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Serum levels of type IIA procollagen amino terminal propeptide (PIIANP) are decreased in patients with knee osteoarthritis and rheumatoid arthritis.

J.-C. Rousseau PhD†*, Y. Zhu PhD‡, P. Miossec MD, PhD†, E. Vignon MD§, L. J. Sandell PhD‡, P. Garnero PhD†,¶ and P. D. Delmas MD, PhD†

† *INSERM Unit 403, Pavillon F, Hôpital E. Herriot, Lyon, France*

‡ *Department of Orthopaedic Surgery, Washington University School of Medicine at Barnes-Jewish Hospital, St Louis, MO, USA*

§ *Hôpital Sud, France*

¶ *SYNARC, France*

Summary

Objective: The aim of this study was to develop a specific immunoassay for PIIANP and measure its serum concentration in healthy controls and in patients with osteoarthritis (OA) and rheumatoid arthritis (RA). In addition, we investigated circulating forms recognized by antiserum IIA in pools of serum from healthy adults, patients with OA and patients with RA.

Design: Using as immunogen and standard the recombinant human Glutathione S-Transferase (GST)-exon 2 fusion protein of type II collagen, we developed a competitive polyclonal antibody-based ELISA. We compare serum PIIANP levels in 43 patients with knee OA (23 women and 20 men; mean age: 62.6±9.6 yr), 63 women with RA (mean age: 54±16 yr) and 88 healthy controls (67 women, mean age: 53±13 yr and 21 men, mean age: 63±7 yr). We randomly selected serum in each group for analyze circulating forms.

Results: The immunoassay we developed demonstrated adequate intra and inter-assay precision (CV<10%) and dilution recovery (mean: 96%), allowing accurate measurements of serum PIIANP from 1.13 to 40 ng/ml. No significant cross-reactivity of the ELISA was observed with purified intact human procollagen type I N-propeptide, circulating thrombospondin and von Willebrand factor, proteins which exhibit significant sequence homology with PIIANP. Western blot analysis showed that antiserum IIA recognized two circulating immunoreactive forms of approximately 80 and 100 KDa respectively in serum from healthy adults, patients with OA and RA but also in a pool of synovial fluids from patients with OA.

Serum PIIANP levels were markedly decreased in patients with knee OA (12.0±3.2 vs 25.8±7.5 ng/ml for OA and controls respectively, $P<0.0001$) and RA (14.1±2.5 ng/ml vs 21.7±7.6 ng/ml for RA and controls respectively, $P<0.0001$). In patients with RA, serum PIIANP levels were higher in those taking low-dose prednisone compared to non-users (15.0±2.4 vs 13.5±2.4 ng/ml, $P<0.05$).

Conclusions: We have developed the first specific immunoassay for serum PIIANP which exhibits adequate technical performances. This assay detects specifically two immunoreactive forms both in healthy adults and patients with arthritis and does not cross react with other proteins with sequence homology with PIIANP. Levels of PIIANP were significantly decreased in patients with knee OA and RA suggesting that type IIA collagen synthesis may be altered in these arthritic diseases. The measurement of type IIA collagen synthesis with this new molecular marker may be useful for the clinical investigation of patients with joint diseases.

Key words: Type II collagen, N-propeptide IIA, Osteoarthritis, Rheumatoid arthritis.

Introduction

Destruction and remodelling of articular cartilage are features of both rheumatoid arthritis (RA) and osteoarthritis (OA)[1]. However, in such diseases, catabolic processes are combined with alterations in synthesis[2–9]. Thus, it is important to gain access to sensitive tools for assessing cartilage synthesis, in addition to degradation markers.

In this aim, we focused on the synthesis of type II collagen, the most abundant cartilage protein representing 80–95% of the total collagen content. Like fibril-forming collagens I, III, V and XI, type II collagen is synthesized as a procollagen form[10]. These procollagens are secreted from the cell into the extracellular matrix where extension propeptides (N-propeptide and C-propeptide) are removed by specific proteinases before the mature molecules are incorporated into fibrils in matrix[10]. Type II procollagen is synthesized in two splice forms, type IIA and type IIB. Type IIA contains an additional 207 base pair exon (exon 2) encoding the 69 amino acid cysteine-rich domain of the N-propeptide (Fig. 1).

