Antiperinuclear factor in the polyarticular form of juvenile chronic arthritis: are use of frozen material or age of patients relevant?

We read with interest the work of Gabay and colleagues1 and their exchange of letters with Dr Nester and colleagues.2 We would like to try to reconcile the two points of view regarding antiperinuclear factor (APF) results in juvenile rheumatoid arthritis (JRA), first by drawing attention to an important technical detail which seems to have been neglected until now, and then by suggesting an alternative explanation for this discrepancy between results.

Our experience corroborates that of Nester’s group, as we noted APF-IGG in four of 16 cases of JRA of polyarticular onset but in no other form; however, interestingly, three of these four patients were young adults at the time of APF testing (personal unpublished results). When we used the exact methodology described by Younou1, especially the 10% positive cell criterion and a 1:80 threshold dilution, the titres of these four sera were evaluated as 1:200 (twice), and 1:500 (twice). Thus we can also agree with Gabay and colleagues that the suggested procedure1 is relevant, especially the 10% positive cell criterion. Accordingly, Nester’s explanation for the lower prevalence found in Gabay’s study is apparently not correct.

How could such a misunderstanding occur? We had the opportunity several years ago to study all the APF parameters in Brest. At that time we observed (personal unpublished results) that the slides (even if frozen) had to be used quickly to avoid a dramatic loss of sensitivity. We recently reproduced this phenomenon blindly using four different sera against APF at various times of positivity. When the slides were not frozen, the titre decreased from three dilutions (at least) after one week. A decrease of one dilution (at least) at one week and of three dilutions after two weeks was still observed even when the slides were frozen at −80°C. We also wish to emphasise the importance of another time related phenomenon which is especially evident for APF, namely the fact that the serum titre usually decreases dramatically after two years of freezer storage.

Though such technical ‘details’ do not usually appear in the Materials and Methods section of papers dealing with the APF test, they are very important and should be requirements for publication to avoid further unnecessary disputes.

Thus it would not be surprising that teams using fresh slides and sera obtain results different from those using frozen material. However, even if the study by Gabay and colleagues had been conducted with frozen material, the general conclusions would remain true: why is APF (and antikeratin antibody (AKA)) positivity not as frequent in these polyarticular forms of JRA as in adult RA (sensitivity of 75%),1 and should this lead to the conclusion that this form of JRA is distinct from RA?

The target antigen for AKA has been recently identified as filaggrin;3 that for APF remains unknown, but it has already been hypothesised that a similar antigen may be detected in thymus.3 This would be in keeping with the fact that, in buccal cells, APF target colocalises exactly with profilaggrin4 which is also expressed in Hassall’s corpuscles of human thymic medulla.5 Interestingly, the number of Hassall’s corpuscles (which is very low in children) dramatically increases with age.6 While there is still not any proof that this phenomenon is implicated in the different prevalences of (and AKA) and polyarticular JRA and RA, it would be interesting to know if the ages of patients studied by Gabay and colleagues1 and Nester and colleagues2 were similar. Indeed, both the prevalence and titre of these antibodies could increase in the sera of children with the polyarticular form of JRA when they grow older.

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Authors’ replies:
We read with interest the letter of Dr Berthelot. In a previous unpublished study we have compared the sensitivity of our assay for the detection of antikeratin factor (APF) in sera assessed on fresh and frozen slides, but we did not detect any difference. For our study on juvenile chronic arthritis (JCA),1 as for other previous studies on serum from patients with rheumatoid arthritis (RA),2 we used frozen slides and frozen sera, and found that the occurrence of antiperinuclear factor (APF) was clearly lower in JCA than in RA. We therefore do not think that this particular technical detail is able to explain the discrepancy between the results of Nester and colleagues1 and our own results. We have already reviewed in detail the numerous other methodological differences in APF assays than may interfere in the sensitivity and specificity of this test and emphasise the fact that standardisation is needed in the future.

As indicated in our paper,1 the vast majority of our patients were under the age of 16 when serum was collected; Nester and colleagues did not give any information on the age of their patients. However, we do not think that variation in occurrence of APF between the two studies or between JCA and RA are related to patient age. Patients with JCA are a heterogeneous group with different clinical presentations and different serological characteristics. Only 10% of those with JCA have clinical and biological features comparable to those found in adult RA. It is therefore, not unexpected to find that sera from patients with JCA have different autoantibody characteristics than sera of adult RA. Furthermore, we have shown that variation in the occurrence of serological findings included only that between antikeratin and anti-RA 33 antibodies.

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We thank Dr Berthelot for trying to reconcile the varied results regarding antiperinuclear factor (APF) testing in patients with juvenile rheumatoid arthritis (JRA).

