Serum Monocyte Chemotactic Protein-1 Concentrations Distinguish Patients With Ankylosing Spondylitis From Patients With Mechanical Low Back Pain

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Objectives: This study aimed to identify potential blood-derived biomarkers distinguishing patients with ankylosing spondylitis from those with mechanical low back pain.

Methods: Serum and synovial fluid samples from our cohorts were assayed by using enzyme-linked immunosorbent assay for the following inflammatory biomarkers: interleukin (IL)-1α, IL-6, IL-8, IL-17, IL-23, monocyte chemotactic protein (MCP)-1, macrophage inflammatory proteins (MIP)-1α, MIP-1β, tumor necrosis factor-α (TNF-α), interferon-α (IFN-α), IFN-β, metalloproteinase (MMP-3), and bone morphogenetic protein 7 (BMP-7).

Results: After screening, a panel of serum and synovial fluid samples with a series of potential biomarkers, cytokines including IL-6, IL-8, MMP-3, and MCP-1 were selected for additional testing because they exhibited higher concentrations than paired serum samples in the synovial fluid. Sera obtained from 50 patients with ankylosing spondylitis and 27 patients with mechanical low back pain were measured for these biomarkers.

Conclusions: The MCP-1 serum was identified as a biomarker candidate, distinguishing ankylosing spondylitis from mechanical low back pain with a sensitivity of 96% and a specificity of 83.3%.

Key Words: ankylosing spondylitis, low back pain, cytokines, monocyte chemotactic protein-1

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Ankylosing spondylitis (AS) is a chronic inflammatory arthritis predominantly affecting the axial skeleton.[1] A major symptom of this disease is low back pain. However, because low back pain is a common symptom among patients suffering from spondyloarthropathy (SpA) family and rheumatology practices, it is important to mention that diagnosis of AS cannot be based on this symptom alone.[2] To a certain extent, the low back pain of AS can be distinguished from other causes because in AS, the onset of back pain is usually insidious; comes at an early age; is associated with morning stiffness; wakes patients up from sleep in the second half of the night; and improves with exercise but not with rest. This type of low back pain is known as "inflammatory low back pain," whereas pain from other causes is denoted as "mechanical low back pain."[2–4] However, only 5% of the patients suffering low back pain meet the criteria for AS.[5,6] In addition, specialized clinical and imaging evaluations made by rheumatologists are required to improve the accuracy of the diagnosis.[7,8] The accuracy of diagnosis by nonspecialists would be much higher if there were useful blood-derived biomarkers. Although frequently used, acute phase reactants have been found to have low sensitivity and specificity when used alone.[9] Currently, the most useful blood-derived biomarker is HLA-B27. It is present in more than 90% of AS patients of most ethnicities.[10] However, because it is also present in 6% to 9% of most normal populations, the combination of having inflammatory low back pain and HLA-B27 raises the probability of AS to only about 11% to 40%.[6] Clearly, additional sensitive and specific blood-derived

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biomarkers are needed to evaluate the clinical activity of AS.[11]

Several studies have been published in attempts to identify specific blood-derived biomarkers for AS.[11] Unfortunately, almost all of these studies compare AS patients against healthy individuals rather than patients with mechanical low back pain. To our knowledge, this paper is one of the first that aimed at comparing a number of both arthritis-related, blood-derived candidate biomarkers in an attempt to distinguish AS from mechanical low back pain patients.

We adopted a 2-stage strategy, based on the premise that most blood-derived biomarkers probably originate at the local inflammation as joint and enthesis. In the first stage, we collected both serum and synovial fluid samples from patients suffering from SpA, including AS, undifferentiated spondyloarthritis (USpA), and reactive arthritis (ReA), and, as a comparison, also rheumatoid arthritis (RA). We tested these samples by using the enzyme-linked immunosorbent assay (ELISA) test for 13 biomarker candidates and identified 4 of them, which showed higher levels in the synovial fluid compared with the serum samples. The tests for these 4 biomarker candidates were then conducted in a second, larger cohort of AS patients and compared with patients having mechanical low back pain as well as healthy individuals. We identified serum monocyte chemotactic protein-1 (MCP-1) as a promising candidate biomarker, meriting inclusion in future multicenter biomarker studies.

