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Serum amino terminal type III procollagen peptide and serum hyaluronan in rheumatoid arthritis: relation to clinical and serological parameters of inflammation during 8 and 24 months' treatment with levamisole, penicillamine, or azathioprine

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SUMMARY Increased serum levels of the amino terminal type III procollagen peptide and serum hyaluronan were demonstrated in patients with rheumatoid arthritis. In patients with active disease a significant correlation was shown between serum levels of the propeptide and hyaluronan and the clinical signs of synovitis reflecting the extent of synovial inflammation. During recovery the serum propeptide and serum hyaluronan showed a delayed decline as compared with the clinical signs of synovitis and the acute phase protein response. This probably reflects the presence of persistent subclinical chronic inflammation. Normal serum propeptide levels in rheumatoid arthritis were associated with a good prognosis without progression of erosive joint lesions. Azathioprine reduced the number of patients with progression of erosive joint lesions and caused a more marked suppression of the serum propeptide than levamisole and penicillamine.

Key words: procollagen type III N-peptide, serum analyses, disease modifying antirheumatic drugs.

Measurement of the serum concentration of the procollagen metabolite, amino terminal type III procollagen peptide (PIIINP),[1 2] and of the polysaccharide, hyaluronan (hyaluronic acid, HA),[3 4] has made it possible to monitor changes in the metabolism of matrix constituents in acute and chronic fibrotic diseases.[5]

Assays for these metabolites have previously been carried out in patients with connective tissue diseases, including rheumatoid arthritis (RA)[1 6–8] and systemic sclerosis.[9 10]

Cross sectional studies have shown that serum PIIINP (S-PIIINP) and serum HA (S-HA) reflect disease activity in RA.[6 8] The inflamed synovium seems to be the main source of increased S-PIIINP.[6] A significant correlation has been shown between the acute phase proteins and S-PIIINP[6] and S-HA.[8] A preliminary prospective study showed that S-PIIINP may be a useful prognostic marker in RA.[6]

In the present study the relation between S-PIIINP, S-HA, and the established clinical, biochemical, and radiological variables reflecting synovitis activity was evaluated during a two year, double blind trial of levamisole, penicillamine, and azathioprine in patients with RA.[11–14] The purpose of the study was to compare the changes in the connective tissue metabolites with the clinical signs and the serological parameters of inflammation to find out whether S-PIIINP and S-HA had a predic-

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tive value for clinical remission and progression of joint erosions, and to investigate the influence of drug treatment on the serological connective tissue metabolites.

Patients and methods

PATIENTS AND CONTROLS

Patients with active classical or definite RA according to the American Rheumatism Association criteria[15] were included in a 24 month trial of levamisole, penicillamine, and azathioprine.[12] All patients had active synovitis fulfilling at least three of the following four criteria[16]: three or more swollen joints, six or more tender joints, morning stiffness of 45 minutes' duration or more, and erythrocyte sedimentation rate (ESR) of 30 mm/h or more. The exclusion criteria were as follows: functional class IV,[17] age of 16 years or less, pregnancy, concurrent diseases with poor life expectancy, clinical sign of abnormal thyroid function, impaired liver function (S-aspartate aminotransferase >40 U/l, S-alkaline phosphatase >275 U/l, and S-bilirubin >17 μmol/l) or kidney function (S-creatinine >120 μmol/l), patients who refused to participate after receiving information about the trial, and patients who had received levamisole, penicillamine, azathioprine, cyclophosphamide, chloroquine, gold salts, or steroids within the last three months. The study was designed as a two year, double blind trial. Non-responders to treatment were withdrawn from the study after eight months.

Sixty six patients, 45 women and 21 men, aged 31–77 years (median 62 years) with a disease duration of 5-530 months (median 60) were included in the study. Fifty eight patients had erosive disease, and 61 were seropositive (Rose-Waaler test). Sixteen patients had extra-articular manifestations. All had subcutaneous rheumatoid nodules, two had pleurisy, and one polyneuropathy. There was no evidence of disturbed pulmonary function in the remaining patients. Twenty patients allocated to treatment with levamisole were given 50 mg daily for one week, 100 mg daily on the following week, and 150 mg daily as a maintenance dosage. Twenty four patients allocated to penicillamine received 150 mg daily for three weeks. The dosage was raised by increments of 150 mg every three weeks until a dosage of 600 mg daily was reached. Twenty two patients allocated to azathioprine were given 2·5 mg per kilogram body weight daily. All patients were given 1600 mg ibuprofen daily. Other non-steroidal anti-inflammatory drugs were not allowed during the study.

Clinical evaluation of synovitis activity was performed by a 'blind' observer (the same rheumatologist throughout the study) at months 0, 4, 8, and 24.[11] The patients were questioned about the duration of morning stiffness. The total number of swollen and tender joints, corrected for joint size according to Lansbury,[18] was recorded, and the average grip strength of both hands was measured. The following serological variables were measured at months 0, 4, 8, and 24: ESR, C reactive protein (CRP), orosomucoid, haptoglobin, total IgG, IgM rheumatoid factors, IgG rheumatoid factors,[19] and Clq. Circulating immune complexes were detected by two methods: complement consumption test[20] and platelet aggregation test.[21]

The evaluations at month 4 were not performed in two of the patients who completed eight months of treatment. Serum analysis for IgG rheumatoid factors were missing in three patients at month 4 and eight patients at month 8.

