Urinary Type II Collagen Helical Peptide (HELIX-II) as a New Biochemical Marker of Cartilage Degradation in Patients With Osteoarthritis and Rheumatoid Arthritis

Nadine Charni, Fabrice Juillet, and Patrick Garnero

Objective. Type II collagen, which consists of a large helical domain and telopeptides at each end, is the most abundant protein of cartilage matrix. The aim of this study was to develop a biochemical marker reflecting the degradation of the helical region of type II collagen and to evaluate its clinical performance in patients with osteoarthritis (OA) and rheumatoid arthritis (RA).

Methods. We developed a competitive polyclonal antibody–based enzyme-linked immunosorbent assay (ELISA) using the 622–632 peptide derived from the sequence of the α1 chain of human type II collagen (HELIX-II) as immunogen and standard. We measured urinary levels of HELIX-II peptide and C-terminal crosslinking telopeptide of type II collagen (CTX-II) in 90 patients with knee OA (73% women; mean ± SD age 63.0 ± 8.0 years, mean ± SD disease duration 6.1 ± 6.8 years), 89 patients with early RA (disease duration <3 years) (79% women; mean ± SD age 48.7 ± 11.6 years), 25 patients with Paget’s disease of bone (HELIX-II only), and 162 healthy controls. In RA patients, we investigated the relationships between baseline urinary HELIX-II and CTX-II levels and the progression of joint destruction as measured by the changes in the total Sharp score (average from 2 independent readers) over 1 year.

Results. The intraassay and interassay variations of the HELIX-II ELISA were lower than 13% and 15%, respectively. The HELIX-II ELISA showed no significant cross-reactivity with human intact or denatured type II collagen, with the homologous peptides from human type I or type III collagens, or with HELIX-II peptides elongated (by 1 amino acid) or shortened (by 1 or 2 amino acids) at the C-terminal end, indicating that the HELIX-II ELISA recognized a neoepitope from the α1 chain of type II collagen. Median urinary HELIX-II levels were increased in patients with knee OA (by 56%; P < 0.0001) or early RA (by 123%; P < 0.0001) compared with those in age- and sex-matched healthy controls. Baseline urinary HELIX-II levels in the highest tertile were associated with an increased risk of radiographic progression in RA (increase in the total Sharp score ≥0.5 units/year), with an odds ratio (OR) of 5.9 (95% confidence interval [95% CI] 2.0–17.2) after adjustment for serum C-reactive protein (CRP) levels and baseline joint damage. Patients with increased levels of both urinary HELIX-II and CTX-II had the highest risk of progression (OR 17.5 [95% CI 3.1–99]).

Conclusion. The HELIX-II ELISA is specific for type II collagen degradation, has adequate technical performance, and can distinguish patients with knee OA or RA from healthy controls. Elevated HELIX-II levels are associated with increased risk of radiographic progression in RA independently of CRP levels, baseline joint damage, and urinary CTX-II levels. The HELIX-II ELISA should be useful for the clinical investigation of patients with arthritis and for identifying RA patients at higher risk of progression.

One of the major clinical manifestations of rheumatoid arthritis (RA) and osteoarthritis (OA) is abnormal and degraded cartilage in affected joints. The presence and extent of cartilage destruction in RA and OA is mainly monitored by plain radiography, although magnetic resonance imaging is currently being optimized for this purpose (1). Because alterations of cartilage matrix turnover occur early in the disease both in RA and in OA, long before significant damage can be detected radiographically, it is of critical importance that
clinicians gain access to sensitive noninvasive biochemical markers of cartilage turnover (2,3).

Type II collagen is the major fibrous collagen of cartilage, representing 80–90% of the collagen in this tissue (4). Type II collagen fibers make up 40–50% of cartilage dry weight and give articular cartilage its tensile strength (4,5). Degradation of type II collagen is considered to be the hallmark of cartilage degradation in arthritis, frequently resulting in irreversible damage. Because of the abundance of type II collagen, its specificity for cartilage tissue, and the fact that its degradation is an early sign of damage in the disease process both in OA and in RA (6–8), the detection of type II collagen-derived fragments in synovial fluid, serum, and urine has been a field of extensive research for several years (2,3,9).

