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Correlation of serum neopterin concentrations with disease activity in juvenile dermatomyositis

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Abstract

Serum neopterin concentrations were measured in 15 patients with juvenile dermatomyositis and their correlation with the severity of muscle impairment was evaluated. Neopterin concentrations were measured by radioimmunoassay. When compared with controls (median 0·75 ng/ml; range 0·33–1·18), serum neopterin concentrations were increased in patients with active juvenile dermatomyositis (median 3·21 ng/ml; range 0·95–13·81), but not in patients during remission (median 0·81 ng/ml; range 0·55–1·34). In horizontal and longitudinal studies, serum neopterin was significantly correlated with the severity of muscle strength impairment, whereas serum muscle enzyme values were not. These data suggest that measurement of serum neopterin may be used, together with clinical examination, to guide the treatment of patients with juvenile dermatomyositis.

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Neopterin (6-D-*erythro*-trihydroxypropylpterin) is a low molecular weight compound derived biosynthetically from guanosine triphosphate. Neopterin is synthesised essentially by monocytes activated by interferon gamma and is considered to be a marker of activated cellular immune responses.[1] Increased concentrations of neopterin in serum or urine, or both, have been reported in a variety of diseases characterised by the activation of cellular immune responses, such as allograft rejection, viral and bacterial infections, and autoimmune diseases, including rheumatoid arthritis, chronic inflammatory bowel disease, autoimmune thyroditis, and early onset insulin dependent diabetes mellitus (reviewed by Fuchs *et al* [1]).

Juvenile dermatomyositis is an inflammatory disease affecting muscle, skin, and more rarely other organ systems.[2] Although histology and laboratory data suggest that the disease may be of autoimmune origin,[3 4] the aetiopathogenesis of juvenile dermatomyositis is still unknown. Myones *et al* have reported that serum neopterin concentrations appear to correlate with disease activity in patients with juvenile dermatomyositis.[5] In this study we measured serum neopterin in patients with juvenile dermatomyositis and evaluated their correlation with the severity of muscle impairment.

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Patients and methods

PATIENTS

Fifteen patients fulfilling the diagnostic criteria for dermatomyositis proposed by Bohan and Peter[6] were included in the study. At each visit a serum sample was taken and stored, serum muscle enzyme values were measured, and the clinical assessment included the measurement of a disease activity score using a standardised form that was filed in the records of the patients.

The disease activity score is based on a functional and a muscle strength score. The functional score, which assessed the ability to perform 11 everyday activities (climbing stairs, dressing and undressing, walking on tiptoe, etc), was graded (1, impossible; 2, possible with difficulty; 3, performed without difficulty) and the score was calculated as the total of the grade for each activity (normal 33). Muscle strength score was recorded as the muscle strength in nine muscle groups and was graded according to the Medical Research Council scale[7] (0, no contraction; 1, flicker or trace of contraction; 2, active movement with gravity eliminated; 3, active movement against gravity; 4, active movement against gravity and resistance; 5, normal power), and the score was calculated by adding the grades for each muscle group (normal 45). The disease activity score was calculated by adding the differences from the normal values of the two previously described scores. Patients presenting an abnormal disease activity score (≥ 1) were considered to have clinically active disease whereas patients with a normal disease activity score (0) were considered to be in remission.

All patients were evaluated at least twice. Twelve patients were evaluated during active disease, and six of these were also evaluated during a subsequent remission. Samples were obtained from three patients only during remission. A follow up study was performed in three patients from whom 11, eight, and seven samples were obtained at intervals ranging from two to 12 months. The median age was 8·5 years (range 2·5–16·5 years) and the median disease duration was 18·5 months (range 2–110 months). At the time of sampling all patients with active disease, except three who were receiving no treatment, were treated with steroids. In addition to steroids, two patients were receiving cyclosporin or azathioprine.