Dr Berthelot’s data further emphasise our concern of the need to form universal criteria regarding the definition of positivity in children.1 Using fresh buccal cells and 10% positive cells at 1:80 or greater dilution as criteria for APF positivity, this group documented the prevalence of APF in 25% of children with polyarticular onset JRA. This prevalence is greater than reported by Gabay and colleagues,2 but still less than the prevalence reported by our group.1 Using fresh buccal cells but different criteria for APF positivity (>5 cells per slide and 1:5 or greater dilution), we documented APF presence in 14 of 28 patients with polyarticular onset JRA. None of the 24 control subjects and only 8% of lupus patients were APF positive using these criteria. Using the criteria suggested by Younou et al,2 we noted that the occurrence of APF in these children with polyarticular onset JRA decreased to 11%. It is not clear from Dr Berthelot’s letter if lower serum dilutions were tested and, if so, the prevalence of APF positivity at these dilutions.

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We agree with Dr Berholtz that technical details, such as storage time of samples, should be included in APF studies. It is nevertheless difficult to evaluate retrospectively the effect of storage time or patient’s age on APF positivity. For two of our patients, we assayed three serum samples collected over a period of four years and stored for one to five years. In each case, the initial APF was negative, but the following two were positive. However, it is difficult to conclude if this is a result of a shorter storage time, the patients growing older, or other variables. In our study, APF was most prevalent in the polyclonar onch, seropositive JRA group, which typically consists of older children, mostly teenagers. However, it is not clear if this is related to the patients’ ages or, most likely, to their type of JRA, which closely resembles adult RA. We did not find any difference in APF occurrence between younger and older JRA patients with pauciarticular and seronegative polyarticular onset diseases.

These data underline the observation that APF testing can be influenced by many variables, especially in children, thus emphasizing the need to define APF positivity in JRA on the basis of universal criteria.

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LETTERS TO THE EDITOR

Chronic calcifying pancreatitis in rheumatic diseases

We describe chronic calcifying pancreatitis (CCP) in two patients with rheumatic diseases.

A 61 year old female presented in May 1991 with acute pancreatitis caused by cholecystitis; she underwent a cholecystectomy. Six months later, because of increased alkaline phosphatase concentrations an abdominal computed tomography (CT) scan was undertaken and revealed dilatation of bile and pancreatic ducts. This observation was confirmed by endoscopic retrograde cholangiopancreatography (ERCP) (fig 1); in addition, pancreatic tests were subnormal. However, the patient remained asymptomatic. In May 1992 she was evaluated in the rheumatology service where the diagnosis of systemic lupus erythematosus (SLE) and Sjogren’s syndrome (SS) were made. Prednisone 5 mg/day was given for two months and chloroquine thereafter; diabetes was diagnosed in August 1992. In April 1993 the patient reported a mild epigastric pain and an abdominal CT scan revealed pancreatic calcification and a pseudocyst in the tail.

The second patient was a 51 year old female followed in the rheumatology service since 1978 with the diagnosis of scleroderma. She received as treatment indomethacin, metoclopramide, and ranitidine; she required the introduction of insulin in 1983, but never received steroids. In August 1992, after an episode of mechanical lumbar pain, calcifications were noted at the level of the pancreas on plain radiographs. Evaluation in the gastroenterology service revealed subnormal pancreatic tests, and pancreatic calcifications on abdominal CT scan (fig 2). ERCP revealed dilations and stenoses of the main pancreatic duct, with normal bile ducts.

Acute pancreatitis is the commonest clinical pancreatic manifestation in SLE. Corticosteroids or immunosuppressive therapy are believed to be the causes, but immunological and vasculitic mechanisms are also important. Chronic pancreatitis in SLE has been reported only recently.

Pancreatic problems have been reported rarely in SS, though there is evidence of subclinical insufficiency in SS, ranging from 7% to 60%. Pancreatic tests in sclero-derma have shown insufficiency in about 30% of patients but the nature of the disease is at best equivocal, as malabsorption may have other causes which cannot be distin- guished easily by available tests; for example, hyperperistalsis with bacterial overgrowth or even reflux of duodenal contents into the pancreatic duct may affect the pancreatic exocrine function without necessarily indicating primary pancreatic involvement.

The pancreas has a potential for calcification in chronic pancreatitis, especially in alcohol or nutritionally-induced disease. The mechanism is not clear, but it seems that calcium may precipitate when the concen-tration of lithostatin, which maintains calcium solubility in the pancreatic juice, is reduced, or when there is a decrease in volume flow with a high protein content of the pancreatic juice. Theoretically, either or both mechanisms could be mediated by the pathogenetic processes of the collagen diseases.

Our first patient displayed several features that deserve remark: (a) the association of CCP with two conditions (SLE and SS) that independently predispose to pancreatitis; (b) onset of acute pancreatitis one year before the onset of SLE and SS; (c) mildness of SLE and SS; and (d), in common with our patient with scleroderma, a nearly asymptomatic course to calcification. It is possible that the modest dose of prednisone given for two
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