Type II collagen is constituted by the association of 3 identical α1 chains in triple helix except at the 2 ends, in the N- and C-telopeptides. Degradation of type II collagen takes place via different enzymatic processes that involve primarily the interstitial collagenases (matrix metalloproteinase 1 [MMP-1], MMP-8, MMP-13, and membrane type 1 MMP) (7,10), which cleave the triple-helical region of type II collagen at a single site between residues 778 and 776, generating 2 fragments representing three-quarters and one-quarter, respectively, of the intact collagen molecule. These fragments of triple helix carry neoepitopes that have been used to develop antibodies to type II collagen (11,12). It has been demonstrated that the Col2-3/4 epitope from the larger three-quarter fragment, but not the Col2-1/4 epitope, can be found in the circulation, probably due to its higher resistance to proteolysis (13). The three-quarter fragment is then further degraded to release several smaller fragments.

Recently, immunoassays detecting C-terminal crosslinking telopeptide of type II collagen (CTX-II) have been developed, and increased levels of CTX-II have been reported in the synovial fluid soon after knee injury (14) and in the urine of patients with OA and RA, elevated levels being associated with more rapid progression (15,16). Although the exact structure of the various type II collagen fragments and the enzymatic processes involved in their release remain to be investigated, it is possible that they reflect different molecular mechanisms of type II collagen degradation, as has been recently suggested not only for the collagenase-generated Col2-3/4C epitope and the telopeptide fragment CTX-II (8), but also for different bone type I collagen fragments (17). Thus, the availability of different molecular markers of type II collagen degradation, and, more specifically, those originating from the helical region (which constitutes the major part of the protein), could be useful for a better understanding of the cartilage degradation processes in arthritis.

In this report, we describe the development and technical evaluation of an enzyme-linked immunosorbent assay (ELISA) for the measurement in urine of a new specific fragment arising from the degradation of type II collagen helical domain. We also compare its clinical performance in RA and OA patients with that of urinary CTX-II, which is currently one of the most promising markers of cartilage turnover both in OA and in RA (9,18).

**PATIENTS AND METHODS**

**Patients with knee OA, early RA, and Paget’s disease of bone, and healthy controls.** Patients with knee OA. We investigated 90 patients (73% of whom were women) with a mean ± SD age of 63.0 ± 8.0 years (range 48–84 years) and a mean ± SD disease duration of 6.1 ± 6.8 years who met the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) criteria for primary knee OA (19). These patients were randomly selected from a total of 537 patients with knee OA who were participating in a larger randomized, multicenter, double-blind study of an investigational drug (20). The demographics of that subset of patients were similar to those of the whole population (76% women; mean ± SD age 63.0 ± 8.0 years, mean ± SD disease duration 6.6 ± 6.0 years).

Inclusion criteria were as follows: adult male or female age ≥50 years, clinically confirmed OA of the knee for at least 3 months prior to the study, pain in or around the knee most of the time, <30 minutes of morning stiffness, crepitus on active motion, radiographically confirmed osteophytes of the knee of grade 2 or 3 according to the Kellgren/Lawrence scale (21), and a Western Ontario and McMaster Universities Osteoarthritis Index (22) total score of ≥50 mm on a 100-mm visual analog scale (VAS). Patients were excluded from the study if they had current or previous acute inflammatory joint disease or acute major trauma; treatment within the 3 months before inclusion with any investigational drugs, alkylating agents, growth factors, biologic agents, or immunosuppressive drugs; intake of any OA-directed medication within 1 week prior to inclusion; intake of >10 mg/day oral steroids within 4 weeks before the study; treatment with intraarticular corticosteroid or hyaluronic acid injections within the previous 2 months; advanced disease defined by grade 3 joint space narrowing (JSN) (assessed from a radiograph using visual scoring according to the Osteoarthritis Research Society International protocols [23]); or joint replacement planned within the next 6 months. All patients had normal renal function as documented by normal creatinine clearance. In this study, we only analyzed samples collected at baseline before any administration of the investigational drug.

Patients with early RA. We investigated 89 patients (79% of whom were women) with a mean ± SD age of 48.7 ± 11.6 years (range 23–75 years) who met the ACR 1987 revised
criteria for RA (24). None of these patients had juvenile idiopathic arthritis. These patients were randomly selected from a total of 632 patients with early RA who were participating in a larger randomized, multcenter, double-blind study comparing the efficacy of etanercept and methotrexate (25). This patient cohort has been described previously (26).

