

**MARKERS OF COLLAGEN
METABOLISM IN THE
ASSESSMENT OF
RHEUMATOID ARTHRITIS.**

**SARI
ÅMAN**

Department of Internal Medicine

With special reference to cross-linked
carboxyterminal telopeptide of type I collagen (ICTP)

1999



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Abstract

The purpose of the present study was to investigate the value of different markers of collagen metabolism in assessing the disease process and further disease progression in patients with inflammatory arthritis, mainly rheumatoid arthritis (RA).

In a series of 59 patients with RA and knee joint effusion, the level of synovial fluid (SF) carboxyterminal telopeptide of type I collagen (ICTP), a marker for type I collagen degradation, was associated with the Larsen's grade of the corresponding joint ($p < 0.001$). The mean SF concentrations of ICTP and the markers of type I and III collagen synthesis (the aminoterminal propeptides of type I and III procollagens, PINP and PIIINP) were higher than those in serum. In addition, the levels of these markers correlated with each other in both serum and SF ($p < 0.001$ in both occasions).

In a three-year follow-up study of 44 RA patients from the abovementioned series and 11 patients with other chronic arthritides, a high SF ICTP level turned out to reflect accelerated radiological progression in the assessed joint ($p < 0.05$). Contrary to this, the results on the SF leukocyte level were contradictory.

In a population-based cross-sectional series of 90 patients with advanced RA, elevated baseline serum ICTP levels discriminated the patients with a need for total joint replacement surgery from those with milder disease ($p = 0.001$) in a three-year follow-up.

In a study of 52 patients with recent onset RA, the changes in BMD during a two-year follow-up were not associated with the serum level of markers of type I collagen metabolism. In this series, however, the decrease in BMD (measured in the spine and the femoral neck) was smaller than earlier reported.

In a three-year follow-up series of 63 patients with early RA, the patients with simultaneously elevated serum ICTP ($> 4.6 \mu\text{g/l}$) and RF positivity at baseline, had an increased risk for progressive joint disease (an increase in Larsen's score > 20 as assessed from radiographs of hands and feet) with an odds ratio of 9.1 (95% CI 2.5 to 32.9). A risk profile of this kind may be useful in early disease assessment to identify the patients who will need the most active drug therapy.

Keywords: synovial fluid analysis, osteoporosis, PINP, PIIINP

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Abbreviations

ACR:	American College of Rheumatology
ARA:	American Rheumatism Association
BMD:	bone mineral density
BMI:	body mass index
BSP:	bone sialoprotein
BUA:	broadband ultrasonic attenuation
CI:	confidence interval
COMP:	cartilage oligomeric matrix protein
CrossLaps™:	carboxyterminal telopeptide (synthetic peptide EKAHDGGR) of type I collagen
CRP:	C-reactive protein
DPYD:	deoxypyridinoline (correct name lysylpyridinoline)
DPA:	dual photon absorptiometry
DXA:	dual x-ray absorptiometry
ELISA:	enzyme-linked immunosorbent assay
ESR:	erythrocyte sedimentation rate
FN:	femoral neck
HAQ:	Health Assessment Questionnaire
HPLC:	high-pressure liquid chromatography
ICTP:	carboxyterminal telopeptide (authentic, trivalently cross-linked peptide) of type I collagen
IP:	interphalangeal
LS:	lumbar spine
MMP:	matrix metalloproteinase
NSAID:	non-steroidal anti-inflammatory drug
NTx:	aminoterminal telopeptide (authentic, trivalently cross-linked peptide) of type I collagen

OR:	odds ratio
PICP:	carboxyterminal propeptide of type I procollagen
PINP:	aminoterminal propeptide of type I procollagen
PIINP:	aminoterminal propeptide of type III procollagen
PYD:	pyridinoline (correct name hydroxylslypyridinoline)
QCT:	quantitative computed tomography
RA:	rheumatoid arthritis
RF:	rheumatoid factor
S:	serum
SAARD:	slow-acting anti-rheumatic drug
SPA:	single photon absorptiometry
SD:	standard deviation
SF:	synovial fluid
TJRS:	total joint replacement surgery
T score:	BMD as a fraction of the SD of the mean of the previously published normal values for young adults (20-40 yr) of the same sex
VOS:	velocity of sound
WBC:	white blood cell
Z score:	BMD expressed as a fraction of the SD of the mean of the previously published normal values for the patient's sex and decade of age

List of original articles

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

- I Hakala M, Åman S, Luukkainen R, Risteli L, Kauppi M, Nieminen P & Risteli J (1995) Application of markers of collagen metabolism in serum and synovial fluid for assessment of disease process in patients with rheumatoid arthritis. *Ann Rheum Dis* 54:886-90.
- II Åman S, Hakala M, Risteli L & Risteli J (1996) Increased type I collagen degradation is associated with a need for total joint replacement surgery in rheumatoid arthritis. *Ann Rheum Dis* 55:147.
- III Åman S, Hakala M, Silvennoinen J, Manelius J, Risteli L & Risteli J (1998) Low incidence of osteoporosis in a two year follow-up of early community based patients with rheumatoid arthritis. *Scand J Rheumatol* 27:188-93.
- IV Åman S, Risteli J, Luukkainen R, Risteli L, Kauppi M, Nieminen P & Hakala M. The value of synovial fluid analysis in the assessment of knee joint destruction in arthritis in a 3-year follow-up study. *Ann Rheum Dis*. In press.
- V Åman S, Paimela L, Leirisalo-Repo M, Risteli J, Kautiainen H, Helve T & Hakala M. Prediction of disease progression in early rheumatoid arthritis by ICTP, RF and CRP. A comparative 3-year follow-up study. Submitted.

Contents

Abstract

Acknowledgements

Abbreviations

List of original articles

1. Introduction	13
2. Review of the literature	15
2.1. Rheumatoid arthritis (RA)	15
2.2. Seronegative spondylarthropathies	15
2.3. Markers used in the diagnosis and assessment of RA	16
2.3.1. Genetic markers	16
2.3.2. Immunologic markers	16
2.3.2.1. Rheumatoid factor	16
2.3.2.2. Other marker antibodies	17
2.3.3. Acute phase reactants	18
2.3.3.1. Erythrocyte sedimentation rate (ESR)	18
2.3.3.2. C-reactive protein (CRP)	19
2.3.3.3. ESR versus CRP in inflammatory arthritis	19
2.4. Type I-III collagens	20
2.4.1. Type I collagen	20
2.4.2. Type II collagen	20
2.4.3. Type III collagen	21
2.5. Non-collagenous bone formation markers	21
2.6. Products of type I and III collagen synthesis	22
2.6.1. Carboxyterminal propeptide of type I procollagen (PICP)	22
2.6.2. Aminoterminal propeptide of type I procollagen (PINP)	22
2.6.3. Aminoterminal propeptide of type III procollagen (PIIINP)	23
2.7. Degradation products of type I collagen	23
2.7.1. Collagen cross-links	24
2.7.1.1. Pyridinoline and deoxypyridinoline	25
2.7.1.2. Cross-linked aminoterminal telopeptide of type I collagen	25
2.7.1.3. Cross-linked carboxyterminal telopeptide of type I collagen (ICTP)	25
2.8. Non-collagenous bone resorption markers	26

2.9.	Collagen degradation enzymes	26
2.10.	Synovial fluid analysis	27
2.10.1.	Non-collagenous synthesis and breakdown products of synovial membrane, bone and cartilage in synovial fluid and serum	28
2.11.	Osteoporosis	29
2.11.1.	Bone mineral density and its measurement	30
2.11.2.	Osteoporosis in rheumatoid arthritis	31
3.	Purpose of the present study	33
4.	Subjects and methods	34
4.1.	Patients	34
4.2.	Clinical examinations	36
4.3.	Laboratory examinations	36
4.4.	Measurement of RF	37
4.5.	Radiographic examinations	37
4.6.	Markers of collagen metabolism	37
4.7.	Measurement of bone mineral density	38
4.8.	Statistical analysis	38
5.	Results	40
5.1.	Assessment of the disease process in inflammatory arthritis by serum and synovial fluid analysis (Studies I and IV)	40
5.1.1.	Study I	40
5.1.2.	Study IV	42
5.2.	Relation of serum ICTP to the need for total joint replacement surgery (Study II)	44
5.3.	Incidence of osteoporosis in a two-year follow-up of early RA (Study III)	44
5.3.1.	Bone mineral density values	44
5.3.2.	Serum PINP and ICTP concentrations	46
5.3.3.	Laboratory, clinical and radiographic findings	46
5.4.	Prediction of disease progression by ICTP, RF and CRP (Study V)	46
6.	Discussion	49
6.1.	Assessment of collagen metabolism in serum and synovial fluid	49
6.1.1.	Study I	49
6.1.2.	Study IV	51
6.2.	Type I collagen degradation and need for total joint replacement surgery (Study II)	52
6.3.	Incidence of osteoporosis (Study III)	53
6.4.	Prediction of disease progression by ICTP, rheumatoid factor and C-reactive protein (Study V)	54
6.5.	Biochemical and immunologic markers for diagnosis and outcome measurement in early RA	55
7.	Conclusions	57
8.	References	58

1. Introduction

Impairment and disability in patients with rheumatoid arthritis (RA) mainly result from the destructive power of the arthritic component of the disease (Peel *et al.* 1992). The degree of joint inflammation is usually assessed by clinical and laboratory measures of disease activity, such as the number of swollen joints, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). On the other hand, radiographs only show the end results of tissue destruction (Gabriel & Luthra 1988, Kushner 1991, Sharp 1989, Brower 1990). Much research has been done to develop biochemical assays which would reflect the ongoing pathological processes in the joints (Peel *et al.* 1992, Poole 1994, Wollheim 1994). Such tests include those measuring the synthesis and breakdown of different types of collagen (Peel *et al.* 1992, Poole 1994, Wollheim 1994, Taylor *et al.* 1994). Yet, rheumatoid factor (RF) is still postulated to be the most powerful single predictor of erosive disease in spite of the huge number of laboratory measures evaluated since the 1940s (Young & van der Heijde 1997).

Type I collagen accounts for about 90% of the organic matrix of bone, and is also the major matrix protein in tendons, ligaments and soft connective tissues. Type III collagen is the second most abundant collagen type, and it occurs together with type I in soft tissues (Risteli 1993). Thus, assessment of their synthesis and breakdown might be useful in diseases with connective tissue degradation, such as RA.

It has been shown previously that the increased degradation of type I collagen measured by ICTP was accelerated in patients with advanced RA compared to age- and sex-matched controls, and the maximum values of degradation products were seen in patients with signs of aggressive joint disease (Hakala *et al.* 1993b). In early RA, increased type I collagen degradation is also associated with a more erosive disease course (Paimela *et al.* 1994). Similar results have been found in RA with respect to the serum concentration of the aminoterminal propeptide of type III procollagen (S-PIIINP), which is a marker of type III collagen synthesis (Hørslev-Petersen *et al.* 1986, Hørslev-Petersen *et al.* 1988a). Inflammatory synovial fluid (SF) contains large amounts of PIIINP (Gressner *et al.* 1984, Hørslev-Petersen *et al.* 1988b).

Accelerated bone loss leading to osteoporosis is regarded as a common clinical problem in RA. The bone loss may be periarticular and occur early during the course of the disease prior to erosions, or it may be generalized and occur more gradually (Star &

Hochberg 1994). Periarticular osteoporosis on hand radiographs is one of the American Rheumatism Association (ARA) classification criteria for RA (Arnett *et al.* 1988). The rate of bone loss has been shown to correlate with disease activity (Sambrook *et al.* 1985), with the majority of the effect of RA on bone occurring during the early years of the disease (Gough *et al.* 1994, Shenstone *et al.* 1994).

The aim of the present study was to evaluate the degradative and synthetic processes involving type I and type III collagen in patients with inflammatory arthritis, especially RA, and the relationship of these processes to the patients' clinical state. Collagen metabolism was assessed by measuring the concentrations of ICTP, PINP, and the aminoterminal propeptide of type I procollagen (PINP, an assay of type I collagen synthesis) (Melkko *et al.* 1996) in serum and synovial fluid.

In order to estimate the prediction of disease progression based on these collagen metabolism measurements, the patients were followed up for 2-3 years. In a community-based follow-up of patients with advanced RA, we looked for major signs of morbidity, such as destruction of large joints, trying to correlate type I collagen degradation with this process. In a series of patients with early RA, both bone mineral density and serum markers of type I collagen metabolism were regularly measured. The aim was to find out how often patients develop osteopenia, and whether changes in the serum PINP and ICTP concentrations correlate with a loss of bone mineral density. In another series of early RA, the aim was to compare the predictive power of type I collagen degradation and the more commonly used laboratory measures, such as CRP and RF, in assessing radiological disease progression and to test whether predictive accuracy could be increased by combining these measures.

2. Review of the literature

2.1. Rheumatoid arthritis (RA)

Rheumatoid arthritis is a heterogeneous disease with a variable course ranging from benign non-deforming oligoarthritis to rapidly progressing destructive polyarthritis. In most western countries, the prevalence of RA is about 0.5-1.0% of the adult population, being 0.8% in Northern Finland (Hakala *et al.* 1993a) if the ARA 1987 criteria are applied (Arnett *et al.* 1988). In Finland, Kaipiainen-Seppänen (1996) reported the annual incidence of RA to be 39/100 000 of the adult population, while the corresponding figures for ankylosing spondylitis, psoriatic arthritis and juvenile RA were 7/100 000, 6/100 000 and 14/100 000, respectively.

The major abnormalities caused by RA appear in synovial articulations symmetrically on both sides of the body. The initial changes consist of synovial proliferation, fusiform soft tissue swelling, and periarticular osteoporosis. Somewhat later, the inflamed synovial tissue or pannus extends across the cartilaginous surface, leading to chondral erosions. Small osseous erosions then appear at the margins of the joints, and finally, diffuse loss of joint space develops. At more advanced stages, large marginal and central erosions and cysts appear. In advanced RA, fibrous ankylosis of the joint may be seen. The synovium of bursae and tendon sheaths is also affected (Resnick & Niwayama 1995). The proliferative and heterogeneous mass of rheumatoid synovium has a capacity to erode bone and destroy cartilage and tendons similarly to localized malignancies (Harris 1997).

