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LETTERS

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Disparate results in studies of methotrexate plus corticosteroids in the treatment of giant cell arteritis: comment on the article by Hoffman et al

To the Editor:

We read with interest the report of the multicenter study on the use of adjuvant methotrexate (MTX) therapy in giant cell arteritis (GCA), by Dr. Hoffman and the International Network for the Study of Systemic Vasculitides (INSSYS) (1). The main conclusion from this work was that the use of MTX as an adjunct to prednisone therapy was not justified to control disease activity or to decrease the cumulative dose and toxicity of corticosteroids in patients with GCA.

As Hoffman et al comment on extensively in their Discussion section, we have recently published the results of a randomized, double-blind, placebo-controlled trial showing that the combination of MTX and prednisone was more efficient than prednisone alone in maintaining disease remission, allowing lower prednisone requirements over a 2-year period (2). In our opinion, the differing results of the 2 studies might be explained by differences in patient selection, duration of followup, and steroid therapy. We do not believe differences in the definition of relapses have a significant role in the differences in outcomes. Our definition of relapse did not include change in the erythrocyte sedimentation rate (ESR) and was based exclusively on "recurrence of symptoms of giant-cell arteritis after definite objective improvement followed by symptom reversal upon resumption of or increases in the prednisone dose." This definition, in accordance with Hoffman and colleagues' study, excluded isolated elevation of ESR as a criterion for relapse. In addition, although elevation of ESR was not included as a requisite for the definition of a relapse, all relapses in both the MTX and the placebo groups were in fact accompanied by elevations in ESR. In our opinion, definition and management of relapses were, with small methodologic differences, quite similar in the 2 studies, and it is unlikely that the difference in results lies therein.

Patient selection could make a difference. While a positive biopsy result was required for inclusion in our study, the INSSYS study included patients (up to 17%) without a positive biopsy result, raising the possibility that patients with GCA-like conditions could have been included in the study. Furthermore, the selection process in Hoffman et al's study is unclear: no data are provided regarding the total number of eligible patients, the proportion of eligible patients who were randomized, causes for nonrandomization, or the proportion of patients in each of the 17 participating centers. This makes it difficult to rule out some sort of selection bias.

Although the difference in relapse incidence at 1 year was not statistically significant between groups, a detailed analysis of the results of the INSSYS study reveals a diminished cumulative incidence of each of the specific symptoms of GCA, except tongue or jaw pain, in the MTX group; this achieved statistical significance for both polymyalgia rheumatica and sustained fever. This divergence between clinical findings and statistical results might be caused by the recalculation of the study sample (initially set at 300, and then changed to 84). After the recalculation, the authors determined that a difference in the relapse rate of ≥50% would be necessary for statistical power to be maintained. This is higher than the difference found in our study (39%). Had Hoffman et al preserved the initial sample size, a difference of only 25% in the relapse rate would have become statistically significant. Two other factors might help in the understanding of their results, the first being a followup period of only 12 months, making the possibility of detecting long-term differences in outcomes impossible. The second factor is that, by study design, a second relapse was considered as a definite treatment failure, which prevented some patients from having their MTX adjusted to a higher, and eventually more efficacious, weekly dosage. We think both factors are relevant and might have important consequences in terms of the statistical results, especially in view of survival curves for relapses and treatment failures, which demonstrate a positive effect of MTX.

Finally, we believe the most relevant factor that might explain the relative lack of efficacy of MTX in Hoffman et al's study is the alternate-day corticosteroid regimen, compared with the daily schedule used in our study. Although an alternate-day corticosteroid regimen has shown some success in several autoimmune conditions (3–7), its use in GCA is not widespread, and its efficacy in this disease was shown by Hunder et al to be low (8). In that study, 14 of 20 GCA patients had a relapse after 4 weeks of alternate-day therapy, while this occurred in only 2 of 20 patients receiving daily therapy. Supporting the contention that alternate-day corticosteroid therapy has suboptimal efficacy in GCA is the fact that a significant percentage of patients in Hoffman and colleagues' study developed ischemic visual symptoms after initiation of therapy, while in our study (2) and the study by Spiera et al (9), using different daily regimens of prednisone alone or with MTX, no patient developed this complication.

Taken together, the results of the 3 recent studies on GCA and MTX (1,2,9) indicate that the treatment of patients should be based on high initial doses of corticosteroids and a tapering daily schedule. The use of MTX as adjuvant therapy permits faster steroid withdrawal and a lower cumulative corticosteroid dose, while achieving good disease control. The choice between the use of MTX or a more conservative corticosteroid-tapering schedule should be based on careful weighing of the risks and benefits of each option in individual patients.

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Vision loss in giant cell arteritis patients treated with alternate-day corticosteroids: comment on the article by Hoffman et al

To the Editor:

We read with interest the report of the study by Hoffman et al (1), evaluating the efficacy of methotrexate (MTX) as a potential disease-controlling/steroid-sparing agent in the treatment of giant cell arteritis (GCA). No advantage could be demonstrated in terms of rates of disease relapse, cumulative corticosteroid dose, or disease- or therapy-related morbidity, by the addition of MTX to the treatment regimen. We similarly could not demonstrate benefit with addition of MTX (2), but the power of our study was limited by the smaller sample size. Jover and colleagues (3), in contrast, did find that MTX-treated patients had fewer relapses and required a lower cumulative corticosteroid dose.

What was most striking, however, in Hoffman and colleagues' study was the vision data. The authors report a disturbingly high incidence of vision loss, which of course is the major feared complication of GCA. A total of 8 patients had new vision loss at 1 year (4 in each treatment group), and 3 patients who had vision loss at study entry experienced additional vision loss during the first year after enrollment. This is markedly higher than the rate we have observed in our study or clinical practices. Neither in our study nor in that of Jover et al (3) did late vision loss occur. This was not a factor of underreporting in our study, since patients had meticulous ophthalmologic followup with an ophthalmologist experienced in GCA.

Aiello et al (4) described 245 patients with GCA, in whom only 1 episode of late vision loss occurred in a patient 1 year after completion of 2 years of corticosteroid therapy, and this was not believed to be related to active arteritis. Four patients in this series lost vision while taking corticosteroids, but those events occurred after $\leq$1 month of therapy. Turbin and Kupersmith (5) found no occurrence of late vision loss in 69 patients with GCA at a minimum of 1 year, and a mean of 27 months, of followup.

