphatase levels in systemic inflammatory MSK disease like RA, would not entail a detailed search for liver or bone disease. However, there is one special situation, namely Paget's disease where its rise is due to the bone involvement.

C-Reactive Protein (CRP) and other Acute Phase Proteins

CRP is among the acute proteins that rise during inflammation.[9,11] Its name is derived from its ability to bind and to cause precipitation of the somatic C-polysaccharide of pneumococcus. It is mainly synthesized in the liver. Because its levels rise rapidly in tissue damage and inflammation it is considered more sensitive an indicator of inflammation than ESR. However, the time taken in getting the test results back from the laboratory is longer than for ESR. This offsets its value in routine evaluation of inflammatory MSK diseases in a busy outpatient clinic. Moreover, CRP is an expensive test.

There are a large number of other serum proteins that rise in inflammation.[9,11] However, they are of no practical use in day-to-day clinical practice.

RHEUMATOID FACTORS (RF)

RF is an autoantibody with specificity against

aggregated constant (Fc) region of immunoglobulin G (IgG). RF may belong to any immunoglobulin isotype (IgG, A or M).[10,12,22,23] However, the so-called 'classical', easily detectable RF is of IgM isotype. The standard technique for its detection has been agglutination or aggregation of IgG coated latex particles (latex fixation test). It mainly detects the 'classical' (IgM isotype) RF. More recently, however, nephelometry has become the more popular technique for its detection as it can be used to detect different isotypes of RF. A small proportion of normal persons (~2-5%) may have detectable amounts of RF, but generally in low titers. There are a large number of inflammatory as well as infective conditions where RF can be detected in the blood, occasionally in significant titers. In clinically-diagnosed rheumatoid arthritis it is usually present in 70-80% of patients. Thus, the presence of RF by itself without a clinical diagnosis of RA is not diagnostic of this disease. However, in a clinically-diagnosed RA, the presence of RF (seropositive RA) classifies the subset of the disease, which is more aggressive and generally requires early aggressive treatment with disease-modifying drugs and/or biologicals. Higher titers correlate with severe erosive destructive disease.

ANTI-NUCLEAR ANTIBODIES AND THE SUBSETS

Anti-nuclear antibodies (ANA) are a family of autoantibodies with specificity against the following:

- Nuclear antigens including
  - Chromatin-associated antigens (single and double stranded DNA, histones)
  - Ribonucleo-proteins [Sm, different snRNP, SS-A (Ro) and SS-B (La)];
- Nucleolar antigens (RNA polymerases, ribosomal RNP, topoisomerase 1, others)
- Some cytoplasmic antigens (tRNA, others).[25]

Despite the availability of several newer techniques (mainly ELISA method), indirect immunofluorescence using human epithelial tumor cultured cell line HEp-2 as the substrate remains the 'gold standard' and the recommended technique for ANA screening in routine clinical practice.[23,25] If fluorescence-ANA (FANA) test gives a positive result, further investigations for defining the ANA-subsets may be necessary. A proportion of serum from healthy, normal persons may show the presence of low titers of ANA. Thus Tan, et al[26] reported that in apparently healthy individuals the frequency of a positive ANA titer was 31.7% with Hep 2 cells at a titer of 1 to 40, 13.0% at titer 1 to 80, 5.0% at titer 1 to 160, and 3.3% at titer 1 to 320.[26] However, Thompson and Bird have stated the prevalence of a positive ANA to be 8% in healthy children and 15% in healthy adults.[9] Using the figures of Tan, et al, Lightfoot calculated that the risk that an individual with ANA at a titer of 1 to 320 would develop systemic lupus erythematosus or Sjögren's syndrome during 10 years of follow-up was less than 5%.[27] Lightfoot recommended that ANA testing should be used to confirm a clinical diagnosis rather than simply as a screening tool. It is to be noted that the variable sensitivity of the test used as well as the genetic background of different populations, may produce different results. Thus, in a study from India <1% of normal adults showed ANA positivity.[29] On the other hand, 95-99% patients with SLE usually have high titers of ANA in their blood.[30] Thus, a negative FANA test virtually excludes the diagnosis of SLE. Yet, ANA is non-specific as it is present in varying titers in rheumatoid arthritis as well as in most other connective tissue diseases including scleroderma, Sjögren's syndrome, mixed connective tissue disease, and others.[9] Therefore, the presence of ANA by itself is not diagnostic of SLE.