Serum levels of these propeptides can thus be used as specific markers of collagen synthesis and assays for type I collagen N and C-propeptides have proven to be of significant utility for the clinical investigation of metabolic bone diseases including osteoporosis[11].

*Address reprint requests to: J.C. Rousseau, INSERM Unit 403, Pavillon F, Hôpital E. Herriot, 69437 Lyon Cedex 03, France. Tel: +33-4-72-11-74-31; Fax: +33-4-72-11-74-32; E-mail: rousseau@lyon.inserm.fr

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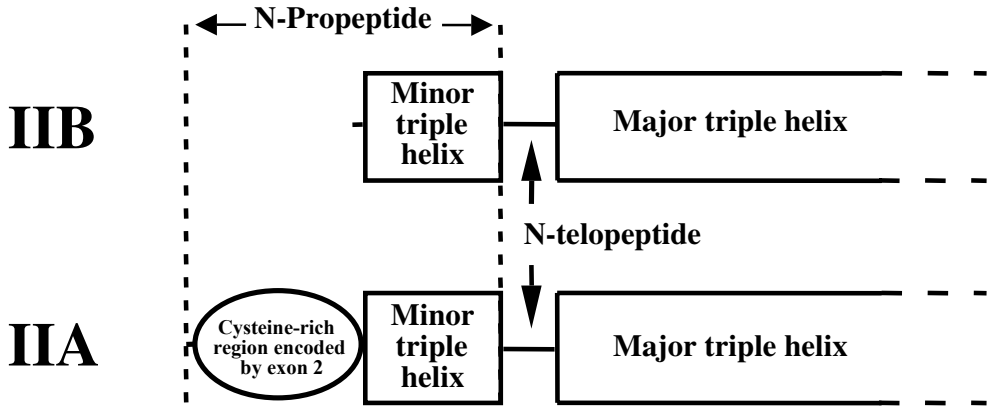

Fig. 1. Schematic representation of type IIA/IIB procollagen. Type II collagen is expressed in two forms of IIA and IIB, as the result of alternative splicing of exon 2 in NH2-propeptide region. Polyclonal antibody IIA raised against the protein part encoded by exon 2 recognizes specifically IIA N-propeptide.

Currently, only assays for the C-propeptide of type II procollagen (PIICP also referred to as chondrocalcin or pCOL-IIC or CPII) have been described to assess type II collagen synthesis[12,13]. Serum PIICP levels have been shown to be increased in patients with RA compared to controls whereas patients with OA exhibited decreased levels[14]. However, there was no data concerning the measurement of the N-propeptide in body fluids in spite of major advances in the knowledge of the IIA form expression that have been achieved in the last few years. This long form was known to be synthesized mainly by skeletal progenitor cells and non cartilaginous embryonic tissues[15–17] but also by a distinct population of cells in osteophytes[18] and chondrocytes in the fracture callus[19] while the IIB form was know to be mainly expressed in adult cartilage[15].

Recently, type IIA procollagen was shown to be re-expressed by adult articular chondrocytes in OA cartilage, suggesting the potential reversion of the cells to a chondroprogenitor cellular phenotype in this disease[20]. Alternatively, type IIA procollagen and type X collagen were shown to be expressed by chondrocytes in the upper zone of moderately and severely affected human OA cartilage suggesting that articular chondrocytes undergo hypertrophic changes in osteoarthritis cartilage[21]. Taken together, these results suggested that IIA form of type II collagen play an important but not clearly established role in the pathogenesis of osteoarthritis.

We anticipated that the propeptide of type IIA procollagen (PIIANP), arising from the maturation of type IIA procollagen, could be found in serum and be used as a specific marker of type IIA procollagen synthesis in articular diseases.

The aim of this study was to develop an immunoassay for PIIANP and investigate serum PIIANP levels in healthy adults and patients with OA or RA and characterize circulating forms recognized by antiserum IIA.

Methods

HEALTHY SUBJECTS

Healthy subjects included 67 women (mean age: 53±13 yr) and 21 men (mean age: 63±7 yr). Healthy women and men were randomly selected from two large population-based cohorts involved in prospective studies on the determinants of bone loss in women (OFELY study, 1039 female volunteers, 31–89 yr of age)[22] and men (MINOS study, 842 healthy male volunteers, 50–85 yr of age)[23]. All healthy subjects came from the same region of France as the patients with knee OA and RA. None of the control subjects had evidence of OA assessed by clinical examination, questionnaire and X-ray films of the thoracic and lumbar spine and were without disease or treatment that could interfere with bone metabolism including hormone replacement therapy in postmenopausal women.