Given the paramount importance of ocular outcomes in GCA, it would be helpful to have more detailed information regarding the vision data in Hoffman and colleagues' study. The definition of GCA-related vision loss used by the investigators would be relevant, as would be the way in which vision loss was documented. In particular, the timing of the episodes of new vision loss would be important to know, since it is recognized that new vision loss within the first 2 weeks of corticosteroid therapy is not rare, as opposed to late vision loss, which is decidedly uncommon. In Hoffman et al's study, corticosteroids were initially administered daily, and after 4 weeks were tapered according to an alternate-day schedule, leading to a prednisone dosage of 60 mg on alternate days by the third month. If indeed the occurrences of vision loss were late events, particularly during alternate-day therapy, this of course raises the question of the efficacy of the corticosteroid regimen utilized. While there is no consensus as to what constitutes the optimal corticosteroid regimen to treat GCA, there have been concerns raised in prior studies as to whether alternate-day regimens adequately control disease activity (6). In the trial reported by Hoffman et al, even with monitoring by physicians who are among the world's most experienced in the treatment of this disorder, a very high rate of vision loss was observed. Moreover, while the total corticosteroid dose and duration of corticosteroid therapy were relatively low, they were not less than in the study by Jover et al (3), who observed no late vision loss in their patients, the main difference being that in Jover's study, corticosteroids were administered daily.

The study by Hoffman et al is the largest prospective placebo-controlled study of adjuvant therapy in GCA to date, and was performed by recognized leaders and experts in the treatment of this disorder. We are concerned that an implicit message that might be gleaned by readers is that the corticosteroid regimen utilized is the standard of care for treating GCA. Perhaps the vision outcomes observed are indeed evidence for the opposite—that alternate-day corticosteroid regimens are insufficient to achieve adequate disease control in this form of vasculitis.

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Reply

To the Editor:

Jover and colleagues have offered several explanations to account for why in their study (1) MTX appeared to be advantageous in the treatment of GCA whereas our study did not support this conclusion. Their suggested explanations include: 1) selection bias; 2) insufficient power; 3) insufficient length of followup; 4) failure to use a high enough MTX dose; and, 5) the use of an alternate-day corticosteroid regimen in our trial after 3 months.

Based on the comments in Jover et al's letter, we agree that the definitions of relapse in their study and in ours appear to be similar. They question whether the 17% of our patients who had negative biopsy findings may have had diseases other than GCA. We do not think so. Even under the most rigorous temporal artery biopsy collection procedures (the performance, by experienced surgeons, of bilateral biopsies at least 2 cm in length [2,3]), the presence of skip lesions (and possibly other factors) indicates that the false-negative rate of this procedure is at least 9%. Thus, a negative result on a temporal artery biopsy (or biopsies) does not exclude the diagnosis, which remains a clinical one. Conversely, the presence of a positive biopsy result may not guarantee an accurate diagnosis of GCA, if care is not exercised to exclude involvement of the temporal artery by conditions such as Wegener's granulomatosis, polyarteritis nodosa, Churg-Strauss syndrome, microscopic polyangiitis, hepatitis-associated vasculitis, and even rheumatoid arthritis–associated vasculitis (4). Most compelling, however, in our study is the fact no patients were diagnosed as having any disease other than GCA to account for their symptoms, despite regular followup for 1 year in a clinical trial setting.

Also with regard to the possibility of selection bias, our colleagues from Madrid suggest that the higher rate of patient refusal to participate in our trial may explain the differences in trial results. In both trials, all consecutive eligible patients were invited to participate in the respective protocols. In our study, two-thirds of patients declined enrollment, which contrasts with the $<5\%$ of eligible patients in the Madrid study who declined to participate. We note, parenthetically, that our enrollment rate of eligible patients should not be viewed as low, particularly since the elderly patient population may inherently be less likely to enroll in clinical trials, at least in the US. Although the observation of different enrollment rates in the 2 countries is interesting for its implications about different cultures, patient perceptions of clinical trials, or enrollment strategies, it is not clear how this difference would have had any impact on the outcomes of the trials.

Jover et al's comments regarding study size are curious insofar as their group enrolled 42 patients and we enrolled 98. Although we enrolled fewer patients than projected, the power (80%) of our trial to demonstrate the effect size originally chosen (50%) was unaltered, because of the higher-than-anticipated relapse rate observed ($>60\%$, a rate similar to that observed in the Madrid study). A larger sample size would certainly have permitted us to detect a smaller effect—if present—but as we noted in the Methods section of our report, $\sim$300 patients would have been needed to demonstrate such an effect.

Jover and colleagues also imply that our results might have been different had the length of followup been longer than a mean of 1 year. It is our impression that if MTX had a substantial effect on the course of GCA, it would have been more apparent within a 1-year (mean) period of followup.

Would our results have been different if the median MTX dose was >15 mg/week? It is intriguing that Jover and colleagues should raise this question in light of the fact that only 15 patients in their MTX group finished the study, 3 having withdrawn due to MTX toxicity while receiving 10 mg MTX/week. Neither the mean nor the median dosage of MTX in their study patients was reported, but we note that the patients started MTX at only 10 mg/week, with no clear plan elaborated for increasing the dosage significantly. The comment that our definition of a second relapse as a treatment failure prevented the achievement of "higher, and eventually more efficacious" doses of MTX is peculiar, since it is quite likely that patients in our trial received higher doses of MTX than those in the Madrid trial. Whether even higher doses of MTX would have had a greater impact on the course of disease in our trial is a matter of speculation.

Both Jover et al and Spiera and colleagues raise important questions about the "proper" corticosteroid regimen for GCA. Hunder et al (5) clearly established that if treatment is initiated with daily high-dose prednisone for 5 days and then immediately switched to an alternate-day schedule (90 mg every other day), alternate-day treatment was less effective than daily therapy at 4 weeks of followup. From that study and others, treatment protocols have evolved that include aggressive, high-dose daily corticosteroids for 1 month, followed by a tapering protocol. Indeed, that was the approach used in our study: all patients received daily prednisone for 3 full months. What Hunder et al's study did not resolve was whether high-dose daily prednisone could be shifted, over several months, to an alternate-day tapering regimen. Protocols of this type have been shown to be effective in controlling Wegener's granulomatosis (6,7) and Takayasu arteritis (8). This approach has also been shown by Bengtsson and Malmvall to be effective in 67% of 27 GCA patients followed up for a mean of 2 years (9). Among treatment failures in that study, none of the patients experienced vision loss. Thus, within the limitations of the literature, our trial protocol was grounded in an evidence-based approach, developed by a consensus of international GCA experts, and designed to determine if the addition of MTX to a reasonable regimen of corticosteroids improved disease outcomes compared with treatment with corticosteroids alone. Our results suggest that it did not.