Serum samples obtained from 28 healthy children (median age 9·5 years, range 5–17 years), admitted to hospital for bone marrow donation or for minor operations, were used as controls. Permission to draw extra blood

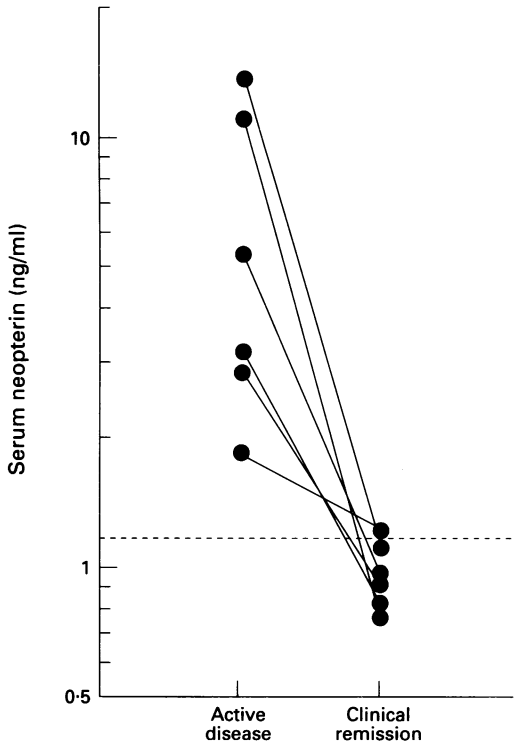

Figure 1 Serum neopterin concentrations in patients with juvenile dermatomyositis tested during active disease and a subsequent clinical remission. The horizontal broken line represents the upper range of control values.

during routine venepuncture was obtained from the parents of all children.

NEOPTERIN MEASUREMENT

Neopterin concentrations were evaluated on stored serum samples obtained at 9.00 am from patients and controls using a commercially available radioimmunoassay according to the instructions provided by the manufacturer (Sorin). Briefly, increasing concentrations of neopterin (range 0·2–200 ng/ml) or serum samples (1:1) were incubated for one hour at 37°C in the presence of neopterin labelled with iodine-125 and a rabbit serum against neopterin. A polyethylene glycol conjugated antirabbit antiserum was added and after a 15 minute incubation at room temperature samples were centrifuged and the pellet was counted in a gamma counter.

STATISTICAL ANALYSIS

Results were analysed using the Mann-Whitney U test, the Wilcoxon test for paired samples, and the Spearman rank correlation coefficient, as indicated.

Results

In patients with clinically active juvenile dermatomyositis (disease activity score ≥ 1) serum neopterin concentrations (median 3·21 ng/ml; range 0·95–13·81) were significantly increased ($p<0·0001$) compared with healthy controls (median 0·75 ng/ml; range 0·33–1·18), whereas during remission patients (disease activity score 0) had serum neopterin concentrations (0·81 ng/ml; range 0·55–1·34) comparable with those of healthy controls. A decrease in serum neopterin concentrations was found in all six patients evaluated during active disease and a subsequent clinical remission ($p<0·03$ by Wilcoxon test for paired samples) (fig 1).

