Review

Serological assessment of type I collagen burden in scleroderma spectrum disorders: A systematic review

M. Dziadzio[1,2], R.E. Smith[1], D.J. Abraham[1], R.J. Stratton[1], A. Gabrielli[2], C.M. Black[1], C.P. Denton[1]

[1]*Centre for Rheumatology and Connective Tissue Diseases, Royal Free and University College Medical School, University College, London, UK;* [2]*Istituto di Clinica Medica, Ematologia ed Immunologia Clinica, Università di Ancona, Italy*

Magdalena Dziadzio, MD, PhD; Roy E. Smith, PhD; David J. Abraham, PhD; Richard J. Stratton, MD, FRCP; Armando Gabrielli, MD, Carol M. Black, MD, PRCP; and Christopher P. Denton, PhD, FRCP.

This work was in part supported by the grant from the Scleroderma Society, UK. We would also like to acknowledge generous support from the Raynaud's and Scleroderma Society, UK and the Arthritis Research Campaign.

Please address correspondence and reprint requests to: Dr Christopher P. Denton, Centre for Rheumatology, Royal Free and University College Medical School, University College London, Rowland Hill Street, London NW3 2PF, U.K. E-mail: c.denton@rfc.ucl.ac.uk

Received on August 1, 2003; accepted in revised form on March 4, 2004.

Clin Exp Rheumatol 2004; 22: 356-367.

Key words: Scleroderma,systemic; Raynaud's disease; collagen type I; biological markers; radioimmunoassay; review literature; confounding factors (epidemiology); severity of illness index; sensitivity and specificity.

ABSTRACT

Rationale: *The aim of the study was to evaluate the validity of collagen type I metabolites as markers of disease activity in scleroderma (SSc), through a systematic review of the literature and by validating the results by measuring collagen type I metabolites in well characterized patients with scleroderma spectrum disorders and in Raynaud's phenomenon.*

Methods: *A systematic review was performed of studies of collagen type I metabolites in scleroderma spectrum disorders published from 1980 to 2003. The collected results from the literature were compared with our own measurements of collagen type I metabolites (PINP and ICTP) in a small number of well characterized patients within the scleroderma spectrum and in patients with primary and "autoimmune" Raynaud's phenomenon. Peptide concentrations from all sources, including the present study, were compared. Reported correlations between peptide concentrations and clinical variables were also analysed.*

Results: *Of 19 papers identified by an extensive Medline search, 12 were eligible for systematic analysis. There was a considerable heterogeneity in the results with a wide range of metabolite concentrations. Values from disease groups and healthy controls overlapped. These findings were confirmed by our study where, similarly, there was a large range of values in all groups, but particularly in the diffuse SSc subset. When the correlation between peptide levels and clinical variables was assessed, large discordance between the studies was observed.*

Conclusions: *We have not found sufficient evidence to support the use of serum markers of collagen turnover in the assessment of scleroderma activity and severity, in view of their low specificity and the heterogeneity of the results of various studies. Lack of standardized routine evaluation of SSc patients in clinical studies might have accounted for the variability of the findings. However, due to the small sizes of most published studies, demonstration of no effect should come from large-scale randomised trials. Longitudinal serial analysis of these molecules in individual patients may play a future role in the evaluation of the response to fibroblast-targeting therapeutic strategies in scleroderma patients.*

Introduction

Type I collagen excess is a hallmark pathological feature of established scleroderma (systemic sclerosis, SSc) (1). Collagens are the major fibrillar proteins in extracellular matrix (ECM) and collagen type I (Col I) is the main component of ECM in the skin, bones and ligaments (2, 3). Increase in Col I synthesis by lesional dermal and lung fibroblasts is characteristic for SSc (4-5). Several efforts have been made to identify the mechanisms of upregulation of Col I in SSc *in vitro* and many signal transduction factors involved in this process have been identified (6-7). Increased rates of collagen gene transcription (6-7) and increased levels of tissue inhibitors of matrix metalloproteinase-1 (TIMP-1) (8) seem also to account for elevated levels of Col I in SSc.

Excessive collagen synthesis and deposition in SSc was a rationale for measurements of Col I turnover in the assessment of SSc activity and severity *in vivo*. Collagen metabolites reflect the dynamic nature of the ECM (2-3). Procollagens are secreted by the fibroblasts in the ECM space where the N-terminal [PINP] and C-terminal [PICP] propeptides are cleaved by specific pro-

teases; N- and C-terminal telopeptides are cross-linked products of collagen degradation, released to the circulation (2,3,9-11). Specific methods have been developed to measure these peptides in body fluids (9-10, 12). Their concentrations have been measured in the serum, plasma, urine, blister fluid or bronchoalveolar lavage of patients with SSc and found elevated by some authors, who suggested their utility as surrogate markers of disease activity (13-25).

Discordant results from earlier studies meant that measurements of collagen metabolites in SSc patient sera have not been recommended for routine evaluation of SSc severity and activity (26-29). In fact, Col I metabolites have not been included in the proposed non-organ based laboratory markers in SSc (26) for clinical investigation studies and clinical practice recommended by the European Scleroderma Study Group (28) and recent Consensus Conference on scleroderma (27).

The purpose of this work was a systematic review of major published studies of collagen type I metabolites in patients with scleroderma spectrum disorders, in order to gain more insight into the significance of Col I measurements *in vivo*. We validated our findings by measuring Col I peptides in a small number of well characterized patients with scleroderma spectrum disorders, stratified by disease subsets, as well as in healthy controls, and rationalized our data in the context of published studies which used high sensitivity radioimmunoassay. We also attempted to validate our measurements against the recent consensus descriptive disease variables (disease activity and severity indices) (27, 29).

Systematic review strategy

The published literature from January 1980 to January 2003 was searched. The search started with a broad Medline search using terms "systemic scleroderma" or "localised scleroderma" and "PINP", "PICP", "ICTP" and "human". The electronic searches were supplemented by a thorough search of the reference lists of all eligible studies. The review process was based on the following pre-defined inclusion criteria: direct assessments expressed as concentration of Col I peptides (PINP, PICP and/or ICTP) in body fluids of patients with a diagnosis of systemic sclerosis, localised scleroderma or Raynaud's phenomenon; research involving only humans (*in vitro* studies were not included); only primary studies (and not reviews of studies) were analysed. Both prospective and retrospective studies were eligible. The presence of a healthy control group was not part of the inclusion criteria. There was no minimum sample size, since most available studies were very small. The main objective of the analysis was to evaluate the validity of Col I peptides as markers of disease process in SSc as well as the assessment of the variability and/or repeatability of the assay results between different studies. The eligible papers were analysed; key data elements included: patient group characteristics, material studied, peptide type, measurement technique used, mean and/or median peptide concentrations, reported clinical and biochemical correlations and conclusions. As there were a limited number of studies with clearly reported peptide concentrations, results that required extrapolations from graphs or derivation from figures or tables were also captured; results that were only given for a single patient were not analysed.

Meta-analysis or systematic literature review are used to examine the relevant literature for general trends and patterns. Meta-analysis refers to the statistical analysis of a large collection of results from preferably homogeneous individual studies for the purpose of integrating the findings. In our case, the heterogeneity of study designs, very small samples, insufficient clinical characterisation of patient groups, lack of control groups in some studies and different materials analysed by various methods meant that meta-analysis was not an adequate tool for our purposes. Systematic literature review was, therefore, chosen as a more appropriate analytical method; it uses a method of qualitative rather than quantitative manipulation of the published information with subjective rather than statistical analysis of the results. We have supplemented our systematic review with an investigation of the differences between the collagen peptide concentrations: mean and/or median concentrations (with range and/or standard deviations, where available) were compared between the sources for PINP, PICP and ICTP and the data expressed in a graphical form. Correlations between the variables (serological versus clinical) were analysed and tabulated for single studies (Table I).