Anti-DNA antibodies could have specificity against native double stranded DNA (nDNA or dsDNA) or denatured single stranded (ssDNA).[9] The latter is non-specific and present in normal persons as well as in a large spectrum of inflammatory and infective diseases. On the other hand, anti-double stranded DNA antibodies are highly specific for SLE and usually correlate with the presence of renal and/or central nervous system involvement of SLE. But, the sensitivity of anti-dsDNA antibody is rather low. Thus, a single test done randomly at any point in time may not yield more than 20% positivity in SLE patients (as some of them could be in remission at that time).[13] Even if repeated testing is done over long follow-up during the course of the disease in patients with SLE, the positivity is only 60-70%.[13] The experience in Kuwait with point-prevalence of anti-dsDNA

antibody among patients with SLE showed only 35.5% positivity.[30] The difficulty is that there are only two reliable techniques for its detection, both of which are cumbersome and expensive. These are Farr radio-immunoprecipitation technique (requires well equipped radioimmunoassay laboratories with highly standardized reagents; very expensive) and the indirect immunofluorescence test on kinetoplast of *Crithidia luciliae* (a hemoflagellate). This requires cultured *C. luciliae* slides and immunofluorescence set up where a well trained experienced person reads the test results visually. Thus, because these two most reliable techniques that are 'gold standard' for detecting anti-dsDNA antibodies are expensive and labor-intensive, most routine clinical service laboratories around the world are not using them. Instead, the cheaper and easy to perform automated ELISA technique has become popular worldwide. Unfortunately, ELISA test results vary widely between kits from different manufacturers,[31] and often give false positive results due to denaturation of dsDNA on binding to plastic material used in ELISA plates.[25] Because of these problems the author has followed the following 'rule of thumb' in clinical practice: never order an anti-DNA test without first getting a positive FANA test (if FANA is negative, theoretically anti-dsDNA test cannot be positive); always get either Farr immunoassay (but the quality of dsDNA has to be ensured otherwise even this test gives false positive results,[25] or even better, to use *C. luciliae* immunofluorescence test for anti-dsDNA antibody. Stringent quality control is essential in anti-dsDNA test as the positive results have serious clinical consequences. A positive anti-dsDNA test, especially in high titers, correlates with SLE disease activity, and more importantly, with CNS and renal involvement, requiring aggressive, often toxic, therapies.

The increasing number of more and more refined immunoassay techniques allows the detection of different subsets of ANA with reactivities against a variety of cellular antigens.[32] These antibodies are commonly referred to as anti-ENA antibodies (antibodies against different extractable nuclear antigens – ENA). Line immunoassay (LIA) and ELISA using highly purified nuclear antigens are the two most commonly used techniques for detecting anti-ENA antibodies; the latter being much more reliable and easy to perform.[33] For detailed discussion of the types of anti-ENA and their clinical significance the reader is referred to standard textbooks or major reviews.[10,12,13,23,25] The four main anti-ENA antibodies are the ones that react with RNA-binding proteins, namely Sm, U1-RNP, Ro (SSA) and La (SSB). In summary, anti-Sm antibodies are considered highly specific for SLE, but seen only in approximately 10-25% of patients, and correlate with more severe disease often with CNS involvement and vasculitis. Anti-U1-RNP antibodies are seen in ~30% of SLE patients, correlate with Raynaud's phenomenon, swollen fingers, arthritis, myositis, and clinical features recognizable as mixed connective tissue disease, a disease in which this antibody is considered a diagnostic feature. Anti-Ro (anti-SSA) and anti-La (anti-SSB) antibodies are seen in ~40% and ~15% of SLE cases respectively. They tend to correlate with photosensitive rash, subacute cutaneous lupus erythematosus (SCLE), neonatal lupus, and the presence of Sjögren's syndrome. These are seen in a much higher proportion of patients with Sjögren's syndrome and tend to correlate with extraglandular disease, vasculitis and lymphoma. It is to be noted that anti-ENA antibodies are mainly used by specialists (rheumatologists) for defining the clinical subsets of systemic connective tissue diseases, especially among early patients where the full clinical picture is still to evolve or in those with overlap syndromes. General physicians would hardly ever need to requisition an anti-ENA test.