MATERIALS AND METHODS

Demographics

Two cohorts of patients participated in the study. The first cohort consisted of the following patients: 10 USpA, 16 ReA, 10 AS, and 12 RA patients. The diagnoses of USpA and ReA were based on the European Spondyloarthropathy Study Group Criteria.[12] All ReA patients developed arthritis within 1 month of an episode of diarrhea. The diagnosis of AS was based on the modified New York Classification Criteria,[13] whereas the diagnosis of RA was based on the American College of Rheumatology Classification Criteria.[14] All patients were considered by their clinicians to have an active form of the disease. In the case of SpA, the ''Ankylosing Spondylitis Disease Activity Index'' (BASDAI) exceeded 4.0 (0-10 scale).[15] The characteristics of these patients are shown in Table 1. The following numbers of patients in this cohort provided both serum and knee synovial fluid samples: 7 USpA, 13 ReA, 2 AS, and 12 RA patients. All patients were on nonsteroidal anti-inflammatory drugs. The majority of the SpA patients was on sulfasalazine, whereas most of the RA patients were on methotrexate. None of the patients were on biologics or corticosteroids.

For a more precise evaluation, serum samples were collected from a second cohort of 35 male and 15 female AS patients for whom detailed clinical evaluations were available. None of these AS patients were on biologics. One patient each was on methotrexate or prednisone at 5 mg/d, whereas 34 were being treated with sulfasalazine. These medications were prescribed even though there was no peripheral joint or enthesitis swelling. All 50 AS patients satisfied the Modified New York Classification Criteria for AS, and showed radiologic sacroiliitis.[13] The expression of HLA-B27 was tested in 47 of these patients and was positive in all of them. The mean and standard deviation (SD) of the AS patients' age was 35.5 ± 14 years. The mean values and (SDs) for erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were 17.2 ± 12 mm/h and 7.6 ± 6.2 mg/L, respectively. The means (and SDs) of the clinical scores were the following: spinal pain 4.0 ± 2.7, BASDAI 3.5 ± 2.1 and BASFI 1.8 ± 2.1, all on a scale of 0 to 10.[15,16] The means (and SDs) of the clinical metrology measurements[17] were as follows: modified Schober, 4.2 ± 3.3 cm; chest expansion, 3.9 ± 2.4 cm; and occiput-to-wall distance, 1.3 ± 3.8 cm. Knee and/or heel swelling was observed in 12 patients.

Twenty-seven patients, 12 male and 15 female, with chronic mechanical lower back pain were recruited from the orthopedic clinic after review by the rheumatologists. All mechanical lower back pain patients satisfied 2 entry criteria. First, based on global clinical evaluation, they were diagnosed by both an orthopedic surgeon and a rheumatologist as not having AS, but mechanical low back pain instead. Second, all of these patients had a history of episodes of pain in the dermatome distribution typical of sciatica.[18] The pain was restricted to 1 side in 21 of these patients and appeared on alternate sides in the other 6. There was no radiologic sacroiliitis in any of these mechanical low back pain patients. In addition, the diagnosis of sciatica was verified in 19 of these 27 patients by magnetic resonance imaging or computed tomography of the lumbar spine or nerve conduction in the lower extremities. The means (and SDs) of patient age, age at onset of pain, and duration of pain were 57.9 ± 11.0

TABLE 1. Demographics of Arthritis Patients in the First Cohort

	No. Patients	Sex	Age (y)	HLA B27	Duration	Inflammatory Back Pain
USpA	10	3M/7F	35.7 ± 9.5	2+/8−	72.3 ± 56	4
ReA	16	13M/3F	24.8 ± 8	10+/6−	8.3 ± 18	5
AS	10	7M/3F	33.5 ± 81	7+/3−	37.2 ± 35.4	9
RA	12	4M/8F	49.1 ± 22.6		72.6 ± 83.7	0

Values are in mean ± SD. Duration is denoted in months. The numbers in the ''inflammatory back pain'' column denote the numbers of patients in each category considered to complain of inflammatory back pain.

