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# Techneium-99m-HMPAO-Labeled Leukocyte Imaging in Patients with Seronegative Spondyloarthropathies

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**Objective:** Gut inflammation is frequent among patients with seronegative spondyloarthropathies (SSp). The purpose of this study was to evaluate the presence of positive abdominal findings in patients with SSp who did not have clinical symptoms or signs of inflammatory bowel disease (IBD). This represents a new indication for abdominal <sup>99m</sup>Tc-HMPAO-labeled leukocyte scintigraphy.

**Methods:** Eighty-six patients (59 with SSp and 27 controls) were prospectively imaged with <sup>99m</sup>Tc-HMPAO-labeled leukocytes.

**Results:** Leukocyte imaging was positive in 33 patients with SSp (56%), 27 of these patients scored between 2 + and 4 + (51%). Four (15%) control patients also had positive findings.

**Conclusion:** These findings provide evidence linking SSp with intestinal inflammation. SSp may be an important new indication for <sup>99m</sup>Tc-HMPAO-labeled autologous leukocyte scintigraphy.

**Key Words:** leukocyte scintigraphy; technetium-99m-HMPAO; seronegative spondyloarthropathies; inflammatory bowel disease; gut inflammation

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Spondyloarthropathies are serious, chronic inflammatory diseases of the joints. Patients experience progressive difficulty with movement and movement is painful. Patients with seronegative spondyloarthropathies (SSp) do not have measurable serum antibody titers that might explain the inflammation. The diseases are more common among men.

The factors that contribute to development of SSp are not completely known. Gut inflammation is frequent among patients with SSp, as demonstrated by colonoscopy and histological examination of biopsy samples. Abdominal scintigraphy using <sup>99m</sup>Tc-HMPAO-labeled autologous leukocytes has been well validated as a tool for evaluating the presence of and the extent of disease in patients with inflammatory bowel disease (IBD) (1–5). The purpose of this study was to evaluate the

presence of positive abdominal scintigraphy in patients with SSp who did not have clinical symptoms or signs of IBD.

## MATERIALS AND METHODS

### Patients

Eighty-six patients were imaged prospectively with <sup>99m</sup>Tc-HMPAO-labeled leukocytes. Fifty-nine patients fulfilled the European Spondyloarthropathy Study Group's 1991 diagnostic criteria. Twenty-three patients had ankylosing spondylitis, 7 patients had psoriatic arthritis, 9 patients had reactive arthritis and 20 patients had undifferentiated SSp (Fig. 1). Thirty-seven (63%) of these patients with SSp were men. The average age was 37 y ± 16 y. Twenty-seven individuals without SSp, who were all taking nonsteroidal, anti-inflammatory drugs (NSAIDs), were used as a control group. Eleven patients had rheumatoid arthritis, 16 patients had chronic low-back pain, 3 patients had lumbar disk herniation, and 1 patient had juvenile chronic arthritis.

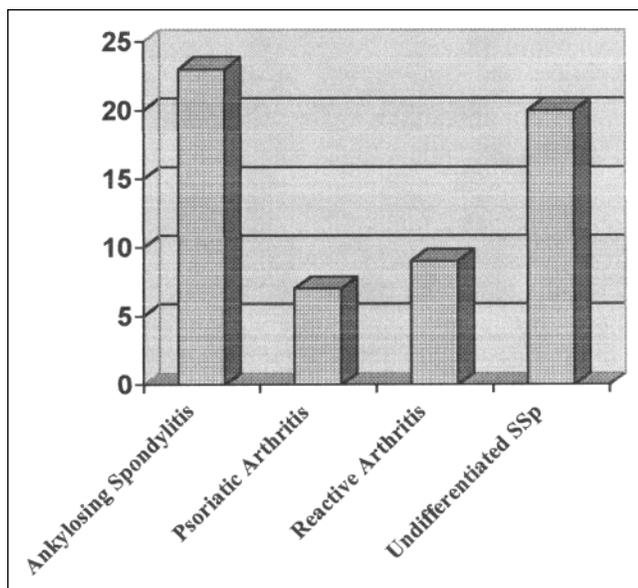
### Leukocyte Labeling

In vitro leukocyte labeling was achieved according to the method of Vorne et al. (6) with some modifications. A 40-mL sample of peripheral blood was taken up in a syringe containing 7.5 mL acid citrate dextrose as the anticoagulant. After spontaneous sedimentation of red cells for 1 h at room temperature, the supernatant was aspirated and centrifuged in sterile tubes at 150 g for 15 min and the mixed leukocyte pellet was then collected. A 10-mL sample of peripheral blood was centrifuged at 2000 g for 10 min to obtain cell-free autologous plasma.

