

Serum Aminoterminal Propeptide of Type III Procollagen in Systemic Sclerosis

A Follow-up - Investigations in Subclasses and during Therapy

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Fifty-seven patients with systemic sclerosis were investigated for connective tissue turn-over related to type III collagen. Sera from 13 patients with diffuse cutaneous systemic sclerosis and 44 patients with limited cutaneous systemic sclerosis were analysed for aminoterminal propeptide of type III procollagen (PIIINP) by a radioimmunoassay based on human propeptide. Increased levels of PIIINP in serum correlated with skin involvement and the clinical course. All patients with diffuse cutaneous systemic sclerosis had levels above the normal range, and in limited cutaneous systemic sclerosis elevated PIIINP levels seemed to be correlated with rapid progression and with extension of lesions. Immunosuppressive drugs, cyclosporin A, and prednisone with or without cyclophosphamide, which were given to patients with rapid disease progression, significantly reduced PIIINP. This was also the case with penicillamine, but to a lesser degree. Our data support the suggestion that immunosuppressive agents are justified in rapidly progressive, life-threatening or disabling disease, when used with the necessary precautions. Serum PIIINP may be utilized as a marker of type III collagen fibrogenesis in systemic sclerosis and be of prognostic value. PIIINP may also be of use in the differential diagnosis between diffuse cutaneous systemic sclerosis and scleredema.

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Systemic sclerosis (SS), or systemic scleroderma, is characterized by progressive fibrosis of the skin and internal organs (1). In the skin the accumulation of type I and III collagen (2,3) seems to arise from increased synthesis rather than from decreased degradation (4). SS consists of diffuse cutaneous SS and a limited cutaneous SS (5). The latter may be subdivided into two groups according to skin involve-

ment (6): type I which has only sclerodactyli with no lesions above the wrists, and type II, an acrosclerosis with additional involvement of areas proximal to the wrists.

The radioimmunoassay for serum aminoterminal propeptide of type III procollagen (PIIINP) was first studied in SS by Krieg et al. in 1986 (7). We published preliminary data on PIIINP in relation to SS and localized scleroderma in 1988 (8). The present paper is a follow-up of this study, after the improved method by Risteli et al. (9) has been used for more than 3 years with all patients with SS consulting our department. We also report on serum PIIINP determinations performed before and during treatment.

PATIENTS AND METHODS

Sera were collected from 57 patients with SS, 13 with diffuse SS, 16 with type II SS, and 28 with type I SS. All patients fulfilled the criteria of the American Rheumatism Association (10). For 26 of the patients, serum PIIINP was determined before therapy or after at least 6 months without treatment. Thirty patients were studied before and after treatment with penicillamine given in dosages from 250 mg to 750 mg per day. Six patients were studied before and after cyclosporin A treatment (1.5 mg/kg to 7 mg/kg) and from nine serum was collected before and after therapy with prednisone with (3 patients) or without (6 patients) cyclophosphamide. Cyclosporin A and prednisone/cyclophosphamide treatment was only used in early and very active SS and in SS with pronounced progression.

All patients were studied with respect to serum creatinine, alkaline phosphatase and aspartate aminotransferase. Patients with suspected kidney involvement were studied with creatinine- and chrom EDTA clearance, and in 6 cases a kidney biopsy was performed. A liver biopsy was done in one patient in which laboratory tests and history indicated possible biliary cirrhosis. Other internal manifestations were diagnosed by lung X-ray, lung function tests, echocardiograms, and studies on esophageal motility. Patients with suspected joint involvement had an X-ray of these joints, in general X-ray of the hands.

Serum PIIINP levels were measured by the radioimmunoassay based upon the human propeptide (9), with the kit from Farnos Diagnostica, Oulunsalo, Finland. The refer-