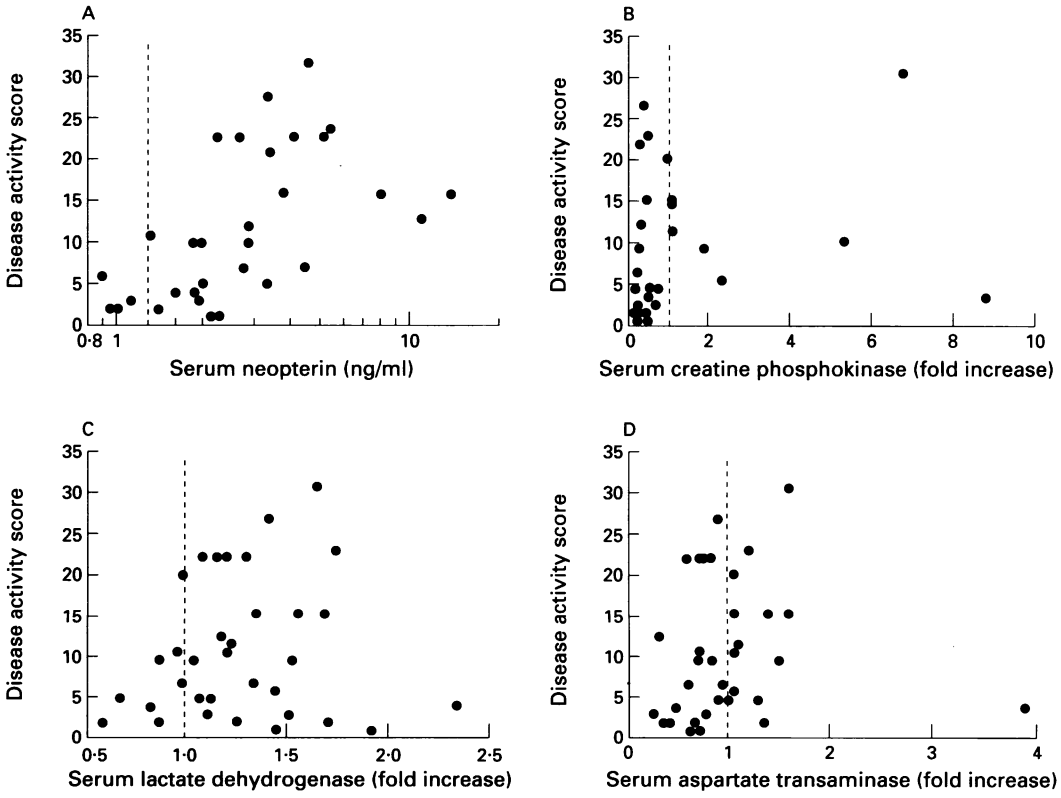

Figure 2 Correlation of disease activity score with (A) serum neopterin concentrations and (B) serum creatine phosphokinase, (C) lactate dehydrogenase, and (D) aspartate transaminase values in patients with active juvenile dermatomyositis (34 samples obtained from 12 patients during active disease). The vertical broken lines represent the upper range of control values for serum neopterin and muscle enzymes.

When all samples obtained from patients with active disease were considered the disease activity score was significantly correlated with serum neopterin concentrations (r_s=0·679; p<0·001), but not with serum values of creatine phosphokinase (r_s=0·192; p=0·29), lactate dehydrogenase (r_s=0·060; p=0·74), or aspartate transaminase (r_s=0·311; p=0·074) (fig 2), with erythrocyte sedimentation rate values (r_s=0·091; p=0·63), or with serum C reactive protein concentrations (r_s=0·075; p=0·69). When the functional score and the muscle strength score were considered independently, similar correlations were found (data not shown). Similar results were obtained in follow up studies of three patients (fig 3). In patient A serum neopterin concentrations paralleled the disease activity score (r_s=0·932; p<0·001), whereas serum creatine phosphokinase or aspartate transaminase values increased during the first few months of disease and were then normal for the rest of the clinical course; lactate dehydrogenase values were slightly increased during active disease but their correlation with the disease activity score was much less significant (r_s=0·705; p=0·017) than that of neopterin concentrations. Similar results were obtained in patient B, in whom the disease activity score was significantly correlated with serum neopterin concentrations (r_s=0,938; p=0·006) but not with serum values of creatine phosphokinase (r_s=0·705; p=0·444), lactate dehydrogenase (r_s=0·705; p=0·07), or aspartate transaminase (r_s=0·366; p=0·395). In patient C, who had two relapses separated by a 14 month period of quiescence, serum neopterin was normal during remission and increased again during relapse with a significant correlation with the disease activity score (r_s=0·839; p=0·014), whereas the correlation of serum creatine phosphokinase, lactate dehydrogenase, or aspartate transaminase values with the disease activity score was not significant (r_s=0·696; p=0·058; r_s=0·476; p=0·216; r_s=0·655; p=0·070 respectively).