We agree with Spiera et al in recognizing that vision loss after initiation of therapy was more common among our patients than in other series. One possible difference between our trial and theirs (10) was the high pre-enrollment cumulative corticosteroid dose received by patients in their trial (mean

of nearly 3 gm). This would suggest a substantially longer period of treatment and/or the frequent use of pulse corticosteroids prior to randomization. In our trial, vision loss after treatment occurred in both groups of (5 of 47 in the corticosteroid plus placebo group, 4 of 51 in the corticosteroid plus MTX group) and was isolated to the first 6 months of followup (1–6 months). Nine new vision loss events occurred, including in 2 patients who originally presented with monocular visual impairment due to GCA. In all but 1 case, other features of GCA were also present, and new vision loss could not be attributed to alternative diagnoses. In the exceptional case, blindness occurred after 4 weeks of therapy, while the patient was still receiving prednisone 60 mg/day. In that patient, it is likely that blindness occurred because of already critically compromised perfusion prior to implementation of effective therapy. In the other cases, it is more likely that blindness was at least a feature of relapsing disease. For our entire cohort, most relapses occurred after an alternate-day prednisone schedule was initiated (51%) or after prednisone was entirely discontinued (34%). Among the 8 patients with delayed vision loss (>1 month from initiation of therapy), prednisone had been tapered and entirely withdrawn in 4. However, 2 patients were receiving 60 mg alternating with 20 or 25 mg (daily therapy), and 2 were receiving 35 mg or 40 mg every other day. Thus, our observations regarding vision loss in GCA differ from those of others who have emphasized the rarity of blindness following corticosteroid therapy, whether therapy is provided daily or tapered to an alternate-day regimen.

At the end of these discussions, we are uncertain as to why the results of our trial, which had more than twice the number of patients and used higher doses of MTX, differed substantially from those of Jover et al. We believe that the benefit of MTX, as an adjunct to corticosteroids, in the treatment of GCA is, at best, modest. Significant advances in the therapy of this disease, for which the conventional therapy carries such a high toll of treatment-related morbidity, will require a different approach.

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Does concomitant osteoarthritis affect histopathologic changes in patients with rheumatoid arthritis? Comment on the article by Kraan et al

To the Editor:

It is still unclear to me whether concomitant osteoarthritis (OA) may affect histopathologic changes in patients with rheumatoid arthritis (RA). If the patients studied by Kraan and colleagues were relatively young, with a minimal amount of OA, then the issue remains moot. However, if the patients had significant OA of the knee (i.e., grades II–IV by Outerbridge classification), then the histopathologic findings are much more important. In other words, the presence of concomitant knee OA has little or no effect on RA histopathology. Therefore, RA tissue could be obtained from any joint for use in clinical trials. Also, it is unclear whether the duration of RA may have an effect on histopathology.

It is often difficult to separate fact from opinion. The following is my opinion, which I hope will be supported by fact in the near future. Rather than relying on surrogate markers of disease activity such as radiographs, 50-foot walking time, joint counts, etc., we should follow Sutton's law. Tissue histopathology is where the action is. The term "arthritis" is derived from the Greek: "arthron," meaning joint, and "itis," meaning inflammation. "Arthritis" does not mean "x-ray of the knee" or "Ritchie-Camp articular index."

I again congratulate Kraan et al on a fine study. It is, as our European colleagues show, relatively easy to obtain tissue samples for use in clinical trials (1,2). This allows for a more rapid interpretation of antirheumatic drug effect, thereby speeding up the process of drug research.

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Reply

To the Editor:

We thank Dr. Wei for his valuable comments regarding our study on the comparison of synovial tissue from the knee joints and small joints of patients with RA. Whether concomitant or secondary OA of the knee influences the histopathologic changes in RA is indeed a challenging question, which is not easy to address in the context of observations that even early primary OA may cause signs of synovial inflammation (1).

It is obvious that the cumulative destruction of bone and articular cartilage could result in the release of fragments that enhance inflammation. In addition, factors other than OA, such as differences in innervation, vascularity, and mechanical strain and stress, may potentially interfere with the histopathologic changes in rheumatoid synovial tissue. A definite conclusion appears even more difficult in the absence of specific (immuno)histologic markers for either RA or OA. This provided the rationale for our recent study comparing knee joints with small joints in patients with RA (2).

Our data suggest that concomitant OA was not a major interfering factor. The average age of the study patients was 55 years (range 21–73 years), including a young patient (age 23 years) with recent-onset disease (disease duration 6 months), allowing assessment of the features of the synovium in the absence of any degenerative changes. In this individual patient, we observed similar changes in the knee joint and the hand joint. Moreover, the immunohistologic features of paired knee joints and small hand joints were comparable in the 2 RA patients with secondary knee OA.

The question about the effect of disease duration is of great interest. To address this question, it is essential to study patient groups that are matched for disease activity and use of medication. Using this approach, synovial tissue samples obtained from patients with early active RA ($<$1 year) were compared with those obtained from patients with longstanding RA ($>$5 years) (3). Results of that study clearly showed that synovial inflammation in early RA is basically the same process as that in late RA, which was later confirmed by other investigators (4). Similar results were obtained when only patients with a disease duration of $<$3 months were included in the early RA group (3). Thus, so-called early RA represents already a chronic phase of the disease, and the histopathology is, on average, not different in longstanding (but still active) disease (5). The results might be slightly different, however, when synovial tissue is acquired at the time of joint replacement surgery from patients with RA who have end-stage, destructive disease (Smeets TJM, et al: Unpublished observations). Such patients are selected on the basis of joint destruction rather than inflammation, and the underlying immune-mediated disease is not necessarily active anymore. Here, factors secondary to bone and cartilage gradation might be more important in determining synovial inflammation.

Because we selected patients with active RA (the group that is eligible for clinical studies on antirheumatic therapy), the effects of concomitant OA appeared very limited and could not, in fact, be demonstrated in our study. The data clearly show that synovial samples from both knee joints and small joints can be used in clinical trials. We completely agree with Dr. Wei that serial synovial tissue samples provide a powerful tool for antirheumatic drug development (6).