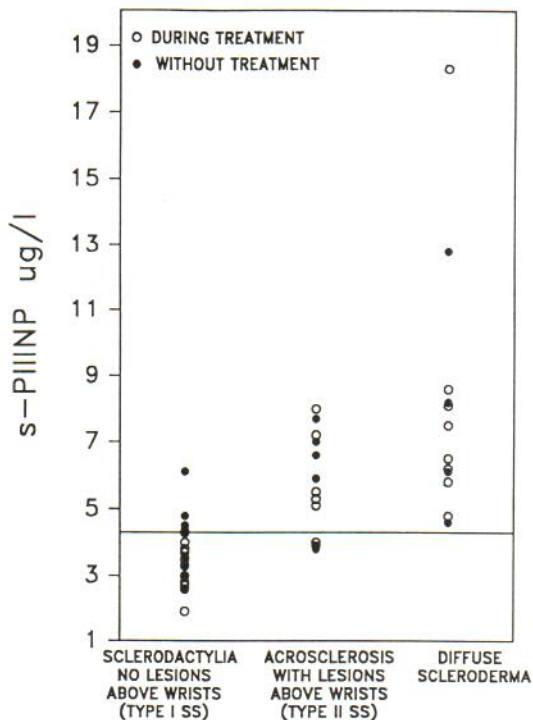


Fig. 1. Serum PIIINP levels in systemic sclerosis. The patients were subgrouped in diffuse cutaneous systemic sclerosis (diffuse scleroderma) and limited cutaneous systemic sclerosis with no lesions above wrists (type I) and with lesions above wrists (type II). ○, patients studied before or at least 6 months after discontinuation of treatment. ●, patients on systemic treatment. A horizontal line indicates upper normal limit.

ence range based upon healthy Finnish blood donors (n = 88) is 1.7 – 4.2 µg/l. Similar results on healthy Danish controls were 2.1 – 4.3 µg/l (n = 39, mean ± 2SD). All sera were stored at -20°C until analysis.

Statistical analyses were performed by the Wilcoxon's test for paired differences and Student's *t*-test (Fig. 1).

RESULTS

All patients with diffuse SS had PIIINP serum values above the upper reference level. The same applies for 9 of 16 patients with type II SS, and 3 of 28 patients with type I SS (Fig. 1 and Table I). Patients with SS as a whole had higher levels than controls ($2p < 0.001$), and this also applies to the subgroups with diffuse scleroderma ($2p < 0.001$) and type II SS ($2p < 0.001$), but not to patients with type I SS. Pretreatment values were significantly higher than values after treatment with cyclosporin A ($2p < 0.05$) (Fig. 2) or prednisone with and without cyclophosphamide ($2p < 0.05$) (Fig. 3). In all cases,

Table I. Mean serum PIIINP in systemic sclerosis (SS) subclasses.

Diffuse cutaneous SS = DS, limited cutaneous SS with no lesions above wrists = type I LSS, and limited cutaneous SS with lesions above wrists = type II LSS.

Patients Type	n	Serum PIIINP µg/l	
		mean	SD
DS	13	7.9	3.7
Type II LSS	16	5.6	1.4
Type I LSS	28	3.6	0.9
All SS	57	5.1	2.7
Controls	39	3.2	0.6

pretreatment values were compared with the first posttreatment investigation.

Serial investigations showed that some patients had a later increase in serum PIIINP, generally corresponding to an exacerbation of their disease. During penicillamine treatment there was an initial decrease in PIIINP ($2p < 0.05$) (Fig. 4).

Four patients had pathological liver tests. One of these was the patient with type II limited SS in which

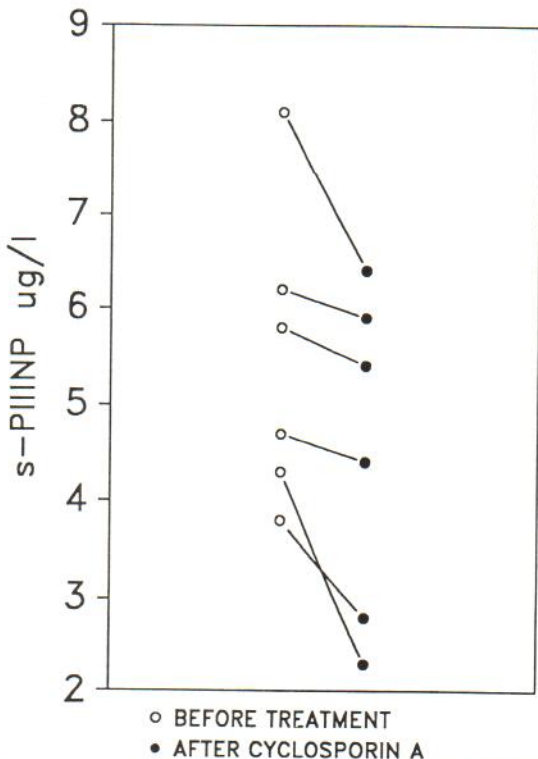


Fig. 2. Serum PIIINP before and following initiation of therapy with cyclosporin A.

