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Serum aminoterminal type III procollagen peptide in inflammatory and degenerative rheumatic disorders

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SUMMARY *Measurement of the aminoterminal type III procollagen peptide in serum has been suggested as a marker of the biosynthesis of collagen type III, a major connective tissue component in repair processes. In the present study the propeptide level correlated with the inflammatory synovial mass in rheumatoid arthritis and osteoarthritis. This implies that the propeptide level reflects the collagen type III synthesis occurring in the synovial repair processes, whether they were caused by inflammatory or degenerative rheumatic disorders. Physical activity did not enhance the transition of the propeptide from the synovial fluid or the inflamed synovial membrane to the blood. Normal serum propeptide values were observed in most patients with ankylosing spondylitis and degenerative diseases of the spine. This may reflect the lower amount of inflammatory tissue in these diseases and hence the sensitivity of the assays.*

Key words: Serum Procollagen Type III N-Peptide. Inflammatory and Degenerative Rheumatic Disorders. Physical Activity.

INTRODUCTION

Repair processes with enhanced collagen synthesis in the synovial membrane are characteristic of inflammatory rheumatic disorders, including rheumatoid arthritis (1), and may also occur in osteoarthritis (2).

The aminoterminal type III procollagen peptide (PIIINP) in serum has been suggested as a marker of the biosynthesis of collagen type III (3,4), one of the major connective tissue components in repair processes (1,5,6). The propeptide is cleaved off in a stoichiometric manner during the conversion of procollagen to collagen (7). The exact metabolic pathway of the propeptide is not known. The intact propeptide, Col 1-3 (45 kD) can be detected in various body fluids (3,4), whereas a smaller fragment, Col 1 (10 kD), presumed to be a degradation product of the intact propeptide, has been demonstrated in serum and urine (3,4). The use of two radioimmunoassays with different affinities to Col 1-3 and to Col 1 allow an estimation of the relative content of intact and degraded propeptide (3,4).

These assays have previously been applied

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to patients with connective tissue diseases, viz. rheumatoid arthritis (RA) (8,9) and systemic sclerosis (10). It was demonstrated that serum PIIINP (S-PIIINP) reflects the disease activity in active RA. In addition, the results indicated that the inflamed synovium is the main source of the increased S-PIIINP values in patients with active disease (8-9).

The purpose of the present study was to evaluate whether S-PIIINP is affected by physical activity and to study the relationship between S-PIIINP and the synovial mass as estimated by Lansbury's swollen and tender joint indices corrigated for joint size (11) in patients with inflammatory and degenerative joint diseases.

MATERIAL AND METHODS

Patients and controls.

All patients and controls had normal liver and kidney function. They were all clinically euthyroid, and none had clinical evidence of neoplasia. The study consisted of two parts. 1) *Influence of physical activities on S-PIIINP level in healthy control subjects and rheumatoid arthritis.*

In one group blood samples were collected at 7 a.m., while the patients were still in bed, and at 12 a.m. after the patients daily physical activities, but before lunch. Fourteen patients with erosive RA, all fulfilling the American Rheumatism Association criteria for classical or definite RA (12), 7 women and 7 men, age 33-83 years (median 67) were included in the study. All patients had active synovitis fulfilling at least three of the following four criteria: three or more swollen joints, six or more tender joints, morning stiffness of 45 minutes' duration or more, and an erythrocyte sedimentation rate (ESR) of 30 mm/hour or more. Treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) was withdrawn 24 hours prior to the study. Eleven patients received penicillamine. The patients had an ESR of 12-58 mm/hour (median 41), a serum haptoglobin of 1.4-6.0 gm/liter (median 3.6), a morning stiffness of 0-360 minutes (median 120), a swollen joint index corrigated for joint size a.m. Lansbury (11) of 22-96 (median 46) and a tender joint index a.m. Lansbury of 36-144 (median 89). Thirteen healthy individuals, 9 women and 4 men, age 31-86 years (median 61) served as controls.