Eligible patients in the initial cohort were at least 18 years old, had no other important concurrent illnesses, had had RA for no more than 3 years, and had not been treated with methotrexate. To recruit patients who were at high risk for radiographic progression, we required that eligible patients have active disease at enrollment, defined as ≥12 tender joints and ≥10 swollen joints. Patients were also required to be rheumatoid factor positive or to have at least 3 bone erosions evident on radiographs of the hands, wrists, or feet. Disease-modifying antirheumatic drugs, including hydroxychloroquine and sulfasalazine, were discontinued at least 4 weeks before the study began. Treatment with stable doses of corticosteroids and nonsteroidal antiinflammatory drugs was permitted. Corticosteroid doses could not exceed the equivalent of 10 mg/day of prednisone. Among the 89 patients analyzed in that study, 33 received methotrexate at a dose which was escalated from 7.5 mg/week to 20 mg/week over the first 8 weeks of the study, 28 received 10 mg etanercept subcutaneously twice a week for 1 year, and 28 received 25 mg etanercept subcutaneously twice a week for 1 year.

Assessment of disease status in RA patients included a complete count of tender and swollen joints (71 joints were assessed; hips and cervical spine were evaluated only for tenderness). All joint assessments were performed by specially trained, independent assessors. The Health Assessment Questionnaire (27), an index of disability, was administered to all patients. Other indices of disease activity included patient’s and physician’s global assessments (on scales ranging from 0 [best] to 10 [worst]), patient’s assessment of pain (on a VAS ranging from 0 [best] to 10 [worst]), and serum level of C-reactive protein (CRP).

Anteroposterior radiographs of the hands, wrists, and feet were obtained at baseline and at 6 and 12 months in all RA patients. Radiographs were analyzed by 2 senior physicians with expertise in musculoskeletal medicine who were trained in the scoring method and who were blinded both to the patient’s treatment assignment and to the chronological sequence of baseline and followup examinations. Radiographs were scored according to the modified Sharp scoring method (28,29). The mean value of the 2 readers’ scores was used at each time point to assess JSN, bone erosion, and total Sharp score (the sum of the JSN and erosion scores). The average progression rate per patient (Sharp units/year) was obtained by linear regression using the 3 available observations in time.

Patients with Paget’s disease of bone. We investigated 25 patients with Paget’s disease of bone (5 women and 20 men; mean ± SD age 70 ± 7 years). The diagnosis of Paget’s disease of bone was documented by radiographs and bone scintigraphy, and all patients had baseline serum total alkaline phosphatase activity at least 2-fold higher than the upper limit in normal adults. None had been treated with calcitonin in the previous 3 months or with bisphosphonate in the previous 6 months.

Healthy controls. One hundred sixty-two healthy subjects (107 women and 55 men) with a mean ± SD age of 55 ± 15 years (range 26–90 years) were included. All subjects were healthy without any disease or treatment that could interfere with bone or joint metabolism, including hormone replacement therapy in postmenopausal women. We also included age- and sex-matched control groups for patients with knee OA (n = 95, 73% female; mean ± SD age 63 ± 10 years [range 48–84 years]), patients with RA (n = 110, 77% female; mean ± SD age 49 ± 12 years [range 26–74 years]), and patients with Paget’s disease of bone (25 men and 8 women; mean ± SD age 68 ± 12 years).

The studies were approved by the local ethics committee. Written informed consent was obtained from all participants.

Fasting second-morning void urine samples were obtained from all OA and RA patients and all patients with Paget’s disease of bone at baseline before treatment. The same types of samples were obtained from controls. All urine samples were kept frozen at –70°C until assayed. Urinary marker levels were corrected by urinary creatinine concentration measured by standard colorimetric assay based on the method of Jaffe (30). Intra- and interassay variations were lower than 2.9% and 3.8%, respectively.

Immunoenasay for type II collagen helical peptide. Materials. All chemicals were purchased as analytic grade from Sigma (St. Quentin Fallavier, France), Merek (Limonest, France), or Euromedex (Strasbourg, France). Intact purified human type II collagen from cartilage was purchased from Chemicon (Temecula, CA). Synthetic peptides, including biotinylated and keyhole limpet hemocyanin (KLH)–coupled peptides, were synthesized to >85% purity by NeoSystmes (Strasbourg, France) using standard 9-fluorenylmethoxy carbonyl solid-phase peptide synthesis (31).