Method

Study of type I collagen peptides

Patients

Patients were recruited from the outpatient clinic at the Rheumatology Department, Royal Free Hospital over a 24-week period. Ten patients for each disease group (primary Raynaud's phenomenon, "autoimmune" Raynaud's phenomenon, limited cutaneous SSc and diffuse cutaneous SSc) as well as for healthy control (HC) group, were enrolled into the study (Tables IIa and IIb). Ethical committee approval was obtained and patients gave written consent to participate.

Systemic sclerosis: Patients were diagnosed with SSc, according to the American College of Rheumatology (formerly, the American Rheumatism Association) preliminary criteria for the classification of SSc (30). According to the classification system proposed by LeRoy *et al.* (31), 10 patients (8 females, 2 male) had diffuse cutaneous systemic sclerosis and 10 (9 female, 1 male) had limited cutaneous systemic sclerosis. Epidemiological and clinical variables of each SSc patient have been investigated, following guidelines formulated recently (29). In particular, the presence of organ involvement, disease activity and severity has been assessed following the recent guidelines (27, 29). Duration of SSc was defined as time from diagnosis (years).

Raynaud's phenomenon: Raynaud's phenomenon (RP) was diagnosed on the basis of a history of episodic digital vasospasm with triphasic colour changes and confirmed by thermography. Capillaroscopic abnormalities were scored using the commonly accepted capillaroscopy scale (32). Patients were

investigated for the presence of antinuclear antibodies, anti-centromere antibodies, anti-dsDNA antibodies, anti-ENA antibodies including anti-Scl-70, anti-PM-Scl, anti-nRNP, anti-Jo-1, anti-Ro and anti-La, anti-Sm, and anti-polymerase I and III antibodies as well as for rheumatoid factor. Patients with RP, no significant nailfold capillaroscopic abnormalities (32) and absent autoantibodies were defined as having primary Raynaud's phenomenon (PRP). Patients with capillaroscopic abnormalities, positive for one or more autoantibodies studied and no clinical signs or symptoms of underlying connective tissue disease were defined as "autoimmune Raynaud's phenomenon" (ARP). Ten patients were included in each group, their characteristics are shown in Table IIa.

Healthy controls: Control samples were obtained from ten healthy age and sex matched volunteers (Table IIa).

Serum markers (samples)

Blood samples were taken from each subject in the morning. Serum was obtained by centrifugation of whole blood at 3000 G for 10 minutes and aliquots were stored at –20ºC until assayed. Levels of type I aminoterminal propeptide of collagen type I [PINP] and carboxyterminal telopeptide of collagen type I [ICTP] were evaluated using commercially available radioimmunoassay (RIA) (Orion Diagnostica, Espoo, Finland). There is no documented cross-reaction with propeptide of type III procollagen in these assays (Orion Diagnostica).

In brief, 50 µl of appropriate standards, controls and serum samples for PINP assay were mixed in 2-ml Eppendorf tubes with 200 µl of PINP antiserum IgG (rabbit) and 200 µl of ^{125}I-labelled PINP. For the ICTPassay, the appropriate samples were of 100 µl, the rest of the procedure was identical. After a 2-hour incubation at 37ºC 500 µl of a second antibody (goat anti-rabbit IgG bound to solid particles in order to precipitate the antigen-antibody complex) was added and tubes were thoroughly mixed and then left to stand for 30 minutes at room temperature. Tubes were subsequently centrifuged for 15 minutes at 2000 G at 4ºC. The supernatant

Table I. Analysis of the published literature.

Reference	Patients						Peptide			Method		Results
	HC	PRP	Suspected SSc	lcSSc	dcSSc	localised	PINP	PICP	ICTP	RIA	ELISA	
Scheja *et al.* (14)	54			30	24			S		✓		SSc = HC dcSSc > lcSSc
Zachariae *et al.* (15)	No			4	3			S		✓		SSc = NR
									S	✓		SSc = NR
Kikuchi *et al.* (16)	30					39		S			✓	Localised > HC GM > LM
Kikuchi *et al.* (17)	21			28	33			S			✓	SSc > HC dcSSc > lcSSc
Kikuchi *et al.* (18)	10		3	8				S Fb			✓	SSc> HC dcSSc > lcSSc
Heickendorff *et al.* (19)	40			25				S		✓		SSc = HC
	11			11				BF		✓		SScuninvolvedskin = HC; SSc involved skin>SSc uninvolved
	36			25					S	✓		SSc = HC
Sondergaard *et al.* (20)	11			10	3			P		✓		SSc = HC
								BF		✓		SSc > HC
Hunzelmann *et al.* (21)	No			10	22	6	S			✓		SSc = NR = localised
									S	✓		SSc > NR
Valat *et al.* (22)	No			22 SSc no ILD; 23 SSc with ILD				S			✓	SSc = NR ILD +ve = ILD -ve
Dziadzio *et al.* (23)	No	25		18	9		S			✓		SSc = PRP
Scheja *et al.* (24)	31		12	23	13		S			✓		SSc = HC
								S		✓		SSc = HC
									S	✓		SSc > HC dcSSc > lcSSc
Allanore *et al.* (25)	25			14	19			S			✓	SSc = HC
									S CTX-I		✓	SSc > HC dcSSc > lcSSc

HC: healthy controls; NR: normal range; GM: generalised morphoea; LM: linear morphoea; ILD: interstitial lung disease; P: plasma; BF: blister fluid; S: Serum; Fb: Fibroblast culture medium; CTX-1: Carboxyterminal telopeptide region of Col I.

Correlations								Conclusions and authors' comments
Disease activity	Disease duration	Organ involvement	Kidney function	Skin score	Extension of skin lesions	Useful	Not useful	
							✓	In HC [PICP] was significantly higher in men than women ($p < 0.001$) and was related to height ($p < 0.001$) and weight ($p < 0.02$).
						✓	✓	PUVAtreatment reduces [PICP]. PUVAtreatment has no effect on [ICTP].
Y			Y	Y	Y	✓		Positive correlation between the presence of autoantibodies and [PICP] in localized scleroderma.
Y			Y			✓		
						✓		Positive correlation between serum [PICP] and [PICP] in the fibroblast culture media (Fb) from the same patient with SSc.
							✓	Treatment with D-penicillamine, steroids and cyclophosphamide reduces [PICP], suggesting their use for SSc treatment.
						✓		
							✓	
						✓	✓	Fibrogenetic process takes place in the transitional zone of SSc skin. Blister fluid (BF) can be used to monitor the progression of SSc skin lesions *in vivo*.
N Y		N Y	N	N	N Y	✓	✓	
		N		N			✓	
						✓		12-week treatment with losartan led to a significant reduction in [PINP], suggesting this drug's disease modifying properties.
N N	N N	N N	N N	N N			✓ ✓	
N	N	Y	Y	N		✓		
N Y		N Y		N Y		✓	✓	No difference in [PICP] and [CTX-I] between patients receiving and those not receiving steroids and/or D-penicillamine.

Correlation between clinical variables and peptide concentration: Y(yes) if found and N(n) if no correlation.

was then removed and the sediment containing the precipitated antibody-antigen complex was counted for 1 minute per tube in a gamma counter (Wallac). Standard curves were produced by calculating the binding of six standards as a percentage of the maximum possible binding and by plotting these values. The sensitivity of this method was 2 $\mu g l^{-1}$ for PINP and 0.5 $\mu g l^{-1}$ for ICTP; the inter- and intra-assay coefficient of variation was about 5% for both assays (Orion Diagnostica).

Statistical analysis

Statistical analysis was carried out on non-transformed data using the Mood median test (non-parametric equivalent of one-way analysis of variance) and pair-wise comparison using Mann-Whitney. Logarithmic transformation was subsequently applied in order to normalize the data. One-way analysis of variance (ANOVA) was carried out and individual group comparisons were made using unpaired t-test assuming unequal variance. Significance was set at 5%. Analysis was performed for HC, RP and SSc as well as for patient subgroups (HC, PRP, ARP, lcSSc and dcSSc), Multiple regression on log-transformed peptide concentration data was used to investigate the correlations between patient demographics (age, duration of Raynaud's phenomenon, duration of disease and patient subgroup) and peptide concentrations. Linear regression was also carried out for clinical variables (defined as disease activity and severity scores (27, 29)) and log-transformed [PINP] and [ICTP]; again, correlations were performed for the scleroderma subsets (lcSSc and dcSSc). Regression was also used to investigate any correlation between [PINP] and [ICTP]; in this analysis all subgroups were pooled together. The Minitab statistics package (Minitab Inc, PA) was used.