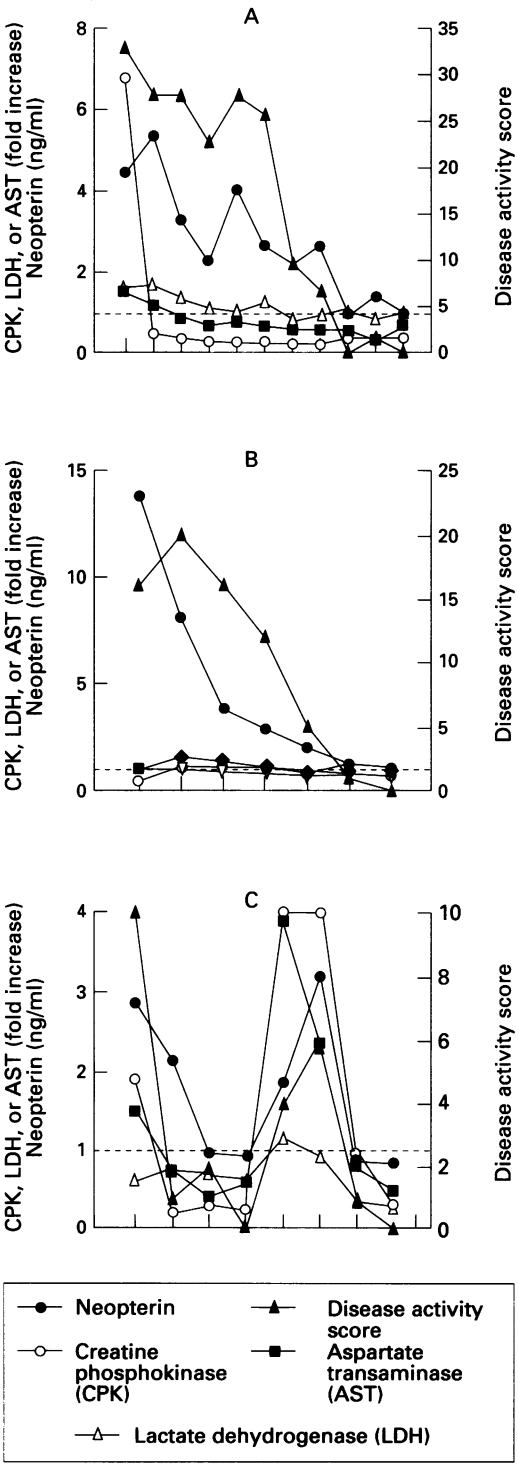

Figure 3 Serial measurements of serum neopterin concentrations, and serum creatine phosphokinase, lactate dehydrogenase, and aspartate transaminase values and of disease activity score in three patients (A, B, C) with juvenile dermatomyositis. The horizontal broken lines represent the normal serum values for the muscle enzymes.

Discussion

In this study we found increased serum neopterin concentrations in patients with juvenile dermatomyositis during active disease, but not during remission. As neopterin production reflects the activation of the cellular immune response,[1] the presence of increased serum neopterin in patients with active disease further supports the role of immune mediated mechanisms in the pathogenesis of juvenile dermatomyositis.

Our results are similar to those of Myones *et al*,[5] who reported that serum neopterin concentrations were increased in patients with active juvenile dermatomyositis and that, in sequential studies, serum neopterin decreased over time with a correlation with disease activity and muscle enzyme values. In their abstract, Myones *et al* did not report the method used to assess disease activity, the duration of the sequential studies, nor the correlation coefficients. In this study, we show that, in horizontal and longitudinal studies, serum neopterin concentrations are significantly correlated with the severity of muscle impairment, much more than muscle enzyme values. The absence of a correlation of muscle disease with serum creatine phosphokinase, lactate dehydrogenase, or aspartate transaminase appears to be due to the presence of normal muscle enzyme values in a significant number of patients with clinically active disease. This is not surprising as in our series most patients were already receiving steroids and muscle enzyme values are often

normal during treatment with steroids.[8] In contrast, in agreement with observations in graft rejections,[9] in patients with juvenile dermatomyositis serum neopterin concentrations do not appear to be rapidly affected by treatment with steroids or immunosuppressants, but decrease gradually when the disease is controlled.

Treatment with steroids induces remission in most patients with juvenile dermatomyositis, although the clinical response of a child with juvenile dermatomyositis to this treatment is not entirely predictable. Moreover, the duration of the disease is variable, with some patients presenting a monophasic course and others presenting recurrences of the disease, which may be secondary to a true polyphasic course or to a premature withdrawal or tapering of steroids.[2 10] The tapering of the dose of steroids is mainly based on the clinical evaluation of muscle strength impairment, because presently available laboratory parameters are generally poorly correlated with disease activity. In fact, although in the most severely affected patients massive fibre death and atrophy may cause muscle weakness to persist for weeks or months after apparent disease remission, in most patients muscle weakness is the best available parameter of disease activity. With respect to laboratory parameters, erythrocyte sedimentation rate or other acute phase reactants are increased in only 25% of patients.[8] Muscle enzyme values, usually increased at onset when they may reflect the degree of muscle inflammation, often decrease to within the normal range after treatment with steroids and are therefore lost as a guide to treatment.[8] Electromyography generally shows signs of inflammatory myopathy throughout the course of the disease, providing little information on disease severity.[8] Therefore the presence of a correlation between serum neopterin concentrations and the severity of muscle strength impairment suggests that the measurement of serum neopterin provides useful information about the grade of disease activity in juvenile dermatomyositis and that serum neopterin concentrations may be used, together with clinical examination, to guide the treatment of patients with juvenile dermatomyositis.

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