Production of polyclonal antibodies against HELIX-II. Rabbits were injected intraperitoneally with 1 ml (0.5 mg/ml of conjugated peptide) of the synthetic helical peptide $^{623}$ERGETGPYhGTS$^{632}$ (where hyp is hydroxyproline) derived from the sequence of the α1 chain of human type II collagen (HELIX-II; SwissProt accession no. P02458) conjugated to KLH using glutaraldehyde (32) emulsified in equal volume with Freund’s complete adjuvant. Immunizations were repeated 3 times every month for 3 months using the same immunogen but emulsified in Freund’s incomplete adjuvant as previously described (32). At each bleeding, antiserum was screened by titration for the presence of anti–HELIX-II antibodies. Titration was performed by investigating the binding of subsequent dilutions of the antiserum on microtiter plates coated with biotinylated HELIX-II peptide (see below).

The titer was defined as the dilution of the antiserum giving 50% of the absorbance of the undiluted antiserum. The antisera with the highest titers were selected for the development of the ELISA.

HELIX-II ELISA. Biotinylated HELIX-II peptide diluted in 1.5 mmoles/liter KH$_2$PO$_4$, 8.5 mmoles/liter Na$_2$HPO$_4$, 2.7 mmoles/liter KCl, 137 mmoles/liter NaCl, 5 gm/liter bovine serum albumin (BSA), and 0.5 gm/liter Tween 20, pH 7.2 (100 μl, 0.25 ng/ml) was pipetted into each well of an avidin-coated plate (Immunotech, Marseilles, France). The plate was incubated for 2 hours at room temperature. The plate was then washed 5 times with 25 mM Tris, 150 mM NaCl, 0.5 gm/liter BSA, and 0.05 gm/liter Tween 20, pH 7.2, and 50 μl of calibrator, control, or unknown samples was pipetted into the
and interassay variations were lower than 8% and 15%, respectively.

**Statistical analysis.** All data are expressed as the mean ± SD unless otherwise specified. Between-group comparisons were performed using the Kruskal-Wallis nonparametric test or the nonparametric Mann-Whitney rank test. Because clinical radiographic indices and biochemical markers were not normally distributed, correlations were estimated by nonparametric Spearman’s rank correlation coefficient. In patients with early RA, progression of joint destruction was defined as an increase in the total Sharp radiographic score of ≥0.5 units/year, as previously suggested (26,33). Relative risks of progression of joint destruction according to levels of molecular markers were estimated by logistic regression analyses after adjustment for treatment allocation and other potential confounding variables. All statistical analyses were carried out using SAS software (SAS Institute, Cary, NC).

### RESULTS

**Analytic performance of HELIX-II ELISA.** We developed a competitive ELISA using a polyclonal antibody raised against the HELIX-II sequence. The detection limit, defined as the concentration corresponding to 3 SD above the mean of 32 determinations of the zero calibrator, was determined to be 0.2 μg/liter. The intraassay variation assessed by 32 measurements of 4 different urine samples (mean levels of 0.57, 1.35, 1.95, and 9.76 μg/liter) in the same run ranged from 7.7% to 12.3%. The interassay variability determined by the measurements of 3 different urine samples (mean levels of 0.55, 1.38, and 1.92 μg/liter) in 10 different runs ranged from 7.3% to 14.5%. The median dilution recovery of urine was 99% (Table 1), and the dose-dilution (from 1:1 to 3 SD above the mean of 32 determinations of the zero calibrator, was determined to be 0.2 μg/liter. The interassay variation assessed by 32 measurements of 4 different urine samples (mean levels of 0.57, 1.35, 1.95, and 9.76 μg/liter) in the same run ranged from 7.7% to 12.3%. The interassay variability determined by the measurements of 3 different urine samples (mean levels of 0.55, 1.38, and 1.92 μg/liter) in 10 different runs ranged from 7.3% to 14.5%. The median dilution recovery of urine was 99% (Table 1), and the dose-dilution

Table 1. Dilution of 3 urine samples in HELIX-II immunoassay*  

<table>
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<th>Sample, dilution</th>
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* HELIX-II = urinary type II collagen helical peptide.
15% to 67%) curves of 2 urine samples, 1 from a healthy subject and 1 from an RA patient, were parallel to the standard curve (not shown). Spiking recovery, determined by addition of known quantities of HELIX-II peptide (1, 2, and 5 μg/liter) into 6 different urine samples (initial concentrations of 1.34, 1.51, 1.93, 3.11, 3.66, and 6.10 μg/liter), ranged from 79% to 131% (median 101%).