2.2. Seronegative spondylarthropathies

Psoriatic arthritis, which is one of the major seronegative spondylarthropathies, produces significant abnormalities of cartilaginous joints and entheses as well as synovial articulations. As in RA, the predominant target area in the synovial joints in seronegative spondylarthropathies appears to be the synovial membrane, while the inflammatory changes are of lesser intensity. Also, the severity and frequency of inflammation in bursae and

tendon sheaths may be somewhat milder than in RA (Resnick & Niwayama 1995). With the exception of psoriatic arthritis, seronegative spondylarthropathies characteristically involve lower limb rather than upper limb joints (Riordan & Dieppe 1989).

2.3. Markers used in the diagnosis and assessment of RA

2.3.1. Genetic markers

Familial aggregation of RA is largely restricted to the families of probands with relatively severe disease, suggesting that the genetic component of the disease may be most relevant for disease severity (Emery 1997). The availability of serological typing showed a genetic association of RA with the major histocompatibility complex Class II HLA-DR4 antigen (Stastny 1974 & Stastny 1978). The HLA-DR4 (and DR1) subtypes associated with an increased prevalence of RA have a common amino acid sequence in the peptide-binding area of the HLA molecule, called shared epitope (Wordsworth & Bell 1990). There are variations in the prevalence of the shared epitope in different racial populations (Emery 1997). Among severely affected hospitalized patients the prevalence of the shared epitope is notably high, having been up to 90% in certain studies (Wordsworth & Bell 1990). There is a hierarchy of prevalence varying from relatively little enrichment in community-based studies of mild disease up to a maximum in inpatients and subjects with severe disease, with the highest prevalence seen in patients with Felty's syndrome (Emery 1997, Hakala *et al.* 1997).

2.3.2. Immunologic markers

2.3.2.1. Rheumatoid factor

Rheumatoid factor is a circulating antibody acting against multiple antigenic determinants on the Fc fragment of the IgG molecule. The conventional agglutination and immunoturbidimetric techniques measure predominantly IgM class RF. However, radioimmunoassay or enzyme-linked immunosorbent assay (ELISA) allow measurement of RF belonging to all the major immunoglobulin classes (IgM, IgG and IgA) (Harris 1997).

In clinical settings, RF can be detected in most cases of RA, but only occasionally in the sera of healthy subjects (Aho *et al.* 1994). However, only a minority, i.e. up to about one third, of those living in community and exhibiting positive RF reactions show clinical and/or radiological evidence of RA (Kellgren 1966). Subjects with RA have higher average RF levels than those with false-positive RF reactions. The proportion of RA cases among RF positives is thus dependent on the sensitivity of the test techniques (Aho *et al.* 1994). Another determinant in this respect is the prevalence of RA in the population. RF often precedes the onset of RA by years (Aho *et al.* 1985, Aho *et al.* 1994). According to

the Mini-Finland Health Survey, healthy subjects with positive sensitized sheep cell agglutination test results had an approximately 40-fold risk of developing RF-positive RA compared to subjects with negative results during a 10-year period (Aho *et al.* 1994).

It has been suggested that RF may have a pathogenetic role in RA. For example, the administration of human IgG or IgM monoclonal RF to mice immunized with collagen type II markedly enhanced the activity and severity of the ensuing collagen-induced arthritis and the levels of circulating anticollagen antibodies (Ezaki *et al.* 1996). In addition, the presence of RF and its serum level associate with the severity of RA, such as radiologic erosions, poor outcome, and extra-articular manifestations, including rheumatoid nodules and vasculitis (Paimela *et al.* 1995, Richardson & Emery 1996, van Zeben & Breedveld 1996, Saraux *et al.* 1997). Although some studies have not shown RF to be predictive of erosions (Fex *et al.* 1996), it should be noted in this regard that RF-positive RA patients may turn seronegative in the course of therapy or vice versa (Smolen & Steiner 1998).

About one third of patients initially presenting with symptoms and signs compatible with RA are seronegative according to the results of conventional RF tests (Masi *et al.* 1976, Heliövaara *et al.* 1993). It is important to remember, however, that these patients have some kind of inflammatory joint disease, whatever it may be. On an average, RF-negative patients tend to have a more favourable disease course than those with positive RF reactions, and many of them end up in complete remission (Isomäki 1987). There remains, however, a variable but relatively small proportion of patients who develop chronic progressive disease.

In an eight-year follow-up of a community-based cohort of 150 patients with recent-onset arthritis, Aho *et al.* (1989) found that the radiological index values in high-titre groups were no higher than the average values for seropositive cases. Thus, no severity gradient based on RF titre could be demonstrated. On the other hand, Paimela *et al.* (1995) followed up 78 patients with early RA for three years and found that initial RF positivity alone was a sensitive predictor for subsequent joint destruction, but quantitative measurement of the initial RF level, and especially repeated measurements of RF, seemed to add significantly to the prognostic value of RF in distinguishing between progressive and non-progressive disease.

2.3.2.2. *Other marker antibodies*

The presence or absence of circulating RF has long been used to divide chronic peripheral arthritis into two categories, seropositive and seronegative (Masi & Feigenbaum 1983). Seropositive RA may represent a single disease, whereas seronegative RA is likely to be a combination of different entities. The initial heterogeneity of seronegative RA diminished when several subsets of it, such as chronic uoarthritis and psoriatic arthropathy, were assigned specific labels on the basis of their clinical and immunogenetic characteristics. It has been proposed that the definition of seropositive RA should be extended to include all patients with RA who have any of the currently known marker antibodies of RA, including the antibody against the perinuclear factor in human buccal mucosa cells, the antibody against the keratin-associated component in the stratum corneum of rat eso-

phagus and possibly also the *anti-RA33* (Aho & Kurki 1994). *Antikeratin antibody* is a highly specific, although not very sensitive, marker of RA. On an average, 40-50% of sera from patients with established RA have been positive. The test for *antiperinuclear factor* is more sensitive but less specific than that for antikeratin antibody; the frequency of positive reactions in RA patients has been about 60-80%. Although the available evidence seems to indicate that both antibodies are associated with erosive chronic RA, they may not define any subgroup with a particularly severe disease among such patients (von Essen *et al.* 1993).

The absence of the above marker antibodies means that there is no humoral evidence of the rheumatoid immunological process, i.e., the disease is seronegative. The presence of one, and preferably more than one, of the marker antibodies is indicative of an underlying immunological process, which shows the disease to be seropositive. With respect to RF alone, it might be appropriate to speak of RF-positive versus RF-negative disease (Aho & Kurki 1994).

2.3.3. *Acute phase reactants*

Inflammation resulting from any form of tissue injury causes an increase in the concentration of a number of liver-derived plasma proteins, which appear to have important functions in the inflammatory process. This physiological event is known as the acute phase response, and it is accompanied by several other systemic responses, such as fever, leukocytosis and muscle proteolysis. The measurement of acute phase proteins in plasma provides a clinically valuable indication of the presence of inflammation and its extent (Whicher & Dieppe 1985).

The most commonly used measurements of the acute phase response are ESR, plasma viscosity, CRP, orosomucoid, haptoglobin and alpha 1-antitrypsin (Whicher & Dieppe 1985). The most potent inducers of hepatic synthesis of the acute phase proteins are the cytokines interleukin 1, interleukin 6 and tumour necrosis factor, which interact mutually in a complicated way (Wollheim & Eberhardt 1992). The various acute phase proteins respond differently to different combinations of cytokines, which may explain the occurrence of different patterns of acute phase response.

2.3.3.1. *Erythrocyte sedimentation rate (ESR)*

ESR is a widely used laboratory measure of disease activity in clinical medicine. It indirectly reflects the potentially increased concentrations of serum proteins, particularly asymmetric molecules, such as fibrinogen, but also other acute phase proteins and immunoglobulins. ESR is also affected by factors independent of inflammation, such as erythrocyte morphology (Wollheim & Eberhardt 1992).

When evaluating the relative efficacy of different laboratory measures in an effort to detect the effects of treatment in RA, Bull *et al.* (1989) found ESR to be the best single measure. Ninety per cent of the information obtained from a total of 12 laboratory tests was obtained from ESR alone. The measures that were compared included plasma visco-

sity, serum orosomucoid, CRP and fibrinogen. Even though ESR has repeatedly been found a poorer indicator of disease activity than CRP in RA (Blackburn 1994), van der Heijde (1991) achieved a 75% success rate in predicting the absence or presence of radiological damage by means of the baseline ESR value and HLA-DR4 status over a follow-up of two years.

2.3.3.2. *C-reactive protein (CRP)*

CRP is the acute-phase protein that has been most widely assessed. It can be measured quantitatively with immunoassays that are both simple and rapid to perform. The prognostic value of CRP measurement has mostly been evaluated for the short-term outcome of RA. McConkey *et al.* (1972) studied 187 patients with RA for 3 years and concluded that acute phase proteins reflected exacerbations and remissions of RA and, overall, reflected the course of the disease. It was also concluded that CRP reflected disease activity better than the other acute phase proteins or ESR. Mallya *et al.* (1982) studied 99 patients with RA in a more quantitative manner. They evaluated disease activity with an index composed of morning stiffness, visual analogue scale, grip strength, articular index, hemoglobin, and ESR. They correlated CRP with the disease activity index and found a good overall correlation. Otterness (1994) found CRP to correlate with clinical disease activity, radiologic progression, and response to therapy. Eberhardt *et al.* (1990) used the baseline CRP value together with RF titre and found an accuracy of 67% when predicting radiological severity in a two-year follow-up of early RA. The prediction was somewhat improved if disease activity during the first 6 months was included (Wollheim & Eberhardt 1992).

2.3.3.3. *ESR versus CRP in inflammatory arthritis*

Amos *et al.* (1977) and Dawes *et al.* (1986) both found that patients with consistently low ESR and CRP levels were less likely to develop progressive radiological damage. Several other authors have found correlations between one or some of the acute-phase reactants and radiological progression (Wollheim & Eberhardt 1992).

Both McConkey *et al.* (1972) and Mallya *et al.* (1982) postulated that CRP is a better indicator of disease activity than ESR in RA. Nevertheless, Wolfe (1997) reported that simple comparisons between ESR and CRP suggested the two tests to be similar, but partial correlation analysis indicated that part of the correlation between ESR and clinical variables came from non-acute phase factors (Wolfe 1997). These factors, in turn, were responsible for most of the discordance between the ESR and CRP results. Thus, CRP appeared to be the better test in view of measurement of the acute phase. Because ESR is sensitive to immunoglobulins, it may measure general severity better than CRP, even though it is a poorer measure of inflammation. This may have accounted for the relative equivalence of the tests. The combination of ESR and CRP is thought to yield useful information that is often not apparent when only a single test is used (Wolfe 1997).

2.4. Type I-III collagens

2.4.1. Type I collagen

Type I collagen is one of the most abundant protein species in the human body, accounting for at least 70% of total collagens, i.e. for 2-3 kg in an adult. Most of this is present in bones, where about 90% of the organic matrix consists of type I collagen. The remainder is found in soft connective tissues all over the body, a lot of it in skin. The type I collagen molecule is a long, rigid rod - a shape necessary for its function as part of the collagen fiber in tissue. Two of the three constituent chains of the normal type I collagen molecule are identical $\alpha 1(I)$ chains, while the third is a different but homologous $\alpha 2(I)$ chain. These chains are all intertwined into a triple helix. The original gene products, the pro- $\alpha 1(I)$ and pro- $\alpha 2(I)$ of type I procollagen, are about 50% longer than the corresponding final products, α chains. The two additional, bulky domains at both ends of the molecule are usually called the aminoterminal (abbreviation PINP) and the carboxyterminal (PICP) propeptide of type I procollagen, despite their relatively large sizes, which are clearly outside the ordinary definition of peptide. These parts are removed *en bloc* from the procollagen by two specific endoproteinases, the N- and C-proteinases, once the molecule has reached the extracellular space. In abnormal situations, a variant form of type I collagen containing three $\alpha 1(I)$ chains can be synthesized (Risteli 1993, Risteli & Risteli 1999).

The synthesis of type I collagen involves the production of specific by-products that, among other things, provide a possibility for elegantly assessing the rate of synthesis of this collagen. Another set of metabolic products is related to the degradation of this collagen, which may occur either together with the dissolution of the mineral phase or independently, when there is increased breakdown of the nonmineralized matrix (Risteli & Risteli 1999).

2.4.2. Type II collagen

Type II collagen is the major fibrous collagen of cartilage, representing 80-90% of the collagen in this tissue. Type II collagen is produced by chondrocytes, and its fibers make up 40-50% of cartilage dry weight. It is closely linked with type XI collagen, with which it has striking sequence homology. The globular domains of type XI and the increased glycosylation of type II collagen compared with the types I and III may have a role in the determination of the fibril diameter. Type II collagen is structurally highly similar to type I collagen and may cause arthritis if genetically susceptible rats and mice are immunized with it. The major function of type II collagen is to provide the tensile strength and toughness of cartilage (Harris 1997).

There are a few reports about measurement specific to the metabolism of type II collagen. Månsson *et al.* (1995) measured elevated levels of serum C-propeptide of type II procollagen, a marker of collagen II synthesis, in RA patients with rapid hip joint destruction, suggesting a selective increase in collagen synthesis.

2.4.3. Type III collagen

The main cells synthesizing type I collagen in soft tissues are fibroblasts, which also always produce significant amounts of type III collagen (Risteli & Risteli 1999). Type III collagen is the second most abundant collagen type in the human body. Its thin fibrils constitute the principal collagen in blood vessels and, together with type I collagen, in newly formed soft connective tissue (Harris 1997). Its relative concentration is particularly large in young, metabolically active connective tissue, e.g. the granulation tissue of a healing wound. During wound healing its proportion decreases, probably due to the half-life, which is shorter for type III collagen than for type I collagen. The type III collagen molecule is a homotrimer of three identical $\alpha 1(\text{III})$ chains. Its fibres are generally thinner than those containing mainly type I collagen (Risteli 1993) and these fibers are covered by type III pN-collagen with retained aminoterminal propeptide. Such molecules are believed to prevent further lateral growth of the fiber.

2.5. Non-collagenous bone formation markers

Alkaline phosphatase is an enzyme synthesized by a number of tissues. The bone-specific isoenzyme is produced by osteoblasts and localized in the matrix vesicles. The serum activity of this isoenzyme correlates with the bone formation rates determined by bone histomorphometry (Eastell *et al.* 1988). The alkaline phosphatase molecules produced in bone, liver and kidney are all formed from an identical gene product, and the three types differ only in their post-translational carbohydrate modifications (Peel & Eastell 1993). Radioimmunoassay for bone alkaline phosphatase can be used to assess bone turnover whenever the total alkaline phosphatase activity is not more than 2.6-fold compared to the upper limit of the normal range (Garnero & Delmas 1993).