Results

Literature review

The search identified nineteen reports. Twelve papers were considered eligible for systematic analysis (14-25). The results are shown in Table I. Studies selected were heterogeneous for pa-

Table II (a). Characteristics of scleroderma patients, Raynaud's phenomenon patients, and healthy controls.

	Healthy Controls	Primary Raynaud's Phenomenon	Autoimmune Raynaud's phenomenon	Raynaud's phenomenon (PRP+ ARP)	Limited cutaneous SSc	Diffuse cutaneous SSc	SSc (lcSSc + dcSSc)
Number of subjects	10	10	10	20	10	10	20
Sex (Female: Male)	8:2	8:2	9:1	17:3	9:1	8:2	17:3
Age (years)							
Median (Range)	42.9 (28-64)	40.3 (24-59)	38.5 (16-58)	39.5 (16-59)	46.4 (22-72)	47.1 (18-73)	45.2 (18-73)
Mean ± SD	41.0 ± 11.8	40.3 ± 12.2	36.1 ± 14.8	39.1 ±13.1	44.6 ± 10.5	48.1 ± 15.6	46.2 ± 12.7
RP Duration (years)							
Median (Range)	NA	11 (1-30)	6 (1-43)	6.5 (1-43)	17.5 (2-50)	3.5 (1-35)	9 (1-50)
Mean ± SD		13.6 ± 10.4	11.3 ± 14.0	12.4 ± 12.1	20.8 ± 14.3	7.9 ± 9.5	14.3 ± 13.6
SSc Duration							
Median (Range)	NA	NA	NA	NA	10 (2-24)	3 (1-24)	6 (1-24)
Mean ± SD					11.9 ± 7.6	6.6 ± 8.7	9.3 ± 8.4
Autoantibody profile							
ANApositive	0	0	10	10	10	10	20
ACApositive	0	0	0	0	8	0	8
Anti-Scl70 positive	0	0	0	0	1	6	7

ANA: antinuclear antibodies; ACA: anti-centromere antibodies; NA: not applicable.

Table II (b). Scleroderma activity and severity scores (29).

Scleroderma activity Active: inactive	Limited cutaneous SSc 3:7			Diffuse cutaneous SSc 4:6			SSc (lcSSc + dcSSc) 7:13		
Organ involvement	Y/N	Severity median (range)	Mean ± SD	Y/N	Severity median (range)	Mean ± SD	Y/N	Severity median (range)	Mean ± SD
General	7/3	1 (0-3)	1.20 ± 1.14	6/4	1 (0-2)	0.70 ± 0.67	13/7	1 (0-3)	0.95 ± 0.94
Peripheral vascular	9/1	2 (0-3)	1.80 ± 0.79	10/0	1 (1-3)	1.50 ± 0.71	19/1	2 (0-3)	1.65 ± 0.75
Skin	9/1	1 (0-2)	1.10 ± 0.57	10/0	2 (1-4)	2.00 ± 0.94	19/1	1 (0-4)	1.55 ± 0.89
Joint/tendon	3/7	0 (0-2)	0.40 ± 0.70	3/7	0 (0-3)	0.60 ± 1.07	6/14	0 (0-3)	0.50 ± 0.89
Muscle	3/7	0 (0-1)	0.30 ± 0.48	1/9	0 (0-1)	0.10 ± 0.32	4/16	0 (0-1)	0.20 ± 0.41
GI tract	9/1	1 (0-3)	1.10 ± 0.74	8/2	1 (0-1)	0.80 ± 0.42	17/3	1 (0-3)	0.95 ± 0.60
Lung	5/5	0.5 (1-2)	0.70 ± 0.82	7/3	1 (0-4)	1.30 ± 1.25	12/8	1 (0-4)	1.00 ± 1.08
Heart	2/8	0 (0-1)	0.20 ± 0.42	1/9	0 (0-1)	0.10 ± 0.32	3/17	0 (0-1)	0.15 ± 0.37
Kidney	1/9	0 (0-3)	0.30 ± 0.95	1/9	0 (0-2)	0.20 ± 0.63	2/18	0 (0-3)	0.25 ± 0.79
Severity score		6 (3-12)	6.80 ± 3.05		7 (4-15)	7.40 ± 3.10		6.5 (3-15)	7.10 ± 3.01

Scleroderma activity is scored between 0-11: active disease is denoted by a score ≥ 3 (27). The severity of organ involvement is scored between 0 and 4, with 0 denoting no involvement. The median and range shown is for all patients in a group. Scleroderma severity score ranges from 0 to 36 (27).

tient characteristics, study design, materials studied (serum, plasma, blister fluid, supernatants), methods used (RIA, ELISA) and the presence of healthy control group as opposed to reference normal values. Healthy control groups were not included in 4 out of 12 studies; in these papers, the reference range provided by the manufacturer of the kit was used as a normal control range. Peptide concentrations have been measured using either RIA (13 out of 19 measurements) or enzyme-linked immunoabsorbent assay (ELISA) (6 out of 19 measurements). There was discordance regarding the correlation(s) between peptide concentrations and different clinical parameters between the studies (Table I). [ICTP], [PINP] and [PICP] in SSc were raised in 2 out of 5, 1 out of 3 and 3 of 9 papers, respectively. For [PICP], the same research group performed all three positive studies (16 - 18). Sondergaard *et al*. (20) found an 87% increase in [PICP] in dermal blister fluid from SSc patients as compared to healthy controls; this was not observed for plasma. Carboxyterminal telopeptide region of Col I (CTX-I), biologically similar to ICTP, was studied by Allanore *et al*. (25) and its levels were found increased in SSc patients.

Comparative analysis of the mean and/or median [PICP], [PINP] and [ICTP] values was performed and is shown in Figures 1 - 3. It was carried out on 11

out of 12 papers; no numeric values were provided for either [PICP] or [ICTP] in one paper (15). Data were presented as median (with range) by most authors due to a non-normal distribution of the values. In fact, very large ranges can be seen (Figs. 1- 3), in particular in the dcSSc groups. In the graphical representation each symbol represents either the mean or the median and the horizontal lines are the group standard deviations (SD) or range; for the "manufacturer's" reference ranges a thick horizontal line is used.

Study of type I collagen peptides

Patients

Characteristics of healthy controls and patients are summarized in Tables IIa and IIb. Groups were matched by age with no significant differences between them (Table IIa). Sex differences were ignored in all analyses as there was a 42:8 F:M ratio, reflecting naturally occurring female preponderance for RP and SSc. All ARP patients had abnormal capillaries on capillaroscopy and had positive autoantibodies (ANA present in a titre ≥ 1:1000 in 8 patients, with a homogeneous pattern in 1 patient, and fine speckled and cytoplasmic pattern in 7 patients, and ≥ 1:100 in 2 patients, with a fine speckled pattern).

PINP and ICTP concentrations

Mean (±SD) and median (and range) serum PINP and ICTP concentrations (μgl^{-1}) are shown in Table III. Single patient measurements are shown in Figure 4 [PINP] and Figure 5 [ICTP]. In Figures 4 and 5 we have shown non-transformed data to allow ease of comparison with other published results; p-values on these graphs derived from the analysis of log-transformed data.