AS indicates ankylosing spondylitis; HLA, human leukocyte antigen; RA, rheumatoid arthritis; ReA, reactive arthritis; USpA, undifferentiated spondyloarthritis.

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years, 53.2 ± 1.6 years and 4.74 ± 4.2 years, respectively. The CRP was 0.23 ± 0.25 mg/L. The age at onset of pain in all except 2 patients was ≥45 years. Nineteen patients were retrospectively tested for HLA-B27, with only 3 being positive. Twenty-four patients responded to a questionnaire consisting of the following 3 questions: (1) Do you have ≥30 minutes of morning stiffness? (2) Does your back pain improve with exercise but not with rest? (3) Does your back pain wake you up during the second half of the night? Fifteen of the 24 patients (62.5%) responded positively to at least 1 question. Six responded positively to 2 of the questions. Most of the patients were on nonsteroidal anti-inflammatory drugs or analgesics.

Serum samples were also collected from 39 female and 14 male volunteers, all of whom were apparently healthy and exhibited no chronic low back pain. The mean (and SD) of their age was 29 ± 5.9 years. This project was approved by the Human Protection Committees of the participating institutions.

ELISA

Serum and knee synovial fluid samples were stored at −80°C. The levels of interleukin (IL)-6, IL-8, MCP-1, and tumor necrosis factor (TNF)-α (BD Biosciences), IL-1α, IL-17, IL-23, macrophage inflammatory protein (MIP)-1α, MIP-1β, metalloproteinase (MMP-3), and bone morphogenetic protein 7 (BMP-7) (R&D Systems), and IFN-α and IFN-β (PBL Biomedical Laboratories) were determined by sandwich ELISA using paired antibodies according to the manufacturers' recommendations. The lower limit of detection for all cytokines was 10 pg/mL. Serum and synovial fluid samples were routinely tested in duplicate. The values of each cytokine in a given group of patients were expressed as the means ± SD in pg/mL.

Statistical Analysis

Degrees of statistical difference were evaluated by the Mann-Whitney *U* test. The *P* values that were shown were not corrected for multiple testing. Diagnostic usefulness was evaluated by the receiver operator characteristic curve (ROC), as well as likelihood and predictive values (Medcalc). The samples derived from the first and second cohorts were assayed on different days. To avoid technical inconsistency, no attempt was made to normalize the data to compare results between the 2 cohorts. Instead, comparisons were made only between samples assayed on the same days.

RESULTS

Screening for Cytokine Biomarkers Differentially Expressed in Serum and Synovial Fluid

Serum and synovial fluid samples collected from our first cohort of 36 SpA and 12 RA patients (Table 1) were tested by using the ELISA test for the following 13 potentially arthritis-related factors: IL-1α, IL-6, IL-8, IL-17, IL-23, MCP-1, MIP-1α, MIP-1β, TNFα, IFN-α, IFN-β, MMP-3, and BMP-7. The results showed that