PATIENTS WITH KNEE OSTEOARTHRITIS

The study group included 43 outpatients suffering from knee OA (23 women and 20 men; mean age: 62.6±9.6 yr). They consulted in the department of Rheumatology of the Centre Hospitalier Lyon Sud (Lyon, France). All patients fulfilled the American College of Rheumatology criteria for primary knee OA[24]. All patients had chronic daily pain of the knee for at least 3 months (median: 10 yr) and radiographic evidence of OA with joint space narrowing (JSN) when using the posteroanterior view of the knees flexed at 30° (schuss view). Patients presenting an advanced stage of OA with a minimum joint space width (JSW) lesser than 1 mm were excluded. All women were postmenopausal and all patients were without treatment that could interfere with bone metabolism including estrogen replacement therapy, dilantin, thyroid replacement therapy and diuretics. Pain and physical function were assessed by the Western Ontario and McMaster Universities multifunctional (WOMAC)[25] index, using a visual analogue scale as a grading system. A subset of 53 healthy age and sex-matched subjects (32 women and 21 men, mean age: 62.4±7.7 yr) was used as controls for patients with knee OA.

PATIENTS WITH RHEUMATOID ARTHRITIS (RA)

Sixty three women suffering from RA were studied (mean age: 54±16 yr; median disease duration: 6 yr). All patients fulfilled the American College of Rheumatology criteria for RA disease revised in 1987[26]. Twenty four were on low-dose corticosteroids (<10 mg/day prednisone). Patients with renal insufficiency were excluded. A subset of 67

healthy age-matched women (mean age: 53±13 yr) was used as controls for patients with RA.

COLLECTION AND PROCESSING OF BLOOD AND SYNOVIAL FLUID SAMPLES

In all healthy adults, patients with knee OA and patients with RA, blood samples were collected fasting in the morning before 10 am. After centrifugation at 1000 ***g*** for 10 min, serum were stored at –70°C until used. Synovial fluids were collected in sterile tubes and stored at –20°C. Before used, synovial fluids were centrifuged at 15 000 ***g*** for 10 min to remove all cells and debris**.**

RECOMBINANT PROTEIN AND SPECIFIC ANTIBODIES

Recombinant fusion protein GST-exon 2 and polyclonal antibody anti-IIA were obtained from Dr. L. Sandell laboratory (St Louis, MO) and have previously been described[20]. The specificity of polyclonal antibody IIA was demonstrated by Western blot against recombinant protein (prior and after cleavage with thrombin) and against IIA procollagen from medium culture of day 54 human fetal ribs[27].

ELISA FOR PIIANP

Maxisorp microtiter plates (Nunc, Roskilde, Denmark) were used throughout the study. Each well was coated overnight, without shaking, at +4°C with 100 µl of recombinant fusion protein GST-exon 2 (10 ng/ml) in PBS. After coating, wells were saturated 2 h at room temperature with 200 µl of PBS, BSA 1% (Sigma, St Louis, MO) on a microwell plate rotation apparatus (40 rpm). Microwells were washed with 200 µl of PBS, 1% BSA, 0.05% Tween (four times between each step of the protocol). After washing, 100 µl of standards (recombinant fusion protein GST-exon 2: 0 (PBS/BSA/Tween), 2.5, 5, 7.5, 10, 15, 20, 30, 40 ng/ml) or serum (samples and controls) were incubated with shaking 4 h at room temperature with 100 µl/well of IIA antiserum (1/1650 final). Serum must be centrifuged 15 min at +4°C (14 000 g) before used. After washing, the peroxidase conjugated anti-rabbit (diluted: 1/8000, Sigma, St Louis, MO) was added (100 µl/well) and incubated at room temperature for 1 h with shaking. After washing, 100 µl H_2O_2/Tetramethylbenzidine substrate-indicator solution (Sigma, St Louis, MO; 1 tablet in 10 ml of 0.05 M phosphate-citrate buffer, pH=5 with 2 µl H_2O_2) was added to each well. After incubation at room temperature for 30 min with shaking, the colour reaction was stopped by addition of 100 µl 2M H_2SO_4/well and the optical density was read at 450 nm in a Dynatech MR 7000. Each sample was run in duplicate.