Osteocalcin (bone Gla protein, BGP) is a vitamin K-dependent protein that has been estimated to account for up to 26% of all noncollagenous protein in bone. It is present in bone and dentin (Cormier 1995). This peptide is produced by osteoblasts during bone formation and incorporated into the bone matrix, where it may have a role in mineralization. A small proportion of newly synthesized osteocalcin is not incorporated into the matrix and is released into serum, where it can be measured by radioimmunoassay (Peel & Eastell 1993). Since intact osteocalcin is not released from the bone matrix on bone resorption, intact osteocalcin levels in serum reflect the bone formation rate and correlate better with the rates measured by bone histomorphometry than bone alkaline phosphate (Eastell *et al.* 1988). However, there are limitations to this marker in some clinical situations, such as in RA patients or in patients receiving corticosteroids. Osteocalcin is also highly unstable, and in order to measure it, serum samples should be frozen within 1 hour of collection (Peel & Eastell 1993). The studies of osteocalcin in RA have been inconclusive, with approximately equal numbers of studies showing increased and decreased serum levels (Sambrook *et al.* 1985a, Gevers *et al.* 1986, Weisman *et al.* 1986, Aroso Dias *et al.* 1989). It is generally accepted that patients receiving corticosteroids, which are known to suppress bone formation, have low serum osteocalcin levels (Peel *et al.* 1992). A possible explanation for the contradictory evidence has been provided by Fairney *et al.*

(1990), whose work suggested that patients with severe RA produce small amounts of active (fully carboxylated) and larger amounts of inactive (non-carboxylated) osteocalcin compared to individuals with osteoarthritis. They postulate that patients with RA may have abnormal osteoblast function.

2.6. Products of type I and III collagen synthesis

2.6.1. Carboxyterminal propeptide of type I procollagen (PICP)

A radioimmunoassay for human PICP was first described in 1974 (Taubman *et al.*). The molecular weight of the propeptide is 100 kd, and two main bands of about 30 kd each appear after reduction. The propeptide was first thought to be derived from the aminoterminal end of type I procollagen, but was later identified as a carboxypeptide. Elevated serum levels have been found in patients with Paget's disease (Simon *et al.* 1984) and various liver diseases (Savolainen *et al.* 1983). The elevated values observed in Paget's disease correlated with the extent and activity of the disease as determined by serum alkaline phosphatase (Partiff *et al.* 1987). Exogenous hormone treatment elevated the serum PICP concentrations in growth hormone-deficient children (Carey *et al.* 1985). A low rate of type I collagen synthesis, as assessed by PICP in serum, has been previously reported in RA (Kröger *et al.* 1993)

2.6.2. Aminoterminal propeptide of type I procollagen (PINP)

The aminoterminal propeptide of type I procollagen contains a collagenous domain and thus has the shape of a short rod with a more globular part at the end. The molecular mass of the propeptide is about 35 kd. The circulating antigenicity related to PINP can be resolved into two peaks with different molecular sizes, the first of which is identical to the free, authentic trimeric antigen, while the latter resembles part of a single domain of the pro- α 1(I) chain of PINP (Melkko *et al.* 1996). The origin of the smaller forms of PINP is most probably the degradation of newly synthesized type I procollagen or type I pN-collagen with the retained propeptide. Thus, the exclusive assay of intact PINP seems to be more sensitive than total PINP for detecting changes in the rate of bone collagen synthesis (Risteli & Risteli 1999). For instance, estrogen treatment in postmenopausal women leads to a 42% decrease in the circulating concentrations of intact PINP, compared with the 30% decrease observed with an assay measuring both antigenic forms (Suvanto-Luukkonen *et al.* 1997). In postmenopausal women with hormone replacement therapy, the changes in intact PINP were twofold compared to those in PICP (Sharp *et al.* 1996). In a study where 206 pre- and postmenopausal breast cancer patients with nonmetastatic disease were followed up for two years, an increase in the concentration of intact PINP at 12 months predicted a further loss of BMD at 24 months (Saarto *et al.* 1998).

2.6.3. Aminoterminal propeptide of type III procollagen (PIIINP)

The aminoterminal propeptide of type III procollagen is an elongated molecule with an overall molecular mass of 42 kd that consists of three identical polypeptide chains linked by disulphide bonds. The elongated shape of PIIINP is due to the fact that the central part of PIIINP contains a collagen-like triple helix, where every third amino acid is glycine and where several hydroxyproline residues are also located. PIIINP resembles PINP in overall structure (Risteli *et al.* 1993).

The helical domain of PIIINP can be digested away with bacterial collagenase. The different parts of PIIINP are conventionally named according to their elution positions in gel filtration after such digestion. The aminoterminal, known as Col 1, is the most immunogenic region in the molecule, while most anti-PIIINP antibodies recognize the antigenic determinants present in this part (Risteli *et al.* 1993).

The serum concentrations of PIIINP were elevated in active RA and showed some correlation with the clinical and serological signs of disease activity (Hørslev-Petersen *et al.* 1986, Hørslev-Petersen *et al.* 1988a). Inflammatory synovial fluid (SF) contains large amounts of PIIINP (Gressner *et al.* 1984, Hørslev-Petersen *et al.* 1988). The high SF:S PIIINP ratios found in such patients suggest local production and release of the protein from the joint via lymphatics into the circulation (Hørslev-Petersen *et al.* 1988b, Jensen *et al.* 1993).

2.7. Degradation products of type I collagen

4-hydroxyproline, an imino acid formed in a posttranslational enzyme reaction from proline, is a necessary requirement for the stability of the triple-helical conformation of the collagen molecule at body temperature. It makes up about 12% of the weight of the collagen molecule, and can thus be used as a measure of the tissue collagen content. Acid hydrolysis followed by a chemical colour reaction to assess this imino acid can be used both for tissue samples and for urine.

Once freed from the helical part of a collagen molecule, *hydroxyproline* can no longer be incorporated into a new protein; yet, exogenous hydroxyproline is absorbed from the diet. Most of the imino acid (up to 90%) is metabolized in the liver, but some is always passed into the urine. The increase of hydroxyproline correlates with growth velocity in children and with the presence of bone degradation processes, e.g. bone metastases of cancers, in adults (Risteli & Risteli 1999). A generally enhanced bone metabolic rate also increases urinary hydroxyproline excretion. Some hydroxyproline is directly derived from collagen synthesis, since it is present in the helical domain of PINP and PIIINP. Although urinary hydroxyproline has been used as a marker of bone resorption for a long time, it is relatively non-specific owing to the fact that it is present in many types of collagen and that only about 10% of hydroxyproline is excreted into urine. Furthermore, in inflammatory conditions, the complement component C1q may elevate the urinary hydroxyproline levels since it contains collagen-like amino acid sequences in the form of a triple helix as part of its structure (Robins 1982, Reid 1982).

In contrast to the 4-hydroxyproline content, which is similar in all type I collagens, the extent of other posttranslational modifications varies from one situation and tissue to another. Such modifications include the hydroxylation of lysine and the hydroxylation of proline at position 3, leading to the formation of 3-hydroxyproline. The presence of a half-way glycosylated hydroxylysine, *galactosylhydroxylysine*, has been suggested to be characteristic of adult bone tissue, and the excretion of this amino acid into urine has been used as a marker of bone collagen degradation (Bettica *et al.* 1996). Nevertheless, it is not suitable for routine measurements because high-pressure liquid chromatography (HPLC), a complex and demanding technique, must be used (Cormier 1995).

2.7.1. Collagen cross-links

The biological function of fibrillar collagen is to provide the tissue with tensile strength. The tensile strength is due to the covalent bonds, known as collagen cross-links, that develop between individual collagen molecules in a collagen fiber. The cross-links are formed in a complicated series of partially alternative chemical reactions that gradually lead, through divalent cross-links joining two polypeptide chains, to multivalent, i.e. tri- or even tetravalent, cross-links (Eyre *et al.* 1984). The continuation of cross-linking explains the fact that older collagen in soft tissues is progressively less soluble when studied in various ways, e.g. by digestion with pepsin or bacterial collagenase. In addition to the intermolecular cross-links essential for tensile strength, intramolecular cross-links also exist (Risteli & Risteli 1999). In soft tissues, conditions associated with rapid remodeling of tissue seem to involve a different route of collagen degradation than does turnover under steady-state conditions (Everts *et al.* 1996).

A characteristic approach in resolving the structure of collagen cross-links has been to isolate these compounds from hydrolyzed tissue samples, i.e. to destroy the information of their original locations in collagen molecules. According to this tradition, the concentrations of either free or total cross-links can be measured in different body fluids, usually urine. This approach is more specific for assessing the breakdown of fibrillar collagen than the measurement of hydroxyproline, as the cross-links can only be derived from the degradation of collagen molecules that have participated in collagen fibers. Furthermore, pyridinoline cross-links are not absorbed from the diet, which means that their excretion is related only to their endogenous formation (Risteli & Risteli 1999). The urinary excretion of cross-links has been validated as a marker of the bone resorption rate (Seibel *et al.* 1992), but a substantial day-to-day variation in the deoxypyridinoline concentrations has been reported (Seyedin *et al.* 1993). Because urinary cross-link excretion decreases by 30% between 8:00 and 11:00 a.m. (Schlemmer *et al.* 1992), sampling time is important for reproducible results.

2.7.1.1. *Pyridinoline and deoxypyridinoline*

Pyridinoline (PYD) and *deoxypyridinoline (DPYD)*, chemically correctly known as hydroxylysylpyridinoline and lysylpyridinoline, respectively, are the two nonreducible trivalent cross-links that stabilize type I collagen chains and are released during the degradation of mature collagen fibrils. Pyridinoline is abundant in bone and cartilage, whereas deoxypyridinoline is largely, although not entirely, confined to bone. Type III collagen also contains pyridinoline cross-links at the aminoterminal. Total PYD and DPYD are measured in hydrolyzed urine samples by fluorimetric detection after reversed-phase HPLC. This technique requires extensive sample pretreatment and purification steps, which limit its clinical application. It is possible to measure only free fractions of pyridinoline by avoiding the hydrolysis steps. During bone degradation, only about 40% of the cross-links are excreted in a free form. The remaining 60% continue to be peptide-bound (Cormier 1995).

The earlier studies to measure collagen degradation in RA with assays of PYD and DPYD have shown controversial results as to the association with radiological progression in hands and feet (Robins *et al.* 1986, Seibel *et al.* 1989).

2.7.1.2. *Cross-linked aminoterminal telopeptide of type I collagen*

It has been established that type I collagen has two cross-link forming sites, one in the aminoterminal peptide region and another in the carboxyterminal region of the molecule. Immunoassays to detect both N-telopeptide of type I collagen and carboxyterminal telopeptide of type I collagen (ICTP) have been described. The use of N-telopeptide of type I collagen as assayed in 24-hour urine samples (NTx) has been suggested to be valuable for monitoring changes in bone turnover in individual patients, for instance, after antiresorptive therapy (Cormier 1995). NTx is an assay recognizing small cross-linked peptides from urine that are derived from type I collagen. The assay is said to be a specific and responsive index of bone resorption activity (Garnero *et al.* 1994). No studies of RA patients had addressed urinary NTx excretion, until St. Clair *et al.* (1998) recently found urinary NTx excretion to be more abundant in patients with RA than in healthy controls.

2.7.1.3. *Cross-linked carboxyterminal telopeptide of type I collagen (ICTP)*

The ICTP antigen prepared *in vitro* is a cross-linked peptide derived from mature type I collagen fibres. A similar, though somewhat larger, antigen seems to be liberated during the turnover of type I collagen fibres *in vivo* and can be found in human serum. ICTP contains a mature trivalent collagen cross-link and adjacent peptide material from three polypeptide chains. Two of these are the carboxyterminal ends of the $\alpha 1$ chains of one type I collagen molecule and the third is derived from either an $\alpha 1$ or an $\alpha 2$ chain of the helical region of another molecule. This structure has been verified by N-terminal pro-

tein sequencing. A compound of this kind can only be derived from the degradation of type I collagen molecules which have participated in the formation of collagen fibres (Risteli *et al.* 1993).

The standard and tracer antigens of the assay are cross-linked ICTP collagen liberated by digestion with bacterial collagenase or trypsin from decalcified human femoral bone. The ICTP assay has been shown to be a reliable marker for increased type I collagen degradation in situations that include local destruction of bone tissue, e.g. multiple myeloma (Elomaa *et al.* 1992), bone metastases from carcinomas (Aruga *et al.* 1997), and both early and advanced RA (Hakala *et al.* 1993b, Kotaniemi *et al.* 1994, Paimela *et al.* 1994). On the other hand, the circulating ICTP antigen levels do not reflect accelerated or retarded physiological bone resorption, such as seen in the postmenopausal state or during the use of estrogen replacement therapy (Hassager *et al.* 1994) or short-term treatment with bisphosphonates (Garnero *et al.* 1994). To determine the reason for this discrepancy, Sassi *et al.* (submitted) designed a study to characterize the antigenic determinant of the ICTP assay. They measured the immunoreactivity of the cleavage products of the major osteoclastic enzyme, cathepsin K. They showed that cathepsin K cleaves the trivalent ICTP structure between the phenylalanine-rich region and the cross-link, destroying the reactivity with ICTP antibodies. They postulated that, *in vivo*, the above process is part of physiologic and postmenopausal bone resorption, whereas the increased circulating concentrations of immunoreactive ICTP found in other clinical situations, as in multiple myeloma and RA, are not primarily derived from physiological cathepsin-K-mediated, osteoclastic bone turnover.

In a recent cross-sectional analysis, where 5 different markers of collagen degradation were tested in patients with advanced RA, serum ICTP together with urinary pyridinoline (PYD) were found superior to the other tested markers (urinary deoxypyridinoline, N-telopeptide and CrosslapsTM assay for the C-telopeptide cross-linking domain) in discriminating patients with RA from healthy controls (St. Clair *et al.* 1998). These measures also had minimal short-term, day-to-day variability, and they were hence suggested to be useful in assessing the effect of potentially disease-modifying drug therapies (Cortet *et al.* 1998).

2.8. Non-collagenous bone resorption markers

Osteoclasts contain a *tartrate-resistant acid phosphatase* that is released into the circulation. The lack of specificity of this enzyme in plasma, its instability in frozen samples, and the presence of enzyme inhibitors in serum limit its clinical usefulness as a bone resorption marker (Cormier 1995).