Radiological examinations were performed of eight regions (shoulders, elbows, wrists, hands, hips, knees, ankles, and feet) at months 0, 8, and 24. The radiological changes in each region were graded as described previously.[22] The grading was based on the most severe destructive changes of each region. In the first 34 patients, who completed eight months of treatment, the radiograms of the hands at months 0 and 8 were read separately, the individual joints being evaluated in detail. The number of joint erosions were counted and the narrowing of the joint spaces was graded.[11] The radiograms from each patient were read at the same time by two radiologists who were unaware of the patients' data. Radiological examinations were omitted in two patients at month 0 and one patient at month 8 owing to incomplete radiograms.

The treatment was stopped owing to development of drug side effects within the first four months in six patients and during the next four months in another six patients. Between months 8 and 24 treatment was withdrawn owing to lack of treatment response, relapse of the disease, or side effects of drugs in 32 patients.

Control sera for determination of S-PIIINP were obtained from 56 healthy individuals, 30 women and 26 men, age 34-82 years (median 52). S-HA was measured in 160 healthy individuals, 91 men and 69 women, age 31–80 years (median 46).

SERUM SAMPLES

Blood samples were collected between 9·00 am and 10·00 am. The blood samples were allowed to clot at room temperature and then centrifuged at 1500 *g* for 10 minutes. Aliquots were immediately frozen and stored at −20°C for up to eight years.

ANALYTICAL PROCEDURE

PIIINP and its degradation products were determined by two radioimmunoassays: the RIA-gnost

procollagen-III-peptide assay system and the Fab-procollagen-III-peptide assay system (Hoechst A G, Frankfurt, West Germany). Both assays were developed by Rohde *et al.*[1 2] The analyses were performed as previously described.[6] The intra-assay and interassay variations determined with a control reference serum were for the RIA-gnost assay (median 6·5 ng/ml) 5% and 8% respectively, and for the Fab assay (median 53 ng/ml) 5% and 9% respectively. Samples to be compared were run simultaneously, and the values given are means of duplicate determinations. Repetitive thawing, and freezing for up to six years did not influence the S-PIIINP measurements. The higher propeptide levels recorded with the Fab assay are due to a 10–20 times higher affinity of the propeptide degradation product, Col 1, to the antibodies used in the Fab assay, as compared with the antibodies in the RIA-gnost technique.[2]

Recovery experiments were performed on serum using RIA-gnost and Fab PIIINP assays. The following components were added to 0·25 ml of serum from eight healthy individuals and from eight patients with active seropositive rheumatoid arthritis included in the prospective study: 0·25 ml of phosphate buffer pH 7·2, 0·25 ml purified human propeptide, Col 1–3, in phosphate buffer pH 7·2 (concentration in RIA-gnost assay 15·6 ng/ml, in Fab PIIINP assay 24 ng/ml), or 0·25 ml human Col 1 in phosphate buffer pH 7·2 (concentration in RIA-gnost assay 3·9 ng/ml, in Fab PIIINP assay 53 ng/ml). The aliquots were mixed, incubated at 4°C for 16 hours, and analysed.

Samples (1–2 ml) were chromatographed by application on a column (1·6×90 cm) of Sephacryl S-300 equilibrated in phosphate buffered saline pH 7·2 containing 0·05% Tween 20, and elution at a flow rate of 14 ml/h. Fractions of 1·9 ml were collected. Each fraction was analysed for the presence of PIIINP immunoreactive material in the RIA-gnost and Fab PIIINP assay. The column was calibrated with labelled intact bovine propeptide, intact human Col 1–3, and human Col 1. The elution volume of the column was determined with dinitrophenylalanine as indicator.

In the prospective study S-PIIINP analyses of one month 0 sample and three month 4 samples were not performed owing to lack of serum.

S-HA was determined by a radioassay for sodium hyaluronate using high affinity binding protein from bovine cartilage according to methods previously described.[3 4] The intra-assay variation was 12% at a serum concentration of 20 μg/l. All samples to be compared were run concurrently, and the values given are means of duplicate or triplicate determinations.

Owing to lack of samples, S-HA analyses were not performed on 11 samples at 0 months, four at four months, four at eight months, and four at 24 months.

STATISTICAL ANALYSIS

Data are expressed as range and median. The statistical analyses were performed using the Mann-Whitney test, the Kruskal-Wallis χ^2 test, and the Fisher exact probability test for unpaired data and the Wilcoxon test for paired data.[23] For these analyses p values $\leq 0{\cdot}05$ (two tailed test) were considered significant. Owing to the distribution of S-HA and IgM rheumatoid factors, these results were transformed logarithmically before analysis with the Wilcoxon test for paired data. The correlations were calculated using the Spearman correlation coefficient.[23] To avoid type I error due to multiple correlation analyses only correlations with $p \leq 0{\cdot}005$ (two tailed tests) were considered significant.