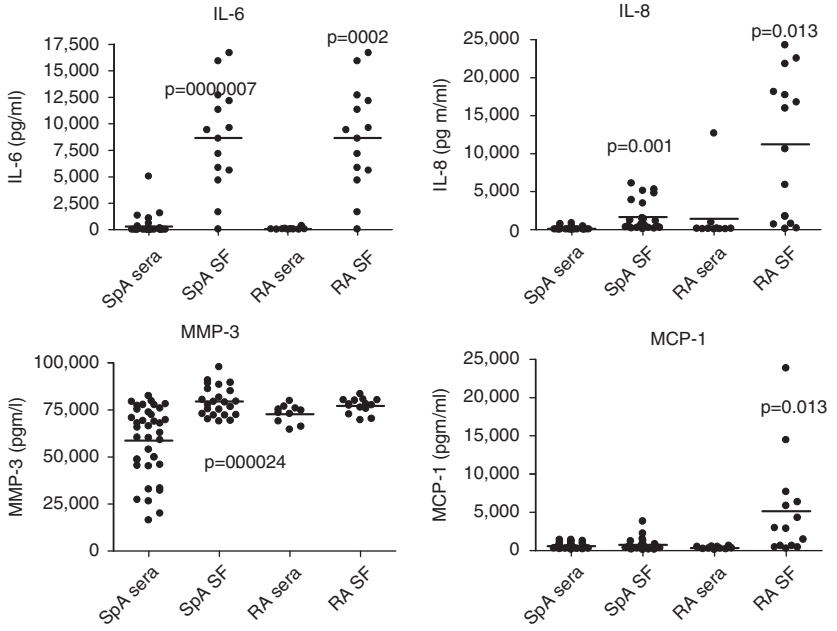

FIGURE 1. Sera and synovial fluids concentrations of interleukin (IL)-6, IL-8, monocyte chemotactic protein (MCP)-1, and metalloproteinase (MMP)-3. Levels of IL-6, IL-8, MCP-1, and MMP-3 in serum and synovial fluid samples of spondyloarthritis (SpA) and in rheumatoid arthritis patients. Each dot represents results from 1 sample. The horizontal bars indicate the mean values. The *P* values in the synovial fluid columns reflect comparisons to the corresponding serum sample values. The mean values of SpA serum and synovial fluid concentration are 582 and 766 pg/mL, respectively.

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only 4 of these candidate biomarkers were significantly higher in the synovial fluid compared with the serum samples, having *P* values ranging from 0.013 to 0.0000007. As shown in Figure 1, levels of IL-6 and IL-8 were higher in the synovial fluid samples of both SpA and RA patients. The level of MMP-3 was higher in the synovial fluid samples of SpA patients, whereas the level of MCP-1 was higher in the synovial fluid of RA patients. As we have observed earlier that MCP-1 transcripts are expressed at a high level in synovial tissue samples of SpA patients,[19] serum MCP-1 was included as a potential candidate biomarker in the subsequent analysis.

Levels of IL-6, IL-8, MMP-3, and MCP-1 in a Second Cohort of 50 AS Patients and 27 Patients With Mechanical Low Back Pain

We next measured the levels of IL-6, IL8, MMP-3, and MCP-1 in the serum samples of the following patients: a second cohort of 50 AS patients, 27 patients with mechanical low back pain, and 53 healthy individuals (Fig. 2). Serum MIP-1α levels were also assessed in comparison. The results for MCP-1 were the most remarkable in that the mean level in AS patients was statistically different than the mean level in patients suffering from mechanical low back pain (P = 5.7E-13) as well as healthy individuals (P = 7.1E-12). As there were more male patients in the AS group compared with the mechanical low back pain group or the group of healthy control individuals, a comparison of MCP-1 levels was also made among the male patients of these 3 groups. The MCP-1 levels of male AS patients were still higher than those with mechanical low back pain (P = 1.7E-14) or healthy controls (P = 3.9E-07). For the male patients, the mean age of the AS group was not statistically different from that of the male healthy controls (P = 0.62).

We then performed a correlation matrix to test whether there was a statistical relationship between serum MCP-1 level and any of the following clinical parameters: sex, age, ESR, CRP, BASDAI, "Bath Ankylosing Spondylitis Functional Index" (BASFI), Schober test, occiput-to-wall distance, chest expansion, finger-to-floor distance, and the extent of left lateral flexion of the lumbar spine. There was no correlation of the MCP-1 level with any of these parameters, including ESR and CRP ($r < 0.4$). There was also no relationship between the MCP-1 level and knee and/or heel swelling.

Finally, we used the ROC curve to evaluate the usefulness of MCP-1 as a diagnostic tool to distinguish AS patients from patients with mechanical low back pain.[20] Using a cutoff of 154.4 pg/mL, the sensitivity and specificity were 96% and 83.3%, respectively; the area under the curve (AUC) was 0.91 confidence interval of 95%: 0.83 to 0.97, P = 0.0001; and the positive and negative likelihood ratios were 5.76 and 0.048, respectively (Fig. 3). Assuming that in the general practice, 5% of the patients complaining of chronic low back pain suffer from AS, the positive and negative predictive values were 99.1% and 52.3%, respectively. These statistical values were based on the comparison between AS patients and patients with mechanical low back pain. The statistical values were very similar when AS patients were compared with healthy controls.