2.9. Collagen degradation enzymes

The sequence of collagenolysis in mammalian tissues begins when active collagenase attaches to individual triple-helical molecules approximately three fourths of the way from the amino-terminal end of the molecule and cleaves through all the three polypepti-

de helical chains. The two fragments that are produced denature at temperatures above 32°C. The fragments lose their helical structure, uncoil into gelatin, and either are degraded by extracellular proteases (e.g. stromelysin, gelatinase) or are endocytosed and degraded within phagolysosomes (Harris 1997).

The initial step at the breakdown of the connective tissue matrix in both physiological and pathological situations is an extracellular process, often involving *matrix metalloproteinases* (MMPs) that function at neutral pH. In certain special environments, *cysteine proteinases* with more acidic pH optima are also active, and in rapid resorption or inflammatory events, *serine proteinases* are released by invading cells. Mechanical disruption and the presence of free radicals may also augment the degradative process. Matrix fragments subsequently undergo phagocytosis for processing intracellularly within the lysosomal system. These processes are normally strictly regulated by a complex interplay of cell-cell and cell-matrix interactions involving the production of proteinases, activators, inhibitors and other regulatory molecules (Murphy & Hembry 1992).

MMPs comprise a multigene family of at least 10 members. They can be grouped into 3 main classes. Group 1 includes MMP-1 (interstitial) and MMP-8 (neutrophil) collagenase, whose major substrates are collagen types I, II and III. Group 2 contains the gelatinases/type IV collagenases. The third group is made up of the stromelysins: stromelysin 1 (MMP-3), stromelysin 2 (MMP-10), and pump-1 (MMP-7). The stromelysins are active against a broad spectrum of substrates, e.g., proteoglycans, laminin, fibronectin, and some collagens (Vincenti *et al.* 1994). Stromelysin 1 is more abundant and is found more consistently in different samples of rheumatoid synovium than either gelatinase or collagenase (Harris 1997).

In a study by Maeda *et al.* (1995), collagenase (MMP-1) levels in the synovial fluid of RA patients correlated positively with the degree of synovial fluid inflammation. Nevertheless, the synovial fluid collagenase concentrations did not correlate with the C-reactive protein levels in serum. The authors suggested that this finding supported the observations that there is often a discordance between the inflammatory and proliferative components of rheumatoid arthritis.

2.10. Synovial fluid analysis

Sampling of synovial fluid (SF) is one of the most useful tests available to the clinician for evaluating a patient with joint complaints, because it may provide a specific diagnosis, such as septic arthritis and gout, and thus expedite the choice of a specific treatment. Arthrocentesis is generally a simple, successful, and relatively noninvasive way to gather critical information that is available in no other way; the difficulties lie primarily in the interpretation of the findings (Shmerling 1994).

Pain and swelling in joints is a fundamental feature of inflammatory arthritis, such as RA, while the amount of synovial fluid often increases at the same time. Synovial fluid analysis is commonly used to diagnose arthritis and to evaluate the inflammatory activity of joint effusion. There is increasing evidence to suggest that SF analysis may help in analysing the further destruction of an individual joint in patients with arthritis (Peel *et al.* 1992, Poole 1994, Wollheim 1994, Taylor *et al.* 1994).

The white blood cell (WBC) count is a helpful test in differential diagnosis, though nonspecific. The number of cells gives an important index of the strength of the inflammation within the joint at the time of aspiration. Cell counts may range from more than 100,000 cells/mm³, as in septic arthritis, gout, reactive arthritis, and severe RA, to less than 200 cells/mm³ in mild trauma or osteoarthritis (Schumacher 1993). The number of WBCs per cubic millimeter has relevance for joint destruction when the counts are high. Active proteases capable of degrading the cartilage matrix and collagen are usually not measurable in synovial fluid because they are inhibited by protease inhibitors. However, collagenase and other proteases can be detected in synovial fluid when the neutrophil count exceeds 50,000 cells/mm³. At these levels, the available inhibitors become saturated (Harris 1997).

Neutrophils may have a turnover rate of more than 1×10^9 cells/day in an inflamed knee joint. Their numbers in fluid can be influenced markedly by large doses of glucocorticoids, but by few other compounds. The differential WBC count in synovial fluid is rarely of help in differential diagnosis, principally because the number of neutrophils as a percentage of the total also increases as the total number of cells increases. In noninflammatory effusions the neutrophil:mononuclear cell ratio may be as low as 1:1, but once the cell number exceeds 5,000/mm³, a 10:1 neutrophil excess over the other cell types can be expected (Harris 1997).

Only a few studies have been performed concerning the prognostic value of routine SF analysis in joint inflammation. In a study by Luukkainen *et al.* (1989), where 30 patients with RA were evaluated, SF leukocytes were higher in patients with progressive radiological destruction in the knee joint compared to patients with no deterioration of the Larsen X-ray grade during a follow-up of about 3.5 years. In another study by Luukkainen *et al.* (1993), where 29 patients with erosive RA and hydropsy in a knee joint were followed for 7.5 years, SF leukocytes and SF polymorphonuclear leukocytes did not have any prognostic value, however.

2.10.1. Non-collagenous synthesis and breakdown products of synovial membrane, bone and cartilage in synovial fluid and serum

Much interest has been shown in the measurement of the synthesis and breakdown of different components of bone and cartilage. The quantification of tissue-derived macromolecules or their fragments in serum is a possible way to assess the destructive potential. The rationale for this is the hypothesis that increased or changing serum levels of a tissue-derived “marker“ reflect changes in tissue turnover. If sustained for a certain period of time, such changes may lead to uncoupling of the normal balance between matrix degradation and repair, and permanent joint damage may ensue (Fex *et al.* 1997). Examples of such tissue-derived macromolecules are *cartilage oligomeric matrix protein (COMP)*, *bone sialoprotein* and *hyaluronate*, which are putative markers for cartilage, bone and synovial tissue metabolism, respectively.

Saxne *et al.* (1987) studied a group of patients with RA from whom knee joint SF had been aspirated 10 years previously and found a correlation between the SF *proteoglycan* concentrations and the degree of subsequent radiographic joint destruction. In two other

studies, an increasing SF bone sialoprotein concentration was accompanied by increasing degrees of knee joint damage (Saxne *et al.* 1995, Månsson *et al.* 1997). Bone sialoprotein is a bone-specific macromolecule which constitutes some 12% of the noncollagenous proteins in bone, being hence a major component of this group of proteins (Fisher *et al.* 1990). This protein is released from bone into the circulation, where it can be quantified by immunoassay (Saxne *et al.* 1995). Serum levels of bone sialoprotein are increased in patients with RA compared with both healthy controls and patients with non-erosive reactive arthritis (Saxne *et al.* 1995).

In a longitudinal study by Månsson *et al.* (1997), the SF aggrecan, i.e. COMP, concentrations were initially highest in the group of RA patients with developing joint destruction. Increased serum concentrations of COMP early after the disease onset have been found in RA patients who developed severe large-joint (hip or knee) destruction leading to joint replacement (Forslind *et al.* 1992) within 2-3 years after the disease onset, though the elevated concentrations markedly decreased during the follow-up.

Serum hyaluronate in RA is believed to originate mainly from the inflamed synovial membrane (Laurent *et al.* 1995). Although serum hyaluronate often correlates markedly well with the levels of CRP or with ESR, additional prognostic value is suggested by a study where serum hyaluronate correlated with the radiological progression of joint lesions in the hands and feet of patients with early RA (Paimela *et al.* 1991). Nevertheless, in another longitudinal study of patients with different outcomes with regard to large-joint destruction, somewhat contradictory results have been obtained (Fex *et al.* 1997).

In a study by Fex *et al.* (1997), serum concentrations of COMP, bone sialoprotein or hyaluronate were not useful for identifying patients prone to small-joint destruction in a 5-year follow-up of early RA. The serum concentration of hyaluronate at inclusion correlated with the radiographic score at follow-up, but was not a better predictor in this respect than the ESR or CRP levels at inclusion.

2.11. Osteoporosis

Osteoporosis is a disorder that involves a diminution of bone mass without a detectable change in the ratio of mineralized to nonmineralized bone (to exclude osteomalacia). The high accuracy of the techniques used to measure bone mineral density (BMD) makes them appropriate for use as a diagnostic test of osteoporosis. The most straightforward way is to define a fracture threshold, namely the cut-off point for BMD which captures most patients with osteoporotic fractures. For adults, the cut-off value of 2.5 standard deviations (SD) below the average range of healthy young adults of equivalent sex is appropriate to satisfy the criteria particularly for hip fracture. The World Health Organization has accepted the following criteria for different BMD thresholds. A value for BMD not more than 1 SD below the average value of young adults is considered normal, while a low bone mass or osteopenia is considered to be indicated by BMD more than 1 SD, but not more than 2.5 SD below that usually seen in young adults. Osteoporosis is defined as a BMD value more than 2.5 SD below the young adult average value, and if there are additionally one or more fragility fractures present, the term “severe” or “established” osteoporosis can be used (Kanis 1994). These SDs from a reference population of equiva-

lent sex can also be expressed as Z scores if the reference population is of approximately the same age. The T score (to which some manufacturers refer as the young-adult Z score) represents the abovementioned numbers of SD above or below the mean peak bone mass of a young normal population of equivalent sex (Levis & Altman 1998).

2.11.1. Bone mineral density and its measurement

Conventional radiography is relatively insensitive in the assessment of osteoporosis, since bone loss is only apparent when bone mass has decreased by about 30-50%. There are, however, several distinctive radiographic features and morphometric techniques which may aid in the assessment of patients (Kanis 1994). With the exception of ultrasound, all bone densitometry procedures consist of a radiation source, either x-rays or radionuclide sources. These methods are based on the principle that the attenuation suffered by x-ray or gamma-ray photons is related to the thickness and composition of the tissues on the attenuation path. At most skeletal sites, the thickness of bone mineral is the dominant cause of attenuation. Using a calibration procedure, attenuation values are converted into equivalent mineral thicknesses and compared with population-based normative curves (Levis & Altman 1998).

Photon or x-ray absorptiometry uses a beam that scans the area of interest in synchrony with a detector or array of detectors. Earlier instruments utilized a single photon beam (*single photon absorptiometry*, SPA) or a single x-ray beam (single x-ray absorptiometry) and could only be used to evaluate peripheral sites, such as the forearm, with small variation in soft tissue thickness (Mazess *et al.* 1989). *Dual photon absorptiometry* (DPA) and *dual x-ray absorptiometry* (DXA) utilize two beams of distinct energies, thus allowing for the correction of soft tissue attenuation, which enables the scanning of both peripheral (forearm) and axial (spine and hip) sites. DPA scanners, which utilize a radioisotope source, are now outdated (Levis & Altman 1998).

DXA is widely used because of its good reproducibility and accuracy, its low radiation dose, and its capability of measuring bone density at both axial and appendicular skeletal sites (Jergas & Genant 1991). The technology involves x-rays of two discrete energies generated by an x-ray tube. Low-energy beams are attenuated to a greater extent than high-energy beams, and attenuation is greater in bone than in soft tissues (Mazess *et al.* 1989). After measuring the absorption of each of the two x-rays, two simultaneous absorption curves are generated, which are then used to calculate the attenuation caused by bone and to cancel out the effect of soft tissue. DXA scanners sample the tissue adjacent to bone and calculate its fat content. It is then assumed that the fatty composition of the soft tissue overlying the bone is the same as that of the adjacent area (Levis & Altman 1998).

The BMD measurements called SPA, DPA and anteroposterior DXA are two-dimensional and express BMD as g/cm^2 . These measurements therefore tend to underestimate BMD in small individuals. Since osteoporotic fractures typically occur in bones composed of a high proportion of trabecular bone, such as the vertebral body, the proximal femur and the distal radius, these sites are commonly chosen for the measurement of BMD. In spite of the good precision, osteophytes, aortic calcifications, degenerative facet

hypertrophy and intervertebral space narrowing in degenerative disc disease may sometimes lead to overestimates of lumbar BMD in anteroposterior scans, especially in elderly patients. The lateral projection overcomes some of these problems and allows estimation of the volumetric density (g/cm^3) which does not seem to be influenced by skeletal size (Duboeuf *et al.* 1994). The formerly poor precision of lateral projection seems to be improving along with the new DXA devices (Duboeuf *et al.* 1994). DXA has become one of the most widely used techniques of bone densitometry today (Hagiwara *et al.* 1994). DXA has a precision error of $\sim 1.2\text{-}3.0\%$. Radiation exposure with DXA is lower than the daily natural background level (Johnston *et al.* 1991). DXA does not selectively measure cortical or trabecular bone, although the trabecular bone component can be enhanced by measuring a trabecular-rich site (Levis & Altman 1998).

True volumetric BMD can only be obtained by *quantitative computed tomography* (QCT), which is not widely available. Only QCT can selectively measure cortical or trabecular bone (Levis & Altman 1998).

Ultrasound provides an alternative means of determining the relative risk of fracture due to osteoporosis. Calcaneus is often chosen for measurement because it is an easily accessible site of weight-bearing trabecular bone. Ultrasound has the advantages of involving no radiation and being portable and relatively inexpensive (Scheiber & Torregrosa 1998). Ultrasound measures broadband ultrasonic attenuation (BUA) and the velocity of sound (VOS) across bone. The former determines the density and the structure of the bone, while the latter evaluates its density and elasticity. Broadband ultrasonic attenuation is based on the principle that the more complex the structure, the greater the attenuation of ultrasound. When the speed of sound is measured, the greater the connectivity of the trabeculae, the faster the sound will go through the bone (Levis & Altman 1998). Although there are studies showing good correlation between the ultrasound results and the lumbar spine/femoral neck BMD measured by DXA (Cunningham *et al.* 1996) and even *in vitro* calcaneal ultrasound results, showing high correlations with calcaneal QCT results ($R^2 = 0.88$) (Laugier *et al.* 1997), the poor reproducibility (coefficient of variation varies from 0.4% to 4.0%) precludes the use of ultrasound in the assessment of response to treatment (Levis & Altman 1998). Currently, ultrasound can be used to discriminate between normal and osteoporotic women, and could be considered an alternative to DXA in the baseline screening and evaluation of fracture risk.

2.11.2. Osteoporosis in rheumatoid arthritis

Accelerated bone loss leading to osteoporosis is regarded as a common clinical problem in RA. It may be periarticular and occur early during the course of the disease or generalized and occur more gradually (Star & Hochberg 1994). The former alternative is one of the classification criteria for RA (Arnett *et al.* 1988). The latter was first recognized as a complication of RA by Barwell as early as 1865 (Barwell 1865). Bone mass in an individual is the outcome of the genetic background and the effect of internal (e.g. hormonal status, presence of RA) and external (e.g. diet, exercise) environmental factors (Sambrook *et al.* 1985b).