Results

In healthy control subjects S-PIIINP as measured by the RIA-gnost assay (S-RIA-PIIINP) was 3·2–11·8 ng/ml (median 6·4) and by the Fab assay (S-Fab-PIIINP) 34–85 ng/ml (median 52). The normal level of S-HA was 12–172 ng/ml (median 42).

In serum samples 88–116% (median 99) and 83–104% (median 92) of Col 1–3 added was recovered using the RIA-gnost assay and Fab-PIIINP assay respectively, whereas 77–103% (median 90) and 89–109% (median 98) of Col 1 added was recovered using the RIA-gnost assay and Fab-PIIINP assay respectively. No differences were observed in the recoveries of Col 1–3 or Col 1 between sera from healthy controls or from patients with rheumatoid arthritis. No relation was shown between the recovery percentages of Col 1–3 or Col 1 and the serum concentrations of total IgG, IgM rheumatoid factors, IgG rheumatoid factors, Clq, or circulating immune complexes in the patients with rheumatoid arthritis.

In the prospective study S-RIA-PIIINP, S-Fab-PIIINP, and S-HA levels at months 0, 4, and 8 were significantly higher than in the healthy controls ($p<0{\cdot}001$) (Table 1). An increase in S-Fab-PIIINP during the first four months was followed by a decrease in S-RIA-PIIINP and S-Fab-PIIINP between months 4 and 8 (Table 1). S-HA decreased significantly between months 0 and 8 (Table 1).

The relation between the pooled observations at months 0, 4, and 8 of S-RIA-PIIINP, S-Fab-PIIINP, and S-HA are shown in Table 2. A correlation between S-RIA-PIIINP and S-Fab-PIIINP was seen

at each observation, whereas the correlation seen at month 0 between S-PIIINP and S-HA disappeared after four and eight months of treatment (Table 3).

The clinical and paraclinical variables at months 0, 4, and 8 are shown in Table 1. These indicators of disease activity showed improvement within the first four months, followed by small changes in the same direction during the subsequent four months. No differences were recorded in the clinical signs of disease activity between the three treatment subgroups throughout the study.

Statistically significant correlations were demonstrated between the pooled observations at months 0, 4, and 8 of S-RIA-PIIINP, S-Fab-PIIINP, and S-HA, on the one hand and the tender and swollen joint indices, the grip strength, CRP, ESR, total IgG, and IgG rheumatoid factors, on the other hand (Table 2). No statistically significant correlations were observed between the pooled observations of S-RIA-PIIINP, S-Fab-PIIINP, and S-HA, on the one hand and morning stiffness, S-orosomucoid, S-haptoglobin, IgM rheumatoid factors, Clq, and circulating immune complexes, on the other hand.

Statistically significant correlations were observed at month 0 between the S-RIA-PIIINP, S-Fab-PIIINP, and S-HA and the tender and swollen joint indices, and CRP. These correlations disappeared after four and eight months of treatment (Table 3). No correlations were observed between the connective tissue related serum markers and morning stiffness, grip strength, ESR, S-orosomucoid, S-haptoglobin, total IgG, IgG rheumatoid factors, IgM rheumatoid factors, C1q, and circulating immune complexes at the individual observations at months 0, 4, and 8.

No statistically significant differences were observed in S-RIA-PIIINP, S-Fab-PIIINP, or S-HA between patients with and without extra-articular manifestations. The three patients with extra-articular manifestations other than cutaneous nodules, however, i.e., the two patients with pleurisy and the patient with polyneuropathy, had S-RIA-PIIINP values above 19·7 ng/ml throughout the eight month study.

Fifty four patients completed the first eight months of treatment. The patients were then

Table 1 *Clinical signs of synovitis, acute phase protein response, and serological connective tissue metabolites in rheumatoid arthritis during eight months of treatment with disease modifying antirheumatic drugs*