We also evaluated the usefulness of HLA-B27 testing in distinguishing patients with AS from our cohort of patients with mechanical low back pain. When tested with ROC, the sensitivity and specificity of this test were 100% and 84.2%, respectively; the AUC was 0.92 (P = 0.0001); and the positive likelihood ratio was 6.33. Assuming a pretest probability of 5% in distinguishing AS from mechanical lower back pain, the post-test

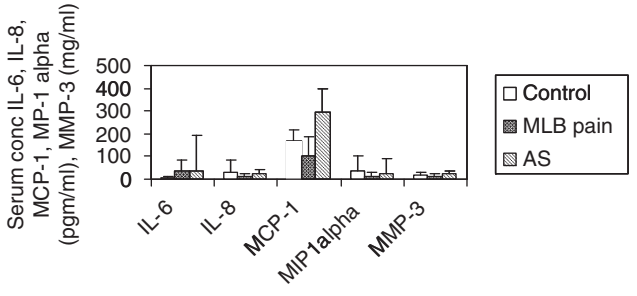

FIGURE 2. Serum concentration of interleukin (IL)-6, IL-8, metalloproteinase (MMP)-3, monocyte chemotactic protein (MCP)-1, and macrophage inflammatory proteins (MIP)-1α in patients with ankylosing spondylitis (AS), mechanical lower back (MLB) pain, and healthy control individuals. Serum levels of IL-6, IL-8, MCP-1, MIP-1α, and MMP-3 in healthy control subjects, MLB pain patients, and patients with AS. Error bars represent SD.

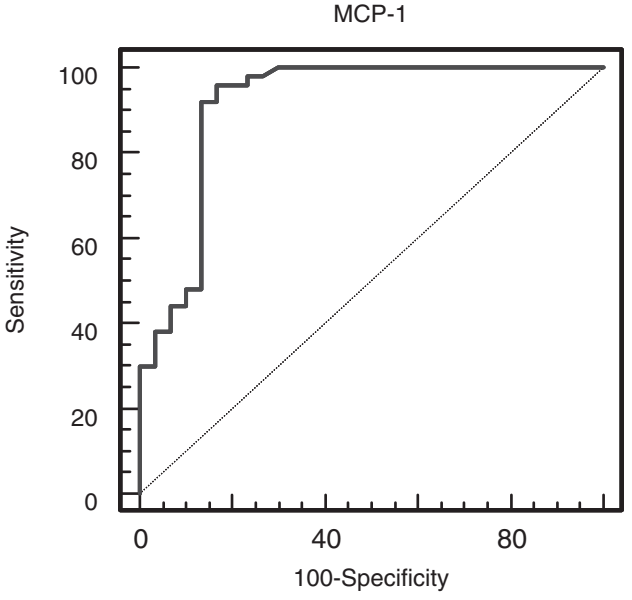

FIGURE 3. Receiver operator characteristic (ROC) curve distinguishing ankylosing spondylitis (AS) from patients with mechanical lower back (MLB) pain. ROC curve showing sensitivity and specificity of using serum monocyte chemotactic protein (MCP)-1 levels to distinguish AS patients from patients with MLB pain. The diagonal represents an AUC of 0.5. Values in the axes are represented as percentages. AUC indicates area under the curve.