In another group of patients and controls blood samples were collected before, immediately after, and 60 minutes after termination of a physical exercise program performed in the afternoon. Thirteen patients with classical or definite RA (9 women and 4 men, age 33-66 years [median 55]) were included. All but two patients fulfilled the criteria for active synovitis. They had an ESR of 9-102 mm/hour (median 39), a serum haptoglobin of 1.6-5.8 gm/liter (median 3.2), a morning stiffness of 0-120 minutes (median 45), a swollen joint index of 0-108 (median 28), and a tender joint index of 0-186 (median 60). They were all treated with NSAIDs and 8 patients received penicillamine. They performed a 60-minute physical exercise program supervised by a physiotherapist. The program included active movements of all muscle groups, and was terminated by active movements of hip and knees, which were performed as long as the patients could endure. Thirteen healthy individuals served as controls. Six women and 7 men, age 20-48 years (median 33) exercised on a test cycle by cycling at least 12 minutes on increasing loads of 50 W per 4 minutes.

2) *Cross-sectional study of patients with ankylosing spondylitis (AS), degenerative diseases localized to the spine, rheumatoid arthritis, and osteoarthritis (OA):*

Thirty patients had AS: four women and 26 men, age 26-69 years (median 46) with 1-47 years of disease (median 23). All fulfilled the New York criteria for AS (13) and all but one were HLA-B 27 positive. Twenty-seven patients had axial arthritis without peripheral joint involvement, and 3 had synovitis of a peripheral joint. The ESR was

1-67 mm/hour (median 23) and the serum haptoglobin 0.4-5.4 gm/liter (median 2.9). All but 3 patients received NSAIDs.

Twelve patients had degenerative diseases localized in the spine. Eight had protrusion of a lumbar intervertebral disc verified by myelography, and 4 had radiologically verified osteoarthropathic changes in the lumbar region: seven women and 5 men, age 19-66 years (median 38). The ESR was 1-23 mm/hour (median 11) and the serum haptoglobin 0.6-2.4 gm/liter (median 1.5). All but 3 patients received NSAIDs.

Sixty patients had active definite or classical RA: forty-two women and 18 men, age 31-77 years (median 62) with a disease duration of 0.5-45 years (median 5). ESR was 12-126 mm/hour (median 60), serum haptoglobin 1.1-6.7 gm/liter (median 3.1) a swollen joint index 4-96 (median 37), and tender joint index 17-198 (median 105). No patients were on disease modifying antirheumatic drugs, but all patients received NSAIDs.

Eighteen patients had radiologically verified primary OA: fifteen women and 3 men, age 51-83 years (median 70) with 3-30 years of disease (median 12). ESR was 4-44 mm/hour (median 14), serum haptoglobin 0.6-3.1 gm/liter (median 1.7), swollen joint index 0-112 (median 36), and tender joint index 16-112 (median 60). All patients received NSAIDs.

Control sera were obtained from 62 healthy blood donors: 37 women and 25 men, age 18-62 years (median 32), who attended the Regional Blood Transfusion Services at Hvidovre Hospital, Denmark.

Serum samples.

The blood samples were allowed to clot for 1 hour at room temperature, and then centrifuged at 1500 g for 10 minutes. Aliquots were immediately frozen and stored at −20°C for up to 1 year.

Analytical procedure:

S-PIIINP radioimmunoassays:

S-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 AG, Frankfurt, West Germany). Both assays were developed by Rohde et al (3,4). The analyses were performed as previously described (8). The intra- and interassay variations as determined using a control reference serum, were in the RIA-gnost assay (mean 6.5 ng/ml) 5% and 8%, respectively, and in the Fab assay (mean 53 ng/ml) 5% and 9%, respectively. All samples to be compared were analysed simultaneously and the values given are means of duplicate determinations. Repetitive thawing, and freezing for up to 6 years did not influence the S-PIIINP concentrations. The higher propeptide levels measured by the Fab assay are due to a 10-20 times higher affinity of the propeptide degradation products to the antibodies in the Fab assay, as compared with the antibodies used in the RIA-gnost technique.