Most studies on bone mass changes in RA have been cross-sectional. Only a few longitudinal investigations have been performed. The rate of lumbar spine (LS) bone loss has been shown to correlate with disease activity (Sambrook *et al.* 1985b), the majority of the effect of RA on bone occurring during the early years of the disease (Gough *et al.* 1994, Shenstone *et al.* 1994). Gough *et al.* (1994) studied 148 patients with early RA over a 3-year period and found that the annual loss of bone mass density in the lumbar spine was 0.2% in males and 1.3% in females. The mean percentage change in BMD over the first year was -1.0 for LS and -2.0 for the femoral neck (FN) in all the patients, and even higher in those with active disease (CRP > 20 mg/L), being -2.1 and -3.6, respectively. In patients with active disease for over 2 years, the mean BMD loss at each site was between 5.5 and 10% ($p < 0.01$ compared to patients with inactive disease). At presentation, the BMDs of patients did not differ from those of controls, but during the next 12 months, BMD loss was greater in RA patients than in controls, significantly so for early disease (e.g. -2.4 vs -0.6 g/cm², $p < 0.05$ in LS and -4.3 vs -0.4 g/cm², $p < 0.001$ in the trochanter). Furthermore, Shenstone *et al.* (1994) found that 16 patients with disease duration < 6 months lost 3.9% of the femoral neck BMD in a year compared with a 0.2% loss in 51 patients with longer disease duration.

3. Purpose of the present study

The purpose of the present study was to investigate the value of different biochemical tests, particularly that of the markers of type I and III collagen metabolism, used to assess disease process and the development of progressive joint disease or osteoporosis in chronic inflammatory arthritis.

The detailed aims of the study were:

1. to examine the relationship of the markers measured in serum and synovial fluid to the patients' clinical state (I).
2. to find out if there is a connection between the SF analysis of a particular joint at the baseline and its radiological status after a three-year follow-up in patients with chronic arthritis (IV).
3. to examine the relationship between important morbidity events, such as destruction of large joints (hip and knee), and a marker for type I collagen degradation (ICTP) in patients with advanced RA (II).
4. to find out how often patients with early RA develop osteoporosis during a two-year follow-up, and whether changes in serum markers for type I collagen synthesis and degradation correlate with the loss of BMD (III).
5. to compare the predictive value of RF, CRP and ICTP in the baseline serum of patients with early RA for radiologic joint damage and to find out the extent to which prediction could be improved if these measures were used in combination (V).

4. Subjects and methods

4.1. Patients

Altogether 275 patients with inflammatory arthritis, mostly RA, were included in four different study protocols. All the RA patients fulfilled the 1987 ARA criteria for RA (Arnett *et al.* 1988). Table 1 shows the baseline demographic findings of the patients covered in the five different studies.

Table 1: Basic demographic findings in the five studies at baseline. Study IV is a follow-up of study I with 44 of the RA patients from study I and 11 patients with other arthritides.

	Study I	Study II	Study III	Study IV	Study V
Number of patients	59	90	52	55	63
Female/male	39/20	59/31	36/16	36/19	52/11
RA patients	100%	100%	100%	80%	100%
Mean age (yr)	58.1	58.7	49.5	51.8	43.5
(range)	(19-82)	(29-84)	(29-73)	(19-82)	(18-64)
Mean disease duration (mo)	162	184	9	131	7.6
(range)	(6-696)	(18-180)	(1-60)	(6-444)	(2-12)
Follow-up study	no	yes	yes	yes	yes
(follow-up time, yr)		3	2	3	3

Study I included 59 unselected patients undergoing treatment for RA and having knee joint effusion. At baseline, they were all outpatients or inpatients in three rheumatism hospitals in Finland. Forty-four of these RA patients together with seven chronic seronegative spondylarthropathy and four juvenile RA patients made up the cohort of study IV. Forty-six (78%) of the patients were being treated with slow-acting anti-rheumatic drugs (SAARDs) and 28 (47%) with peroral corticosteroids at the time of the study. Forty-nine patients (83%) had previously received one or more intra-articular injections of corticosteroids into the knee joint under study: in 23 cases the time between the last intra-articular injection of corticosteroids and the sampling of synovial fluid was ≤ 6 months, and in 12 cases it was ≤ 3 months. Ninety subjects without any signs of joint or metabolic bone

disease recruited from the area around Oulu, Northern Finland, served as a control group in Study I. The patients with a baseline Larsen's grade of 5 (Larsen *et al.* 1977) for the knee joint were excluded from Study IV.

Study II was conducted on 90 patients who had been included in a population-based study on the medico-social aspects of rheumatic diseases carried out in 1989-91 in the Kuusamo area, Northern Finland, with 18,000 inhabitants (Hakala *et al.* 1993a). The patient population included approximately 85% of all subjects with RA in the area, thus representing the whole spectrum of the disease from mild to severe. Five of the 90 patients did not use any medication for RA, 16 had non-steroidal anti-inflammatory drugs (NSAIDs) as the only treatment, 64 used SAARDs and 5 took peroral corticosteroids. In addition, 23 of the 64 patients with SAARDs were also on peroral corticosteroids. The corticosteroid dose was in all cases ≤ 10 mg prednisolone.

In study III, the study group consisted of 52 consecutive unselected patients with recent onset RA visiting the outpatient or inpatient department of Päivärinne Hospital near the City of Oulu. This hospital was a district rheumatism hospital with close co-operation with primary care. Thus, most of the patients were early referrals from primary care physicians. In the three patients who had had symptoms for over two years, the diagnosis of RA was made at the baseline of the study. Twelve patients (23%) had erosions in hand radiographs. Seventeen of the 36 women were either postmenopausal ($n = 15$) or had undergone ovariectomy before menopause ($n = 2$). Eleven (21%) patients had not received any medication before entry into the study. Forty-one (79%) patients used NSAIDs. Three patients had previously received SAARD therapy, one of them also oral corticosteroid therapy. The disease duration of these three patients was 1-10 months. At the start of the study, SAARD monotherapy was started in 43 cases: intramuscular gold in 27 (52%), oral gold in 3 (6%), sulphasalazine in 8 (15%), chloroquine in 2 (4%), methotrexate in 2 (4%) and azathioprine in one (2%) of the 52 patients. A combination of two drugs was instituted in 7 (13%) patients, methotrexate + oral gold in 6 (11%) and methotrexate + chloroquine in one (2%) patient. The mean number of SAARDs used per patient during the follow-up was 1.9 (SD 0.8, range 1 - 4). A prescription of oral corticosteroid (mean and median starting dose 13.6 and 7.5 of prednisolone, respectively) was given to 11 (21%) of the 52 patients at the baseline of the study. Seven of them continued to take it throughout the study. Altogether 25 patients used oral corticosteroids for some time during the study. The mean cumulative corticosteroid dose administered orally, intravenously and intra-articularly was 1374 mg of prednisolone (SD 2224, range 0-9367 mg).

Study V included sixty-three consecutive patients with newly diagnosed RA. At the time of diagnosis, 57% (36/63) had joint erosions either in their hand or feet radiographs or both. None of the patients had previously received any SAARDs. Intramuscular gold was instituted in 83% (52/63), sulphasalazine in 12% (8/63) and hydroxychloroquine in 5% (3/63) of the patients. The mean number of SAARDs used per patient during the 3-year follow-up was 2.3 (range 1-6). Only three of the patients used peroral corticosteroids continuously (5-7.5 mg of prednisolone daily).

All the patients were actively treated with SAARDs. If one SAARD regimen had to be withdrawn during the follow-up because of side-effects or lack of effect, another individually tailored SAARD regimen was instituted (including D-penicillamine).

4.2. Clinical examinations

In studies I, IV and V, the clinical variables of disease activity were assessed at baseline and at the 3-year follow-up visit. In study III, the clinical variables of disease activity were assessed at every 3 months during the first year and at every 6 months during the second year.

The physical examination in studies I and III-V included disease activity evaluation using the Ritchie articular index (Ritchie *et al.* 1968) and a joint swelling score in studies I, III and IV (none = 0, mild = 1, moderate = 2, severe = 3). For the joint swelling score, the same joint areas as in the Ritchie index (hip joint and cervical spine excluded) were assessed. Swelling in individual joints/joint groups was graded into three classes (0-3), and the total score was formed as a sum of the individual scores (Luukkainen *et al.* 1992). A general activity index was formed as a sum of the Ritchie index and joint swelling scores.

To assess disability due to RA in study III, we used the Health Assessment Questionnaire (HAQ) as a 0-3 functional disability index (Fries *et al.* 1980, Wolfe *et al.* 1988). Physical function was assessed on a five-point scale of 1 to 5 (Table 2).

Table 2: Physical function.

Grade	Physical function
1	Normal physical function, capable of running, crouching, walking on toes and heels
2	Slightly deteriorated physical function, no essential inconvenience in everyday life, some limping, difficulty in running or crouching, no need for pain medication or supporting device
3	Considerable difficulties in moving in everyday life and at work, need for supporting device, pain medication occasionally, need for a rest during moving
4	Marked difficulties in moving, need to lean against something nearly all the time, difficulties in moving outdoors or using public transportation, nearly constant need for pain medication
5	Unable to move without a wheelchair, bedridden

4.3. Laboratory examinations

Routine laboratory tests for the assessment of disease activity were performed in all studies. CRP was quantitatively measured with an immunoturbidimetric method (Orion Diagnostica, FIN-02200 Espoo, Finland), with values < 10 mg/l considered as normal. All joint aspirations in studies I and IV were made for therapeutic reasons. The synovial fluids were collected into sterile tubes and stored at -20°C together with the serum samples obtained at the same time. The leukocyte counts of the synovial fluids were done before freezing.

4.4. Measurement of RF

In study III, rheumatoid factor was quantitatively measured with an immunoturbidimetric method (Orion Diagnostica, FIN-02200 Espoo, Finland) at entry and annually thereafter, with values ≥ 25 IU/ml considered as seropositive.

In study V, RF was assayed at entry with a Kone specific automated clinical chemistry analyser (Kone Instruments, Espoo, Finland) for immunoturbidimetric measurement of RF. An assay modification using chemical inactivation of C1q with polyvinyl sulphonate was used as previously described (Nykänen *et al.* 1993). This method showed 95% of healthy subjects to have an RF level < 20 IU/ml, which was used as the cut-off point for seropositivity.

4.5. Radiographic examinations

In studies I and IV, the erosive state of the knee joints was assessed by the same author (R.L.) using Larsen's method (Larsen *et al.* 1977) and comparing the posteroanterior radiographs of the knees with a standard series. Larsen's grades for the knee are: 0 = normal; 1 = slight abnormality with one or more minor lesions (periarticular soft tissue swelling, periarticular osteoporosis, and slight joint space narrowing); 2 = definite early abnormality with erosion (not obligatory) and joint space narrowing; 3 = medium destructive abnormality (erosion obligatory); 4 = severe destructive abnormality (bone deformation present); 5 = mutilating abnormality (gross bone deformation).

Radiographs of the hands were taken at entry and annually thereafter in study III. The erosive state of joint disease was assessed by one of the authors (J.M.) from posteroanterior radiographs of hands using Larsen's method and comparison with a standard series (Larsen *et al.* 1977). The Larsen indices of wrist joints were multiplied by 5. The maximum score for the method was 150.

In study V, radiographs of the hands and feet taken at entry and after 3 years were evaluated with the method of Larsen *et al.* (Larsen *et al.* 1977) by the same observer (T.H.) consecutively. The joints assessed included eight proximal interphalangeal joints, two interphalangeal (IP) joints of the thumbs, 10 metacarpophalangeal joints, the left and right wrists, 10 metatarsophalangeal joints and two hallux IP joints. The scores for wrists were multiplied by five. The maximum possible total score was 210.

The radiographic evaluations in every study were done without knowledge of the clinical data.

4.6. Markers of collagen metabolism

ICTP, PINP, and PIIINP were measured, respectively, in duplicate 50, 100, and 200 μ l aliquots of serum, of undiluted SF in the case of ICTP, and of appropriately diluted (using assay buffer) SF in the case of the procollagen propeptides. Equilibrium radioimmunoassays for human antigens were used (Risteli *et al.* 1993, Melkko *et al.* 1996, Risteli *et al.*

1988) with reagents supplied by Orion Diagnostica (FIN-90460 Oulunsalo, Finland). The intra-assay and inter-assay coefficients of variation of the three markers tested with human serum samples were less than 10%. The intra-assay variation for SF samples was 4.4% for PINP and 5.7% for PIIINP. The SF samples of the series were not centrifuged. We later tested the effect of centrifugation on the results in five additional patients. Centrifugation was found to decrease the levels of the markers of collagen metabolism in SF to the same extent as does centrifugation of plasma into serum. With the exception of one sample each, the level of the markers after centrifugation was lower compared to the native samples by 6.3 to 11.7% for SF-ICTP and by 0.2 to 1.4% for SF-PIIINP.

4.7. Measurement of bone mineral density

The BMD measurements were made by dual energy X-ray absorptiometry (Lunar DPX, Lunar Radiation Corporation, Madison, WI, USA) at 0, 6, 12, 18 and 24 months. The measurement sites were the lumbar vertebrae L2-L4 in the LS and the left femoral neck (FN). Plain radiographs of the lumbar spine were routinely taken at the beginning to detect any possible abnormalities known to falsely elevate BMD values (marked scoliosis and osteophyte formation, and vertebral crush fractures or dense calcification of the abdominal aorta). The measurement site was varied in order to avoid remarkable osteophytes. If possible, the same was done in view of severe aortic calcification. The precision of the method (coefficient of variation) was determined by examining 19 persons twice with an interval of a couple of minutes between the measurements. For BMD in the LS and the FN, the coefficients of variation were 1.38% and 1.61%, respectively. The BMD values of the patients were compared with the previously published Finnish reference values (Kröger & Laitinen 1992a, Kröger *et al.* 1992b). The individual values of BMD were also expressed as fractions of the SD of the mean of the previously published normal value for the patient's sex and decade of age (Z score) and for the young adults (20-40 yr) of the same sex (T score).