PURIFICATION OF INTACT PROCOLLAGEN TYPE I N-PROPEPTIDE (PINP)

The intact *in-vivo*-cleaved PINP was purified from human ascitic fluids as described previously[28]. The purity of PINP was confirmed by SDS-polyacrylamide gel electrophoresis and amino acid sequencing after amino-terminal deblocking. Amino terminal pyroglutamate residues were removed essentially as described by Podell and Abraham[29].

GST-EXON 2 AFFINITY CHROMATOGRAPHY

Recombinant GST-exon 2 protein (0.5 mg) was coupled on a HiTrap NHS-activated HP column (1 ml, Amersham Pharmacia) in 0.2 M $NaHCO_3$, 0.5 M NaCl pH 8.3 for 30 min at room temperature (coupling efficiency: 89%). After washing out the non-specifically bound ligands and deactivation of any excess active groups, antiserum IIA (40 µl diluted in 1 ml 20 mM sodium phosphate pH 7) was applied to the column for 5 h at room temperature. Unbound material was retrieved and the column was washed with 1 ml 20 mM sodium phosphate. Specific antibodies were eluted with 0.1 M glycine pH 2.7 with 1 M Tris added in the collected fractions containing IgG.

CHARACTERIZATION OF CIRCULATING IMMUNOREACTIVE FORMS RECOGNIZED BY ANTISERUM AGAINST IIA

Pools of randomly selected serum from 10 healthy donors, 10 patients with OA and 10 patients with RA and a pool of randomly selected synovial fluids of 10 knee osteoarthritis patients were diluted 10 times in PBS. Serum and synovial fluid pools were boiled 5 min and resolved (15 µl/well) in a 5% Tris/Tricine gel under reducing conditions. After transfer to a nitrocellulose membrane overnight at 30 V in 15 mM Tris, 120 mM Glycine, pH 8.3, containing 20% methanol, membranes were blocked with 5% non fat milk in PBS with shaking. The following antibodies were applied at the appropriate dilution in PBS with 1% non fat milk overnight at +4°C with shaking: IIA antiserum and unbound material after GST-exon 2 affinity chromatography at 1/1000; non immune serum at 1/400; anti-von Willebrand factor (Dako, Carpinteria, CA) at 1/400 and anti-thrombospondin (NeoMarkers, Fremont, CA) at 1/400. After washing, membranes were incubated with anti-rabbit (Jackson ImmunoResearch) peroxidase conjugate in PBS, 1% non fat milk for one hour at room temperature with shaking. After further washing, antibody binding was detected using Western Lightning Chemiluminescent Reagent kit (PerkinElmer, Boston, MA).

STATISTICAL ANALYSIS

Comparison of serum PIIANP levels between healthy controls and patients with OA or RA was assessed by the non-parametric Mann–Whitney U test. The correlation between PIIANP and age was assessed by Spearman rank correlation.

Results

ANALYTICAL PERFORMANCES OF THE PIIANP ELISA

Using as immunogen and standard the recombinant fusion protein GST-exon 2, we developed a competitive polyclonal antibody-based ELISA and a typical standard curve is shown on Fig. 2. Intra and inter-assay precisions were under 10% (Table IA). Mean dilution recovery and mean spiking recovery were 96% and 128% respectively (Table IB and Table IC). The detection limit defined as the concentration 2SDs above that of the lowest calibrator was 1.1 ng/ml. We found no significant cross-reactivity of this assay with purified intact human PINP (data not shown). The stability of serum PIIANP was tested over three freeze-thaw cycles (Table ID). After 2 cycles, no changes of serum PIIANP concentrations were noted. However at the third cycles, serum PIIANP decreased by a mean of 26%.