Parameter	*Month 0*				*Month 4*				*Month 8*			
	*n**	*Range*	*Median*	*p Value† (0 v 4 months)*	*n*	*Range*	*Median*	*p Value (4 v 8 months)*	*n*	*Range*	*Median*	*p Value (0 v 8 months)*
S-RIA-PIIINP‡ (ng/ml)	65	8·0–37·9	16·3	NS	56	8·4–31·3	16·9	0·01	54	8·2–32·3	15·6	NS
S-Fab-PIIINP‡ (ng/ml)	65	41–182	75	0·05	56	54–166	83	0·01	54	45–146	79	NS
S-hyaluronan‡ (ng/ml)	55	23–1045	250	NS	55	45–1025	210	NS	50	21–1325	196	0·05
Tender joints	66	17–198	105	0·001	57	0–173	76	0·001	54	0–173	57	0·001
Swollen joints	66	4–96	37	0·001	57	0–81	17	0·001	54	0–114	10	0·001
x Ray score	64	1–39	10	—	—	—	—	—	53	0–39	12	0·001
Grip strength (mmHg)	66	2·0–61·5	13·0	0·001	57	0·0–60·5	19·0	NS	54	0·5–83·0	19·5	0·001
Morning stiffness (min)	66	0–420	120	0·001	57	0–270	30	0·001	54	0–420	15	0·001
ESR (mm/h)	66	12–126	63	0·001	57	4–130	31	NS	54	3–114	31	0·001
CRP (mg/l)	66	0–1200	340	0·001	57	0–730	150	NS	54	0–750	100	0·001
Orosomucoid (μmol/l)	66	12–67	38	0·001	57	12–48	27	NS	54	14–55	26	0·001
Haptoglobin (μmol/l)	66	15–88	40	0·001	57	8–60	25	NS	54	5–60	24	0·001
IgG (g/l)	66	7·4–27·8	13·2	0·001	57	7·0–24·0	11·7	0·05	54	6·2–24·2	11·1	0·001
IgM RF	66	20–5120	320	NS	57	20–5120	320	NS	54	20–5120	160	NS
IgG RF	66	1·0–15·7	5·1	0·01	54	1·9–11·8	4·6	0·05	46	1·3–8·0	4·1	0·001
C1q	66	0·5–1·6	1·0	NS	57	0·5–1·7	1·0	NS	54	0·5–1·6	1·0	NS

*n=number of patients.
†Statistics: Wilcoxon's test for paired data (two tailed); NS=p>0·05.
‡Normal range (median): S-RIA-PIIINP 3·2–11·8 ng/ml (6·4 ng/ml), S-Fab-PIIINP 34–85 ng/ml (52 ng/ml), S-hyaluronan 12–172 ng/ml (42 ng/ml).

Table 2 *Correlation between the pooled observations at months 0, 4, and 8 of serological parameters of connective tissue metabolites and clinical signs of synovitis and acute phase protein response in rheumatoid arthritis during treatment with disease modifying antirheumatic drugs*

Parameter	*n*	*Months 0+4+8*		
		S-RIA-PIIINP (n=177)	*S-Fab-PIIINP (n=177)*	*S-hya-luronan (n=160)*
S-RIA-PIIINP	177	—	0·69 0·0001	0·31 0·0001
S-Fab-PIIINP	177	0·69* 0·0001†	—	0·25 0·002
S-hyaluronan	160	0·31 0·0001	0·25 0·002	—
Tender joints	177	0·37 0·0001	0·33 0·0001	0·29 0·0002
Swollen joints	177	0·24 0·002	0·21 0·005	0·33 0·0001
Grip strength	177	−0·26 0·0005	−0·12 NS	−0·13 NS
CRP	177	0·22 0·005	0·12 NS	0·34 0·0001
ESR	177	0·20 NS	0·10 NS	0·26 0·001
IgG (total)	177	0·28 0·0002	0·29 0·0001	0·20 NS
IgG RF	166	0·27 0·0005	0·13 NS	0·12 NS

*Spearman's ρ value (two tailed test).
†p Value; NS=p>0·005.

divided into responders and non-responders. Non-responders (n=30) still fulfilled the inclusion criteria for synovitis activity, whereas the responders (n=24) no longer fulfilled these criteria. One non-responder was excluded, because the initial serum sample was missing. No differences were found between responders and non-responders in the changes of the serological connective tissue variables within the first four months, whereas a significant reduction of S-RIA-PIIINP was seen in responders as compared with non-responders between months 0 and 8 (responders −15·5 to 8·3 ng/ml (median −2·1), non-responders −9·6 to 15·0 ng/ml (median 1·8); $p<0·02$), and of S-HA between months 4 and 8 (responders −560 to 379 ng/ml (median −106), non-responders −286 to 400 ng/ml (median −12); $p<0·05$).

To assess the prognostic value of S-PIIINP and S-HA we calculated the correlations between the initial S-RIA-PIIINP, S-Fab-PIIINP, or S-HA and the clinical synovitis scorings as well as the acute phase proteins at months 4 and 8, and the changes in these variables between months 0 and 8. None of the correlations were significant at a level of $p\leq0·005$.

A significant decrease in S-RIA-PIIINP was shown in the 19 patients treated with azathioprine during months 0–8 (−9·6 to 3·4 ng/ml (median −1·9)) as compared with the change in S-RIA-PIIINP in the 34 patients treated with levamisole or penicillamine during months 0–8 (−16·1 to

Table 3 *Correlation between serological parameters of connective tissue metabolites and clinical signs of synovitis and acute phase protein response in rheumatoid arthritis after 0, 4, and 8 months of treatment with disease modifying antirheumatic drugs*

