

This procedure for measuring aggregate represents a modification of methods as described.<sup>1,2</sup> To my knowledge there is no method one can use to obtain pure aggregate. The classical method for obtaining aggregate employing centrifugation in caesium chloride density gradients does not yield 100% aggregate. There are nonaggregatable proteoglycans present in this fraction as well.

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## Chromosomes in rheumatoid arthritis

SIR, Lymphoproliferative disorders are more frequently found in patients with autoimmune disease than in normal persons. Isomaki *et al*<sup>1</sup> showed an increased risk of leukaemia, lymphoma, Hodgkin's disease, and multiple myeloma in rheumatoid arthritis (RA) patients. These investigators postulated that a continuous immunological stimulation in RA could cause proliferation and malignant transformation of the immunologically competent cell clones. Green *et al*.<sup>2</sup> believe that immunodeficiency in patients with autoimmune disease may be a factor in the development of lymphoproliferative disease.

We found marked chromosomal abnormalities in a patient with lymphoma who was treated with intra-articular radioactive gold (<sup>198</sup>Au) for severe RA.<sup>3</sup> Although we were tempted to conclude the chromosomal abnormalities found in our patient were due to her <sup>198</sup>Au treatment, we were unable to exclude the possibility that the phenylbutazone and prednisone treatments or the development of her lymphoma or RA may have been the cause of the chromosomal abnormalities. This communication reports our chromosomal findings of 21 RA patients with various treatments and of 28 controls.

Heparinised peripheral blood was obtained for cytogenetic studies from 21 active RA patients. Five patients received 10 mCi of intra-articular <sup>198</sup>Au; 14 received intramuscular nonradioactive gold; and 2 received full therapeutic doses of aspirin (7800 mg/day). The controls consisted of 28 hospital employees without any known illnesses. Peripheral blood was cultured in TC 199 tissue culture medium, enriched with either autologous plasma or fetal calf serum and stimulated with phytohemagglutinin (PHA) according to the method used in our laboratory.<sup>3</sup> After incubation at 37°C for three days the cultures were exposed to colcemid at a concentration of

0.16 µg/ml for one hour. The cells were then exposed to 0.075 M KCl for half an hour, fixed with acetoalcohol, and the slides made by the air-dried method. The slides were stained with Giemsa and analysed for chromosomal breakages as described.<sup>4</sup>

The mean rate of chromosomal breakages was 8.6 ± 2.4 (SEM) in the 5 RA patients treated with <sup>198</sup>Au; 9.4 ± 1.43 in the 14 treated with nonradioactive gold; and 5.5 in the 2 treated with high doses of aspirin. The 28 controls had a frequency of 3.67 ± 0.56. The mean difference of the chromosomal breakages among the RA patients, treated with radioactive or nonradioactive gold, was not statistically significant. But the difference between the RA patients and the controls was significant.

Acquired chromosomal abnormalities can be seen in patients exposed to radiation, virus, and certain chemicals or in patients with various malignant diseases. The marked chromosomal abnormalities found and reported<sup>3</sup> in our patients with lymphoma, treated with <sup>198</sup>Au for her RA, must not have been caused by the radioactivity of the <sup>198</sup>Au per se. This conclusion is based on our present finding that significant chromosomal breakages are also seen in RA patients treated with nonradioactive gold. Although it is possible that the chromosomal abnormalities found in our RA patients were associated with their primary disease rather than their treatment, we cannot make this conclusion based on our present data, since all our patients were treated with various methods. Also, it is not possible to study RA patients not treated with aspirin to rule out this possibility.

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## Klebsiella, ankylosing spondylitis, and statistics

SIR, Eastmond *et al*.<sup>1</sup> have followed up by post 44 ankylosing spondylitis patients. They obtained monthly a questionnaire about the clinical activity of the disease and a faecal specimen for klebsiella culture. They state that 18 patients on 19 occasions had *K. aerogenes* cultured from

their faeces, when the preceding specimen had been negative (-/+ sequence). Six (31.6%) of these occasions were associated with a deterioration in clinical state compared with a similar deterioration associated with only 17 (9.8%) of the remaining 174 faecal culture sequences (+/+, +/-, -/-). Using the chi-square method, the authors found a p value less than 0.02.

The chi-square method, however, should only be applied to independent variables.<sup>2</sup> Bacteriological sequences from the same patient are not. Chi-square should in this case be calculated on one faecal sample or sequence per patient. Indeed, pooling related data artificially reinforces the strength of an observation. Moreover, if there are seasonal variations in klebsiella prevalence—which we do not know—the comparison has to be done at the same month for all patients and controls.

The same chi-square error is found in the first klebsiella paper,<sup>3</sup> where 433 faecal samples from 163 patients are pooled and subdivided according to clinical activity. So it appears that, even if the klebsiella idea is an interesting one, the evidence thus far of a relationship between klebsiella and activity of ankylosing spondylitis largely rests on unsound statistics.

May we suggest that the editorial board should pay more attention to the validity of statistical procedures used in submitted papers?

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SIR, We read with interest the letter from Drs François and Debouche criticising the use of the chi-square statistics for testing the statistical significance of the association between a particular klebsiella faecal culture sequence (-/+) compared with alternative sequences (-/-, +/-, +/+) and an increase in clinical activity of ankylosing spondylitis in patients. The chi-square statistic is a method of testing the hypothesis that 2 characteristics are independent. If the proportions in the sample tested are significantly different from the expected then the hypothesis that the 2 characteristics are independent is rejected.<sup>1</sup>

In our study<sup>2</sup> the frequency of the faecal culture sequence -/+ was independent of whether the subjects

were patients with ankylosing spondylitis or controls. Nineteen of 193 patients had this sequence compared with 26 of 154 controls giving  $\chi^2_c = 1.36$  (1 DF);  $p < 0.05$ . Patients and controls were contacted on the same consecutive dates, thus excluding any effect of possible seasonal variation in faecal klebsiella carriage.

We believe our results do show an association between the appearance of *Klebsiella aerogenes* in the faeces and an increase in the clinical activity of ankylosing spondylitis, though this does not prove cause and effect or indicate the mechanism of such an effect.

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## Bacteroides causing osteomyelitis in rheumatoid arthritis

SIR, We read with interest the recent case reports of pyogenic arthritis due to bacteroides species complicating rheumatoid arthritis (RA).<sup>1</sup> We recently reported the history of a man with RA who presented with acute haematogenous osteomyelitis of the clavicle due to *Bacteroides fragilis*.<sup>2</sup> There was no evidence of previous bony pathology at a later post-mortem examination, although we had initially suspected a metastasis from a carcinoma of the bronchus. Like Dodd *et al.*'s case<sup>1</sup> our patient was not pyrexial and had not had steroid therapy. He was treated with clindamycin and metronidazole. After daily aspiration of the abscess the pus discharged spontaneously and he made a full recovery. The metronidazole was continued for 8 weeks, again with no evidence of a neuropathy. We did not identify the source of his infection, but he was known to have diverticulosis.

Anaerobic osteomyelitis is not common. Raff and Melo<sup>3</sup> reviewed 193 cases in the literature of which 29 were due to haematogenous spread of the organism. They reported an increased incidence in patients with predisposing factors such as diabetes mellitus, but no patient had RA. Patients with RA may be more susceptible to infection, but it was not clear why our patient developed this unusual presentation of osteomyelitis.

Identification of an anaerobic organism may be delayed because of its slow rate of growth in vitro. The benefits of gas liquid chromatographic (GLC) analysis of septic synovial fluid have been reported,<sup>4</sup> and if anaerobic infection is