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Possible role of shared epitope status in the relationship between matrix metalloproteinase 3 genotype and radiographic progression of rheumatoid arthritis: comment on the article by Constantin et al

To the Editor:

Stromelysin (matrix metalloproteinase 3 [MMP-3]) has an important role in cartilage degradation. Polymorphisms within this gene are clearly good candidates for the part of the genetic contribution to rheumatoid arthritis (RA) that is not encoded by HLA. It is therefore notable that Constantin et al recently reported an association of the 6A/6A MMP-3 genotype with radiographic damage in RA after 4 years of followup (Constantin A, Lauwers-Cancès V, Navaux F, Abbal M, van Meerwijk J, Mazières B, et al. Stromelysin 1 [matrix metallo-

proteinase 3] and HLA–DRB1 gene polymorphisms: association with severity and progression of rheumatoid arthritis in a prospective study. Arthritis Rheum 2002;46:1754–62). Although they caution that further studies are needed to confirm these observations, they conclude that this polymorphism is associated with progression and that its identification could be useful in the management of early RA.

These results are clearly interesting; we would like to draw attention, however, to a potential confounding effect of HLA–DRB1 genotypes, which alone might be able to explain the observations made by Constantin et al. Table 5 of their article shows that the shared epitope (SE) −/−, +/−, and +/+ genotypes are associated with median progression of radiographic damage scores over 4 years of 3, 9.5, and 26, points, respectively. Thus, especially the homozygous SE+/+ group shows a high radiographic progression score. This implies that testing of the effect of the MMP-3 polymorphism on radiographic progression may be confounded by the influence of the HLA–DRB1 genotypes if the percentage of SE+/+ patients varies substantially between the different MMP-3 genotype groups.

The data presented and testing results reported by Constantin and colleagues allow us to calculate the genotype distribution in these 96 patients. It seems as if the test for association between MMP-3 and SE genotypes was performed in the baseline group and not in the group followed up for 4 years. However, Table 1 of the article implies a significant difference in the frequency of the SE in the 2 subgroups, indicating that test results might clearly be different in the group with 4-year followup. Furthermore, a chi-square analysis with 4 degrees of freedom was used to test for overall changes in genotype distribution. This test is relatively insensitive to differences in the percentage of homozygous individuals between the MMP-3 genotype groups followed up prospectively. We calculate the percentage of SE+/+ patients to be 14% in the 5A/5A group, 24% in the 5A/6A group, and 25% in the 6A/6A group. Based on these results, if the MMP-3 polymorphism itself does not influence radiographic progression, we would expect low radiographic progression in the 5A/5A group and higher, approximately equal progression in the 5A/6A and 6A/6A groups, the latter being substantially less than the progression in the SE+/+ group. Those are exactly the results presented in Constantin et al's Table 5.

Since the subject of their study is clearly important, we would like to urge the authors to present more data on the interaction between SE and MMP-3 genotypes in the 96 patients who were followed up for 4 years. Additionally, it would certainly be of interest to see the results of an analysis on the effect of this MMP-3 polymorphism after stratification for SE genotypes.

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Reply

To the Editor:

We thank Drs. de Vries and Tak for their careful reading of our article, and we would like to provide more detailed data on the interaction between SE and MMP-3 genotypes. A total of 103 patients were enrolled in the study, but the final analysis after 4-year followup was based on 96 patients. The 7 patients lost to followup were not statistically different with regard to the frequency of the SE when compared with the group with 4-year followup, as indicated in Table 1 of the article ($P = 0.41$ by Fisher's exact test). However, for each patient lost to followup, based on the combination of their MMP-3 and HLA–DRB1 alleles and on data reported in Table 6 of our article, we assigned the least probable score of radiographic progression. For example, to a patient with no SE and with a 5A/5A MMP-3 genotype, we allocated the median score obtained with the 6A/6A SE+/+ patients, and vice versa. Then we again performed the statistical analysis, and we confirmed our previous finding.

To have a potential confounding effect, the confounder must be related to both the cause and the effect. It is correct that the test for association between MMP-3 and SE genotypes was performed in the baseline group, but when we repeated this test in the group with 4-year followup, the result was the same ($P < 0.83$). However, we had previously analyzed our data after stratification for SE genotypes (Table 1), and because we identified neither interaction nor confounding bias, we presented only results for the total population.

In conclusion, our results cannot be explained by a

Table 1. Influence of MMP-3 genotype on radiographic severity of rheumatoid arthritis after 4 years of followup in patients stratified by SE genotypes*

	SE −/−		SE +/−		SE +/+	
MMP-3 genotype	No. of patients	TDS	No. of patients	TDS	No. of patients	TDS
5A/5A	8	0 (0–10.5)	10	5.5 (0–31)	3	3 (3–31)
5A/6A	15	3 (0–16)	23	10 (4–23)	12	25.5 (10–38.5)
6A/6A	6	9 (5–88)	12	17 (11.5–62)	6	41.5 (5–77)

* Radiographic severity was evaluated on radiographs of the hands and feet, according to the Sharp/van der Heijde method (Van der Heijde DM. How to read radiographs according to the Sharp/van der Heijde method. J Rheumatol 1999;26:743–5). Values are the median (interquartile range). MMP-3 = matrix metalloproteinase 3; SE = shared epitope; TDS = total radiographic damage score.

confounding effect of HLA–DRB1 genotype. MMP-3 gene polymorphism could be an interesting genetic component of RA severity.

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Anti–glomerular basement membrane antibody–associated renal failure in a patient with leflunomide-treated rheumatoid arthritis

To the Editor:

Leflunomide, a pyrimidine synthesis inhibitor, is a novel immunomudulatory agent that has been shown to be effective in treating active rheumatoid arthritis (RA) (1). It mainly acts by reversibly inhibiting dihydroorotate dehydrogenase, an enzyme required for pyrimidine synthesis in dividing cells (2). We present herein the first published report of a patient with RA who, shortly after starting leflunomide treatment, developed pathogenic anti–glomerular basement membrane (anti-GBM) antibodies, leading to renal failure.