As shown in Table 2, urinary HELIX-II levels remained stable after up to 24 hours of incubation at room temperature or at 4°C, with changes compared with nonincubated samples within the analytical precision of the assay for most samples. Similarly, 4 repeated freeze–thaw cycles did not substantially change the urine concentration of HELIX-II (Table 3).

The specificity of the antibody was investigated by experiments involving competitive inhibition between HELIX-II peptide and different synthetic peptides. As shown in Figure 1, there was no significant cross-reactivity of the antibody with the HELIX-II peptides that were shortened by 1 or 2 amino acids at the C-terminal end or with the ERGETGPhypGTSG peptide (which corresponds to the HELIX-II sequence extended by 1 amino acid) up to a concentration of

<table>
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<th>Sample</th>
<th>Freeze–thaw cycle</th>
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<tr>
<td>2</td>
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<td>2</td>
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<td>4</td>
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* Values are levels of urinary type II collagen helical peptide (HELIX-II) in μg/liter. Values in parentheses are the percent change in urinary HELIX-II levels from initial levels.

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Figure 1. Typical standard curve and specificity of the enzyme-linked immunosorbent assay (ELISA) using urinary type II collagen helical peptide (HELIX-II). The graph shows the competitive inhibition of HELIX-II ELISA with HELIX-II synthetic peptide used as standard (ERGETGPhypGTGTS), with HELIX-II peptide in which hydroxyproline (hyp) was replaced by a proline (ERGETGPPGTGTS), with HELIX-II peptide extended by 1 amino acid (glycine) (ERGETGPhypGTSG) or shortened by 1 or 2 amino acids at the C-terminal end (ERGETGPhypGT and ERGETGPhypG, respectively), with the homologous sequence peptide 622–632 derived from the α1 chain of human type I and type III collagens (DRGEPGPhypGPA and ERGETGPhypGPA, respectively), and with intact or denatured human type II collagen. The y-axis shows the relative binding of the HELIX-II antibody on HELIX-II–coated microtiter plates at different concentrations of each peptide (B) expressed as a percentage of the binding with no competitor peptide (BO). The x-axis shows the molar concentration of each peptide for purposes of comparison. The concentration of the HELIX-II standard is shown below.
0.04 μM. The antibody did not demonstrate significant immunoreactivity with human intact or denatured type II collagen or with the peptides DRGEPGPhypGPA or ERGETGPhypGPA, which are the homologous sequences derived from the α1 chain of human type I and type III collagens, respectively. The antibody recognized the HELIX-II sequence in which hydroxyproline was replaced by a proline, although with a lower affinity. Indeed, a 13-fold higher concentration of this peptide compared with the native HELIX-II peptide was needed to displace 50% of the binding of the antibody on the microtiter plate (Figure 1).

Urinary HELIX-II levels in healthy controls, patients with arthritis, and patients with Paget's disease of bone. There was no significant correlation with age in urinary HELIX-II levels in healthy women ages 26–90 years (r = 0.064, P = 0.54) or men ages 26–79 years (r = 0.17, P = 0.24). In healthy individuals, urinary HELIX-II levels were slightly higher in men than in women (median [interquartile range] 228 ng/m mole creatinine [132–336] versus 175 ng/m mole creatinine [107–257]; P = 0.016).

As shown in Figure 2, median urinary HELIX-II levels were significantly increased in patients with knee OA (by 56%; P < 0.0001) and in patients with early RA (by 123%; P < 0.0001) compared with those in age- and sex-matched controls. When women and men were analyzed separately, urinary HELIX-II levels were 75% higher in female patients with knee OA than in healthy age-matched women (median 289 ng/m mole creatinine versus 165 ng/m mole creatinine; P < 0.0001). HELIX-II levels were also increased in the 24 men with knee OA compared with HELIX-II levels in healthy men, although the difference did not reach statistical significance, probably because of small sample size (median 277 ng/m mole creatinine versus 225 ng/m mole creatinine; P = 0.23). Female and male RA patients had significantly higher levels than healthy women (median 384 ng/m mole creatinine versus 180 ng/m mole creatinine; P < 0.0001) and healthy men (median 452 ng/m mole creatinine versus 218 ng/m mole creatinine; P = 0.015), respectively. The proportions of patients with HELIX-II levels above the upper limit in controls (defined by the 95th percentile) were 28% and 55% for patients with knee OA and early RA, respectively. RA patients had significantly higher levels than patients with knee OA (P = 0.0005).