Recovery experiments:

Recovery experiments were performed using RIA-gnost and Fab PIIINP assays on serum. To 0.2 ml of normal serum (PIIINP concentration prior to dilution: RIA-gnost assay 6.8 ng/ml, Fab-assay: 74 ng/ml) 0.2 ml of purified human Col 1-3 or human Col 1 was added in concentrations (as measured by the RIA-gnost assay and the Fab PIIINP assay, respectively) of 0, 15.6, 31.3, 62.5, 125, 250, 500, 1000 ng/ml. The aliquots were mixed, incubated at 4°C for 16 hours, and analysed.

Statistical analysis.

The statistical analyses were performed using the Mann Whitney test for unpaired data and the Wilcoxon test for paired data (14). The correlations were calculated using

the Spearman correlation coefficient (14), with the exception of the recovery experiments. In these experiments the calculations were performed by linear regression analysis (15). Data are expressed as range and median, p values ≤ 0.05 were considered significant.

RESULTS

Recovery experiments

There was a highly significant correlation between the amounts of Col 1-3 or Col I added to serum and those recovered using either assays. RIA-gnost assay: Col $1\text{-}3_r$ (recovered) = $0.92 \times$ Col $1\text{-}3_a$ (added) + 0.3, R = 1.000, Col 1_r = $0.84 \times$ Col 1_a + 2.1, R = 0.998. Fab PIIINP assay: Col $1\text{-}3_r$ = $0.87 \times$ Col $1\text{-}3_a$ - 4, R = 1.000, Col 1_r = $1.00 \times$ Col 1_a - 1, R = 1.000.

Controls.

In healthy individuals, S-PIIINP measured by the RIA-gnost assay (S-RIA-PIIINP) was 3.2-11.8 ng/ml (median 6.5) and by the Fab-assay (S-Fab-PIIINP) it was 34-85 ng/ml (median 49).

Influence of physical activities on S-PIIINP level in healthy control subjects and rheumatoid arthritis

The S-RIA-PIIINP and S-Fab-PIIINP levels decreased between 7 a.m. and 12 a.m. in healthy individuals as well as in patients with active RA. However, only the decline in S-Fab-PIIINP was statistically significant (Table I). No significant change could be demonstrated in the S-PIIINP levels during or after muscle exercise (Table II). The changes were not related to the duration of hip and knee movements in RA patients or in controls. The individual changes in S-PIIINP were in both studies relatively small, and no patients or controls showed more than 18% change from their initial values. The total changes in S-PIIINP seen in RA did not differ from those seen in healthy individuals (Table I and II). The changes in S-PIIINP during physical activities did not correlate with the clinical or serological parameters of disease activity and no differences were observed between RA patients treated with and without penicillamine.

Cross-sectional study of patients with ankylosing spondylitis, degenerative diseases localized to the spine, rheumatoid arthritis, and osteoarthritis

The individual S-RIA-PIIINP and S-Fab-PIIINP values in patients with AS, degenerative diseases localized to the spine, RA, and OA are shown in Figure 1. The median values in each group were in S-RIA-PIIINP: AS, 8.6 ng/ml, degenerative spine diseases, 6.4 ng/ml, RA, 16.5 ng/ml, and OA, 11.2 ng/ml, and in S-Fab-PIIINP: AS, 58 ng/ml, degenerative spine diseases, 46 ng/ml, RA 73 ng/ml and OA, 82 ng/ml.

The S-RIA-PIIINP and S-Fab-PIIINP levels were significantly higher in AS, RA, and OA as compared with healthy individuals, whereas patients with degenerative diseases localized to the spine revealed normal S-PIIINP levels.

There were no differences in the S-PIIINP values between AS patients with and without peripheral synovitis (Fig. 1). However, only three patients had peripheral synovitis, and none of them had involvement of more than one joint. We could not demonstrate differences between patients having protrusion of a lumbar intervertebral disc and patients with osteoarthropathic changes in the lumbar region.

In patients with active RA, S-RIA-PIIINP correlated with the tender joint index ($r = 0.53$, $p < 0.0001$) and the swollen joint index ($r = 0.32$, $p < 0.01$), and S-Fab-PIIINP correlated with the tender joint index ($r = 0.46$, $p < 0.0005$) and the swollen joint index ($r = 0.33$, $p < 0.01$).