The patient, a 60-year-old woman, was hospitalized with a 3-month history of lethargy and a 1-month history of fever. Four years previously, she had been diagnosed as having erosive, rheumatoid factor–positive RA and psoriasis. Antinuclear antibody (ANA), antibodies to Sm, Ro, La, Jo-1, Scl-70, and RNP, anti–double-stranded DNA (anti-dsDNA) antibody, and antineutrophil cytoplasmic antibody (ANCA) were not detected at that time. Upon diagnosis, sulfasalazine (SSZ) therapy had been instituted. Within 2 weeks of treatment initiation, she developed a rash and SSZ was discontinued. It was replaced with methotrexate (MTX), 7.5 mg administered orally once weekly. Multiple side effects to this treatment developed, such as dizziness, swollen eyes, light-headedness, and nausea. Despite a switch to subcutaneous administration of MTX, the adverse effects persisted. MTX was administered for more than a year, but was eventually discontinued. Thereafter, she received only nonsteroidal antiinflammatory drug therapy for ~1 year. Four months prior to admission, leflunomide treatment was started because of persistent arthritis at 100 mg daily for 3 days and then 20 mg daily. Concomitant medication consisted of naproxen 500 mg twice daily and an alternating etidronate/calcium carbonate regimen (i.e., etidronate 400 mg daily for 2 weeks followed by calcium carbonate 500 mg daily for 11 weeks, in a continuing cycle).

Physical examination at the time of admission revealed no abnormalities except for a temperature of 39°C. Laboratory studies revealed an elevated erythrocyte sedimentation rate (90 mm/hour), a C-reactive protein level of 170 mg/liter (normal <5), microcytic anemia with a hemoglobin level of 4.8 mmoles/liter (normal 8.7–11), a white blood cell count of 9.5×10^9/liter, a platelet count of 502×10^{12}/liter, a creatinine level of 175 μmoles/liter (normal <95), and a blood urea nitrogen level of 12.1 mmoles/liter (normal <8). Results of liver function tests, electrocardiography, and chest radiography were normal. Microscopic urine examination revealed erythrocytes and leukocytes. Urine protein output was 0.7 gm/24 hours.

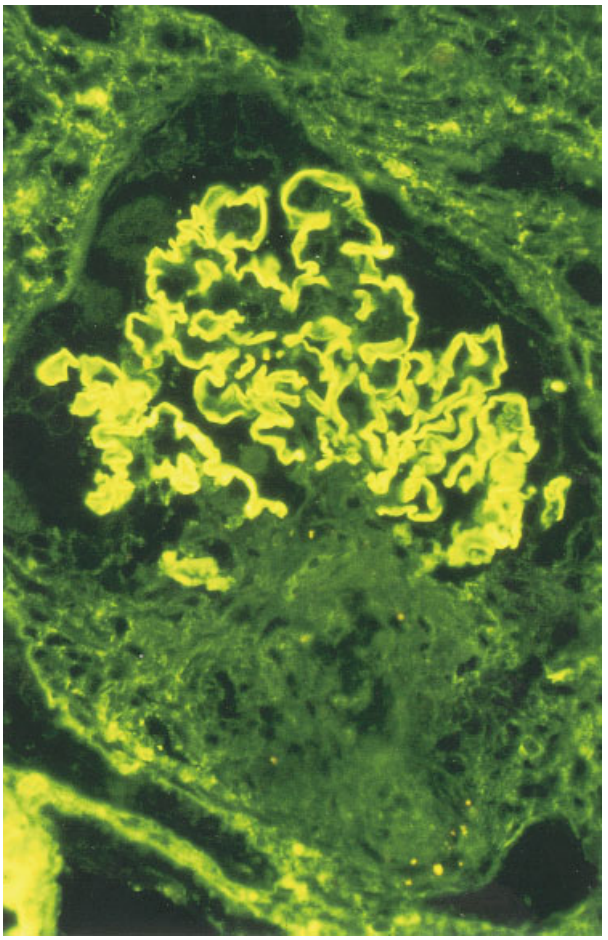

Figure 1. Direct immunofluorescence of a glomerulus specimen, showing linear deposition of IgG antibodies along the basement membrane.

Leflunomide was discontinued, and the serum creatinine level rose rapidly. Hemodialysis had to be instituted 2 weeks after admission because of anuria. Testing for ANCA by indirect immunofluorescence using ethanol-fixing neutrophils yielded negative results, as did testing for anti–proteinase 3 and antimyeloperoxidase antibodies by enzyme-linked immunosorbent assay. Anti-GBM antibody was strongly positive (>1,000 units/liter), and ANA was weakly positive (1:40). No antibodies against dsDNA, extractable nuclear antigen, antistreptolysin O, or DNase B were found. Hepatitis C virus antibodies and hepatitis B surface antigen were negative. Complement testing revealed normal C3 and C4 levels.

Ultrasound of the kidneys excluded postrenal obstruction. A renal biopsy was performed. Silver-stained sections showed a diffuse extracapsular proliferative glomerulonephritis with cellular crescents in all 15 glomeruli. Direct immuno-

fluorescence of the biopsy samples revealed linear deposition of IgG and C3 along the GBM (Figure 1).

Plasma exchange, cyclophosphamide (3 mg/kg/day), and prednisone (1 mg/kg/day) were initiated. The titer of anti-GBM antibodies rapidly decreased to <50 units/liter. However, the patient remained dialysis-dependent.

Anti-GBM antibody disease is an autoantibody-mediated disorder that typically presents as rapidly progressive glomerulonephritis. If pulmonary hemorrhage is present, it is called Goodpasture's syndrome. These antibodies have proven pathogenicity and bind the noncollagenous domain of the $\alpha 3$ chain of type IV collagen (3). The question in the present case is whether such antibodies have been induced by administration of leflunomide?

From experimental studies, leflunomide is thought to act as an immunoregulatory agent by preferentially causing cell arrest of dividing autoimmune lymphocytes (in contrast to other dividing lymphocytes or cells of the hematopoietic lineage or gastrointestinal tract). Cell arrest of activated autoimmune lymphocytes decreases the autoimmune response. Immunoregulatory drugs themselves are thought to induce autoimmune phenomena by 2 mechanisms: modification of autoantigens due to the presence of highly activated chemical groups, such as the thiol group of D-penicillamine, and interference with the lymphoid cells involved in suppressor or effector lymphocyte cellular cooperation.

Serious adverse events with leflunomide have mostly been reported to be associated with hepatic cell necrosis, occurring in 17 patients over a total of 1,040,000 patient-years; 9 of them had a fatal outcome (4). It is not known whether the necrosis is the result of a direct toxic effect on hepatocytes, an allergic phenomenon, or an autoimmune-mediated phenomenon. Vasculitis has been reported in far fewer patients receiving leflunomide (4,5), of whom 2 had a fatal outcome over 1,040,000 patient-years (5).