In the same individuals, median urinary CTX-II levels were increased by 185% in patients with knee OA and by 90% in patients with early RA (P < 0.0001 for both), and 56% and 28% of patients with knee OA and RA, respectively, had CTX-II levels above the 95th percentile of age- and sex-matched controls. When all patients were analyzed together, there was a significant but modest correlation between urinary HELIX-II levels and urinary CTX-II levels (r = 0.56, P < 0.001). Similar associations were observed in each population considered separately (r = 0.60, r = 0.56, and r = 0.64 for...
controls, patients with knee OA, and patients with RA, respectively; $P < 0.0001$ for all). In the 25 patients with active Paget’s disease of bone, urinary HELIX-II levels were similar to those in healthy age-matched controls (median [interquartile range] 218 ng/m mole creatinine [153–303] versus 230 ng/m mole creatinine [146–305], respectively; $P = 0.87$). This was in contrast to a 10-fold increase in urinary nonisomerized CTX-I, a specific and sensitive biochemical marker of bone type I collagen degradation (34), in patients with active Paget’s disease of bone ($P < 0.0001$) (data not shown).

### Relationships between urinary HELIX-II levels, disease activity, and joint damage in RA patients

In patients with early RA, we found modest associations of urinary HELIX-II levels with physician’s global assessment ($r = 0.26, P = 0.02$) and with serum CRP levels ($r = 0.30, P = 0.006$) at baseline. Urinary levels of HELIX-II and CTX-II correlated significantly with changes in total Sharp score over 12 months ($r = 0.30, P = 0.007$ and $r = 0.22, P = 0.05$, respectively). Patients were categorized as having low and high cartilage turnover using the highest tertile level as a cutoff, and progression was defined as an increase in the total Sharp radiographic score of $\geq 0.5$ units/year, as previously suggested (26,33). Patients with baseline levels of urinary HELIX-II or CTX-II in the highest tertile had a significantly higher risk of progression compared with the other patients (relative risks of 6.9 and 3.1, respectively) (Table 4). After adjustment for baseline radiographic score and/or CRP levels, increased levels of urinary HELIX-II still predicted the risk of progression, with slightly decreased relative risks (Table 4). Adjustment of the CTX-II level for baseline radiographic score did not modify its association with progression, but adjustment for the baseline CRP level weakened the relationship, which was no longer significant. Further adjustments for age, body mass index, and clinical indices of disease activity did not alter the results significantly (data not shown).

When baseline urinary levels of HELIX-II and CTX-II were both included in a multivariate logistic regression model, each marker significantly ($P = 0.0004$ and $P = 0.03$, respectively) and independently predicted progression (an increase in the total Sharp radiographic score of $\geq 0.5$ units/year). Patients with urinary HELIX-II and CTX-II levels that were both in the highest tertile had a higher rate of progression than did patients with either high urinary HELIX-II levels or high urinary CTX-II levels (Figure 3) and had a relative risk of progression of 17.5 ($95\%$ confidence interval 3.1–99) compared with patients who had low urinary levels of both HELIX-II and CTX-II.

### DISCUSSION

In the present report, we describe the development of a new urinary molecular marker for assessing

![Figure 3. Rates of radiographic progression associated with 2 biochemical markers of type II collagen degradation (urinary HELIX-II and C-terminal crosslinking telopeptide of type II collagen [CTX-II]) in patients with early RA. Patients were categorized as having high (highest tertile) or low (tertile 1 or 2) baseline levels of HELIX-II and CTX-II. Radiographic progression was assessed by the changes in total Sharp score over 1 year in each group of patients. Values are the mean and SEM. The differences in the rates of progression associated with levels of biochemical markers at baseline were statistically significant ($P = 0.007$). See Figure 2 for other definitions.](image-url)
type II collagen degradation. This marker detects a specific sequence arising from the degradation of the helical region of type II collagen which represents the major part of the molecule. We found that urinary levels of HELIX-II were significantly increased in patients with knee OA and early RA, and were predictive of the progression of the disease in RA. This new cartilage marker provides information about the progression of RA that is additional and complementary to that provided by urinary CTX-II, a type II collagen telopeptide degradation marker.