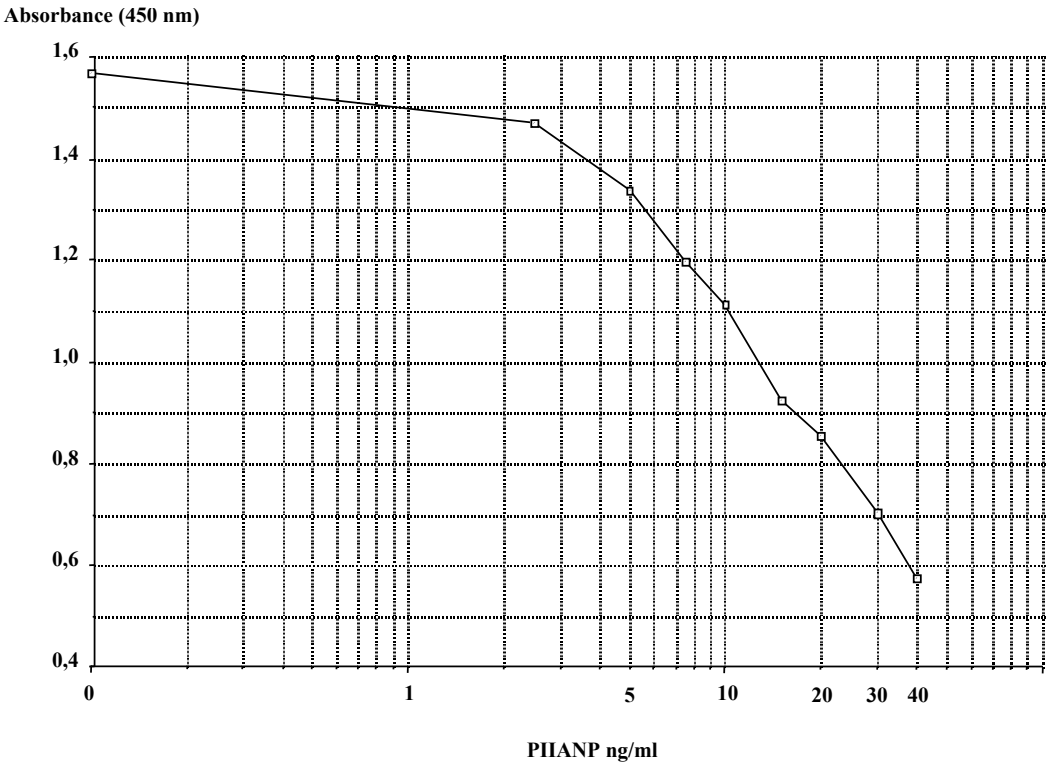

Fig. 2. Standard curve produced by duplicate measurements of the mean optical density at 450 nm of 9 PIIANP standards.

CHARACTERIZATION OF CIRCULATING IMMUNOREACTIVE FORMS RECOGNIZED BY ANTISERUM AGAINST IIA

Western blot analysis showed that IIA antiserum recognizes two immunoreactive bands with molecular weights of 80–100 KDa in the serum of healthy adults, patients with OA and patients with RA (Fig. 3, lanes 1,2,3). Analysis of synovial fluids from patients with knee OA showed the same two bands which were detected in serum (Fig. 3, lane 4). The specificity of antibody binding was verified by preabsorbing antiserum IIA with recombinant GST-exon 2 protein (affinity column) which resulted in the loss of immunoreactivity (Fig. 3, lanes 5,6,7,8). We then verified that these 2 bands were not recognized by anti-thrombospondin and anti-von Willebrand factor antibodies (Fig. 3, lanes 9,10) and additionally that antiserum IIA did not recognize bands corresponding to thrombospondin and von Willebrand factor (Fig. 3, compare lanes 1,2,3,4 with lanes 9,10). As negative control, a non-immune serum showed no cross-reactivity with the two bands recognized specifically by IIA antiserum (Fig. 3, lane 11).

AGE-RELATED CHANGES OF SERUM PIIANP IN HEALTHY ADULTS (TABLE II)

Serum PIIANP levels were measured in 88 healthy subjects including 21 men (mean age: 63±7 yr), 29 pre-menopausal women (mean age: 39±4 yrs) and 38 post-menopausal women (mean age: 63±7 yrs). Serum PIIANP increased significantly with age in women (r=0.63; P<0.001) with a significant difference between pre and post menopausal women (P<0.001). We found no significant difference in serum PIIANP between age-matched men and women (P=0.17).