A large range and standard deviations were observed for both [PINP] and [ICTP]. The largest scatter was seen for [ICTP] in the dcSSc group. One-way ANOVAon log-transformed data (in order to normalise the data) was significant for PINP ($p < 0.006$). Serum levels of PINP were significantly lower in PRP than in healthy controls ($p < 0.0002$). No other group differed significantly from healthy controls, however [PINP] in lcSSc was significantly higher than

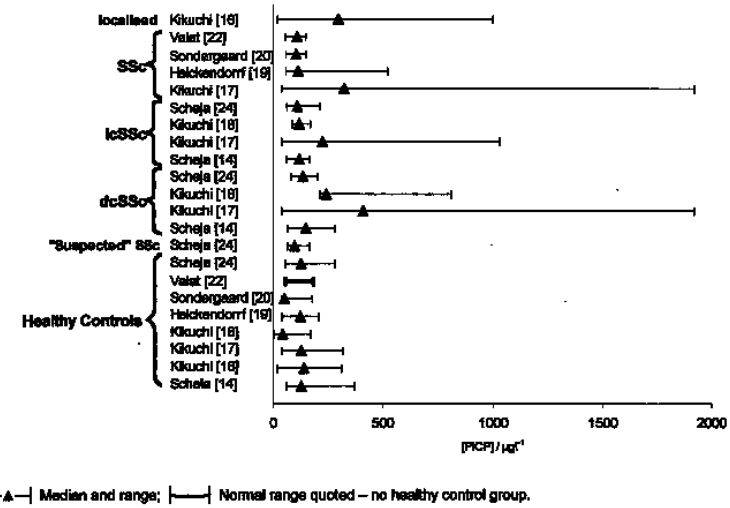

Fig. 1. Comparison of the concentrations of PICPreported by different authors.

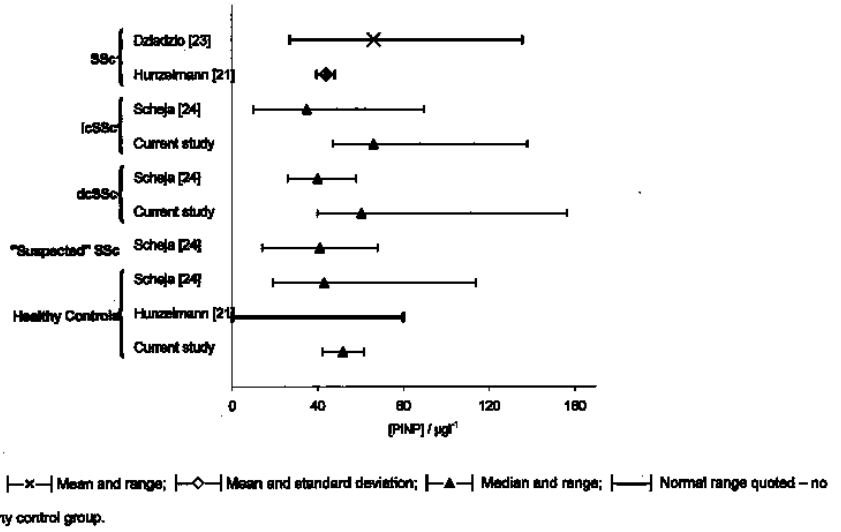

Fig. 2. Comparison of the concentrations of PINPreported by different authors.

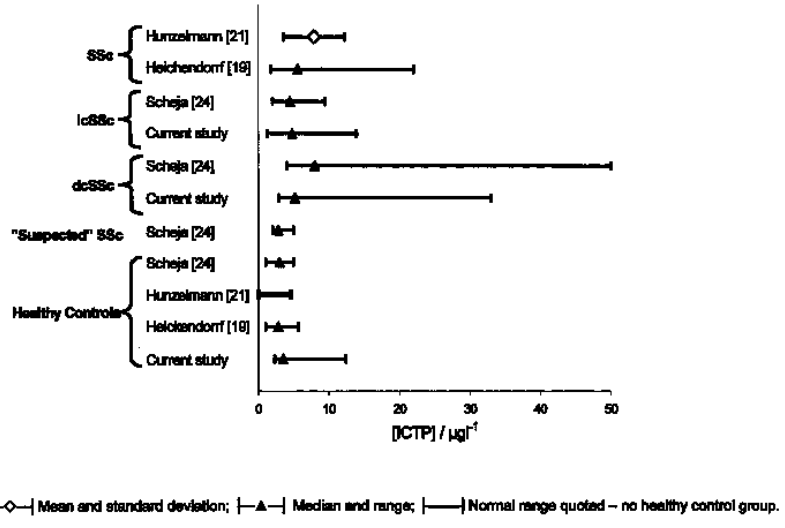

Fig. 3. Comparison of the concentrations of ICTPreported by different authors.

Table III. PINPand ICTPconcentrations in serum (μg/l).

	Healthy Controls (HC)	Primary Raynaud's Phenomenon (PRP)	Autoimmune Raynaud's Phenomenon (APR)	Raynaud's Phenomenon (PRP+ ARP) (RP)	Limited cutaneous SSc (lcSSc)	Diffuse cutaneous SSc (dcSSc)	SSc (lcSSc + dcSSc)
PINP							
Mean ± SD	52.7 ± 6.6	39.8 ± 4.3	50.7 ± 15.2	45.2 ± 12.2	70.1 ± 26.2	72.7 ± 35.2	71.4 ± 30.2
Median	51.8	40.3	49.3	42.0	66.1	60.4	62.9
range	42.2 - 61.5	34.2 - 47.7	31.1 - 85.6	31.1 – 85.6	47.4 - 137.8	39.9 - 156.5	39.9 – 156.5
		$p < 0.0002$ versus HC		$p < 0.02$ versus HC	$p < 0.0003$ versus PRP; $p < 0.03$ versus ARP	$p < 0.01$ versus PRP	$p < 0.02$ versus HC; $p < 0.002$ versus RP
ICTP							
Mean ± SD	5.4 ± 3.6	3.4 ± 2.8	5.2 ± 5.0	4.2 ± 4.0	5.1 ± 3.5	13.4 ± 12.9	9.5 ± 10.1
Median	3.5	2.4	3.3	2.5	4.8	5.2	4.8
range	2.3 - 12.4	0.9 - 8.2	0.6 - 14.7	0.6 – 14.7	1.3 - 13.9	2.9 - 33	1.3 - 33
						$p < 0.05$ versus ARP; $p < 0.02$ versus PRP	$p < 0.01$ versus RP

Note: all group t-tests performed on log-transformed data.

for PRP ($p < 0.0003$) and ARP ($p < 0.03$), whereas in dcSSc [PINP] was higher than in PRP only ($p < 0.01$). One-way analysis of variance on log-transformed data was significant for [ICTP] ($p < 0.02$). Serum levels of ICTP were significantly higher in dcSSc patients than in PRP ($p < 0.02$) and ARP($p < 0.05$).

For the non-parametric statistical analysis similar significance levels were obtained for PINP, however, for ICTP, the Mood test was not significant. We have therefore reported the analysis on the transformed data, since, for ICTP, good normalization occurred and parametric analysis is more robust.

When multiple regression was carried out, there was no correlation for the pooled patient group (all except HC) between [ICTP] and patient age, and age at onset of RP. For the SSc group (lcSSc plus dcSSc) there was no relationship between [ICTP] and duration of SSc. In contrast, when [PINP] was analysed for the SSc group, there was a significant positive correlation between [PINP] and age of onset of RP ($p < 0.02$) and SSc disease subset ($p < 0.02$). As for [ICTP], there was no correlation between [PINP] and duration of SSc.

When all patient groups and HC were pooled, there was a positive correlation between [ICTP] and [PINP], $r^2 = 0.175$, $p < 0.003$ (Fig. 6).

For the SSc group there was no significant correlation between disease activity and severity scores and both log transformed and raw peptide concentrations. There was no difference between mean peptide concentration for active disease (activity score ≥3) and inactive disease (activity score <3) (27). The analysis of subsets (lcSSc and dcSSc) also showed non-significant findings.