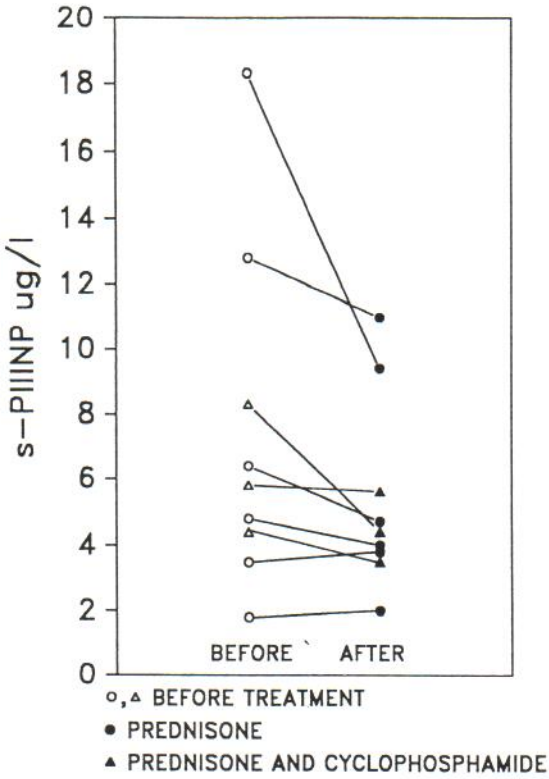


Fig. 3. Serum PIIINP before and following initiation of therapy with prednisone with and without cyclophosphamide. △, prednisone with cyclophosphamide, and ●, prednisone alone.

a liver biopsy was performed showing biliary cirrhosis. As her liver status worsened PIIINP rose from 4.3 µg/l to 10.6 µg/l. All patients had normal pre-treatment kidney function as judged by clearance investigations and/or creatinine in serum. During cyclosporin A, treatment serum creatinine increased in all patients but returned to normal levels after discontinuation of the drug. Kidney biopsies displayed minor increases of interstitial fibrosis in 2 patients after approximately one year's treatment. In the patient with the highest PIIINP serum value (18.3 µg/l), the clinical status rapidly deteriorated, and the patient died two months later in her local hospital. Unfortunately no follow-up on PIIINP and no autopsy was done.

DISCUSSION

The close correlation of serum PIIINP levels and skin involvement in SS is in good accordance with our preliminary data (8) and those of other workers (7,11,12). We found no correlation to the presence

of Raynaud's phenomenon, lung function status or decrease in esophageal motility. Joint involvement was too rare to be evaluated. Only in one patient a rise in PIIINP levels and clinical status suggested increased fibrogenesis of internal organs. This case was the type II SS patient with biliary cirrhosis. Increased serum PIIINP has been found in a number of liver diseases with fibrosis and/or cirrhosis (13,14). Our study indicates that PIIINP may be of use to distinguish between diffuse scleroderma and scleredema. In scleredema PIIINP serum levels are normal (Zachariae & Heickendorff unpublished data).

The mounting evidence that humoral and cell-mediated immune abnormalities play an important role in the pathogenesis of SS, and especially the possibility of inhibiting fibroblast function by alterations of the interaction between immunocompetent cells and fibroblasts would indicate a place for immunosuppressive therapy in SS (5). Our data on patients treated with cyclosporin A and prednisone with and without cyclophosphamide support this suggestion, although they do not allow any prefer-

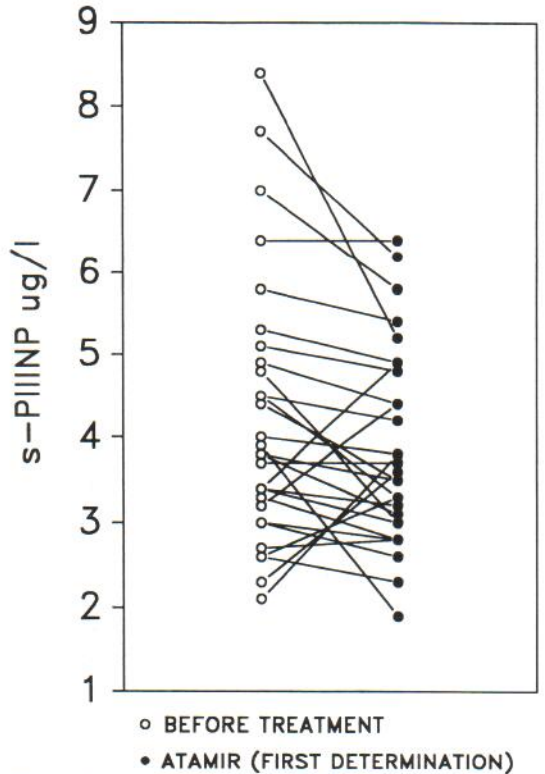


Fig. 4. Serum PIIINP before and following initiation of therapy with penicillamine.

ence of one of these drugs. Penicillamine affects collagenolysis and cross linking (15). This, together with the fact that penicillamine was our drug of choice in type I and type II SS with a slow pace of extremity skin thickening, makes an evaluation and comparisons between penicillamine and the other treatment modalities difficult. The observed lower serum values of PIIINP following penicillamine treatment could be due to the additional immunosuppressive effect of the drug.