CONCENTRATIONS OF SERUM PIIANP IN PATIENTS WITH KNEE OA AND PATIENTS WITH RA

The mean serum concentration of PIIANP was significantly decreased (P<0.0001) in patients with knee OA (12.0±3.2 ng/ml vs 25.8±7.5 ng/ml, Fig. 4a) and RA (14.1±2.5 ng/ml vs 21.7±7.6 ng/ml, Fig. 4b) compared to sex and age-matched healthy controls. When using the mean – 1 SD of the controls as the lower limit of the normal range, 97% and 57% of patients with knee OA and RA respectively, had serum levels below that limit. The distribution of PIIANP serum levels was more narrow in patients with OA or RA than in healthy controls (Fig. 4a and b).

In patients with RA treated with low-dose corticosteroids, serum PIIANP was slightly but significantly higher than in untreated patients (15.0±2.4 vs 13.5±2.4 mg/ml, P<0.05).

Table IA
Intra and inter-assay precisions

Sample	Serum PIIANP (ng/ml)	CV (%)
Intra-assay variability was tested by measuring 3 samples 10 times in the same run		
A	9.6	7.4
B	12.8	8.5
C	25.4	9.0
Inter-assay variability was tested by measuring 3 samples in duplicate in 5 different runs		
Sample	Serum PIIANP (ng/ml)	CV (%)
D	9.0	8.6
E	27.0	6.0
F	36.3	1.5

Table IB
Dilution recovery. Samples with high levels were serially diluted with ELISA buffer

Sample	Dilution	Serum PIIANP expected (ng/ml)	Serum PIIANP measured (ng/ml)	Recovery (%)
G	Undiluted	–	25.9	–
	80%	20.7	21.1	102
	60%	15.5	13.3	86
	40%	10.3	9.5	92
H	Undiluted	–	24.2	–
	80%	19.3	14.0	72
	60%	14.5	13.9	96
	40%	9.7	12.4	128
I	Undiluted	–	30.4	–
	80%	24.3	23.2	95
	60%	18.2	17.8	98
	40%	12.2	12.1	99

Table IC
Spiking recovery. Known quantities of recombinant GST-exon 2 protein (50 µl) were added to human serum (50 µl)

Sample	Standard	PIIANP expected (ng/ml)	PIIANP measured (ng/ml)	Recovery%
J	Undiluted	–	25.8	–
	+5 ng/ml	15.4	16.8	109
	+10 ng/ml	17.9	22.8	127
K	Undiluted	–	14.3	–
	+2.5 ng/ml	8.4	10.3	123
	+10 ng/ml	12.1	19.8	164
L	Undiluted	–	10.6	–
	+5 ng/ml	7.8	8.5	109
	+10 ng/ml	10.3	14.1	137

Table ID
Stability of serum PIIANP. Samples were tested after 1, 2 and 3 freeze/thaw cycles

Sample	freeze/thaw cycle	Serum PIIANP (ng/ml)
M	1	20.2
	2	23.4
	3	14.5
N	1	26.9
	2	28.3
	3	20.8
O	1	32.5
	2	30.5
	3	24.7

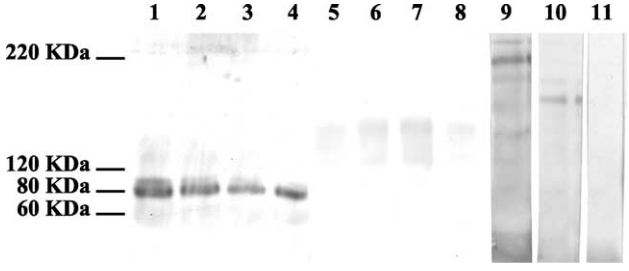

Fig. 3. Western blot analysis. Pools of randomly selected serum from healthy donors (lanes 1,5,9,10,11), patients with OA (lanes 2,6), patients with RA (lanes 3,7) and a pool of randomly selected synovial fluids of knee osteoarthritis patients (lanes 4,8) were probed with the following antibodies: anti-IIA (lanes 1,2,3,4), anti-IIA pre-absorbed with PIIANP (lanes 5,6,7,8), anti-von Willebrand factor (lane 9), anti-thrombospondin (lane 10) or non-immune serum (lane 11).