Discussion

The impetus for this study was the availability of high quality RIA to detect collagen pro- and telo-peptides in scle-

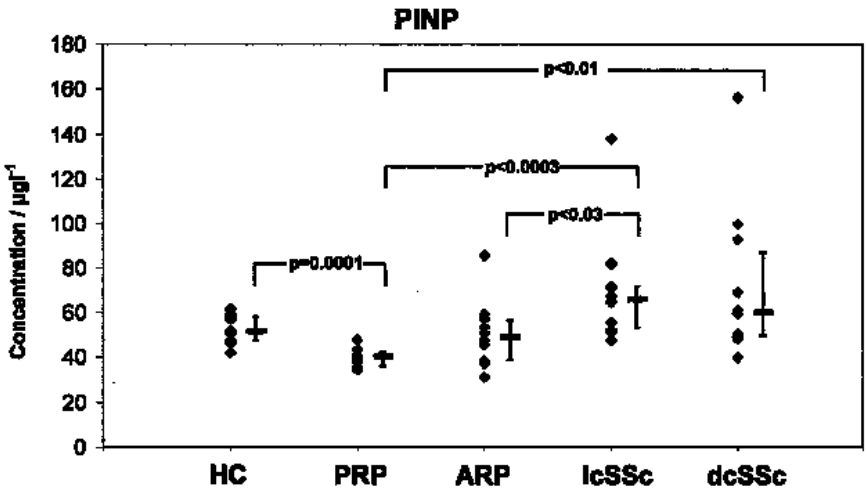

Fig. 4. PINPlevels of individual subjects.
Key: ✧ = individual subjects; horizontal bar = median; long vertical bar = interquartile range.
Ten subjects in each group: HC = healthy controls, PRP = primary Raynauds's phenomenon; ARP = autoimmune Raynaud's phenomenon; lcSSc = limited cutaneous systemic sclerosis; dcSSc = diffuse cutaneous systemic sclerosis. For explanation of p-values, see text.

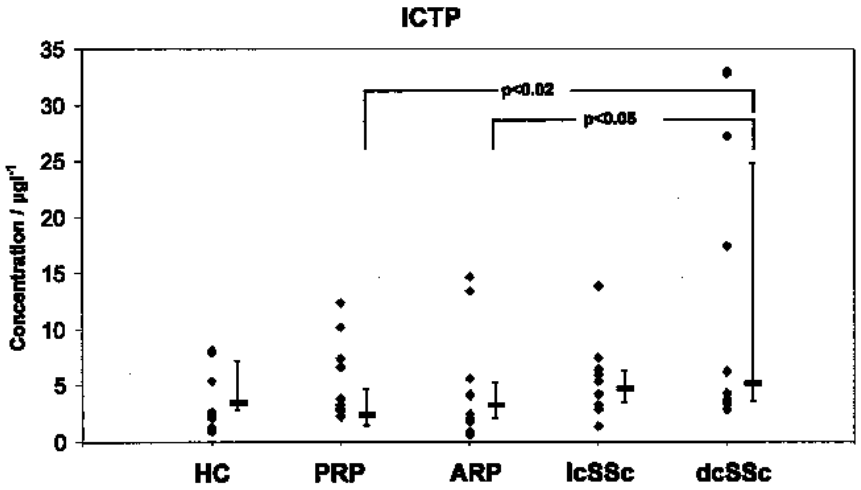

Fig. 5. ICTPlevels of individual subjects.
Key: ◇ = individual subjects; horizontal bar = median; long vertical bar = interquartile range.
Ten subjects in each group: HC = healthy controls, PRP= primary Raynauds's phenomenon; ARP = autoimmune Raynaud's phenomenon; lcSSc = limited cutaneous systemic sclerosis; dcSSc = diffuse cutaneous systemic sclerosis. For explanation of p-values, see text.

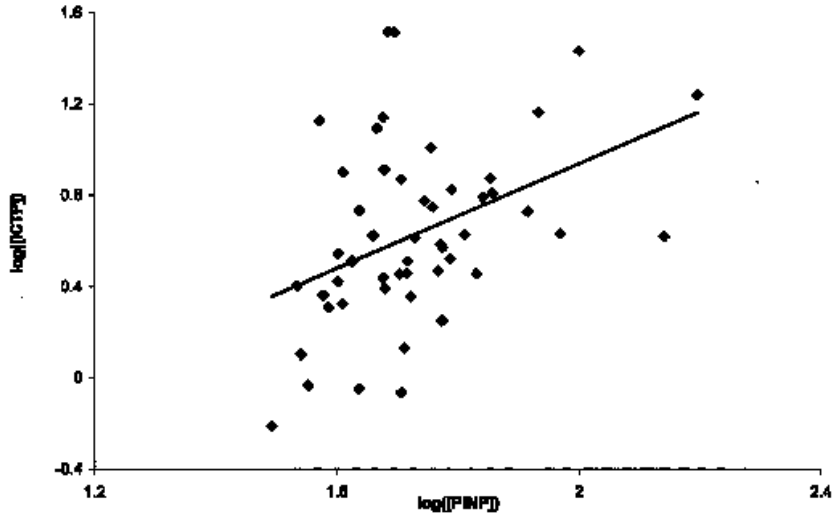

Fig. 6. Correlation between ICTPconcentration ([ICTP]) and PINPconcentration ([PINP]) in the current study, shown as a log-log plot.
Notes: Pre-transform units: µg/l. Best fit straight line shown; correlation $r^2 = 0.175$, $p < 0.003$.

roderma sera. However, due to large variability in the data from previous studies looking at collagen metabolites in SSc, these molecules are not currently recommended as standard laboratory markers of disease activity (26), despite their obvious relevance to the burden of fibrosis. With clear criteria for descriptive studies in SSc we felt confident in adding our own dataset using optimal assays in very well defined distinctive disease groups, including "autoimmune" Raynaud's, not studied previously. For educational purposes, we discuss briefly the principles of collagen metabolism, its alterations in SSc, the significance and clinical application of collagen pro- and telopeptide measurements. Subsequently we discuss the results from the published clinical studies and compare them to our findings.

Type I collagen biosynthesis and metabolism

Collagen type I (Col I) is one of the most abundant components of the extracellular matrix (ECM). Col I is a heterodimer of two alpha1(I) and one alpha2(I) collagen polypeptides (chains). Each of these chains is coiled into a left-handed helix; the three helical chains are then twisted around each other into a right-handed super-helix. The collagen molecule is rich in proline and hydroxyproline, which are rigid, cyclic aminoacids and contribute to the stability of the triple helix. The synthesis of both chains is highly regulated by different cytokines at the transcriptional level. Col I is first synthesized intracellularly as a longer molecule, procollagen, which contains additional "propeptides" (2-3) (Fig. 7). The procollagen polypeptide chains are produced by the fibroblasts and then secreted in the extracellular space where the N-terminal [PINP] and C-terminal [PICP] propeptides are cleaved by specific amino- and carboxy- proteases (Figure 7)(11). Mature collagen molecules aggregate into stable, cross-linked collagen fibrils and form the ECM. Col I is degraded in normal remodelling associated with physiological processes such as morphogenesis, growth, wound healing and physiological bone turnover (2,3). Also, cell–cell and cell–matrix interactions include ECM proteolysis. There are four major enzyme classes involved in Col I degradation: aspartate, cysteine, serine proteases and matrix metalloproteinases (MMPs). Proteolytic activity of these enzymes leads to the formation of various degradation fragments including several triple-helical peptides as well as telopeptides, which are small amino-acid sequences originating from the non-helical ends of collagen molecules. Normal Col I production, deposition in ECM and its degradation is regulated at many levels. Abnormalities at any level may cause defective synthesis, accumulation or degradation leading to fibrosis (3,6,7).

Collagen type I in scleroderma

In fibrotic diseases there is a disturbed balance between the process of synthesis and degradation, with net gain in collagen and fibrotic tissue (6-7). Scleroderma fibroblasts *in vitro* continue to synthesize increased amounts of colla-

gen for several passages (4). Elevated levels of Col I in scleroderma skin (5) is primarily due to the increased rate of collagen gene transcription, regulated by various factors including PDGF, TGF-beta and CTGF to name but a few (4, 6-7). The transcriptional rate of genes encoding pro-alpha2(I) collagen is increased in SSc fibroblasts, suggesting alterations in transcription factors. Data regarding the catabolism of collagen in SSc are heterogeneous. Tissue inhibitors of metalloproteinase 1 (TIMP-1) may contribute to increased collagen burden in SSc; in fact serum concentrations of TIMP-1 were found raised in SSc (8).