In the patient described herein, it is not known whether leflunomide was definitely the cause of the glomerulonephritis. However, the close temporal relationship between the drug administration and the observed renal failure does signal a potential causal association. Prognosis of anti-GBM antibody renal function depends largely on rapid intervention (6). Unfortunately, our patient appeared to have renal crescents in all glomeruli in the biopsy samples. Physicians treating RA patients with leflunomide should be aware of this possible complication.

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Etanercept-induced lupus-like syndrome in a patient with rheumatoid arthritis

In an editorial, Dr. Pisetsky addressed the induction of anti-DNA autoantibodies during treatment with tumor necrosis factor α blockers and some of the potential benefits and risks of these agents in patients with systemic lupus erythematosus (1). We report the case of a 45-year-old woman diagnosed with seropositive rheumatoid arthritis (RA) in April 1990, who developed a lupus-like syndrome after etanercept treatment.

Laboratory data at the time of RA diagnosis included positive rheumatoid factor (RF), weakly positive antinuclear antibody (ANA), negative anti-Ro, anti-La, anti-Sm, anti-RNP, and anticardiolipin antibodies, and normal complement levels. She was treated unsuccessfully with multiple nonsteroidal antiinflammatory drugs, hydroxychloroquine, steroids given intermittently in tapering dosages, and methotrexate. Etanercept was initiated in October 1999, resulting in complete resolution of the synovitis.

In December 2001, the patient presented with a 2-week history of diffuse hair loss and multiple palpable, nonpruritic crops of purple skin lesions on her extremities. Laboratory results included positive RF (titer 1:160), ANA (titer 1:5,120; homogeneous pattern), anti–double-stranded DNA (anti-dsDNA) 145 IU/ml (normal <30), anti-Ro >100 units/ml (normal <20), anti-La 29 units/ml (normal <20), anti-Sm 73 units/ml (normal <20), anti-RNP 93 units/ml (normal <20), C3 36 mg/dl (normal 67–154), C4 <10 mg/dl (normal 16–66), erythrocyte sedimentation rate 46 mm/hour (normal 0–20), normal results of a complete blood cell count, normal C-reactive protein, blood urea nitrogen, and creatinine levels, and negative anticardiolipin antibodies and lupus anticoagulant. Urinalysis revealed 8–10 red blood cells and no casts. Blood pressure was 180/100 mm Hg. Renal and skin biopsies were not performed. Etanercept was discontinued and prednisone 40 mg/day was started.

Seven months later, there has been hair regrowth with no recurrence of skin lesions. The anti-dsDNA level has normalized and the C3 and C4 levels are slowly moving toward the normal range. She no longer has red blood cells in her urine. The ANA (1:2,560) and anti-Ro (64 units/ml) have persisted. She is currently receiving prednisone 17.5 mg a day and enalapril 10 mg twice daily.

Two premarketing clinical trials of etanercept have shown increased induction of autoantibodies (11% of etanercept-treated versus 5% of placebo-treated patients de-

veloped newly positive ANA; 15% of etanercept-treated versus 4% of placebo-treated patients developed newly positive anti-dsDNA), but no evidence of clinical disease (2,3). Three reports suggesting that etanercept may induce lupus-like symptoms and autoimmune skin rashes have since been published (4–6). In the 5 cases described, there was resolution of skin lesions with discontinuation of etanercept. None of these patients had positive anti-Ro, anti-La, anti-Sm, or anti-RNP antibodies or active urine sediment.

Our patient's condition has improved with discontinuation of etanercept, and her autoimmunity markers are showing a trend toward normal values. Unlike previously described patients receiving etanercept, she developed antibodies to extractable nuclear antigens. Although a renal biopsy was not pursued, she did have evidence of persistent red blood cells in her urine sediment; this has resolved with blood pressure management, prednisone therapy, and the discontinuation of etanercept. We believe there is a probable association between the onset of her lupus-like disease and etanercept therapy.

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Reply

The case presented by Drs. Carlson and Rothfield is of particular interest since the patient developed autoantibodies to a variety of nuclear antigens following treatment of RA with etanercept. Models for the induction of systemic lupus erythematosus (SLE) by tumor necrosis factor (TNF) blockers have in general focused on anti-DNA, and it is speculative how an autoimmune response to antigens such as Sm, RNP, Ro, and La, in contrast to nucleosomal components, is generated. While the possible induction of SLE by a TNF blocker is of concern, this finding does not mean that this agent would exacerbate SLE. I remain concerned that extrapolation from an animal model or the occurrence of a possible drug-induced lupus syndrome will discourage investigation of a potentially useful agent in a disease that needs better treatment.

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Chondrocyte apoptosis in osteoarthritis: comment on the letter by Kouri and Abbud-Lozoya

To the Editor:

I thank Drs. Kouri and Abbud-Lozoya (1) for their interest in our article on apoptotic cell death in normal aging and osteoarthritic (OA) human knee cartilage (2). In fact, there is widespread interest, for many reasons, in the entire area of cell death during cartilage degeneration, and many different methods to address various aspects of this issue are currently available (for review, see ref. 3). Overall, no conclusive picture has yet evolved from the different studies, and in fact a recent study showed how different approaches used in parallel might yield contradictory results (4,5). Because I have recently reviewed the topic in greater detail (3), I would like to address only a few main points raised by Kouri and Abbud-Lozoya.

Certainly, the use of strict criteria for assessing apoptosis can lead to significant underestimation of the phenomenon; nevertheless, the numbers of apoptotic cells reported in various previous publications are far too high. As correctly stated by Kouri and Abbud-Lozoya (1), we were clearly in error when we stated that Kouri et al (6) "found no apoptotic cells throughout the entire depth of OA cartilage specimens obtained at arthroscopy" (2), and I apologize for this. However, this strengthens the point that the high rates of TUNEL positivity found by many colleagues are rather artificial, at least as indicators of actual apoptotic cell death. I believe the reason for this might be that no appropriate controls are used to properly titrate the reaction conditions of the TUNEL experiments. In this respect, fetal growth plate cartilage is certainly a very suitable control tissue in that it shows a spatial distribution of cellular events. It seems very unlikely that the methodologic inconsistencies noted by Kouri and colleagues (6) derive from the arthroscopic procedure because, if properly performed, arthroscopic biopsy material should enter the fixative within 1 or 2 minutes (and, to my knowledge, significant apoptotic DNA fragmentation does not occur within this time frame).