Discussion

In that study, we developed for the first time an immunoassay for serum PIIANP and investigated its value to detect abnormalities of systemic type IIA collagen synthesis in patients with OA or RA. The present study showed that the PIIANP ELISA demonstrated adequate technical performances with respect to precision (intra and inter assay variation <10%), dilution of serum samples and specificity, suggesting that this assay allows accurate measurement of serum PIIANP levels.

The region encoded by exon 2, specific of the IIA form of the N-propeptide of type II collagen, is absent in the IIB form. It has the highest sequence homology (68%) with the interstitial collagen chains α1(I), but also present significant homologies with the extracellular matrix proteins thrombospondin 1 and 2 and von Willebrand factor C domain[27]. Consequently, we analyzed the specificity of the PIIANP polyclonal antibody with these circulating proteins.

We found no significant cross-reactivity of the PIIANP antiserum with purified intact human N-propeptide of type I collagen (PINP) composed of two pro-α1(I) chains and one pro-α2(I) chain, in agreement with previously reported findings[27] and with circulating thrombospondin and von Willebrand factor. Thus, these data suggest that indeed the ELISA we have developed is specific for circulating PIIANP.

In Western blot, circulating PIIANP was present in at least two immunoreactive forms with molecular weights of about 80 and 100 KDa respectively, in serum from healthy adults and patients with RA but also in serum and synovial fluid from patients with OA. Antiserum IIA pre-incubated with recombinant GST-exon 2 protein did not anymore recognize these two bands indicating that these circulating immunoreactive forms are indeed related to PIIANP. The molecular weights of these two circulating forms are however higher than the estimated molecular weight of the

Table II
Serum PIIANP levels in healthy men and women

	Men (*N*=21)	Pre-menopausal women (*N*=29)	Post-menopausal women (N=38)
Age (yrs)	63±7	39±4	63±7
Mean±SD (ng/ml)	27.7±7	17.6±5	24.8±7

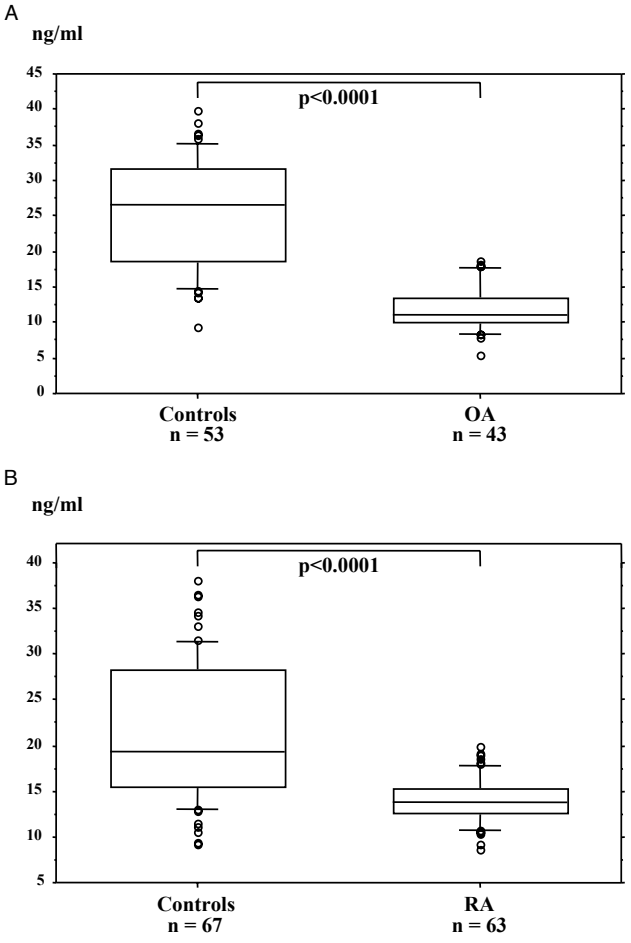

Fig. 4. Box plots distribution of serum PIIANP levels in healthy controls and in patients with OA (A) or RA (B). From the bottom up, the box indicates the 25th, 50th (median) and 75th percentiles, while the bars indicate the 10th and 90th percentiles respectively.

monomeric N-propeptide IIA, i.e. 15–20 KDa. It is unlikely that these two forms correspond to PIIANP bound to unknown serum proteins because the experiments were performed under reducing conditions on boiled samples, a procedure which would have disrupted these aggregates. Alternatively, these large molecular forms could be the trimeric N-propeptide IIA or multiple propeptides which include triple helical regions bound each other in the N-telopeptide region by cross-linking molecules such as pyridinoline[30], although further experiments will be necessary to confirm that hypothesis.