Type I collagen propeptides and telopeptides: assessment of the rate of Col I synthesis and degradation

Procollagen type I contains N-terminal and C-terminal propeptides (PINP and PICP) which are cleaved off by specific proteases belonging to a family of zinc-dependent metalloproteinases in a stechiometric relationship with collagen biosynthesis (2,11). PINP has a molecular weight of 35 kDa and contains three distinct structural domains: a globular amino-terminal domain, a central collagen-like domain and another short-globular domain. PICP has a molecular weight of about 100 kDa and globular conformations without any collagen-like domains. Both propeptides contain cysteine and sugars, not found in type I collagen. The propeptides account for one-third of the bulk of the procollagen molecule. They prevent premature fibril formation and help to direct assembly of the protein into fibrils. After they are cleaved from the molecule they also seem to play a role in the control of the amount of procollagen synthesized by the cells (33). PINP is degraded via the scavenger receptors of liver endothelial cells, which are not hormone–sensitive and therefore the regulation of the clearance of PINPis less dependent on different factors including sex, age and treatment(s) (33). In contrast, PICP is degraded in the liver through the mannose-6-phosphate receptors on endothelial cells. Despite the fact that PICPand PINPare derived from the same molecule, no constant temporal or disease-specific correlation exists between these molecules (3). Recently, extremely high PICPlevels have been described as a familial case and determined genetically as an apparent autosomal dominant condition, with no effect on survival and normal concentrations of PINP and ICTP (34). The assays (RIA or ELISA) use polyclonal antibodies against the propeptides for PINP and PICP and have been widely used to assess the rate of collagen synthesis (9-10).

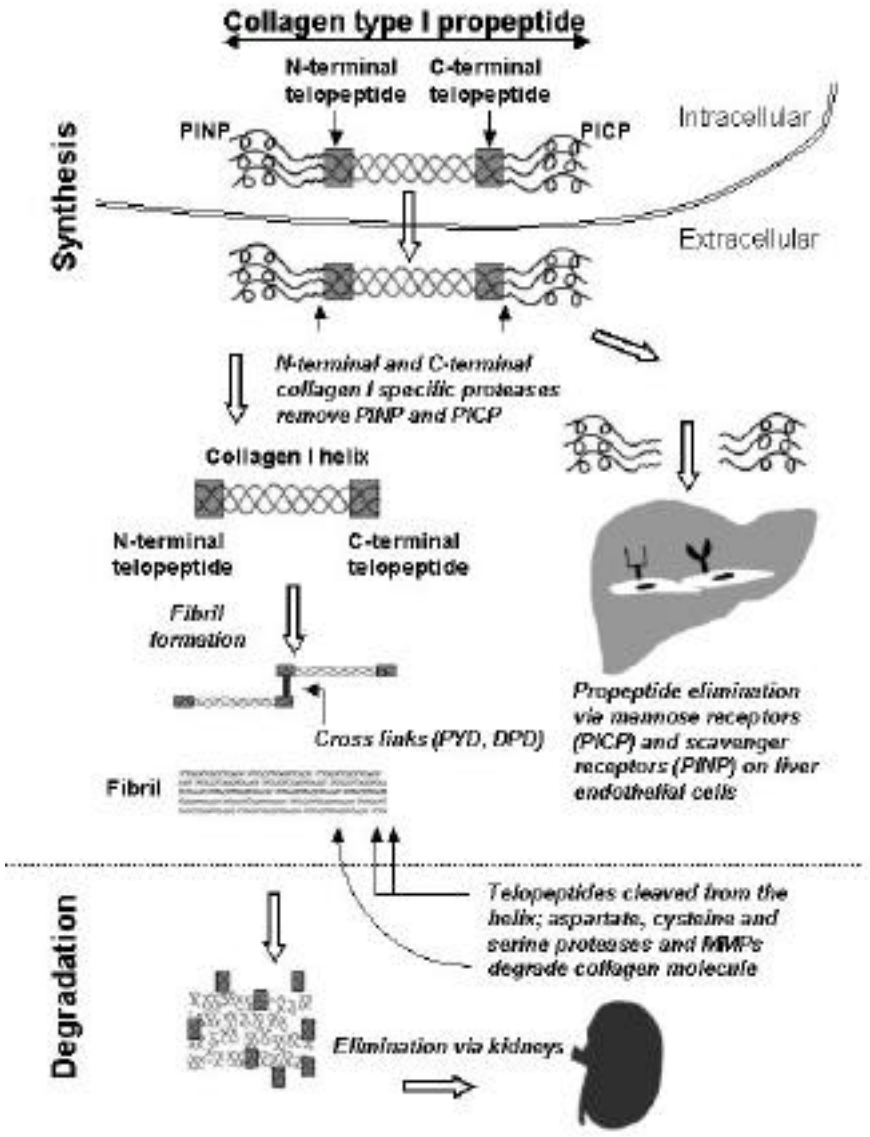

Fig. 7. Schematic representation of type I collagen synthesis and degradation.
Key: PINP: N-terminal propeptide of type I collagen; PICP: C-terminal propeptide of type I collagen; MMPs: matrix metalloproteinases; PYD: pyridinoline; DPD: deoxypyridinoline.

Telopeptides are small aminoacid sequences originating from the nonhelical ends of collagen molecules (10, 34). They are the most immunogenic parts of the type I collagen and play a crucial role in fibrillogenesis. They are primary sites of covalent cross-linking which stabilises the fibrils. N-terminal telopeptide is cleaved by cathepsin K (a cysteine protease) that is also involved in generating a C-terminal telopeptide [ICTP]. MMPs are not implicated in the release of either N-terminal telopeptide or ICTP (35). One molecule of telopeptide is composed, of either an N- or a C-terminal fragment from the alpha1 (I) chain, one helical segment from the alpha1 or alpha2 chain of collagen and a cross-linker (pyridinoline

(PYD) or deoxypyridinoline (DPD)). N-terminal and C-terminal telopeptides are small molecules (about 10 kDa) and are released into the circulation after the degradation of several mature collagen molecules. They are eliminated by the kidneys and tend to accumulate in patients with renal failure (36).

Old methods for evaluating collagen degradation include measurements of urine hydroxyproline and urine and serum cross-link analyses (PYD and DPD). Hydroxyproline estimation is limited by the fact that it is derived from any collagen type; only 10% of hydroxyproline is excreted in the urine and a proportion of it derives from PINP, which contains a collagenous domain. Similarly, assays of PYD and DPD cross-links are not specific for Col I and require tedious HPLC analysis (10). About ten years ago new immunological assays have been developed for the measurements of ICTP antigen (10). These assays are superior to the previous ones, as they are Col I specific and, because the ICTP antigen is cross-linked, it is known to derive from collagen fibrils.

Measurements of collagen type I metabolites in clinical studies in SSc

Several groups have measured the concentrations of collagen metabolites in the serum, plasma, bronchoalveolar lavage or blister fluid from SSc patients with heterogeneous results; correlation with disease subset, disease activity or organ involvement also varied between the studies (Table I). There was a wide range of values, especially in the dcSSc groups and for high concentrations (Figs. 1-3).