Human tissue is not easily accessible for experimental procedures and time-course studies. Thus, animal models—if shown to reflect the human situation—are of high experimental value. However, they cannot replace studies in human tissues and cannot even "correct" them, despite the hazards and difficulties associated with the use of human material. Unfortunately, Kouri and colleagues (6) published their original work in a journal that was not listed in PubMed at that time (7) so the data were not accessible using routine literature search methods. Also, their results do not sound very reliable as far as the detection of TUNEL-positive cells is concerned.

They report on morphologically normal cells with TUNEL positivity and on disrupted chondrocytes without TUNEL staining, and it appears as if cells that were originally TUNEL positive could recover after a certain time. All of this suggests that the TUNEL technique is not reliable at all, or at least not in the experiments reported; again, appropriate controls such as fetal cartilage were missing. This confirms some of the concerns raised in our report.

There is at present little significance to the claim that apoptotic morphology in chondrocytes is not comparable with that in other cell types. Studies from my group and others have indicated that fetal chondrocytes exhibit classic morphologic features in the lower growth plate and that in adult degenerative articular cartilage also, apoptotic bodies can be observed very rarely (2,8). Thus, there is so far no good experimental evidence to back up Kouri and Abbud-Lozoya's assertion concerning apoptotic morpology in chondrocytes, and one might view this claim as an attempt to avoid otherwise straightforward criticisms of some of the work published in this context.

Cell degeneration undoubtedly plays an important role in OA cartilage degradation, but this is not likely to be predominantly apoptosis, but rather some sort of cell (pre)senescence (ref. 9 and Aigner T, et al: unpublished observations). Much remains to be learned in terms of the cell biology of chondrocytes. In fact, in the Bone and Joint Decade (10), the cell biology of the chondrocyte should be a major focus in the attempt to understand this living compartment of articular cartilage.

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Aquaporins in primary Sjögren's syndrome: comment on the articles by Steinfeld et al

To the Editor:

Steinfeld et al report interesting findings on the clinical usefulness of the tumor necrosis factor α (TNFα) inhibitor infliximab in the treatment of primary Sjögren's syndrome (SS) (1), and the redistribution of aquaporin 5 (AQP-5) molecules in acinar cells in salivary gland biopsy specimens from patients with primary SS treated with infliximab (2).

The degree of salivary dysfunction in patients with primary SS does not correlate with the degree of acinar destruction in the salivary glands (3). We have therefore proposed that a functional defect in the salivary and lacrimal glands, rather than acinar destruction, may cause the sicca symptoms (4–6). The role of TNFα in primary SS as an inhibitor of salivary function or as a mediator of tissue destruction is not well established. One study demonstrated that TNFα together with interferon-γ, but not alone, caused an *increase* in noradrenaline secretion from rat neurons (7); this would enhance, not inhibit, salivary secretion (8). Furthermore, salivary secretion is principally controlled by parasympathetic rather than sympathetic neurons (8). Investigations of the distribution of TNFα in the salivary glands of patients with primary SS have yielded variable results, and the majority of studies have measured messenger RNA (for example, ref. 9), which does not correlate with the level of functional protein. Furthermore, the role of TNFα in acinar cell atrophy (10) and defective cell signaling (11) has been questioned. Given the lack of strong evidence for a role of TNFα in the pathogenesis of primary SS, it is perhaps not surprising that unstimulated salivary flow in the infliximab-treated patients was still highly abnormal ($<$1 ml/minute), although there was an improvement in subjective measures of disease (1).

There has recently been considerable interest in the possibility that altered distribution or expression of AQP water channels may contribute to sicca symptoms in primary SS. Steinfeld and colleagues (12) reported a change in AQP-5 distribution in the acinar cells of salivary glands in patients with primary SS. However, we do not agree with these findings (13,14). The negative control used by Steinfeld et al to measure background staining was a slide in which the primary antibody was omitted (12). The appropriate control is primary antibody that has been preabsorbed with peptide. Our work has demonstrated that the basolateral staining observed using immunoperoxidase methods remains when the AQP-5 antibody is preabsorbed with the human AQP-5 peptide (13,14); we therefore conclude that basolateral staining is in fact background staining. On this basis, one could argue that the reported results (2) reflect a change in the level of background staining.

Whereas we have been unable to demonstrate that

AQP-5 expression is altered in the salivary glands of patients with primary SS, we have recently shown that expression of another isoform, APQ-1, is significantly down-regulated (15). AQP-1 is present in the myoepithelial cells that surround the acinus (15,16), and regulation of myoepithelial cell volume is likely to be important in enabling these cells to assist salivary flow (15,17). We therefore propose that dysregulation of myoepithelial cell function may be one mechanism resulting in salivary gland dysfunction in primary SS. In addition, functional autoantibodies that inhibit M_3-muscarinic receptor–mediated neurotransmission are likely to play an important role in inhibiting salivary flow (6,18). Treatment of sicca symptoms in primary SS may thus require a multifaceted approach.

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Use of an artificial neural network to predict cancer development in patients with inflammatory myopathy: comment on the letter by Selva O'Callaghan et al

To the Editor:

We read with great interest the comments of Selva O'Callaghan et al (1) pertaining to our report on use of the artificial neural network to improve differentiation between Churg-Strauss syndrome and Wegener's granulomatosis (2). The authors describe the application of a neural network to predict the development of cancer in patients with idiopathic inflammatory myopathy (IIM).

From the methodologic point of view, the approach is very convincing, particularly the feature selection and the employment of cross-validation. We assume that the authors chose the smallest admissible network size that provides a solution, so that regularization methods or early stopping were not needed in order to avoid overfitting. The accuracy obtained is very impressive. As a suggestion for further work, one might calculate a receiver operating characteristic curve for explicitly adjusting the sensitivity and specificity (3).

Nevertheless, we have some concerns with respect to the results. In Selva O'Callaghan et al's study, 13 patients developed cancer. The authors obtained a sensitivity of 98.46%. They probably correctly classified 12 of the 13 patients with cancer; however, 12 of 13 is 92.3%. How do the authors explain the difference? Perhaps they should recalculate this

point. Moreover, the percentage of men with cancer in relation to the sex is inconsistent. In their Table 1, if there are 6 men with cancer (of 19), then the percentage should be 32%. This percentage still seems to be very high, higher than that described among male patients in other studies (4,5). In this connection it would be of interest to know when these patients developed their cancer. The highest risk for cancer in patients with IIM has been described to occur around the time of diagnosis, i.e., within the first year since the diagnosis was established. The risk for malignant disease diminished with time and disappeared beyond 5 years (4,5). This point seems to be of great practical importance in determining the extent of the search for malignancy at the time of diagnosis of an IIM. The occurrence of cancer beyond the first year of diagnosis of polymyositis/dermatomyositis could also be associated with the type of therapy.