Parameter	*Month 0*			*Month 4*			*Month 8*		
	S-RIA-PIIINP (n=65)	*S-Fab-PIIINP (n=65)*	*S-hya-luronan (n=55)*	*S-RIA-PIIINP (n=56)*	*S-Fab-PIIINP (n=56)*	*S-hya-luronan (n=55)*	*S-RIA-PIIINP (n=54)*	*S-Fab-PIIINP (n=54)*	*S-hya-luronan (n=50)*
S-RIA-PIIINP	— —	0·61* 0·0001†	0·45 0·0005	— —	0·82 0·0001	0·19 NS	— —	0·70 0·0001	0·24 NS
S-Fab-PIIINP	0·61 0·0001	— —	0·45 0·0005	0·82 0·0001	— —	0·13 NS	0·70 0·0001	— —	0·21 NS
S-hyaluronan	0·45 0·0005	0·45 0·0005	— —	0·19 NS	0·13 NS	— —	0·24 NS	0·21 NS	— —
Tender joints	0·53 0·0001	0·46 0·0001	0·46 0·0004	0·28 NS	0·31 NS	0·15 NS	0·25 NS	0·29 NS	0·24 NS
Swollen joints	0·38 0·002	0·31 NS	0·37 0·005	0·15 NS	0·24 NS	0·27 NS	0·13 NS	0·16 NS	0·36 NS
CRP	0·31 NS	0·43 0·0005	0·44 0·001	0·25 NS	0·10 NS	0·40 0·005	0·19 NS	−0·11 NS	0·11 NS
Orosomucoid	0·21 NS	0·19 NS	0·07 NS	0·18 NS	0·11 NS	0·36 0·005	0·13 NS	−0·03 NS	0·02 NS
IgG	0·27 NS	0·25 NS	0·28 NS	0·34 NS	0·38 0·005	0·25 NS	0·19 NS	0·26 NS	−0·01 NS

*Spearman's ρ value (two tailed test).
†p Value; NS=p>0·005.

15·0 ng/ml (median 0·2)) ($p<0·05$). No differences were shown between the levamisole and the penicillamine groups. No differences were seen in the changes in S-Fab-PIIINP and S-HA between the treatment groups.

The relation of S-RIA-PIIINP to treatment, treatment duration, and response is shown in Table 4. In patients treated with levamisole and penicillamine a significant decrease of S-RIA-PIIINP was seen in responders compared with non-responders, whereas no difference was seen between responders and non-responders treated with azathioprine. The responders showed no changes of S-RIA-PIIINP related to treatment, whereas non-responders to azathioprine treatment had a significant decrease in S-RIA-PIIINP for months 0–4 and 0–8 as compared with the non-responders treated with levamisole or penicillamine. Similar calculations were performed with S-Fab-PIIINP and S-HA, but no relation could be found between these variables and treatment, treatment duration, and response.

There were no significant differences in S-RIA-PIIINP, S-Fab-PIIINP, and S-HA at months 0, 4, 8, or 24 between patients with progressive erosive joint changes and those without progression. Within the first eight months, however, only one of 11 patients with an average S-RIA-PIIINP (month 0+4+8) within the normal range showed radiological evidence of progressive bone damage, whereas 17 of 39 patients with an average S-RIA-PIIINP above the normal range developed further bone destruction as shown by the general joint examinations. In the detailed evaluation of the hands none of seven patients with an average S-RIA-PIIINP within the normal range showed progressive bone erosions as compared with 12 of 27 patients with an average S-RIA-PIIINP above the normal range, who developed further bone destruction. The two radiological evaluations (the general joint examination and the detailed evaluation of the hands) showed that none of seven patients with an average S-RIA-PIIINP within the normal range developed new bone erosions as compared with 16 of 27 patients with an average S-RIA-PIIINP above the normal range, who developed further bone destruction ($p<0·02$) (Fig. 1). Between months 8 and 24 none of six patients with an average S-RIA-PIIINP (month 8+24) within the normal range developed further bone destructions as shown by examination of all the joints, whereas progression of bone damage was found in 10 of 16 patients with an average S-RIA-PIIINP above the normal range ($p<0·05$) (Fig. 1).

General joint examination and detailed evaluation of the hands showed that only two of 12 patients treated with azathioprine developed new joint erosions during the first eight months, as compared with 14 of 22 patients treated with levamisole or penicillamine ($p<0·05$).

Fifteen of 22 patients who completed 24 months' treatment fulfilled the remission criteria after eight and 24 months. A significant decrease in the clinical signs of synovitis and a normalisation in the acute

Table 4 *Changes in the serum procollagen type III amino terminal peptide (S-RIA-PIIINP* based on analysis in the RIA-gnost procollagen-III-peptide assay) level in relation to treatment (azathioprine, levamisole, and penicillamine, Kruskal-Wallis (two tailed) test) and treatment response (non-responders still fulfilled the inclusion criteria for synovitis activity after eight months of treatment, whereas responders no longer fulfilled these criteria; Mann-Whitney (two tailed) test for unpaired data)*

Treatment	*Δ4–0 months*			*Δ8–0 months*		
	Responders	*Non-responders*	*Mann-Whitney p value*	*Responders*	*Non-responders*	*Mann-Whitney p value*
Levamisole			0·05			0·05
No of patients	8	6		8	6	
Median	1·1	2·7		−1·6	2·2	
Range	−10·5–12·7	−4·8–5·9		−15·5–8·3	0·1–5·2	
Penicillamine			0·05			0·01
No of patients	12	6		13	7	
Median	−0·2	5·3		−2·0	3·2	
Range	−7·2–4·9	−2·2–11·6		−16·1–5·1	−4·9–15·0	
Azathioprine			NS†			NS
No of patients	9	10		9	10	
Median	−2·7	−1·3		−1·8	−1·9	
Range	−6·3–4·6	−2·8–2·8		−7·5–1·4	−9·7–3·4	
Kruskal-Wallis p value	NS	0·05		NS	0·02	