Similar strong correlations were seen in patients with OA. S-RIA-PIIINP correlated with the tender joint index ($r = 0.81$, $p < 0.001$) and swollen joint index ($r = 0.85$,

Table I:

	Patients	Methods (ng/ml)	7 a.m. values median	(range)	Change from 7 a.m. to 12 a.m. median	(range)	Wilcoxon p value
Controls	13	S-RIA-PIIINP	8.5	(6.5-11.7)	−0.5	(−2.2-0.7)	NS
		S-Fab-PIIINP	48	(34-65)	−2	(−9-4)	0.05
Rheumatoid arthritis	14	S-RIA-PIIINP	13.1	(8.0-22.8)	−0.5	(-3.7-1.8)	NS
		S-Fab-PIIINP	61	(48-83)	−4	(-9-4)	0.05
Rheumatoid arthritis vs. controls (Mann Whitney) (p value)		S-RIA-PIIINP		0.01		NS	
		S-Fab-PIIINP		0.01		NS	

Table II

	Patients	Methods (ng/ml)	Before exercise median	(range)	Change during exercise median	(range)	p value	Change 1 hour after exercise median	(range)	Wilcoxon p value
Controls	13	S-RIA-PIIINP	7.0	(4.6-11.6)	0.4	(-0.4-2.9)	NS	-0.1	(-2.7-0.5)	NS
		S-Fab-PIIINP	48	(27-63)	2	(-3-10)	NS	0	(-6-7)	NS
Rheumatoid arthritis	13	S-RIA-PIIINP	10.5	(6.2-20.4)	-0.6	(-3.8-2.4)	NS	-1.3	(-4.2-1.3)	NS
		S-Fab-PIIINP	57	(39-66)	0	(-4-9)	NS	-1	(-6-5)	NS
Rheumatoid arthritis vs. controls (Mann Whitney) (p value)		S-RIA-PIIINP		0.01		NS			NS	
		S-Fab-PIIINP		0.05		NS			NS	

$p<0.001$), and S-Fab-PIIINP correlated with the tender joint index ($r=0.74$, $p<0.01$) and swollen joint index ($r=0.67$, $p<0.01$). Patients who had OA with involvement of the knees with clinical signs of synovitis and/or joint effusions had significantly higher S-PIIINP values than patients without knee joint complaints ($p<0.01$) (Fig. 1).

In active RA a weak, but statistically significant correlation was seen between S-RIA-PIIINP and ESR ($r=0.31$, $p<0.02$) and between S-Fab-PIIINP and ESR ($r=0.27$, $p<0.05$). No correlations were demonstrated between the S-PIIINP levels and ESR or serum haptoglobin in the other three diseases.

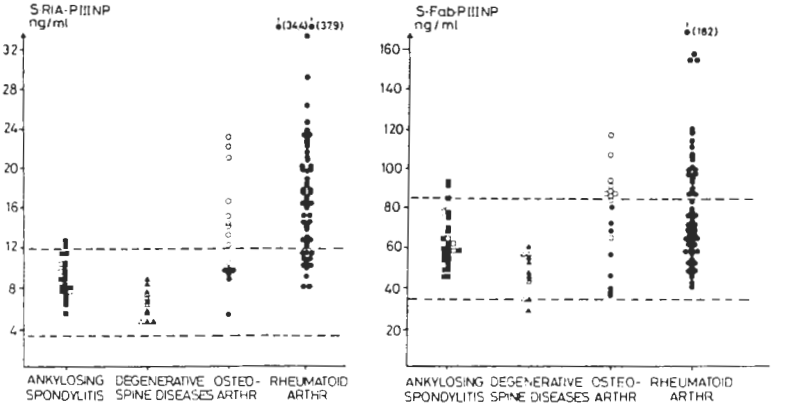

Fig. 1: Serum procollagen type III aminoterminal peptide measured using two radioimmunoassays (S-RIA-PIIINP and S-Fab-PIIINP) in patients with ankylosing spondylitis (with (○) and without (■) peripheral synovitis), patients with degenerative diseases localized to the spine (protrusion of a lumbar intervertebral disc (▲) or osteoarthropatic changes in the lumbar region (△), patients with active rheumatoid arthritis and patients with osteoarthrosis (patients with (o) and without (•) active involvement of the knees). Stippled lines indicate normal range.