The <sup>99m</sup>Tc-HMPAO complex was formed by adding 600 MBq free pertechnetate in 6 mL isotonic saline to a commercial kit containing HMPAO (Nycomed-Amersham, Ibérica, Spain). The cell pellet was washed twice in autologous plasma and incubated 30 min at room temperature in 2 mL plasma and 2 mL (440 MBq [12 mCi]) <sup>99m</sup>Tc-HMPAO. After this incubation, the unbound <sup>99m</sup>Tc-HMPAO was removed by centrifugation at 900 g for 10 min. Cells were washed twice and resuspended in 4 mL plasma and reinjected intravenously. The mean amount of radioactivity injected was 220 MBq (6 mCi).

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**FIGURE 1.** Seronegative spondyloarthropathies distribution. Fifty-nine patients fulfilled the European Spondyloarthropathy Study Group's 1991 diagnostic criteria.

#### Imaging and Interpretation

Scintigraphy was performed using a large field-of-view gamma camera equipped with a low-energy, parallel-hole collimator. Anterior abdominal images were obtained 30 min and 2 h after leukocyte reinjection. For each planar image 800 K counts were accumulated. Caudo-cranial, right and left oblique and pelvic views were acquired to localize tracer uptake when necessary.

Scintigraphic images were independently evaluated by 2 nuclear medicine physicians without knowledge of the clinical history. Any intestinal uptake was interpreted as a positive study when areas of increased abnormal concentration of the tracer were seen in the early images and remained or became more intense at delayed imaging. Tracer uptake was scored from 1 (lowest uptake) to 4 (highest uptake) in comparison with iliac crest uptake:

- I = higher than background;
- II = less than iliac crest uptake;
- III = equal to iliac crest uptake;
- IV = more than iliac crest uptake.

The bowel was divided into 5 segments: rectosigmoid colon; descending colon; transverse colon; ascending colon; and terminal ileum.

#### Statistical Analysis

Data were analyzed using the Chi-square test with Yates' correction, when appropriate, and Fisher's 2-tailed exact test. The cross-products (odds ratios) and the 95% confidence intervals (CI) were calculated. The odds ratios were obtained by entering the explanatory variable in the rows and entering the

outcome event in the columns to measure the degree of association between the variables. A  $P < 0.05$  was considered statistically significant.

