MATTERS ARISING

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 Nesher 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 Nesher'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other form; however, interestingly, three of these four patients were young adults at the time of APF testing (personal unpublished results).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 Youinou,³ 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 procedure³ is relevant, especially the 10% positive cell criterion. Accordingly, Nesher'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 At that time we observed (personal unpublished results) that the slides (even if frozen) had to be used quickly to avoid a dramatic loss of sensitivity.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 sera with APF at various titres 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%),³ 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;⁴ that for APF remains unknown, but it has already been hypothesised that a similar antigen may be detected in thymus.⁵ This would be in keeping with the fact that, in buccal cells, APF target colocalises exactly with profilaggrin⁶ which is also expressed in Hassall's corpuscles of human thymic medulla.⁷ Interestingly, the number of Hassall's corpuscles (which is very low in children) dramatically increases with age.⁸ While there is still not any proof that this phenomenon is implicated in the different prevalences of APF (and AKA) in polyarticular JRA and RA, it would be interesting to know if the ages of patients studied by Gabay and colleagues¹ and Nesher 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 antiperinuclear 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 always 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 Nesher and colleagues³ and our own. However, 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.^{1,4}

As indicated in our paper,¹ the vast majority of our patients were under the age of 16 when serum was collected; Nesher 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 other markers, such as 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 conclusion 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 presence 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.³ 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 Youinou *et al.*,⁴ 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.