4.8. Statistical analysis

In studies I, II and IV, the data were recorded and calculated using the SOLO statistical software. In study I, the distributions of SF-ICTP and SF-PIIINP by Larsen's grade were visualized with box plots. In the comparison of means, statistical significance was evaluated by analysis of variance. Statistical significance was evaluated by the Mann-Whitney test when comparing medians. The SF:S ratios were calculated for each patient, and the mean values with confidence intervals were reported as Larsen's grade values. The correlations between the levels of the different markers of collagen metabolism and disease activity were calculated using Spearman's rank correlation coefficient. When the significance of the differences between two parameters was assessed by dividing them into two groups, Pearson's χ^2 -test or Fisher's exact test were used as appropriate. In study II, Fisher's exact probability test was used to evaluate statistical significance when comparing the proportions of patients with or without a need for total joint replacement surgery. In

study IV, medians were used to describe the average value of markers of collagen metabolism. Statistical significance was evaluated by the Kruskal-Wallis or Mann-Whitney test when comparing medians. The χ^2 -test was used when comparing the proportions of patients who needed one or more corticosteroid injections by stable or deteriorated Larsen's grade.

In study III, the data were recorded and analyzed on a microcomputer using the SPSS statistical software. The significances of the differences between the measurements were determined by Wilcoxon's test for paired data. When the significance of the differences between two parameters was assessed by dividing them into two groups, crosstabs and Pearson's χ^2 -test or Fisher's exact test were used as appropriate. The correlations were calculated using Spearman's rank correlation coefficient. The confidence intervals of Spearman's rank correlation coefficients were calculated with the Confidence Interval Analysis version 1.0 (CIA) in study III.

In study V, the BMDP statistical software was used for statistical analysis. The significance of the differences in Larsen's score values between the groups formed according to the baseline laboratory values was determined by the Mann-Whitney rank-sum test with Bonferroni correction. Score 20 was used as the cut-off point for the change in Larsen's score from baseline to 36 months. The age- and sex-adjusted associations of different abnormal laboratory tests or their combinations with radiologic progression were also expressed as odds ratios (OR) with 95% confidence intervals (CI) calculated by logistic-regression analysis.

For all the tests in all the studies, p-values < 0.05 were considered significant.

5. Results

5.1. Assessment of the disease process in inflammatory arthritis by serum and synovial fluid analysis (Studies I and IV)

5.1.1. Study I

Table 3 shows that the mean concentrations of the three markers of collagen metabolism were higher in the sera of the patients with RA than in the controls.

*Table 3: Median concentrations of three markers of collagen metabolism in the serum of 59 patients with rheumatoid arthritis (RA) and 90 healthy age- and sex-matched controls. f = tested in 57 patients, S-ICTP = serum cross-linked carboxyterminal telopeptide of type I collagen, S-PINP and S-PIIINP = serum aminoterminal propeptides of type I and type III procollagens, respectively. ** = $p < 0.01$, *** = $p < 0.001$, between groups.*

Concentration ($\mu\text{g/l}$)	RA patients		Controls
S-ICTP	5.3		3.0
Value > (mean + 2SD) (% of patients)	58	***	
S-PINP f	42.7		33.0
Value > (mean + 2SD) (% of patients)	19	**	
S-PIIINP f	4.6		3.1
Value > (mean + 2SD) (% of patients)	42	***	

The median (SD, range) SF concentrations were 16.7 (18.1, 2.8-96.9) $\mu\text{g/l}$ for ICTP, 782 (903, 118-5017) $\mu\text{g/l}$ for PINP, and 1478 (857, 278-4781) $\mu\text{g/l}$ for PIIINP. The SF:S ratios for the individual markers of collagen metabolism were 4.0 (95% CI 3.3-4.8) for ICTP, 25.2 (95% CI 19.7-30.8) for PINP, and 340 (95% CI 295-385) for PIIINP.

Because the volume of joint effusion may change rapidly, making the assessment of concentrations inappropriate, we also calculated the values as ratios of one compound to another, which are shown in relation to the radiographic state (Larsen's grade) of the corresponding joint in Table 4.

Table 4: Mean (SE) ratios of the three markers of collagen metabolism in synovial fluid in relation to radiographic findings in the joint (Larsen's grade).

Larsen's grade	Marker ratio		
	PINP:ICTP	PIIINP:ICTP	PIIINP:PINP
0-1	71.3 (14.9)	120.4 (21.0)	1.7 (0.4)
2	61.1 (12.4)	95.2 (17.6)	2.1 (0.3)
3	61.8 (15.4)	92.9 (21.8)	2.1 (0.4)
4-5	36.2 (17.6)	62.2 (24.9)	1.7 (0.4)

Table 5 gives the correlations observed between the levels of the different markers of collagen metabolism in the serum and synovial fluid of patients. The strongest correlations emerged between PIIINP and ICTP in serum and between PINP and PIIINP in SF. In addition, the two markers of synthesis in SF had distinct correlations with SF-ICTP. A significant correlation was also found between the synovial fluid and serum concentrations of each individual marker.

Table 5: Correlations between the concentrations of the three markers of collagen metabolism in the synovial fluid (SF) and serum (S) of patients with rheumatoid arthritis.

		SF (p)			S (p)	
		ICTP	PINP	PIIINP	ICTP	PINP
SF-PINP	r_s	0.69 (<0.001)				
SF-PIIINP	r_s	0.65 (<0.001)	0.86 (< 0.001)			
S-ICTP	r_s	0.55 (<0.001)	0.32 (0.016)	0.34 (0.010)		
S-PINP	r_s	0.42 (0.001)	0.29 (0.027)	0.33 (0.013)	0.50 (<0.001)	
S-PIIINP	r_s	0.54 (<0.001)	0.39 (0.003)	0.39 (0.003)	0.81 (<0.001)	0.53 (<0.001)

In the controls, the following correlations were found between the serum concentrations of the markers of collagen metabolism: $r_s = 0.48$, $p < 0.001$ for PINP vs ICTP; $r_s = 0.51$, $p < 0.001$ for PIIINP vs ICTP; $r_s = 0.34$, $p = 0.001$ for PINP vs PIIINP. The balance of type I collagen metabolism, as assessed by the ratio (mean(SD)) of S-PINP:S-ICTP, was in favour of degradation in the patients compared with the controls: 9.4 (5.4) and 12.1 (4.9), respectively ($p < 0.001$).

Table 6 shows the correlations between the indicators of disease activity and those of collagen metabolism. Both S-ICTP and S-PIIINP showed positive correlations with the disease activity markers. None of the markers in SF correlated with the number of leukocytes or the number of polymorphonuclear leukocytes in SF.

The S-PINP values did not differ relative to menopausal state, but increased S-ICTP and S-PIIINP concentrations were more common among the postmenopausal women, who also more often had signs of active disease (increased CRP values and high joint swelling scores), which are known to be associated with both increased S-ICTP and S-PIIINP.

Table 6: Correlations between the markers of disease activity and collagen metabolism in patients with rheumatoid arthritis. ESR = erythrocyte sedimentation rate, CRP = C-reactive protein.

Marker of disease activity	Marker of collagen metabolism ($r_s(p)$)		
	S-ICTP	S-PINP	S-PIIINP
ESR	0.43 (< 0.001)	-0.07 (NS)	0.23 (NS)
CRP	0.59 (< 0.001)	0.09 (NS)	0.48 (< 0.001)
Ritchie index	0.46 (< 0.001)	0.06 (NS)	0.37 (0.005)
Joint swelling score	0.65 (< 0.001)	0.21 (NS)	0.60 (< 0.001)

Of the markers of collagen metabolism, SF-ICTP was most closely related to the erosive state of the knee joint (Larsen's grade). The mean SF-ICTP concentrations in Larsen's grades 0-1, 2, 3, and 4-5 were 14.7, 17.5, 22.7, and 43.6 $\mu\text{g/l}$, respectively; that in the combination of Larsen's grades 4-5 differed significantly from that in the other groups ($p < 0.05$ in multiple comparisons). The corresponding values for SF-PIIINP were 1305, 1314, 2058 and 2023 $\mu\text{g/l}$ ($p = 0.014$), and the SF-PIIINP values in Larsen's grades 3 and 4-5 differed significantly from those in the grades 0-1 and 2 ($p < 0.05$ in multiple comparisons). The mean SF-PINP concentrations were 821 $\mu\text{g/l}$ in Larsen's grades 0-1, 855 $\mu\text{g/l}$ in group 2, 1531 $\mu\text{g/l}$ in grade 3 and 1353 $\mu\text{g/l}$ in grades 4-5 (NS).

The S-ICTP and S-PIIINP values were significantly lower in the patients currently receiving a SAARD compared with those not taking these drugs: the median values were 4.9 $\mu\text{g/l}$ vs 8.7 $\mu\text{g/l}$ for S-ICTP ($p = 0.010$), and 4.5 $\mu\text{g/l}$ vs 5.5 mg/l for S-PIIINP ($p = 0.021$). The corresponding values for S-PINP were 40.6 $\mu\text{g/l}$ vs 68.4 $\mu\text{g/l}$ ($p = 0.055$). No significant differences in the mean serum levels of the markers were found between the patients taking or not taking peroral corticosteroids, but the SF levels of all the markers were significantly higher in the patients taking peroral corticosteroids than in those not receiving this treatment. The explanation for this difference may be multifactorial, but the patients on oral corticosteroids had significantly more often higher Larsen's grades in the knee under study than did those not taking these drugs. There was an association between the time elapsed since the last intra-articular injection of corticosteroids and the SF-ICTP levels ($p = 0.007$); the patients who had recently received an intra-articular injection more often had lower levels of the marker.

5.1.2. Study IV

At entry, SF ICTP correlated positively with Larsen's grade. Table 7 shows the distribution of Larsen's grades at entry and at the three-year visit. The median SF-ICTP concentrations in the baseline Larsen's grade groups 0-4 were 10.8, 15.5, 12.5, 18.3 and 33.8 $\mu\text{g/l}$, respectively ($p = 0.009$). The radiological grade deteriorated in 22 (40%) patients, but remained stable in 33 patients (60%).

Table 7: Distribution of Larsen's grades of knee joints at entry and at the three-year visit.

Larsen's grade	Baseline n (%)	3 years n (%)
0	12 (21.8)	7 (12.7)
1	15 (27.3)	16 (29.1)
2	14 (25.5)	11 (20.0)
3	11 (20.0)	13 (23.6)
4	3 (5.5)	7 (12.7)
5	0 (0)	1 (1.8)
Total	55 (100)	55 (100)

Table 8: Serum and synovial fluid markers of collagen metabolism and synovial fluid leukocytes in patients (44 RA, 7 chronic seronegative spondylarthropathy and 4 juvenile RA) with stable and deteriorating Larsen's grade of knee joint at 0 and 3 years' visit. S = serum, SF = synovial fluid, ICTP = cross-linked carboxyterminal telopeptide of type I collagen, PINP = aminoterminal propeptide of type I procollagen, PIIINP = aminoterminal propeptide of type III procollagen. * = $p < 0.05$.

Variable	Patients with stable Larsen's grade median (range)	Patients with deteriorating Larsen's grade median (range)	p value of Mann-Whitney test
S-ICTP ($\mu\text{g/l}$)	4.1 (1.7-14.8) (n = 33)	5.2 (1.1-24.2) (n = 21)	0.456
SF-ICTP ($\mu\text{g/l}$)	14.0 (2.8-42.3) (n = 33)	17.6 (7.1-64.6) (n = 22)	0.035*
S-PINP ($\mu\text{g/l}$)	41.3 (15.0-111.3) (n = 32)	44.2 (23.8-90.70) (n = 20)	0.458
SF-PINP ($\mu\text{g/l}$)	731.0 (118.0-5017.0) (n = 31)	869.5 (147.0-2462.0) (n = 20)	0.345
S-PIIINP ($\mu\text{g/l}$)	3.9 (1.8-7.4) (n = 30)	4.4 (2.1-11.7) (n = 20)	0.209
SF-PIIINP ($\mu\text{g/l}$)	1099.0 (438.0-4781.0) (n = 31)	1378.0 (278.0-3905.0) (n = 21)	0.412
SF leukocytes ($10^9/\text{l}$)	11.2 (3.6-54.8) (n = 32)	5.2 (0.1-18.7) (n = 21)	0.012*
SF polymorphonuclear leukocytes ($10^9/\text{l}$)	7.1 (1.0-45.5) (n = 32)	2.7 (0-14.1) (n = 21)	0.018*

Table 8 shows the median baseline values of the different markers in both groups. The median SF-ICTP concentration was significantly higher in the patients with a deteriorating Larsen's grade than in those with a stable value ($p = 0.035$). When tested only in the patients with RA ($n = 44$), an equal difference in SF-ICTP was found between the above groups; i.e. the median level of ICTP was $14.0 \mu\text{g/l}$ (range $2.8\text{-}42.3 \mu\text{g/l}$) for the 25 RA patients with stable disease and $18.3 \mu\text{g/l}$ (range $7.1\text{-}64.6 \mu\text{g/l}$) for the 19 subjects whose disease was deteriorating, though the difference did not reach statistical significance ($p = 0.063$). Instead, the median levels of both SF total leukocytes ($p = 0.012$) and polymorphonuclear leukocytes ($p = 0.018$) were statistically higher in the patients with a stable grade. A comparison of the diagnostic subgroups showed that the association did not hold in the RA group, but was more pronounced in the other inflammatory arthropathies, i.e., the median was $9.8 \times 10^9/\text{l}$ for stable disease and $11.0 \times 10^9/\text{l}$ for progressive disease in

the RA group, while the corresponding values in the other diagnostic groups were $12.6 \times 10^9/l$ and $2.2 \times 10^9/l$. There was no statistically significant difference for S-ICTP, S-PII-INP, SF-PINP or SF-PIIINP between the above groups either in the whole series or in the diagnostic subgroups. The mean SF:S ratio of ICTP was 3.1 (SD 1.8) for the patients with a stable Larsen's grade, and 4.5 (3.3) for those with a deteriorating Larsen's grade (NS).

There was no statistical difference between the patients with a stable or deteriorating Larsen's grade with regard to treatment with DMARDs or peroral corticosteroid therapy during the follow-up.

5.2. Relation of serum ICTP to the need for total joint replacement surgery (Study II)

The original study group comprised 90 patients with advanced RA, of whom 88 were followed up for two years and 85 for three years. During the three-year follow-up, nine (26%) of the 35 patients with initial serum ICTP values above the upper limit of the reference range ($4.6 \mu\text{g/l}$) required TJRS of at least one joint (six of them underwent two operations and one underwent three) compared with one (2%) of the 50 patients with normal serum concentrations of ICTP ($p = 0.001$) (table 9). This was already apparent after follow-up for two years after the ICTP test. During that period, seven patients underwent TJRS of large joints. All these patients were included in the group of 38 patients with baseline ICTP values above the upper limit of the reference interval, while none of the 50 patients with normal ICTP needed this operation ($p = 0.002$).