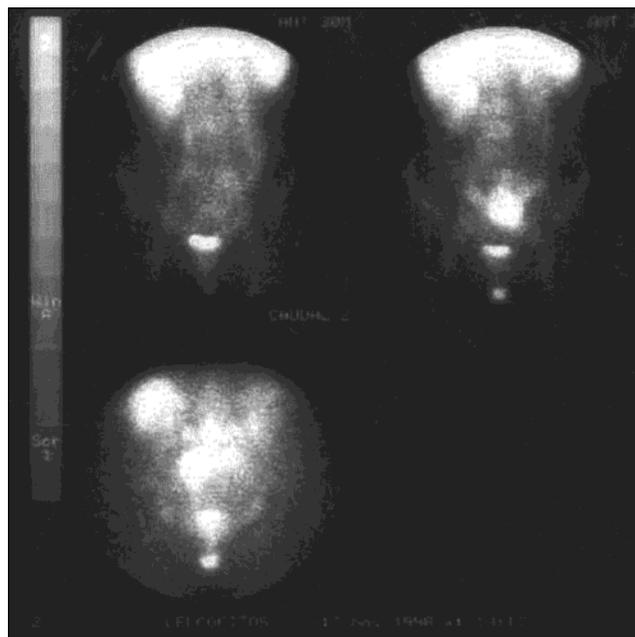
#### RESULTS

The imaging studies were positive in 33 patients with SSP (56%) (Fig. 2). Twenty-seven (51%) of these patients were scored from 2+ to 4+. Four (15%) control group patients also had positive imaging findings. Only 1 (4%) had greater than 2+ uptake. Other statistics were:  $\chi^2 = 12.78$ ;  $P = 0.00035$ ; odds ratio = 7.3; and confidence interval at 95% = 2.24–23.74. For patients who were scored from 2+ to 4+ the relationship was  $\chi^2 = 13.07$ ;  $P = 0.0003$ ; odds ratio = 21.94; and confidence interval at 95% = 2.79–172.46.

#### DISCUSSION

Technetium-99m-HMPAO-labeled leukocyte scintigraphy is a simple and effective tool for evaluating disease extent and activity, treatment response and relapse of patients with IBD (7). The affected gut regions seem to be slightly larger with abdominal leukocyte imaging than on radiographs or by endoscopy. This is probably due to the scintigraphy being performed during the acute phase of the disease.

The concept of spondyloarthropathy (SpA) gathers together a group of chronic diseases with common clinical, biological, genetic and therapeutic characteristics. The concept forms a distinct entity, different from other rheumatic diseases. The target organs are not only the joint, but also the axial skeleton, entheses, eye, gut, urogenital tract, skin and, sometimes, the heart. The prevalence of this entity in the general population is



**FIGURE 2.** Leukocyte images from a 34-y-old man with ankylosing spondylitis. This patient had never had intestinal signs or symptoms of inflammatory bowel disease. Increased uptake of leukocytes was observed over the left colon on both the early and 2-h delayed images.

estimated to be 1%, equal to the prevalence of rheumatoid arthritis. Genetic predisposition (HLA-B27) is one of the clues to the pathogenesis of SpA. Reactive arthritis is induced by specific urogenital or enterogenic bacteria. It is clear that the gut might play an important role by permitting exogenous factors to enter the body. The gut is implicated in various spondyloarthropathies and especially in IBD. This hypothesis was the rationale for investigating the gut in patients with spondyloarthropathies by performing ileocolonoscopies (8).

The intestine plays a significant role in the pathogenesis of SSp. Recent studies strongly support the concept that gut and joint inflammation are closely related (9). Progress also has been made in identifying individual mechanisms that contribute to the pathogenesis of joint disease in IBD and in undifferentiated SpAs. The inter-relationship of the mechanisms that result in chronic disease symptoms at a site distant from the initiating event remains elusive. The absence of homing molecule receptors in the gut wall combined with the expression of these receptors in a target organ may be responsible for transforming the synovial membrane and/or the enthesis into an aberrant tertiary lymphoid organ of the gut (9). Many authors have tried to elucidate the association between the intestine and arthritis. The issue is whether the bowel lesion occurs before or after the appearance of peripheral arthritis (2,5,10,11). Ileocolonoscopy and histological studies have demonstrated gut inflammation in patients with SSp, even in the absence of clinical IBD. This subclinical gut inflammation may represent one end of the IBD spectrum or perhaps it is a different process (12).

In our study, 56% of patients with SSp, under NSAID treatment, had a positive <sup>99m</sup>Tc-HMPAO-labeled leukocyte study. A control group of patients without SSp, who also were taking NSAIDs, was included in the study to evaluate the suggested role of these drugs in the development of gut uptake. There were 4 positive abdominal studies in this group. Our previous data suggest that NSAIDs do not alter the scintigraphic results of leukocyte imaging (13).

Intestinal inflammation can be assessed easily by endoscopy, however, this is usually not well tolerated by patients. Abdominal imaging with labeled leukocytes offers the ability to determine which patients are suitable candidates for treatment with sulfasalazine. This simple and well-tolerated diagnostic procedure also permits repeat assessment for treatment effectiveness and relapses.

## CONCLUSION

These findings provide evidence, as did our previous studies, for linking SSp with intestinal inflammation. Imaging SSp patients with <sup>99m</sup>Tc-HMPAO-labeled autologous leukocytes may be an important new indication for this radiopharmaceutical.

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