*Values of S-RIA-PIIINP in ng/ml.
†NS=$p>0·05$.

phase proteins were seen during the first eight months. A small, but significant additional decrease was shown in the swollen and tender joint indices between months 8 and 24, whereas no changes were observed in the acute phase proteins (Table 5). No statistically significant changes were demonstrated in the serological connective tissue parameters within the first eight months, whereas all three markers showed decreasing levels after two years of treatment (Table 5). Only the changes in S-RIA-PIIINP and S-HA were statistically significant, however. No statistically significant correlations were seen between S-RIA-PIIINP, S-Fab-PIIINP, and S-HA and the clinical signs of synovitis or the acute phase proteins after 24 months of treatment.

Serum samples from a woman, age 60, with erosive seropositive classical RA of 13 years' duration were subjected to molecular exclusion chromatography on a Sephacryl S-300 column before and after eight and 24 months of azathioprine treatment. The acute phase reactants normalised within the first four months, whereas the clinical variables and the serological connective tissue metabolites improved throughout the two years of observation (S-RIA-PIIINP, month 0: 23·4 ng/ml,

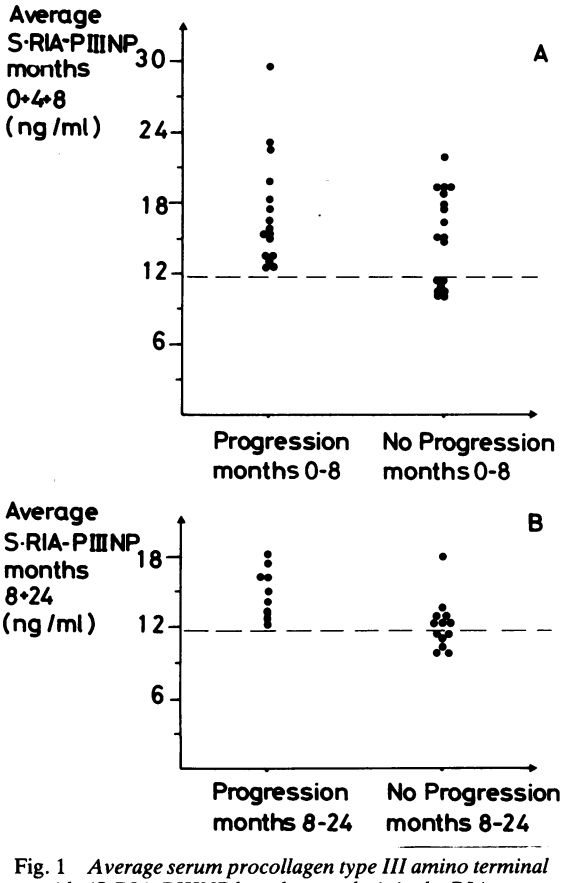

Fig. 1 *Average serum procollagen type III amino terminal peptide (S-RIA-PIIINP based on analysis in the RIA-gnost procollagen-III-peptide assay) level in relation to development of new joint erosions. A=average S-RIA-PIIINP months 0+4+8 in relation to progressive bone damage months 0–8 evaluated by general joint examination and detailed hand evaluation; B=average S-RIA-PIIINP months 8+24 in relation to progressive bone damage months 8–24 evaluated by general joint examination. Stippled lines indicate upper normal range.*