Table 9: Relation between the serum concentrations of ICTP at the baseline of a three-year follow-up and the number of total joint replacement operations. Altogether 18 total joint replacement were made on 10 patients. ICTP = cross-linked carboxyterminal telopeptide of type I collagen.

Serum ICTP concentration	Number of operations		
	0-12 months	13-24 months	25-36 months
Increased	4	8	5
Normal	0	0	1

5.3. Incidence of osteoporosis in a two-year follow-up of early RA (Study III)

5.3.1. Bone mineral density values

Table 10 shows the BMD values and T and Z scores at 0 and 24 months in LS and FN. Neither the mean T scores nor the mean Z scores for LS and FN differed significantly between the two measurements (0 and 24 months). Osteoporosis was determined both as a Z score value of -1.0 or less and a T score value of -2.5 or less. By the Z score definiti-

on, eight patients had osteoporotic BMD values at baseline in LS and nine in FN. During the follow-up, their Z scores did not deteriorate significantly. Two additional patients developed osteoporotic BMD values in LS and three other in FN. As defined by the T score, three patients had osteoporosis at baseline, one in LS, one in FN and one at both sites; and none developed osteoporosis during the two-year follow-up.

Table 10: BMD values (g/cm^2) with Z and T scores in 52 patients with early rheumatoid arthritis (RA) at 0 and 24 months. ^a = based on the Finnish normal data by Kröger et al. (1992a, 1992b), BMD = bone mineral density, Z score = value of BMD as a fraction of the SD of the previously published normal value for patient's sex and decade of age, T score = value of BMD as a fraction of the SD of the previously published normal value for the young adults (20-40 y) of the same sex.

	0 months	24 months
	means, (SD) range	means, (SD) range
BMD lumbar spine	1.14 (0.14) 0.88-1.54	1.12 (0.14) 0.83-1.54
BMD femoral neck	0.92 (0.13) 0.61-1.22	0.91 (0.13) 0.66-1.15
Z score ^a , lumbar spine	0.12 (1.04) -2.26-3.09	0.07 (1.20) -2.79-3.94
Z score ^a , femoral neck	-0.16 (1.05) -2.38-2.15	-0.18 (1.11) -2.57-2.56
T score ^a , lumbar spine	-0.55 (1.09) -2.63-2.80	-0.69 (1.10) -3.05-2.79
T score ^a , femoral neck	-0.67 (1.07) -3.21-2.13	-0.80 (1.02) -2.73-1.55

The mean percentages (SD; range) for the alteration of BMD values from 0 to 12 months in LS and FN were -0.91 (2.9; -7.4 to 6.1) and -0.76 (3.7; -8.9 to 5.9) and the corresponding values from 0 to 24 months were -1.3 (3.7; -10.1 to 7.9) and -0.8 (4.7; -10.5 to 10.8), respectively. The changes in BMD did not correlate with the individual clinical parameters of disease activity. The frequency of bone loss > 5% per year did not differ significantly between the patients with elevated (≥ 10 mg/L) and normal CRP. The percentage change in BMD of FN, but not in that of LS, between 0 and 24 months correlated with the cumulative corticosteroid dose (sum of p.o, i.v. and i.a. use) ($r = -0.31$, 95% CI -0.54 to -0.04, $p < 0.05$, and $r = 0.01$, respectively). The patient with a 60 mg starting dose of prednisolone for thrombocytopenia did not develop osteoporosis as defined by either the Z or the T score. BMD loss (>5% per two years in FN or LS) was not found significantly more often in postmenopausal women compared with the other female subjects.

Generally, the changes in the markers of collagen metabolism did not correlate with the changes in BMD during the two-year period, but there was a correlation between the ICTP and lumbar spine BMD changes ($r = -0.40$, 95% CI -0.62 to -0.12, $p < 0.01$). At baseline, the BMD values of FN, but not those of LS, correlated with the body mass index (BMI) ($r = 0.40$, 95% CI 0.14 to 0.61, $p < 0.01$, and $r = 0.15$, respectively).

5.3.2. Serum PINP and ICTP concentrations

Table 11 shows the mean (SD) concentrations of the two markers of type I collagen metabolism in the sera. At baseline, 21 (40.4%) of the 52 patients had ICTP concentrations exceeding the upper limit of the reference interval (4.6 µg/l; 12). Only five patients (9.6%) had PINP concentrations exceeding the upper limit of the reference interval (67.9 µg/l; 14). The correlation coefficients between the PINP and ICTP concentrations were 0.31 ($p < 0.05$, 95% CI 0.03 to 0.54) and 0.29 ($p < 0.05$, 95% CI 0.02 to 0.52) for the baseline and the mean values, respectively.

Table 11: Laboratory, clinical and radiographic findings of the patients during a 2-year follow-up. ICTP = cross-linked carboxyterminal telopeptide of type I collagen in serum (upper limit of reference interval 4.6 µg/l, Hakala et al. 1993), PINP = aminoterminal propeptide of type I procollagen in serum (upper limit of reference interval 67.9 µg/l, Study I), HAQ = Health Assessment Questionnaire, n.d. = not done.

Index	Mean (SD) of variables			
	0 month	3 months	1 yr	2yr
ICTP (µg/)	4.6 (2.0)	3.8 (1.7)	4.1 (1.7)	4.0 (1.5)
PINP (µg/)	44.9(25.7)	42.6 (18.6)	45.0 (18.3)	43.6(17.2)
ESR (mm/h)	28.7 (21.8)	20.8 (19.6)	16.3 (16.5)	19.5 (21.2)
CRP (mg/l)	14.9 (17.6)	9.8 (13.0)	8.7 (10.8)	14.2 (28.0)
Joint swelling score	9.4 (5.9)	5.5 (5.1)	4.1 (3.6)	4.7 (4.5)
Ritchie score	10.5 (8.1)	6.7 (5.9)	5.2 (4.8)	5.9 (5.3)
Larsen's score	15.5 (8.9)	n.d.	19.6 (12.3)	19.4 (14.2)
Morning stiffness (min)	138 (97.6)	87.7 (106.1)	73.3 (90.7)	68.1 (88.2)
HAQ	0.34 (0.37)	0.36 (0.35)	0.29 (0.32)	0.29 (0.39)
Physical function grading	1.96 (0.79)	n.d.	1.79 (0.74)	1.82 (0.81)

5.3.3. Laboratory, clinical and radiographic findings

There was a tendency for improvement in most clinical and laboratory variables of disease activity during the two-year follow-up (Table 11). As assessed on a five-point scale, physical function did not deteriorate (mean 1.96 and 1.82 at baseline and after two years, respectively).

5.4. Prediction of disease progression by ICTP, RF and CRP (Study V)

In study V, altogether 38% (24/63) of the patients appeared to have had a progressive erosive disease course during the follow-up (Δ Larsen > 20). Table 12 shows the relation of the initial CRP, ICTP and RF values and their combinations to Larsen's score at baseline

and to the change of Larsen's score from 0 to 3 years. All the single tests (normal/negative vs elevated/positive) categorized patients into two different prognostic groups with regard to the median change of Larsen's score during the follow-up. This effect was further enhanced when S-ICTP was used in combination with RF or CRP.

*Table 12: Relation of some laboratory tests and their combinations at baseline to Larsen's score. RF = Rheumatoid factor, CRP = C-reactive protein, ICTP = cross-linked carboxyterminal telopeptide of type I collagen, IQR = Inter-quartile range, * = Mann-Whitney rank-sum test with Bonferroni correction.*

Baseline value	Baseline Larsen's score Median (IQR)	Change of Larsen's score from baseline to 36 months Median (IQR)	p-value*
ICTP ($\mu\text{g/l}$)			
≤ 4.6 (n = 28)	2 (0-5)	6 (1-18)	0.041
>4.6 (n = 35)	3 (0-8)	21 (7-31)	
RF present			
No (n = 17)	0 (0-4)	2 (0-18)	0.005
Yes (n = 46)	2 (1-8)	19 (7-32)	
CRP (mg/l)			
< 10 (n = 27)	1 (0-4)	4 (0-22)	0.056
≥ 10 (n = 36)	3 (1-10)	20 (8-31)	
ICTP > 4.6 and RF present			
No (n = 37)	1 (0-5)	6 (0-18)	< 0.001
Yes (n = 26)	3 (2-10)	28 (15-36)	
ICTP > 4.6 and CRP ≥ 10			
No (n = 35)	2 (0-5)	6 (2-19)	0.008
Yes (n = 28)	3 (0-10)	23 (14-35)	

Table 13 shows the age- and sex-adjusted risks (OR) (95% CI) of different abnormal laboratory tests and their combinations for progressive joint disease.

Table 13: Age- and sex-adjusted risk (OR) of different abnormal laboratory tests for progressive joint disease (change in Larsen's score > 20) calculated by logistic regression analysis. CI = Confidence interval. For abbreviations, see Table 12.

Baseline value	OR (95% CI)
ICTP > 4.6 µg/l	3.9 (1.3 to 11.9)
RF present	3.9 (1.0 to 15.5)
CRP ≥ 10 mg/l	2.6 (0.9 to 7.5)
ICTP > 4.6 µg/l and RF present	9.1 (2.5 to 32.9)
ICTP > 4.6 µg/l and CRP ≥ 10 mg/l	6.2 (1.8 to 21.3)

6. Discussion

There are some data to show that the markers of collagen synthesis and degradation measured in serum and synovial fluid reflect the grade of synovitis and tissue breakdown in RA (Greenwald 1996). We used assays for the markers of synthesis and degradation of type I collagen (PINP and ICTP) and for synthesis of type III collagen (PIIINP). Type I collagen is one of the most abundant protein species in the human body. Most of it is present in bones, where it accounts for about 90% of the organic matrix, and it is also the major matrix protein in tendons, ligaments, and soft connective tissues. Type III collagen is the second most abundant collagen type and is found in soft connective tissues.

6.1. Assessment of collagen metabolism in serum and synovial fluid

6.1.1. Study I

In study I, which represented patients with RA and knee joint effusion, high concentrations of all these collagen-derived substances were found in synovial fluid, and their concentrations correlated with the grade of joint destruction. The high SF:S ratios, particularly for PIIINP and PINP, suggest that these antigens are produced locally in the inflamed joints and then released into the circulation. Jensen *et al.* (1993) investigated the transport of PIIINP from the knee cavity into the circulation in conscious pigs after an intra-articular injection of radiolabelled PIIINP. Sequential sampling of thoracic duct lymph, serum, and urine suggested that PIIINP is most probably transported from joint space by bulk flow. Degradation in lymphatics cannot be completely excluded, but a major part of PIIINP reaches the thoracic duct, and thereby the circulation, in an intact form.

In study I, the concentrations of S-PIIINP and S-ICTP were increased in about 50% of the patients. These concentrations correlated with each other, and they also correlated with markers of disease activity, such as CRP and the joint swelling score. These results are in accordance with the earlier findings (Hakala *et al.* 1993b, Paimela *et al.* 1994, Hørslev-Petersen *et al.* 1988, Kotaniemi *et al.* 1994). In contrast, S-PINP was increased in

only a minority of the patients and did not correlate with disease activity. It is probable that, though PINP is released freely from the joints, the amount is small in comparison with that released from the other parts of the skeleton.

A shift in the balance of type I collagen metabolism in RA towards the direction of collagen breakdown was demonstrated in study I by the decreased ratio S-PINP:S-ICTP in patients compared with healthy control subjects. Similarly, a low rate of type I collagen synthesis, as assessed by PICP in serum, has been reported previously in RA (Kröger *et al.* 1993).

The SF-PINP and SF-PIIINP concentrations correlated strongly. The synthesis of both type I and type III collagen is obviously increased in the synovial tissue of a rheumatoid joint, from where propeptides are released into SF. High-grade synovitis in a rheumatoid joint is usually accompanied by a destructive process in the joint. Accordingly, SF-PINP and SF-PIIINP showed a distinct correlation with SF-ICTP. The assessment of the synovial fluid constituents as concentrations is, however, somewhat inaccurate; there is a bidirectional fluid flux across the synovial lining (McDonald & Levick 1992), and the amount of effusion may vary rapidly. This is why we also compared the ratios between various markers with the radiographic findings in the joint. The ratios suggested more collagen degradation in cases with more severe radiographic damage.

Radiographic images record past destructive changes, while it can be postulated that the markers of collagen metabolism in SF reflect the current balance between destructive and reparatory processes. SF-ICTP correlated most closely with joint destruction. However, SF-ICTP showed notable variation at higher Larsen's grades, reflecting the varying activity of collagen degradation. It is evident that the markers of collagen metabolism, such as SF-ICTP or the ratio SF-PINP:SF-ICTP, provide information on the disease process in the joint additional to that obtained from radiographs alone.

In interpreting the results in study I, certain confounding factors which might have influenced the results should be considered. Previous intra-articular injections are among the most important factors of this kind, and some patients in our study population had recently been treated in this way. In addition, we found that the serum levels of the markers of collagen metabolism were significantly lower in the patients currently taking SAARDs than in those not receiving these drugs. Our cross-sectional study was not planned to investigate the effect of treatments on these markers. However, some longitudinal studies have indicated that S-PIIINP and S-ICTP decrease more slowly than the acute phase reactants after the commencement of SAARDs (Hørslev-Petersen *et al.* 1988, Paimela *et al.* 1994). It should also be noted that the biological variation in the serum level of these markers of collagen metabolism is about 20-25%, the least significant change for an individual being smaller than that of corresponding urinary markers (up to 50%) (Blumsohn *et al.* 1994). To be useful in the follow-up of individual patients in clinical practice, the change in the concentration of a marker in response to treatment should exceed the least significant change. Further longitudinal studies are, however, required to clarify this question.