Also, there was no clear separation of the values between healthy controls and disease groups, with overlapping values in all studies. Although it is known that radioimmunoassay loses its sensitivity with low levels of radioactivity, which, in the case of collagen metabolite measurements using competitive assays, corresponds with high concentrations, other factors can also play a role. Clinical parameters such as skin score, disease subset, disease stage, number of organ-based complications or treatment(s) might have accounted for the variability of the results. For example, corticosteroids, commonly used in scleroderma, decrease the rate of collagen synthesis (2,3,37-39). Also, collagen telopeptides are cleared by the kidneys (10, 36) but the presence or absence of SSc-renal involvement was not discussed in the series of patients in most studies (Table I). Renal impairment is not rare in SSc: glomerular filtration rate was found reduced in about 60% of SSc patients and the prevalence of renal crisis is about 10% in patients with SSc (40). Correlation patterns between collagen metabolite levels and various clinical parameters were very heterogeneous between the studies. Diffuse SSc was most commonly associated with high [PINP], [PICP] or [ICTP]. Discordant results were found for the correlation between peptide levels and disease activity, organ involvement and the extension of skin involvement (Table I). Substantial heterogeneity, both among different patients at presentation and among diverse phases of the disease course in the single patient, is a basis for these inter-individual variabilities; however, lack of standardization in the evaluation of these epidemiological and clinical features of SSc patients in the studies analysed here makes the interpretation of these results difficult. Efforts by the scientific community to identify a core set of clinical and laboratory variables for organ assessment and for their inclusion in research studies should promote future production of high-quality data (26-27).

The results of our study had a similar trend to the studies reviewed in this work. Although [PINP] and [ICTP] were elevated in SSc, there was a large inter-individual variation in the values, especially in the dcSSc group, which also overlapped with healthy controls (Figs. 4, 5). Interestingly, we have found positive correlation between [PINP] and the age of onset and the duration of RP; these findings should be confirmed in a larger study. Collagen peptide levels were not increased in patients with ARP; lower levels of both [PINP] and [ICTP] were found in the PRP group. Therefore it does not appear that collagen peptides can be used as predictive markers of progression to CTD in patients with ARP.

The evidence for the connective tissue constantly undergoing remodelling is clearly demonstrated by the heterogeneity of the findings in the literature. Collagen turnover seems to be diverse among patients with scleroderma spectrum disorders and is related to disease course, severity and many phenotypical features and possibly genetic factors yet to be well characterised. The positive correlation between lung fibrosis and raised levels of collagen metabolite concentrations has been suggested as a reflection of lung involvement; similarly, extent of skin involvement and raised collagen concentrations were suggested as a reflection of disease activity. In our view this interpretation is not that straightforward: assessment of disease activity is complex and should be based on more than a few variables, as recently indicated (27).

Pitfalls of the clinical studies of collagen metabolites in SSc

SSc is a rare disease (with a prevalence of 12.4/100,000 in the UK (41)) and it is not surprising that most published results derived from small or very small studies. Also, the presence of "publication bias" indicates cautious interpretation of the results. The small number of patients studied did not permit an analysis with stratification by potential confounding factor(s). Various studies showed skewed data: stratification by disease category has not eliminated the heterogeneity of the results. Also, statistical subgroup analysis (based on the disease subset) lacks in precision and can be misleading, producing spurious results, which can be at risk of both type I or type II errors. However, demonstration of no effect should come from large-scale randomised trials.

Type III collagen

Type III collagen (2, 3) is not a subject of our analysis. Briefly, unlike Col I metabolites, N-terminal type III collagen peptide (PIIINP) has been proposed by the Consensus conference (26) as one of the candidate non-organ based laboratory markers in SSc. Although collagen type III has been considered more specific than type I collagen for connective tissue turnover due to its

absence from bone, it is not a specific marker for scleroderma (2). Recently, PIIINP has been used as an alternative to liver biopsy in the longitudinal monitoring of patients with psoriasis treated with methotrexate and therefore at risk of developing hepatic fibrosis (42).

Collagen metabolites as serial markers of disease?

It seems plausible that serum collagen metabolites should be best used in serial investigations of individual patient progression or response to treatment rather than as a diagnostic marker in series of patients. In diseases such as bone metastases [ICTP] (43), rheumatoid arthritis [ICTP] (44), multiple myeloma [ICTP] (45), Paget's disease [PINP] (46) or osteoporosis [PINP] [ICTP] (33,37), the efficacy of specific therapies have been monitored by measuring their serum levels in individual patients. Zachariae *et al*. (15) used PICP, ICTP and PIIINP to assess longitudinally the response of SSc patients to photophoresis; readings obtained every 4 weeks showed large variability. We might hypothesize that the assessment of serial measurements for single patients, possibly integrated as "area under the curve" could correlate with clinical variables such as disease activity or severity scores.

Conclusions

In summary, we have not found sufficient evidence that collagen type I peptides are good serological markers for the assessment of SSc activity or severity. Our study confirmed a large range of values for PINP, PICP and ICTP concentrations in a small well-characterised SSc population studied.

The available literature on Col I peptides in SSc is represented by few relatively small studies often lacking control groups and stratification for disease subset and showing large heterogeneity in the results and low specificity for collagen type I metabolites in SSc. Reliable demonstration of "no effect" should come from meta-analysis of large-scale randomised trials which is plausibly difficult to achieve in the field of rare diseases such as SSc. Standardised evaluation of SSc patients enrolled into the clinical studies proposed recently (27) should improve the quality of the results and enable published results to be combined for the purpose of integrating the findings.

We propose that longitudinal analysis of collagen metabolites in individual patients may show if these molecules can be of clinical use in the long-term follow-up of single SSc patients. Perhaps the use of Col I peptides will be justified in the evaluation of the response to the new therapeutic strategies which target fibroblasts in SSc.