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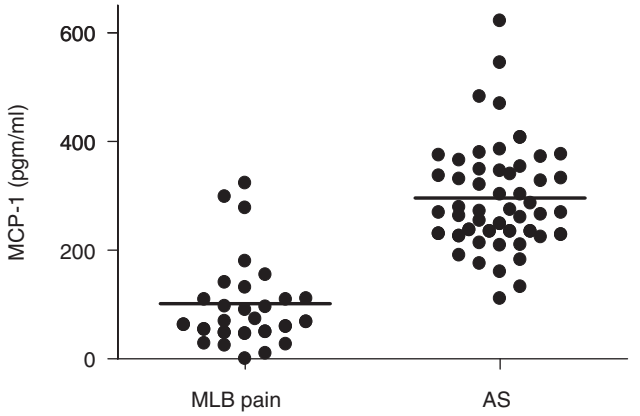

FIGURE 4. Dot plot of serum concentration of monocyte chemotactic protein (MCP)-1 in patients with ankylosing spondylitis (AS) and patients with mechanical lower back (MLB) pain. Serum MCP-1 levels of patients with AS and patients with MLB pain.

probability of AS for those having both positive HLA-B27 and high serum MCP-1 was 65.8%. However, if the patients also provided positive answers to questions of inflammatory back pain, in which the pretest probability of AS was 15%, positive tests for both MCP-1 and HLA-B27 raised the post-test probability of AS to 86.6%. Finally, because CRP is frequently used in the clinics, we also assessed the usefulness of CRP in the diagnosis of our cohort. The AUC was very low at 0.69.

DISCUSSION

Chronic mechanical low back pain patients make up a heterogeneous group ranging from those with completely normal spinal and neurologic images to those with severe noninflammatory pathologies.[6] This report compares blood-derived biomarkers that may help distinguish AS patients from normal participants and patients with chronic mechanical low back pain. Specifically, we focused on patients who had clinically been diagnosed as having mechanical lower back pain and a history of episodes of pain (characteristic of sciatica) by both orthopedic surgeons and rheumatologists. In more than half of the patients with mechanical low back pain, the attending physicians requested magnetic resonance imaging, computed tomography, or nerve conduction studies that verified the diagnosis of sciatica. None of these patients showed sacroiliitis on imaging. The duration of pain was more than 3 months, and their ages of onset, for all except 2 patients, were ≥ 45 years. When submitted to a questionnaire consisting of 3 questions as described in the Materials and Methods section of this study (in which patients addressed whether the quality of the pain had the same characteristics as inflammatory back pain), most of the responding patients gave positive answers to, at most, 2 questions. By incorporating these findings, it was very unlikely that any of these mechanical low back pain patients were misdiagnosed and actually had AS instead. For the AS patients, we recruited a group of patients that satisfied the Modified New York Criteria for AS.[13] All patients were positive for HLA-B27 and showed sacroiliitis on x-ray. Thus, these were classic AS patients.

On the basis of our initial ELISA screening results derived from synovial fluid samples, the serum samples of the AS and mechanical low back pain patients were tested by ELISA for 4 candidate biomarkers. We observed that the mean serum MCP-1 levels of AS were different, to a statistically high degree, from patients with mechanical low back pain and from healthy participants. Unlike earlier reports comparing AS to healthy participants, serum IL-6 was not a distinguishing biomarker in our cohorts.[11] Similar to earlier reports comparing AS patients with healthy participants, CRP was a poor biomarker of both sensitivity and specificity.[5]

We used several statistical methods to help us evaluate how useful MCP-1 is as a biomarker in practice. Using ROC, we discovered that MCP-1 showed high sensitivity and specificity of 96% and 83.3%, respectively. The positive likelihood ratio was 5.8. However, this assessment did not take into account the prevalence of AS in patients with chronic low back pain in general practice. Inflammatory low back pain has been reported to be present in only 5% of patients with chronic low back pain. On the basis of this information, we discovered that the positive predictive value was high at 99%, but the negative predictive value was low at 52.3%.