Developing specific antibodies against the helical part of collagens is challenging because of its highly conserved sequence, both between species and between collagen types. In the present study, we selected the HELIX-II sequence, since a systematic search of 2 referenced databases revealed that it is only found in the \( \alpha_1 \) chain of type II collagen, although it presents 64% and 82% sequence homology with the corresponding peptides in the \( \alpha_1 \) chain of type I and type III collagens, respectively. We demonstrated that the antibody raised against HELIX-II does not cross-react with the homologous type I and type III collagen synthetic peptides. The absence of elevation of urinary HELIX-II levels in patients with Paget’s disease of bone, which is characterized by a marked increase in bone resorption, further provided in vivo evidence that HELIX-II is not released during degradation of bone type I collagen. This sequence was also selected because it is within the three-quarter fragment that results from the initial cleavage of intact type II collagen molecule by collagenases and that is subsequently degraded by gelatinases such as MMP-9, generating type II collagen neoepitopes. Our competitive inhibition experiments showed that the HELIX-II antiserum does not recognize intact or denatured human type II collagen, and that the elongation (by 1 amino acid) or shortening (by 1 or 2 amino acids) of the HELIX-II sequence blunted its recognition.

All of these data support the hypothesis that the HELIX-II ELISA recognizes a neoepitope generated by cleavage of the \( \alpha_1 \) chain of type II collagen. However, the structures of the naturally occurring urinary epitopes and the enzyme(s) involved in their release remain to be determined.

One of the important features of markers of cartilage degradation for their clinical use in arthritides is their ability to distinguish patients from healthy controls (36). In large populations of well-characterized patients with knee OA or early RA, we found that urinary HELIX-II levels were increased compared with those in age- and sex-matched controls, which is consistent with the increased cartilage and type II collagen degradation that are the hallmarks of these 2 diseases. We did not perform radiographic evaluations of joints in the healthy individuals, and thus we cannot exclude the possibility that a few of them had preclinical signs of OA, which would actually have resulted in an underestimation of the differences in HELIX-II levels between patients with arthritis and controls.

Urinary HELIX-II levels were higher in patients with early RA than in patients with knee OA, which probably reflects the larger number of joints involved and the faster rate of joint destruction in RA. However, and as has been shown in previous studies with other markers including urinary CTX-II, significant proportions of OA and RA patients had values within the normal range, indicating that these markers probably have limited diagnostic value. It is likely that a more relevant application of molecular markers in the clinical setting would be their use in identification of patients who are likely to develop progressive joint destruction and who would benefit from more aggressive treatment very early in the disease course. In early RA, urinary HELIX-II levels were significantly associated with the risk of radiographic progression over 1 year independently of serum CRP levels, indicating that this biochemical marker of type II collagen degradation is likely to reflect processes other than inflammation, which has been shown to be partly uncoupled from joint damage in RA (37–39).

In the present study, we showed for the first time that 2 different markers of type II collagen degradation, namely, HELIX-II and CTX-II, were predictive of radiographic progression in RA independently of each other and that the combination of the 2 tests allowed the identification of patients at highest risk more accurately than either marker alone. Although the enzymatic pathways of cartilage type II collagen catabolism are poorly understood, they are complex and involve several enzymes with different cleavage specificity which may be activated in different compartments of the cartilage and at different times (40). Thus, it would not be surprising to find that HELIX-II and CTX-II fragments, which arise from different parts of the type II collagen molecule, are released by different pathways and consequently react differently according to the type and stage of the arthritis, a concept that has recently been suggested for different markers of type I collagen breakdown in metabolic bone diseases (17). This concept is also supported by the fact that although the increase in urinary CTX-II levels in patients with knee OA was...
larger than the increase in urinary HELIX-II levels, the opposite was observed in patients with RA.

In summary, we have developed a new molecular marker detecting a neoepitope generated from the cleavage of the α1 chain of type II collagen within its helical domain. Urinary levels of this marker were significantly increased in patients with knee OA and early RA, and high levels were associated with an increased risk of progression independently of disease activity and inflammation in patients with RA. This new marker appears to provide information about type II collagen degradation processes that is both additional and complementary to that provided by urinary CTX-II, suggesting that it may be useful, either alone or in combination with CTX-II, for the clinical investigation of patients with OA and for predicting disease progression in RA.

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REFERENCES