COMPLEMENT COMPONENT C3 AND C4 LEVELS

Complement factor C3 and C4 are proteins of the complement system, which is part of the innate immunity of the defense organization of the body, mediating and amplifying non-specific inflammation and phagocytic activity.[10,12,34,35] Complement system consists of more than 30 proteins with C3 and C4 being the most abundant complement proteins in the serum. Complement components including C3 and C4 usually behave as acute phase proteins and often show a rise in blood levels during infection and non-specific inflammation. However, in SLE (immune-complex mediated systemic diseases par excellence) and a few other immune-complex mediated diseases

(infective endocarditis, post-streptococcal glomerulonephritis) there seems to be peripheral consumption without compensatory increase in synthesis of at least these 2 complement components with consequent fall in their blood levels. This occurs most prominently in SLE with active renal disease. But, low C4 occurs in almost all the patients with active SLE. The levels rise to normal range once the condition starts to improve and the disease goes into remission. Because of these characteristics, measurement of serum C3 and C4 levels are routinely used in managing SLE patients in day-to-day clinical practice. C3 and C4 serum levels are easily measured by radial immunodiffusion test or, nowadays using automated nephelometric method. There are also some uncommon inherited deficiencies of some of the complement components with their characteristic clinical features that are beyond the scope of this discussion. But, it is to be noted that persons with deficiency of C1, C2, and C4 levels show increased susceptibility for developing SLE.[35]

ANTIBODIES TO CYCLIC CITRULLINATED PEPTIDE (ANTI-CCP)

In the recent past test for antibodies against cyclic citrullinated peptides (anti-CCP antibodies) has gained rapid popularity among clinical rheumatologists. This autoantibody has been shown to be highly specific for RA.[36-38] The presence of anti-CCP antibodies in the serum in significant titers strongly correlates with the presence of active RA, aggressive and destructive RA and, if detected in healthy persons, predicts future appearance of RA. This has made anti-CCP antibody test as one of the most sought after tests in rheumatology in recent years. Besides being highly specific (if positive in significant titers the patient is most likely to have RA), it has good sensitivity of >70-75% (better than RF). Because of these characteristics anti-CCP antibody test has become extremely popular for early inflammatory polyarthritis for identifying aggressive destructive RA at an early stage for aggressive treatment. However, it must be noted that its sensitivity is not near 100% and therefore, anti-CCP test cannot be used for screening out RA. In established RA its positivity classifies the patients as having progressive destructive disease, who would be candidates for most aggressive therapeutic regimens. In some of the polymyalgic or fibromyalgic presentations of RA, anti-CCP may help in diagnosing RA and save them from getting labeled as 'psychogenic rheumatism'. In overlap syndrome patients, where RA could be one of its components (e.g. rheupus), the severity of RA could be gauged by the presence or absence of anti-CCP antibodies. The levels of anti-CCP antibodies correlate with disease activity, further helping the clinician keen to induce clinical remission.

ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCA)

ANCA are autoantibodies with reactivity against primary granule components of granulocytes and monocytes.[10,12,23] Using human peripheral blood ethanol fixed granulocytes as substrate in indirect immunofluorescence test, two distinct staining patterns can be recognized, namely cytoplasmic (called c-ANCA) and perinuclear staining (called p-ANCA). This is due to their distinct reactivities against separate antigens, namely serine protease-3 (PR3) and myeloperoxidase (MPO) respectively. There are several other types of ANCA with reactivities against other granule components (e.g. lactoferrin, elastase, cathepsin G, lysozyme), most of them giving perinuclear staining pattern. (Note: p-ANCA staining pattern is actually an interesting artefact of ethanol fixation. It causes positively-charged cytoplasmic granules to move and rearrange themselves around the negatively-charged nuclear membrane giving the characteristic perinuclear staining pattern. Some of the ANAs also give true perinuclear staining that can be differentiated using formalin fixed substrate neutrophils that would show diffuse cytoplasmic staining while ANA would give a true perinuclear staining). Despite early excitement regarding ANCA test, over a period of time it has been shown to be of limited value in clinical practice. C-ANCA in significant titers correlates with active widespread Wegener's granulomatosis (WG) but less so with its limited forms. The more serious problem, however, is that the test is often positive in other rheumatic diseases and thus has lost its specificity for WG as was claimed in the early stages.[12,39,40] Presently it remains only as a confirmatory test in the clinically-diagnosed systemic form of WG. p-ANCA with MPO specificity is characteristi-