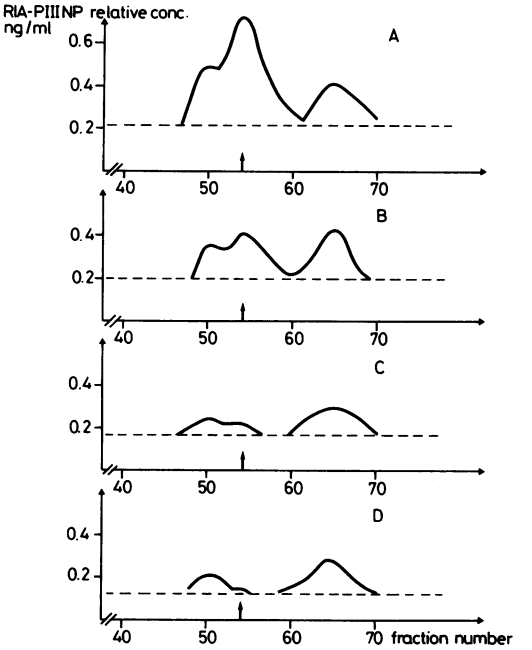

Fig. 2 *Chromatography on a Sephacryl S-300 column (1·6×90 cm, elution flow rate 14 ml/h, collected fractions 1·9 ml) of serum samples from a 60 year old woman with active rheumatoid arthritis treated with azathioprine for 24 months. A=0 month (S-RIA-PIIINP 23·4 ng/ml, S-Fab-PIIINP 78 ng/ml), B=8 months (S-RIA-PIIINP 15·9 ng/ml, S-Fab-PIIINP 62 ng/ml), C=24 months (S-RIA-PIIINP 9·8 ng/ml, S-Fab-PIIINP 48 ng/ml), and D=a 44 year old apparently healthy woman (S-RIA-PIIINP 4·9 ng/ml, S-Fab-PIIINP 40 ng/ml). The position of the radioiodinated standard procollagen type III amino terminal peptide (PIIINP) is indicated by an arrow. The relative concentration of the inhibitor in the different fractions was calculated using an RIA-gnost assay with the standard PIIINP as reference. The elution of human propeptide, Col 1–3, degraded human propeptide, Col 1, and dinitrophenylalanine corresponded to fractions 53, 65, and 122 respectively. Stippled lines indicate the lower detection limit in the RIA-gnost procollagen-III-peptide assay.*

Table 5 *Clinical signs of synovitis, acute phase protein response, and serological connective tissue metabolites in patients with rheumatoid arthritis responding to two years of treatment with disease modifying antirheumatic drugs*

Parameter	*Month 0*				*Month 8*				*Month 24*			
	*n**	*Range*	*Median*	*p Value† (0 v 8 months)*	*n*	*Range*	*Median*	*p Value (8 v 24 months)*	*n*	*Range*	*Median*	*p Value (0 v 24 months)*
S-RIA-PIIINP‡ (ng/ml)	15	9·0–34·4	15·1	NS	15	9·9–20·3	14·1	0·05	15	9·8–16·3	11·6	0·02
S-Fab-PIIINP‡ (ng/ml)	15	44–182	73	NS	15	45–146	76	NS	15	45–100	58	NS
S-hyaluronan‡ (ng/ml)	14	23–800	285	NS	12	88–540	168	NS	11	50–520	124	0·05
Tender joints	15	41–160	85	0·01	15	2–151	27	0·01	15	0–126	1	0·01
Swollen joints	15	8–96	31	0·01	15	0–27	1	0·05	15	0–13	0	0·01
Grip strength (mmHg)	15	6·5–61·5	14·5	0·05	15	5·0–83·0	33·0	NS	15	0·0–85·0	36·5	0·05
Morning stiffness (min)	15	15–390	90	0·01	15	0–60	0	NS	15	0–45	0	0·01
ESR (mm/h)	15	12–103	44	0·01	15	3–38	19	NS	15	4–32	16	0·01
CRP (mg/l)	15	0–730	280	0·01	15	0–360	0	NS	15	0–260	10	0·01
Orosomucoid (μmol/l)	15	16–51	36	0·05	15	14–45	20	NS	15	15–26	20	0·05
Haptoglobin (μmol/l)	15	17–88	37	0·01	15	5–30	15	NS	15	3–63	17	0·01

*n=number of patients.
†Statistics: Wilcoxon's test for paired data (two tailed); NS=p>0·05.
‡Normal range (median): S-RIA-PIIINP 3·2–11·8 ng/ml (6·4 ng/ml), S-Fab-PIIINP 34–85 ng/ml (52 ng/ml), S-hyaluronan 12–172 ng/ml (42 ng/ml).

month 8: 15·9 ng/ml, month 24: 9·8 ng/ml; S-Fab-PIIINP, month 0: 78 ng/ml, month 8: 62 ng/ml, month 24: 48 ng/ml). The chromatograms showed three peaks containing PIIINP immunoreactive material (Fig. 2). A fraction corresponding to the intact propeptide, Col 1–3, dominated before treatment. As the acute inflammation subsided the Col 1–3 fraction, and to some extent the high molecular weight fraction, was reduced. The three fractions had almost equal activity after eight months. After 24 months the antigen size distribution was similar to that seen in normal serum showing the lowest activity in the Col 1–3 fraction and the highest activity in the Col 1 fraction. No alterations were seen in the Col 1 concentration throughout the observation period. For comparison a chromatogram of a serum sample from a healthy woman, age 44 (S-RIA-PIIINP 4·9 ng/ml, S-Fab-PIIINP 40 ng/ml), is shown. No additional peaks containing PIIINP immunoreactive material were detected by the Fab-PIIINP assay in any of the sera.