As the markers of collagen metabolism are not tissue-specific, it is not possible to trace their origin in synovial fluid. However, the results of study I and some earlier reports (Hørslev-Petersen *et al.* 1988a, Gressner & Neu 1984, Hørslev-Petersen *et al.* 1988b) suggest that PIIINP and PINP are synthesized in synovial tissue. The strong correlation between these two propeptides and their stable ratio regardless of the degree of radiological

damage in the knee joint suggests that most of the SF-PINP is derived from synovial tissue, and only a small part from articular bone. SF-ICTP, a breakdown product of type I collagen, may reflect the degradation of this collagen in synovial tissue. Actually, necrotic areas may be found in active synovitis, sometimes shown as “rice bodies“ in synovial fluid. However, type I collagen is most abundant in mineralized bone, and in our patients with the highest Larsen’s grades of the assessed joints associated with high SF-ICTP levels, most of the ICTP was most probably derived from periarticular bone.

6.1.2. Study IV

The markers of collagen synthesis and degradation in serum and SF reflect the grade of synovitis and tissue breakdown in RA (Peel *et al.* 1992, Poole AR 1994, Wollheim 1994, Taylor *et al.* 1994, St. Clair *et al.* 1998). Earlier measurement of type I collagen degradation was based on the use of urine samples, including assays of imino acid 4-hydroxyproline, pyridinoline and deoxypyridinoline cross-links (Robins *et al.* 1986, Seibel *et al.* 1989). In study IV, which was a follow-up study of patients with chronic arthritis, we assayed the relation of the markers of synthesis and degradation of type I collagen (PINP and ICTP) and the markers of synthesis of type III collagen (PIIINP) measured in both serum and SF to the progress of radiological knee joint damage in patients with inflammatory arthritis. The diagnosis varied, but no patients with primary osteoarthritis were included.

During the 3-year follow-up, Larsen’s grade in the knee joint deteriorated in 22 (40%) patients. These patients had a significantly higher SF ICTP level at entry than those who turned out to have stable Larsen’s grade. Serum ICTP did not have the same predictive significance, nor did the serum or SF assays for PINP and PIIINP. Serum ICTP reflects the type I collagen metabolism in the whole body, but in our earlier community-based RA series, 26% of the patients with initially elevated serum ICTP values required total joint replacement surgery of at least one joint during a three-year follow-up, compared with 2% of the patients with initially normal values (Study II). However, when estimating the outcome of one particular joint, such as a knee in this study, it seems more logical to measure ICTP from SF than from serum.

Interestingly, the total number of synovial fluid leukocytes and the number of polymorphonuclear leukocytes were here higher in the patients with a stable erosive grade than in those with a progressive one. An analysis of the diagnostic subgroups shows that the association did not hold in the RA group, but was more pronounced in the other groups of inflammatory arthropathies. The non-RA group represented different arthritides and included a small number of patients (n = 11), which detracts from the reliability of the findings. It is to be noted, however, that a previous prospective study by Luukkainen *et al.* (1993), where 29 patients with erosive RA and hydropsy in a knee joint were followed up for 7.5 years, SF leukocytes and SF polymorphonuclear leukocytes did not differ significantly between the patients with and without radiologically detected joint destruction. In another study by our group, where we tested the value of synovial fluid

analysis in estimating the efficacy of intra-articular corticosteroid injections in 30 patients with RA, the percentage of SF polymorphonuclear leukocytes correlated with the decrease in knee joint circumference after six months (Luukkainen *et al.* 1992).

At entry in study IV, SF ICTP correlated positively with Larsen's grade, and those with the highest SF ICTP levels most often deteriorated during the follow-up. The patients with a deteriorating Larsen's grade were more often treated with intra-articular corticosteroids than those with a stable grade. Thus, once the breakdown process has started, it is seldom stopped by the treatment alternatives currently available. The role of local corticosteroids in accelerating joint destruction is speculative. Although local corticosteroids could be expected to accelerate destruction, it is probably the activity of the inflammatory joint disease that produces recidivous effusion, which requires repeated corticosteroid injections.

Our preliminary results indicate that a high SF leukocyte level is not necessarily associated with a poor prognosis. Furthermore, a recent study on the relation of synovial biopsy findings to SF analysis in 33 patients with inflammatory arthritis suggested that SF cell counts reflect the activity of acute synovial inflammation but not that of chronic inflammation (Luukkainen *et al.* 1999).

We conclude that synovial fluid analysis may help in assessing the disease process of a particular joint in patients with inflammatory arthritis. A high SF ICTP level seems to reflect accelerated synovial tissue and bone degradation with further radiologic destruction.

6.2. Type I collagen degradation and need for total joint replacement surgery (Study II)

In the series of advanced RA, we found that a single serum level of ICTP had some prognostic value as to the subsequent need for total joint replacement surgery. This was shown during a follow-up of three years after the baseline ICTP test in a series of 90 patients with advanced RA. After three years, nine (26%) of the 35 patients with elevated ICTP at the beginning *vs* one (2%) of the 50 patients with a normal value needed total joint replacement surgery.

It can be postulated that in RA patients with central joint affliction, a large periarticular area is available for accelerated bone resorption and consequent liberation of ICTP into circulation as compared with patients with merely small joint affliction. The finding by Kotaniemi *et al.* (1994) concerning the association of increased type I collagen degradation with widespread joint affliction in RA (measured by the Lansbury index) supports the above theory.

Interestingly, in the present series, increased CRP had an equivalent prognostic power as to the subsequent need for total joint replacement surgery. We did not make sequential analyses of the laboratory tests for this study, but it has been shown that even when the current drug treatment reduces the acute phase response, radiographic progression continues (Scott *et al.* 1985). The few findings on increased serum ICTP reported previously were similar (Paimela *et al.* 1994), though the decline of ICTP levels was slower and less remarkable than that of CRP.

In this series of patients with advanced RA, higher serum levels of ICTP were associated with progressive disease course as judged by the need for total joint replacement surgery. Ideally, the measurement of outcome in RA should estimate the outcome of disease during the early reversible stages rather than the later stages.

6.3. Incidence of osteoporosis (Study III)

In our series of early RA, only a few patients developed osteoporosis as shown by individual LS or FN Z and T scores during the two-year follow-up. Altogether 12 (23%) of the 52 patients lost > 5% of their BMD - eight at FN and four additional subjects at LS - during the first year. During the second year, however, bone loss was not progressive among these patients, indicating osteoporosis (defined by Z Score for LS or FN) in only one of them.

As regards the mean percentage BMD change, our patients seemed to have less bone loss during the early years of RA than has been earlier reported (Gough *et al.* 1994, Shenstone *et al.* 1994). The results may diverge for a number of possible reasons. In our cohort, all patients, regardless of disease activity, were early referrals by primary care physicians to the district rheumatism hospital. Hence, this group represents more a community-based than a tertiary-based sample of RA patients who met the ACR 1987 criteria (Arnett *et al.* 1988). Accordingly, half of our patients had mild RA with normal CRP at entry. In addition, our patients were actively treated with SAARDs, and there was a tendency for improvement of the clinical findings during the follow-up period (Table 11). Furthermore, when assessed by a five-point scale, the patients' physical function did not deteriorate during the follow-up.

The role of low-dose glucocorticoid therapy in enhancing the risk of generalized osteoporosis in rheumatoid arthritis is controversial, as the frequency of osteoporosis and its localization vary widely between different studies (Star & Hochberg 1994, Sambrook *et al.* 1986, Kröger *et al.* 1994). On the other hand, it has been postulated that by reducing the disease activity and improving mobility, glucocorticoids would decrease bone loss in active RA (Gough *et al.* 1994, Sambrook *et al.* 1989). However, there is no doubt that large doses of corticosteroids inhibit bone formation effectively, leading to rapid development of osteoporosis. In our series, prescriptions for oral corticosteroids were not common. Although 25 patients took oral corticosteroids at some point during the study, only seven of them (13%) used these drugs throughout the study period. There was, however, a correlation between the percentage change of BMD in FN and the cumulative corticosteroid dose administered. Because RA patients with active disease are likely to be treated with corticosteroids, the confounding effect of disease activity on this correlation cannot be excluded.

We looked for the possible association between the changes in BMD and the serum markers of type I collagen metabolism. At baseline, the serum level of ICTP was more often elevated than that of PINP, indicating the predominance of type I collagen breakdown, while these markers correlated only weakly with each other. There was some correlation between the change of ICTP and BMD in LS during the two-year period ($r = -0.40$). However, no correlations were detected between the collagen markers and BMD

changes in FN. On the other hand, we confirmed our earlier finding (Paimela *et al.* 1994, Studies I & II) that S-ICTP correlates with the markers of disease activity and the progress of erosive joint disease (data not shown).

Based on our results, early community-based RA cases with predominantly mild disease rarely develop generalized osteoporosis in a two-year follow-up. Against that background, the ability of markers of type I collagen metabolism to reflect BMD changes could not be tested in this series.

6.4. Prediction of disease progression by ICTP, rheumatoid factor and C-reactive protein (Study V)

There has been an intensive search for different biochemical markers, such as tests measuring bone and cartilage degradation, to distinguish the early RA cases with an aggressive disease course from those with a milder course. The ideal marker should be reliable, reproducible, sensitive, simple and inexpensive. Yet, there is no consensus so far about the tests which should be generally used for disease prediction in RA. The lack of agreement concerning the current gold standard against which any new prognostic indicator should be compared further complicates the situation (Young & van der Heijde 1997). Thus, our main guides to therapy at the present are the standard clinical and laboratory tests, such as ESR and CRP, and radiographs.

In study V, it was shown that progressive joint disease (change of Larsen's score > 20 during 3 years) was more likely to occur in a patient who had elevated baseline S-ICTP (> 4.6 µg/l) combined with either positive RF or elevated CRP (≥ 10 mg/l) than in one without these changes. When the tests were used alone, the associations were weaker, and in the case of CRP the relation was not statistically significant.

The treatment schedule used in the present series was a typical "saw-tooth strategy". Accordingly, a SAARD initially instituted was replaced by another in case of inefficacy or side-effects. Despite the relatively good clinical response, however, which was reflected in the rapid mean decline of CRP, ICTP dropped more gradually and most patients showed radiologic progression (Paimela *et al.* 1994). A randomized study of early RA from Finland showed that combination therapy retards radiological progression more effectively than monotherapy (Möttönen *et al.* 1999). How this is reflected in tissue-derived degradation products is not yet known. Although a prescription of SAARD is given to nearly every RA patient at the time of diagnosis in Finland, the present risk profile could, however, be useful in differentiating the patients who will need the most aggressive therapy from those with a mild disease course who can be treated with a single drug.

Several studies have also shown that CRP correlates with radiologic progression (Otterness 1994). On the other hand, RF is still postulated to be the most powerful single predictor of erosive disease in spite of the huge number of laboratory measures tested since the 1940s (Young & van der Heijde 1997). Other factors, including poor function, female gender, articular index, HLA-DRβ1 shared epitope and acute-phase reactants, may influence the outcome adversely, but are either no better than RF or unreliable when used

alone (Young & van der Heijde 1997). The results of the present series show that a combination of baseline RF or CRP with a tissue degradation product, ICTP, gives a more satisfactory prediction of subsequent radiologic progression.

Type I collagen accounts for about 90% of the organic matrix of bone, and is the major matrix protein in tendons, ligaments and soft connective tissues. Thus, an assessment of its breakdown is thought to be useful in diseases with connective tissue degradation, such as RA. No comparative studies of the different measures of type I collagen degradation have been performed in early RA, but a recent cross-sectional analysis of these markers in advanced RA showed serum ICTP and urinary pyridinoline to be superior to urinary excretion of deoxypyridinoline, aminoterminal cross-linked telopeptide of type I collagen and CrossLapsTM in discriminating between patients with RA and controls (Cortet *et al.* 1998). ICTP and PYD also have minimal short-term, day-to-day variability, and the authors proposed that testing of these measures in the assessment of the effect of new, potentially disease-modifying therapies (Cortet *et al.* 1998) may be useful. In contrast to urinary pyridinoline excretion, serum ICTP assay is readily available and easy to perform (Cortet *et al.* 1998).

Serum ICTP has been shown to correlate with the extent of joint inflammation as measured by the Lansbury index in early RA (Kotaniemi *et al.* 1994). In addition, compared to other collagen-derived peptides, the synovial fluid:serum ratio of this collagen marker is rather low (Study I). Hence, it seems that the elevation of ICTP in the sera of patients with RA is mostly due to its direct liberation from the periarticular bone and synovial tissue of inflamed joints into the circulation (Hakala *et al.* 1993b, Cortet *et al.* 1998).

It can be concluded that initially elevated serum ICTP combined with abnormal RF or CRP could serve as a predictive combination for an aggressive disease course in early RA.

6.5. Biochemical and immunologic markers for diagnosis and outcome measurement in early RA

Table 14 shows a summary of the laboratory markers that are thought to have a potential for the diagnosis and prediction of the outcome of early RA. Only a few of these tests, namely RF, CRP and ESR, are routinely used in clinics. The results are contradictory as to the predictive potential of many of these markers.

Taulukko 14: Biochemical and immunologic markers for diagnosis and outcome measurement in early RA. RF = rheumatoid factor, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, COMP = cartilage oligomeric matrix protein, BSP = bone sialoprotein, ICTP = cross-linked carboxyterminal telopeptide of type I collagen, PYD = pyridinoline, DPYD = deoxypyridinoline.

Marker	Prognostic	Disease-specific
RF	Yes	Yes
CRP, ESR	Yes	No
Antifilaggrin antibody	?*	Yes
Shared epitope/HLA-DR4	?	Yes
COMP	Yes	No
Hyaluronate	Yes	No
BSP	Yes	No
ICTP	Yes	No
PYD	Yes	No

? = Few or conflicting data, * Aho *et al.* 1999.

7. Conclusions

1. There are markers of collagen metabolism that can be measured in serum and synovial fluid to provide an assessment of the disease process in patients with RA. Hitherto, ICTP and PIIINP have been the most informative.
2. According to the results of this study, which mainly focused on patients with RA, synovial fluid analysis may help in assessing the disease process of a particular joint in patients with inflammatory arthritis. The results indicate that a high SF leukocyte level is not necessarily associated with a poor prognosis. Instead, a high SF-ICTP level seems to reflect accelerated radiological progression of the assessed joint.
3. An elevated serum ICTP level in patients with advanced RA seems to discriminate between cases with mild and destructive joint disease and the need for total joint replacement surgery.
4. In discordance with the previous findings of early RA, generalized osteoporosis was rarely seen during the first two years. The possible explanatory factors are that the present series was community-based and the patients had predominantly mild disease, were actively treated and showed no deterioration of their their physical function. Against this background, the power of the markers of type I collagen metabolism to reflect BMD changes could not be tested.
5. Initially elevated serum ICTP combined with either RF positivity or increased CRP may serve as a predictive combination for an aggressive disease course in early RA.

8. References

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