cally seen in microscopic polyangiitis. However, it is also seen in other forms of vasculitis, inflammatory bowel disease, several autoimmune diseases, infections and due to drugs.[40] Development of ELISA assays using purified PR-3 and MPO as antigen substrates has also not solved this problem of false positivity as the commercially available ELISA kits often give false positive results, further confusing the situation. The author has followed the following algorithm: clinical diagnosis of severe systemic necrotizing vasculitis of some type → request immunofluorescence ANCA-screening test. If positive (irrespective of the fact whether it is c- or p-ANCA) → request PR3 and MPO anti-body test with ELISA technique. If result is positive for PR3 it is likely to be WG, and if it is positive for MPO it is likely to be microscopic polyangiitis. It is important to emphasize that ANCA by ELISA test should not be requested as the first step because of the serious problem of false positivity in ELISA assays.

ANTI-STREPTOLYSIN O ANTIBODY (ASO) TITER

Testing for ASO titer is primarily to demonstrate an antecedent streptococcal infection.[41-43] In some children and juveniles antecedent streptococcal infection may lead to the development of rheumatic fever, and antistreptolysin O has been the most widely used test to demonstrate it. But, there are serious problems with this test because the titers of antistreptolysin O vary with age, season, and geographical region. They reach peak levels in the young, school age population. Titers of 200–300 Todd units/ml are common, therefore, in healthy children of elementary school age (false positive).[42] On the other hand, up to 20% of documented rheumatic fever cases may not show a rising titer (false negative). It is also to be taken in account that non-rheumatic normal individuals in an area with a high incidence of rheumatic fever have inordinately elevated levels of streptococcal antibodies in serum. The presence of elevated streptococcal antibody titers in such a population, which probably reflects a high background prevalence of streptococcal infections, should be taken into consideration when evaluating the role of the group A Streptococcus in non-purulent complications of infections.[45] It is to be noted that it takes at least 4-5 weeks from the episode of acute Streptococcal pharyngitis for ASO titers to peak. Therefore, for the diagnosis of acute rheumatic fever a rising titer must be demonstrated requiring 2 test samples done at an interval of 4-5 weeks.[12] Moreover, it is to be noted that persistent arthritis or back pain never occurs in rheumatic fever. Another important feature of rheumatic fever is almost a universal occurrence of carditis even at an early stages, easily demonstrable with sensitive new (echocardiographic and/or electrocardiographic) techniques in most patients. Lastly but not the least, the first episode of rheumatic fever almost always occurs below 15 years of age.[46] In developing countries the age of onset could be even lower.[47] Therefore, in routine rheumatological practice the following points may be noted regarding ASO titer and its relevance: Firstly, never requisition this test in patients above 15 years of age; never requisition this test if the patient is having persistent arthritis (>6 weeks). Never requisition this test in patients with back pain, as back pain is never a manifestation of rheumatic fever. Lastly, even in the presence of significant titers of ASO the diagnosis of rheumatic fever cannot be made in the absence of clinical features, among which the most important component is carditis. The practice of initiating treatment with long-acting penicillin injections in a child, juvenile or an adult simply because there is a positive ASO test is to be strongly discouraged. Similarly, penicillin treatment in a child, juvenile or an adult with persistent arthritis, just because ASO result has been reported as positive must be strongly discouraged as it delays diagnosis and treatment of the actual disease (in most cases juvenile idiopathic arthritis), with disastrous outcome.