Discussion

It has been suggested that measurement of S-PIIINP may be a useful monitor of disease activity in various fibrotic diseases.[5 24] The increase in S-PIIINP reflects the collagen type III synthesis and fibrillogenesis in developing granulation tissue.[25] The inflamed synovium in RA is characterised by such a stimulated synthesis and accumulation of collagen type III.[26–28] Collagen type III is particularly abundant around the inflammatory cell infiltrate.[29] As the inflammatory changes progress to chronic fibrosis the cellular infiltration slowly disappears leaving a matrix composed predominantly of collagen types I and III.[29] In accordance with these immunohistological findings, we have shown that patients with rheumatoid arthritis have higher S-PIIINP levels than patients with inactive disease, who still present higher levels than normals.[6]

The correlation between S-PIIINP and the clinical signs of synovitis and the acute phase protein response (Tables 2 and 3) supports the assumption that the inflamed synovium is the predominant contributor to the increased S-PIIINP values in active RA. Accordingly, up to 1000 times higher PIIINP values may be demonstrated in synovial fluid than in serum.[30] Significant differences were seen, however, between the serological connective

tissue metabolites and the other clinical and serological variables during treatment with disease modifying antirheumatic drugs. The correlations between the collagen metabolites and the clinical signs of synovitis recorded in the active phase of the disease probably reflect the enhanced collagen type III formation elicited by the acute inflammation (Table 3). The delay in normalisation of the collagen metabolites (Table 1) may reflect continuing chronic inflammation similar to that previously reported in follow up studies of patients with acute viral hepatitis.[31]

In the present study S-PIIINP correlated with S-HA only in the initial active phase of synovitis (Table 3). S-HA decreased throughout the first eight months, whereas S-PIIINP increased slightly at month 4 followed by a significant decline during the next four months. These changes in circulating matrix elements accord with the temporal pattern of extracellular matrix formation in developing granulation tissue, in which hyaluronic acid dominates in the early stages followed by type III collagen proliferation.[26 32–34]

The S-PIIINP antigen profile in active RA and during recovery (Fig. 2) was very similar to that seen during recovery from an episode of alcoholic hepatitis,[35] implying that this peptide pattern represents changes which are common to procollagen/propeptide metabolism during non-specific acute and chronic inflammation: the Col 1–3 fraction dominates in the acute inflammatory phase. The Col 1–3 and the high molecular fraction are prevalent during the chonic inflammatory phase, whereas the Col 1 fraction dominates after complete remission and also in healthy individuals.

The initial S-PIIINP and S-HA were not useful as predictors of the outcome of the clinical or serological variables of inflammation. This finding supports the assumption that the serological connective tissue metabolites reflect alterations in the connective tissue metabolism caused by the inflammatory process, and not factors initiating the inflammation.

In a previous prospective pilot study we showed that normal S-RIA-PIIINP in patients with active RA indicated a good prognosis without progression of erosive joint lesions.[6] The present more extensive study further substantiated this observation. In patients with normal averag̈ S-RIA-PIIINP during the follow up new joint erosions were only recorded in one patient within the following two years. On the other hand, 44% (59% when both radiological evaluation methods were used) of the patients who had raised average S-RIA-PIIINP values within the first eight months, and 63% of patients with raised average S-RIA-PIIINP between months 8 and 24 developed new erosions. Apparently this finding reflects a linkage between collagen fibrogenesis and matrix destructive mechanisms in the rheumatoid joints. We were not able to distinguish between patients with and without progressive bone damage using the Fab-PIIINP or the HA assays. The explanations may be that S-Fab-PIIINP detects mainly propeptide degradation products and therefore is more susceptible to alterations in the metabolism of circulating propeptides. S-HA in contrast with S-PIIINP shows variation in relation to physical activities (unpublished data). This may influence the S-HA values even though the sampling time was standardised.

Theoretically, the presence of aggregated IgG and rheumatoid factors in serum could interfere directly with the assays, and C1q, which has a collagen-like fragment, could cross react with PIIINP. We only recorded a correlation between S-RIA-PIIINP and total IgG and IgG rheumatoid factors at month 0 (Tables 2 and 3). As the recovery of added Col 1–3 or Col 1 was not influenced by the presence of IgG, including IgG rheumatoid factors, this correlation probably indicates that S-RIA-PIIINP, total IgG, and IgG rheumatoid factors are all related to the inflamed synovial mass. The recovery experiments also speak against an influence of circulating anticollagen antibodies on the S-PIIINP levels in RA.[36] These antibodies are directed mainly against covalent structural determinants present on denaturated collagens, and, in contrast with S-PIIINP, they show no correlation with disease activity in RA.[36]

The present study has confirmed the previously observed suppressive effect of azathioprine on the S-RIA-PIIINP level in active RA.[6] Apparently, this suppression does not represent a non-specific effect on protein synthesis because no differences were observed in serum albumin between the three treatment groups.[11 14] The decreased S-PIIINP level in azathioprine non-responders may reflect an azathioprine induced specific suppression of the collagen type III synthesis elicited by acute inflammation. Immunosuppressive treatments leading to declining S-PIIINP values in treatment responders have also been shown in cryptogenic fibrosing alveolitis[37] and chronic active hepatitis,[38 39] but appear not to influence the S-PIIINP level in primary biliary cirrhosis.[40 41]

The importance of suppressing the S-PIIINP level in chronic fibrosing conditions remains to be elucidated. The propeptide, however, seems to be closely related to the pathogenetic processes in the inflamed synovium: RA patients with normal S-RIA-PIIINP have a favourable prognosis as regards progression of joint erosions and there was a minimal progression of joint damage during azathioprine treatment.

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