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Reply

To the Editor:

We are grateful to Linder et al for their appropriate comments on our previous letter; nevertheless, some issues need to be clarified. In our study, 13 patients developed cancer: 7 women and 6 men. Of course, the 5 patients who developed breast cancer (2 patients) and ovarian cancer (3 patients) were female, but it should not be assumed that all of the other 8 patients were male. Indeed, there was 1 female patient with colon carcinoma, so the total number of female patients with cancer was 7. In our study, 6 of 19 male patients and 7 of 43 female patients (total 13 of 62 patients [21%]) developed a neoplasm, so the percentages of male/female patients in relation to cancer development are not inconsistent. In a recent study, 32% of patients with dermatomyositis developed cancer (198 of 618 cases) (Hill CL, Zhang Y, Siguregeirsson B, Pukkala E, Mellemkjaer L, Airio A, et al. Frequency of specific cancer types in dermatomyositis and polymyositis: a population based study. Lancet 2001;357:96–100); in our series, 11 of the 13 patients with cancer had dermatomyositis, which would explain the high percentage obtained. Finally, although cancer developed mostly in the first year, it is known that the overall incidence of cancer is increased in patients with IIM. To our knowledge, there have been no studies demonstrating that immunosuppressive treatment in IIM is related to an increased prevalence of cancer.

Regarding sensitivity, although the figure of 98.46% is correct, the methodology used should be explained in more detail in order to answer the question posed by Linder et al. Every "leave-one-out" cross-validation procedure was repeated 5 times for every different training set. This alleviates the effect of the randomness of the selection of some parameters. The values shown in our letter were the mean from all 5 cross-validation procedures. With respect to the sensitivity, the system correctly classified 64 of 65 (13×5) cases.

We would also like to add some information with regard to the comments on the size of the network and the overfitting. As noted in our letter, features in real-world data sets may be "noisy," irrelevant, or redundant. A priori, when many variables are present, there may be many different solutions capable of learning the same training set, but only a few of these solutions will lead to good generalization, and there is no reason to assume a good one will be selected. If the system gives some importance to noisy or irrelevant variables in order to learn the data set, it will use this information for new data, probably leading to poor generalization even if one tries to control the overfitting. Imagine that we have collected a database where there is an irrelevant variable for the problem. Since initially we know nothing about the importance of this attribute in order to predict the desired behavior, this variable will have the same a priori probability to appear in the resulting solution as the rest of the variables. This is more likely to happen when only a small number of examples is available.

In this context, it is convenient to use a feature selection procedure. If the system uses an irrelevant variable to learn the data set, generalization should improve without this variable. In previous experiments (with other data sets), we observed that this effect is more evident if we try to fit the training set as much as possible, that is, when there is a large degree of overfitting. An intuitive justification of this could be that, if we try to perfectly adjust the data set, we are forcing all of the variables to be used as much as possible in the resulting solution. Therefore, information indicating which variables are useless will emerge if generalization improves when the system is not allowed to use them. Ironically, generalization is improved if overfitting is forced at every step. Another justification comes from the bias/variance decomposition of the generalization error (Geman S, Bienenstock E, Doursat R. Neural networks and the bias/variance dilemma. Neural Comput 1992;4:1–58), which suggests that optimal performance is obtained when a tradeoff between the quality of the approximation to the training set and the complexity of the solution is achieved. When many variables are present, although the training set can be approximated almost perfectly, the system is too complex. As variables are eliminated, the complexity of the

system is reduced together with the capacity of approximation. This allows detections of variables that are irrelevant and noisy for the problem at hand. After these variables have been discarded, a different approach to the problem can then be undertaken, since we can consider (of course, with a certain probability of error) that all remaining variables are useful. This does not necessarily imply that the system will not suffer negative effect of overfitting with the selected variables, but the damage caused will probably be lower. A standard technique that tries to control the overfitting (regularization or early stopping, for example) would be expected to obtain better results with this reduced number of variables.

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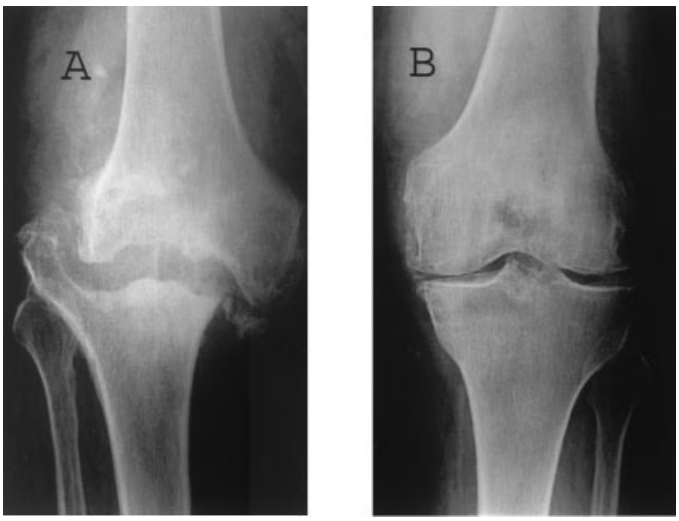
Clinical Images: Neuropathic joint in a patient with calcium pyrophosphate deposition disease



The patient, an 81-year-old woman, had osteoarthritis of the knees since 1980; during the last 2 years the pain on the left side worsened, with progressive severe disability. Radiography of the left knee showed a destructive process with large femoral and tibial erosions **(A)**; the right knee exhibited calcification of menisci in addition to osteoarthritic changes **(B)**. Synovial fluid culture of the left knee yielded negative results, but histologic examination of synovial membrane showed foreign body granulomas with giant cells surrounding crystalline deposits. Findings of a tuberculin skin test were negative. Primary hyperparathyroidism and latent syphilis were found. We diagnosed calcium pyrophosphate deposition disease, which may be associated with hyperparathyroidism, in the form of pseudoneuropathic joint. This rare manifestation usually affects elderly women and preferably involves the shoulder, knee, or hip, with rapid destructive changes. It is more frequent among patients with syphilis, even in the absence of tabes dorsalis.

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