SYNOVIAL FLUID ANALYSIS

Unlike effusions in other body spaces (pleural, pericardial or peritoneal) synovial fluid examination is not mandatory in most patients with joint disease. Moreover, unlike other body fluids, only a few investigations are of relevance in synovial fluid. These include leukocyte count (even differential count is usually not necessary), smear (for gram stain) and culture of the fluid for microbes, and examination for the presence of crystals. Joint aspiration and examination of synovial fluid is mandatory in patients presenting with monoarthritis.

Firstly, the leukocyte count helps in distinguishing inflammatory from non-inflammatory joint effusions. A leukocyte count of >2000/cmm is indicative of an inflammatory process. If the fluid is inflammatory in nature, it further helps in distinguishing between septic inflammation, inflammation due to the presence of crystals or neither of these causes (i.e. sterile inflammation due to autoimmune etiology; the majority of the patients belongs to this category). However, demonstrating septic infection in the joint is somewhat difficult. This is mainly because most patients reach the rheumatologist after having been given one or the other antibiotic. Also, some of the microbes may require special procedures and culture media for their demonstration (e.g. gonococcus, acid-fast bacilli and fungus). For demonstrating crystals in synovial fluid a good microscope fitted with the facility to examine specimen in polarized light is essential. Secondly, it requires a person trained and experienced in examining specimens for crystals. In routine practice, only one crystal disease is of importance, namely gout, which shows negatively birefringent needle-shaped crystals in synovial fluid. The crystals are easy to recognize and usually present in abundance in the majority of the gouty specimens. An important practical point is that crystal diseases occur, by and large, in men over 40 years of age. Therefore, except in the rarest of rare cases, crystal examination of the joint fluid is generally requisitioned if the patient is a male above 40 years of age with clinical features suggestive of gout. It is hardly ever that one requires analyzing joint fluid in a patient with polyarthritis. Thus, polyarticular gout is relatively uncommon. Moreover, its clinical features (gouty tophi) are rather typical and easy to recognize. Occasionally, a patient with a polyarticular disease (e.g. rheumatoid arthritis) may show complete improvement in all the joints but one joint does not respond or flares up during follow up. In such a clinical setting it may become necessary to exclude secondary septic arthritis requiring examination of the synovial fluid from that joint.[48-51]

HLA B27 Screening

All the diseases within the category of spondyloarthritis [SpA, i.e. ankylosing spondylitis, psoriatic spondyloarthritis, inflammatory bowel disease related spondyloarthritis, juvenile spondyloarthritis, enterogenic as well as urethritic form of reactive arthritis; also included in the SpA-concept are undifferentiated spondyloarthritis, synovitis acne pustulosis hyperostosis osteomyelitis (SAPHO) syndrome, non-granulomatous acute anterior uveitis] show strong association with one of the B locus genes on the major histocompatibility complex, namely human leukocyte antigen (HLA) B27.

A recent study has shown that at Primary Care level it may be difficult for the physicians to clinically distinguish non-specific non-inflammatory biomechanical low back pain from very early stages of one of the diseases of spondyloarthritis group.[52] Routine radiological examination is also negative at this early stage. In such a situation screening for HLA B27 could be of great help. It may be one of the most important methods of detecting the disease at an early stage when it can be satisfactorily controlled with the available drugs before permanent damage sets in. There are, however, some technical problems. The technique of microlymphocytotoxicity in microtiter plates using rabbit complement and specific antisera has been the 'gold standard'. More recently, polymerase-chain reaction using purified DNA from the patient is becoming more popular because of its increased sensitivity as well as specificity.[53,54] However, both of these tests are labor-intensive, require special equipment and high degree of expertise not feasible in routine clinical laboratories. Therefore, many clinical laboratories have started to use flowcytometric method for the screening of HLA B27. Unfortunately, being a less sensitive technique it often gives a false negative result. Therefore, if the clinician is strongly suspicious of the presence of one of the spondyloarthritides the patient must be referred to a specialized laboratory for the screening of HLA B27 gene. At All India Institute of Medical Sciences, New Delhi in India the reference laboratory prefers the single tube PCR-SSP protocol.