The low negative predictive value described above is not too surprising. There was considerable overlap in the serum MCP-1 levels between AS patients and those with mechanical low back pain (Fig. 4). Currently, there is no laboratory or clinical parameter that is single handedly capable of diagnosing AS with high positive and negative predictive values. In practice, each patient is assigned a degree of probability of having AS based on a combination of clinical, laboratory, and imaging parameters.[7] For practical purposes, in general practice, history and blood tests would be the preferred first steps toward making a diagnosis. From the history, one can decide whether a particular patient has inflammatory low back pain or not. Currently, the most useful blood test is HLA-B27. If a patient has both inflammatory low back pain and HLA-B27, there is about 30% probability that the patient has AS.[6,8] Our preliminary assessment showed that if these patients also have high serum MCP-1 levels, the probability increased to 86.6%. One possible drawback of our study was that the age and sex distributions of our AS cohort were different from those in the patients with mechanical low back pain and those in the healthy controls. Among the AS patients, 75% were male, versus 44.4% and 26.4% in the groups with mechanical low back pain and healthy controls, respectively. However, sex was probably not an important factor. When the MCP-1 levels of the male patients in all 3 groups were compared, the mean level was still statistically much higher in the AS group. In addition, in the AS cohort, the MCP-1 serum levels did not show a correlation either with age or with sex. Future multicenter studies will be required to determine the usefulness of MCP-1 as a

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possible biomarker for AS. As a candidate, MCP-1 has the promising advantage of being easily measured by standard, commercially available ELISA assays.

REFERENCES

1. Braun J, Sieper J. Ankylosing spondylitis. *Lancet*. 2007;369:1379–1390.
2. Underwood MR, Dawes P. Inflammatory back pain in primary care. *Br J Rheumatol*. 1995;34:1074–1077.
3. Calin A, Porta J, Fries JF, et al. Clinical history as a screening test for ankylosing spondylitis. *JAMA* 1977;237:2613–2614.
4. Rudwaleit M, Metter A, Listing J, et al. Inflammatory back pain in ankylosing spondylitis: a reassessment of the clinical history for application as classification and diagnostic criteria. *Arthritis Rheum*. 2006;54:569–578.
5. Rudwaleit M, Khan MA, Sieper J. The challenge of diagnosis and classification in early ankylosing spondylitis: do we need new criteria? *Arthritis Rheum*. 2005;52:1000–1008.
6. Rudwaleit M, van der Heijde D, Khan MA, et al. How to diagnose axial spondyloarthritis early. *Ann Rheum Dis*. 2004;63:535–543.
7. Rudwaleit M, Feldtkeller E, Sieper J. Easy assessment of axial spondyloarthritis (early ankylosing spondylitis) at the bedside. *Ann Rheum Dis*. 2006;65:1251–1252.
8. Rudwaleit M, van der Heijde D, Landewe R, et al. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part II): validation and final selection. *Ann Rheum Dis*. 2009;68:777–783.
9. Dougados M, Gueguen A, Nakache JP, et al. Clinical relevance of C-reactive protein in axial involvement of ankylosing spondylitis. *J Rheumatol*. 1999;26:971–974.
10. Brown MA. Human leucocyte antigen-B27 and ankylosing spondylitis. *Intern Med J*. 2007;37:739–740.
11. Chen CH, Yu DT, Chou CT. Biomarkers in spondyloarthropathies. *Adv Exp Med Biol*. 2009;649:122–132.
12. Dougados M, van der Linden S, Juhlin R, et al. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. *Arthritis Rheum*. 1991;34:1218–1227.
13. Van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum*. 1984;27:361–368.
14. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum*. 1988;31:315–324.
15. Garrett S, Jenkinson T, Kennedy LG, et al. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. *J Rheumatol*. 1994;21:2286–2291.
16. Calin A, Garrett S, Whitelock H, et al. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. *J Rheumatol*. 1994;21:2281–2285.
17. Jenkinson TR, Mallorie PA, Whitelock HC, et al. Defining spinal mobility in ankylosing spondylitis (AS). The Bath AS Metrology Index. *J Rheumatol*. 1994;21:1694–1698.
18. Vroomen PC, de Krom MC, Wilmink JT, et al. Diagnostic value of history and physical examination in patients suspected of lumbosacral nerve root compression. *J Neurol Neurosurg Psychiatry*. 2002;72:630–634.
19. Gu J, Rihl M, Marker-Hermann E, et al. Clues to pathogenesis of spondyloarthropathy derived from synovial fluid mononuclear cell gene expression profiles. *J Rheumatol*. 2002;29:2159–2164.
20. Rao G. What is an ROC curve? *J Fam Pract*. 2003;52:695.

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