DISCUSSION

Recently, S-PIIINP has been suggested as a useful serological parameter in monitoring the activity in fibrotic diseases (5,8-10,16). At least three immunoactive PIIINP related fragments have been demonstrated in serum. The PIIINP antigen profile varies in the different stages of acute and chronic inflammation (9,17). The PIIINP variants have different affinities to the antibodies used in the two PIIINP assays (3,4). By application of both assays the relative concentration of intact and degraded propeptide has been estimated (8). The recovery percent of human Col I and Col I-3 in serum was 84-100%. Thus there is no evidence of significant nonspecific interference by serum.

The concentration of PIIINP in synovial fluid is up to 1000 times higher than that found in serum (18). Therefore physical activity, including active joint movement and muscle activity might lead to increased S-PIIINP values, via an enhanced lymphatic drainage from the joints, or via filtration from the synovial fluid during joint movement (19-20). Thus, it has been proposed that hyaluronan, another extracellular connective tissue constituent, which appears in especially high concentrations in the synovial membrane and the synovial fluid, is accumulated there at rest and carried by the lymph to the general circulation during physical activity (21).

We could not demonstrate any statistically significant influence of muscle exercise or active joint movements on the S-PIIINP levels in patients with RA or in healthy controls. The minor changes in S-PIIINP seen during and after exercise were not related to the duration of the active hip and knee movements, and the changes during physical activities were not related to treatment with penicillamine or NSAIDs in the RA patients.

Thus during physical activities PIIINP appears not to be released in excess from the synovial fluid, or from accumulated PIIINP in the inflamed synovial tissue. The finding supports the hypothesis that the increasing propeptide levels found in the blood in RA predominantly reflect PIIINP released from the synovial tissue immediately after synthesis of collagen type III (8).

The decline in the S-Fab-PIIINP level between 7 a.m. and 12 a.m. was of the same degree in healthy individuals and in patients with active RA, and was not related to disease activity in RA, i.e. the inflammation of the synovial membrane. Only S-Fab-PIIINP declined significantly from 7 a.m. to 12 a.m., indicating a decrease primarily in serum Col 1. The decrease in Col 1 may indicate an enhanced elimination and degradation of propeptide metabolites in the daytime. Thus, in conclusion the changes in S-PIIINP during the morning were modest, and require no standardization of blood sampling in clinical studies.

The correlations between S-PIIINP and the synovial mass as estimated by the joint indices in patients with peripheral joint involvement support the previous observations that S-PIIINP reflects the non-specific inflammation of the diseased synovial tissue (8,9). Accordingly S-PIIINP was elevated in joint inflammation in RA as well as OA, in spite of pathogenetic differences. These observations are in agreement with the similarities between RA and degenerative joint diseases concerning the immunohistochemical findings in synovial tissue (1,2) and the PIIINP levels in synovial fluids (18).

No patients with degenerative diseases localized to the spine or the small peripheral joints, and only two patients with ankylosing spondylitis showed SPIIINP values above the normal range. However, none of the patients with degenerative diseases of the spine showed evidence of joint inflammation and only 3 out of 30 patients with AS had signs of peripheral joint involvement with active synovitis in only one joint. In previous studies we have demonstrated, that the sensitivity of S-PIIINP to minor increases in collagen type III synthesis is limited (6).

Acute phase proteins reflect to some extent the disease activity in RA (22,23), but are of little value in AS (24) and degenerative joint diseases. This probably explains the correlation between S-PIIINP and ESR in active RA, in contrast to the lack of correlation in AS and OA.

In RA the propeptide has been proved to be a more sensitive indicator of subclinical chronic joint inflammation than the acute phase proteins (9), and normal propeptide levels in RA are associated with a good prognosis without erosive progression (8,9).

So far, no serological parameters have proved useful as markers for the inflammatory and repair processes in degenerative joint diseases. S-PIIINP, which reflects a specific step in the repair cascade, may be of value in monitoring joint inflammation also in OA.

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