References

1. BLACK CM, DENTON CP: Systemic sclerosis and related disorders in adults and children. *In* MADDISON PG, ISENBERG DA, WOO P and GLASS DN (Eds): *Oxford Textbook of Rheumatology*, 2nd ed., Oxford University Press, Inc. 1998; Vol. 2: 1217-47.
2. PROCKOP DJ, KIVIRIKKO KI, TUDERMAN L, GUZMAN NA: The biosynthesis of collagen and its disorders. First of two parts. *N Engl J Med* 1979; 301: 13-23.
3. PROCKOP DJ, KIVIRIKKO KI, TUDERMAN L, GUZMAN NA: The biosynthesis of collagen and its disorders. Second of two parts. *N Engl J Med* 1979; 301: 77-85.
4. LEROY EC: Increased collagen synthesis by scleroderma fibroblasts *in vitro*. *J Clin Invest* 1974; 54: 880-9.
5. SCHARFFETTER K, LANKAT-BUTTGEREIT B, KRIEG T: Localization of collagen mRNA in normal and scleroderma skin by *in situ* hybridization. *J Invest Dermatol* 1988; 90: 48-50.
6. GHOSH AK: Factors involved in the regulation of type I collagen gene expression: implication in fibrosis. *Exp Biol Med* 2002; 227: 301-14.
7. DENTON CP, ABRAHAM DJ: Transforming growth factor-beta and connective tissue growth factor: cytokines in scleroderma pathogenesis. *Curr Opin Rheumatol* 2001; 13: 506-11.
8. YOUNG-MIN SA, BEETON C, LAUGHTON R *et al.*: Serum TIMP-1, TIMP-2 and MMP-1 in patients with systemic sclerosis, primary Raynaud's phenomenon, and in normal controls. *Ann Rheum Dis* 2001; 60: 846-51.
9. RISTELI L, RISTELI J: Biochemical markers of bone metabolism. *Ann Med* 1993; 25: 385-93.
10. RISTELI J, ELOMAA I, NIEMI S, NOVAMO A, RISTELI L: Radioimmunoassay for the pyridinoline cross-linked carboxyterminal telopeptide of type I collagen. A new serum marker of bone collagen degradation. *Clin Chem* 1993; 39: 635-40.
11. PROCKOP DJ, SIERON AL, LI SW: Procollagen N-proteinase and procollagen C-proteinase. Two unusual metalloproteinases that are essential for procollagen processing probably have important role in development and cell signalling. *Matrix Biol* 1998; 16: 399-408.
12. TAHTELAR, TURPEINEN M, SORVA R, KARONEN SL: The aminoterminal propeptide of type I procollagen: evaluation of a commercial radioimmunoassay kit and values in healthy subjects. *Clin Biochem* 1997; 30: 35-40.
13. LAMMI L, RYHANEN L, LAKARI E *et al*.: Type III and type I procollagen markers in fibrosing alveolitis. *Am J Respir Crit Care Med* 1999; 159: 818-23.
14. SCHEJA A, HELLMER G, WOLLHEIM A, AKESSON A: Carboxyterminal type I procollagen peptide concentrations in systemic sclerosis: higher levels in early diffuse disease. *Br J Rheumatol* 1993; 32: 59-62.
15. ZACHARIAE H, BJERRING P, HEICKENDORFF L, MOLLER B, WALLEVIK K, ANGELO H: Photophoresis in systemic sclerosis: clinical and serological studies using markers of collagen metabolism. *Acta Derm Venereol (Stockh)* 1993; 73: 356-361.
16. KIKUCHI K, SATO S, KADONO T, IHN H, TAKEHARA K: Serum concentration of procollagen type I carboxyterminal propeptide in localized scleroderma. *Arch Dermatol* 1994; 130: 1269-72.
17. KIKUCHI K, IHN H, SATO S *et al*.: Serum concentration of procollagen type I carboxyterminal propeptide in systemic sclerosis. *Arch Dermatol Res* 1994; 286: 77-80.
18. KIKUCHI K, KADONO T, FUJIMOTO M, IHN H, SATO S, TAKEHARA K: Elevated procollagen type I carboxyterminal propeptide production in cultured scleroderma fibroblasts. *Dermatol* 1995; 190: 104-8.
19. HEICKENDORFF L, ZACHARIAE H, BJERRING P, HALKIER-SORENSEN L, SONDERGAARD K: The use of serologic markers for collagen synthesis and degradation in systemic sclerosis. *J Am Ac Dermatol* 1995; 32: 584-8.
20. SONDERGAARD K, HECKENDORFF L, RISTELI L *et al*.: Increased levels of type I and III collagen and hyaluronan in scleroderma skin. *Br J Dermatol* 1997; 136: 47-53.
21. HUNZELMANN N, RISTELI J, RISTELI L *et al.*: Circulating type I collagen degradation products: a new serum marker for clinical severity in patients with scleroderma ? *Br J Dermatol* 1998; 139: 1020-5.
22. VALAT C, DIOT P, DIOT E: Serum III procollagen, but not serum I procollagen, is predictive of lung involvement in systemic sclerosis. *Clin Exp Rheumatol* 1998; 16: 517-8.
23. DZIADZIO M, DENTON CP, SMITH R *et al*.: Losartan therapy for Raynaud's phenomenon and scleroderma: clinical and biochemical findings in a fifteen-week, randomized, parallel-group, controlled trial. *Arthritis Rheum* 1999; 42: 2646-55.
24. SCHEJA A, WILDT M, WOLLHEIM A, AKESSON A, SAXNE T: Circulating collagen metabolites in systemic sclerosis. Differences between limited and diffuse form and relationship with pulmonary involvement. *Rheumatology* 2000; 39: 1110-3.
25. ALLANORE Y, BORDERIE D, LEMARECHAL H, CHERRUAU B, EKINDJIAN OG, KAHAN A: Correlation of serum collagen I carboxyterminal telopeptide concentrations with cutaneous and pulmonary involvement in systemic sclerosis. *J Rheumatol* 2003; 30: 68-73.

26. MCHUGH NJ, DISTLER O, GIACOMELLI R, RIEMEKASTEN G: Non-organ based laboratory markers in systemic sclerosis. *Clin Exp Rheumatol* 2003; 21 (Suppl. 29): S32-S38.
27. VALENTINI G, MEDSGER TAJR, SILMAN AJ, BOMBARDIERI S: Conclusion and identification of the core set of variables to be used in clinical investigations. *Clin Exp Rheumatol* 2003; 21 (Suppl. 29): S47-56.
28. VALENTINI G, DELLA ROSSA S, BOMBARDIERI S *et al*.: European multicentre study to define disease activity criteria for systemic sclerosis. Identification of disease activity variables and the development of preliminary activity indexes. *Ann Rheum Dis* 2001;60:592-8.
29. APPENDIX: Manual of signs, symptoms, methods and procedures for the assessment of the patient with SSc. *Clin Exp Rheumatol* 2003; 21 (Suppl. 29): S49-56.
30. SUBCOMMITEE FOR SCLERODERMA CRITERIAOF THE AMERICAN RHEUMATISM ASSOCIATION DIAGNOSTICAND THERAPEUTIC CRITERIA COMMITTEE: Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980; 23: 581-90.
31. LEROY EC, BLACK CM, FLEISHMAJER R *et al*.: Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988; 15: 202-5.
32. MARICQ HR: Wide-field capillary microscopy. Technique and rating scale for abnormalities seen in scleroderma and related disorders. *Arthritis Rheum* 1981; 24: 1159-65.
33. HASSAGER C, RISTELI J, RISTELI L, JENSEN SB, CHRISTIANSEN C: Diurnal variation in type I collagen synthesis and degradation in healthy premenopausal women. *J Bone Miner Res* 1992; 7: 1307-11.
34. SORVA A, TAHTELA R, RISTELI J *et al*.: Familial high serum concentrations of the carboxy-terminal propeptide of type I procollagen. *Clin Chem* 1994; 40: 1591-3.
35. VITAGLIANO L, NEMETHY G, ZAGARI A, SCHERAGA HA: Structure of the type I collagen molecule based on conformational energy computations: the triple-stranded helix and the N-terminal telopeptide. *J Mol Biol* 1995; 247: 69-80.
36. URENA P, DE VERNEJOUL M: Circulating biochemical markers of bone remodelling in uremic patients. *Kidney Int* 1999; 55: 2141-56.
37. HASLING C, ERIKSEN EF, MELKKO J, RISTELI L, CHARLES P, MOSEKILDE L: Effects of a combined estrogen-gestagen regimen on serum levels of carboxy-terminal propeptide of human type I procollagen in osteoporosis. *J Bone Miner Res* 1991; 6: 1295-300.
38. AUTIO P, RISTELI J, KIISTALA U, RISTELI L, KARVONEN J, OIKARINEN A: Serum markers of collagen synthesis and degradation in skin diseases. Altered levels in diseases with systemic manifestation and during systemic glucocorticoid treatment. *Arch Dermatol Res* 1993; 285: 322-7.
39. WOLTHERS OD, HEUCK C, HANSEN M, KOLLERUP G: Serum and urinary markers of types I and III collagen turnover during short-term prednisolone treatment in healthy adults. *Scand J Clin Lab Invest* 1997; 57: 133-40.
40. KINGDON EJ, KNIGHT CJ, DUSTAN K *et al*.: Calculated glomerular filtration rate is a useful screening tool to identify scleroderma patients with renal impairment. *Rheumatology (Oxford)* 2003; 42: 26-33.
41. HOPKINSON ND, BURNS AM: The frequency of progressive systemic sclerosis in a non-industrialised, semi-rural population in Southern England. *Rheumatology* 2000; 39S: 253.
42. ZACHARIAE H, HEICKENDORFF L, SOGAARD H: The value of amino-terminal propeptide of type III procollagen in routine screening for methotrexate-induced liver fibrosis: a 10-year follow-up. *Br J Dermatol* 2001; 144: 100-3.
43. KOIZUMI M, OGATAE: Bone metabolic markers as gauges of metastasis to bone: a review. *Ann Nucl Med* 2002; 16: 161-8.
44. HAKALAM, RISTELI L, MANELIUS J, NIEMINEN P, RISTELI J: Increased type I collagen degradation correlates with disease severity in rheumatoid arthritis. *Ann Rheum Dis* 1993; 52: 866-9.
45. ELOMAA I, VIRKKUNEN P, RISTELI L, RISTELI J: Serum concentration of the cross-linked carboxyterminal telopeptide of type I collagen [ICTP] is a useful prognostic indicator in multiple myeloma. *Br J Cancer* 1992; 66: 337-41.
46. PONS F, ALVAREZ L, PERIS P *et al*.: Quantitative evaluation of bone scintigraphy in the assessment of Paget's disease activity. *Nucl Med Commun* 1999; 20: 525-8.