SERUM URIC ACID

Estimation of serum uric acid has little role in the routine clinical practice of rheumatology.[55,56] There is widespread misunderstanding among doctors that aches and pains anywhere in the body associated with coincidental finding of high serum uric acid is diagnostic of gout. This is not only wrong but borders on the

absurd, especially because many of these persons are prescribed allopurinol with the wrong notion that asymptomatic hyperuricemia requires drug treatment. There is no valid reason for doing so, as asymptomatic hyperuricemia does not cause any ill effect directly. High serum uric acid is seen in varying proportions of normal adult population, especially among those with tendency for metabolic syndrome. It correlates with obesity, dislipidemia and hypertension.[57] All cause mortality, and put such individuals at high risk for ischemic heart disease.[58] Occasionally, asymptomatic hyperuricemia could be due to uncommon situations, e.g. underlying hematological malignancies, polycythemia vera, vitamin B_{12} deficiency, pre-eclampsia, lead exposure or lead nephropathy.[12] It is now also known that uric acid is an endogenous antioxidant.[59] Therefore, its rise in blood could be Nature's way of compensating for the poor lifestyle of the individual who is a candidate for metabolic syndrome and its complications. Gout, on the other hand, is a clinical diagnosis [recurrent acute attacks of severely inflammatory monoarthritis classically in the first metatarsophalangeal joint called 'podagra' or in any of the other joints in the ankle/feet region (> 80% of attacks occur in these joints)] in the male above 40 years of age often with family history of gout. Once suspected, demonstration of monosodium urate crystals on polarized light microscopy in the joint aspirate or even in a small drop of tissue fluid aspirated from the inflamed periarticular tissue is required for confirmation of diagnosis. In summary, serum uric acid level should not be used as a screening test for any musculoskeletal disease. On the other hand, in patients with recurrent attacks of gouty arthritis or those with tissue deposits (e.g. gouty tophi, gouty nephritis or other manifestations) taking serum uric acid lowering drugs, serial measurement of serum uric acid is required for monitoring drug response.

CONCLUSION

Rheumatology is a strongly clinical subject where 80% of the diagnostic information comes from clinical history, 15% from physical examination and only 5% from the laboratory investigations. Therefore, it is common sense that a provisional diagnosis is first made based upon reliable history and accurate physical examination. Then, a short list of relevant investigations should be drawn up to confirm or exclude a specific diagnostic possibility. Reliability of the test procedure is essential. If laboratory reports show gross variance from the provisional diagnosis, a discussion with the concerned laboratory person as well as a re-evaluation of clinical history and physical examination may be required. Finally, the patient with an isolated abnormal laboratory test result but with no clinical illness should not be treated.

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CME/CPD Questions

After you have completed reading the article *Clinically pertinent and cost-effective use of laboratory investigations in musculoskeletal diseases*, take the test given below. Circle T (True) or F (False) in the answer sheet (page 104) to show the correct answer to each question. Questions 1 to 10 are related to the content in this article.

1. ESR is a useful laboratory investigation for distinguishing inflammatory from non-inflammatory musculoskeletal diseases.
2. Most patients with chronic inflammatory rheumatic diseases have low platelet count.
3. In chronic inflammatory rheumatic diseases polyclonal diffuse increase in total serum globulin levels is commonly observed.
4. Serum alkaline phosphatase is invariably high in active rheumatoid arthritis because of increased osteoblastic activity in the bone marrow.
5. Classical rheumatoid factor is an autoantibody of IgM class reactive against the Fab-portion of aggregated IgG immunoglobulin.
6. Systemic lupus erythematosus (SLE) would be a highly UNLIKELY diagnosis if the fluorescent anti-nuclear antibody test (FANA) is NEGATIVE.
7. Despite its high specificity, most of the routinely available laboratory techniques for performing tests for anti-DNA antibody make it unreliable.
8. Anti-DNA antibody test may be negative even when a patient has active SLE.
9. A patient with small finger-hand joint swelling and tenderness only of a few days' duration can still be diagnosed as having definite rheumatoid arthritis if antibodies to cyclic citrullinated peptide (anti-CCP) are positive.
10. In the presence of low-back pain a positive test for HLA B27 is still considered non-specific for early ankylosing spondylitis (AS).