The significant levels of PIIANP measured in the serum of controls may be surprising because it is believed that type IIA collagen is predominantly expressed in embryonic and not mature normal tissues[15–17]. Although immunohistochemistry studies were unable to detect type IIA procollagen in mature cartilage of healthy controls even after extensive protease treatments[20], animal studies showed however mRNA expression of type IIA procollagen in culture of adult bovine chondrocytes[31] and in articular cartilage of healthy dogs[32] and mice[33]. In addition, detectable concentrations of type IIA procollagen protein were shown in the pellets of adult bovine chondrocytes[34]**.** Apart from articular cartilage, type IIA may arise in part from other body areas such as adult vitreous or intervertebral disc tissues[35–37]. It remains however unclear whether these non-articular cartilage tissues continue to synthesize type IIA in adults. Additional studies are needed to elucidate the relative contribution of articular cartilage and others tissues to the global serum levels of PIIANP.

In OA patients, we found decreased levels of serum PIIANP compared to controls which is in apparent contradiction with the observed reexpression of type IIA collagen by adult articular chondrocytes in OA cartilage[20]. One of the reasons for this discrepancy could be related to the fact that serum PIIANP levels in OA may reflect systemic alterations of type IIA collagen metabolism which may not be related to abnormalities of cartilage metabolism of the signal joint. This hypothesis has also been suggested to explain the decreased serum PIICP levels observed in patients with OA[14], which is likely to reflect synthesis of both IIA and IIB collagens. The clinical significance of decreased serum PIIANP and PIICP in OA should be interpreted with caution. Indeed, measurement of PIICP in cartilage tissue extract[14] and synovial fluid[6] were shown to be increased in patients with knee OA compared to healthy controls. However, these increased levels are mainly found in early and mid stages of the disease, and with advancing OA, synovial fluid levels of PIICP progressively decrease probably due to decreased cartilage mass and chondrocytes end stage failure. In our study, we only analyzed serum and thus it remains to be determined whether the conflicting data between synovial fluid and serum PIICP levels will also apply to PIIANP. Nevertheless the findings of our present study are likely to be of clinical relevance for the investigation of patients with OA. Indeed, we recently confirmed decreased serum PIIANP levels in another independent population of patients with knee OA, and lower levels were associated with a more rapid progression of cartilage damage assessed prospectively over one year by either radiography or chondroscopy of the signal knee[38]. Our study showed that serum PIIANP levels are also markedly decreased in RA patients compared to controls. Conversely, levels of serum PIICP were found to be increased in serum[14] without significant differences between aggressive and non-aggressive disease[13]. The reasons of this discrepancy between serum levels of PIIANP and PIICP in patients with RA is unclear, but may result from differences in populations characteristics, our patients being characterized by a long standing disease. Alternatively, these differences may also result from the fact that PIICP reflects the synthesis of both IIA and IIB procollagen molecules whose regulation may differ in RA. It seems unlikely that the decrease of PIIANP in RA was the result of increased proteinase degradation of propeptide at the tissue level. Indeed, Fukui[30] showed recently that the

antibody raised against IIA which was used in this study still recognizes recombinant N-propeptide IIA even after cleavage by the different MMPs know to be involved in the destruction of articular cartilage.

Decreased PIIANP levels in RA are more likely to reflect in part the inhibitory effects of inflammatory cytokines such as interleukin-1 and tumour necrosis factor-α on type II collagen synthesis[39–41]. In support of this hypothesis, we found that patients treated with steroids, which are potent anti-inflammatory agents, are characterized by higher serum PIIANP levels compared to non-users. Thus serum PIIANP could potentially be a useful marker to investigate the effects of drugs aimed at increasing the capacity of cartilage repair.

In summary, we have developed the first ELISA for serum procollagen type IIA N-propeptide and characterized its circulating immunoreactive forms in humans. Serum PIIANP levels were decreased in patients with OA or RA suggesting that this new molecular marker may be useful to investigate the alterations of cartilage metabolism probably in combination with markers of type II collagen degradation as recently reported[38] and also to monitor efficacy of disease modifying therapy, especially anabolic treatments.

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