Our study only reports on fibrogenesis related to type III collagen, but we believe that serum PIIINP may be utilized as a marker of this type of fibrogenesis in SS and therefore is of prognostic value. Hermann et al. (16) recently investigated PIIINP and laminin P1 serum levels together with acid lysosomal beta-galactosidase in SS and silicone-associated scleroderma. Increased PIIINP strongly correlated with enhanced activity of beta-galactosidase and the clinical course in both groups, but although serum levels of laminin P1 were also elevated, there was no correlation with the severity of the disease. Recently, it has also become possible to study fibrogenesis of type I collagen by analysis of serum samples (17) for carboxyterminal propeptide of type I procollagen (PICP). We are at present studying the relationship between PIIINP and PICP in the different subsets of SS and the relation to therapy.

REFERENCES

- Haustein U, Herrmann K, Böhme H. Pathogenesis of progressive systemic sclerosis. *Int J Dermatol* 1986; 25: 286-293.
- Le Roy C. Increased collagen synthesis by scleroderma fibroblasts in vitro. *J Clin Invest* 1974; 54: 880-889.
- Fleischmajer R, Perlish J, Krieg T, Templ R. Variability in collagen and fibronectin synthesis by scleroderma fibroblasts in primary culture. *J Invest Dermatol* 1981; 76: 400-403.
- Welgos H, Stricklen G. Biology and pathophysiology of collagen, elastin, and glycosaminoglycans. In: Soter N, Baden H, eds. *Pathophysiology of dermatological disorders*. New York: McGraw-Hill, 1984.
- Medsgert T. Treatment of systemic sclerosis. In: Trentham D, ed. *New directions in antirheumatic therapy. Rheumatic disease clinics of North America* 15/3. Philadelphia. W. B. Saunders, 1989.
- Arbeitsgruppe Sklerodermie der Arbeitsgemeinschaft Dermatologische Forschung (ADF). Klinik der Progressiven Systemischen Sklerodermie (PSS). *Hautarzt* 1986; 37: 320-324.
- Krieg T, Langer G, Gerstheimer H, et al. Type III collagen aminopeptide levels in serum of patients with progressive systemic scleroderma. *J Invest Dermatol* 1986; 87: 788-791.
- Zachariae H, Halkier-Sørensen L, Heickendorff L. Serum aminoterminal propeptide of type III procollagen in progressive systemic sclerosis and localized scleroderma. *Acta Derm Venereol (Stockh)* 1989; 69: 66-70.
- Risteli J, Niemi S, Trivedi P, et al. Rapid equilibrium assay for the amino terminal propeptide of human type III procollagen. *Clin Chem* 1988; 34: 715-718.
- Subcommittee for Scleroderma Criteria of the ARA Diagnostic and Therapeutic Criteria Committee: preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980; 23: 581-590.
- Majewski S, Skiendzielewska A, Makiella B, et al. Serum levels of type III collagen aminopropeptide levels in serum of patients with systemic scleroderma. *Arch Dermatol Res* 1987; 279: 484-486.
- Hørslev-Petersen K, Ammitzbøll T, Engström-Laurent A, et al. Serum and urinary aminoterminal type III procollagen peptide in progressive systemic sclerosis. Relation to scleroderma involvement, serum hyaluronan, and urinary collagen metabolite. *J Rheumatol* 1988; 15: 460-467.
- Frei A, Zimmermann A, Weigand K. The N-terminal propeptide of collagen type III in serum reflects activity in degree of fibrosis in patients with chronic liver disease. *Hepatology* 1984; 4: 830-834.
- Zachariae H, Søgaard H, Heickendorff L. Serum aminoterminal propeptide of type III procollagen - A non-invasive test for liver fibrogenesis in methotrexate-treated psoriatics. *Acta Derm Venereol (Stockh)* 1989; 69: 241-242.
- Niemi M. A defect in the intermolecular and intramolecular cross-linking of collagen caused by penicillamine: I Metabolic and functional abnormalities in soft tissues. *J Biol Chem* 1968; 243: 1457-1466.
- Herrmann K, Schultze E, Heckmann M, et al. Type III collagen aminopropeptide and laminin P1 levels in serum of patients with silicone-associated and idiopathic systemic scleroderma. *Br J Derm* 1990; 123: 1-7.
- Meeke J, Niemi S, Risteli L, Risteli J. Radioimmunoassay for the carboxyterminal propeptide of human type I procollagen (PICP). Submitted for publication.