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TRACKING CARTILAGE TURNOVER: URINARY CTX-II AS A BIOMARKER FOR EARLY RHEUMATOID ARTHRITIS PROGRESSION AND ANTI-TNF THERAPY RESPONSE

KIKIRDAK YIKIMININ İZLENMESİ: İDRAR CTX-II DÜZEYLERİNİN ERKEN ROMATOİD ARTRİT İLERLEMESİ VE ANTİ-TNF TEDAVİSİNE YANIT İÇİN BİYOBELİRTEÇ OLARAK KULLANIMI

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ABSTRACT

Introduction: This study aimed to evaluate urinary C-telopeptide of type II collagen (CTX-II) as a biomarker of cartilage turnover, disease activity, and anti-TNF therapy response in rheumatoid arthritis (RA), with a focus on early disease.

Methods: In a prospective cohort study (June–October 2010), 39 RA patients initiating anti-TNF therapy (adalimumab, etanercept, or infliximab) and 36 age- and sex-matched healthy controls were enrolled. Baseline and 3-month assessments included urinary CTX-II (corrected for creatinine), Disease Activity Score in 28 joints (DAS28), Health Assessment Questionnaire (HAQ), and van der Heijde modified Total Sharp Score (mTSS). Correlations between CTX-II and disease activity or radiographic damage were analyzed using Spearman's rank correlation. Changes in CTX-II were assessed with the Wilcoxon signed-rank test, and subgroup analyses compared early (<3 years) versus established RA.

Results: At baseline, RA patients had significantly higher urinary CTX-II levels than controls (447.8 ± 359.3 vs. 233.8 ± 122.4 ng/mmol, p<0.005). CTX-II correlated positively with DAS28 (ρ=0.609, p<0.001), HAQ (ρ=0.493, p=0.001), and joint counts, and was twofold higher in RF-positive patients (557.0 ± 395.0 vs. 252.8 ± 159.6 ng/mmol, p=0.018). After 3 months, early RA patients (n=11) showed a significant 45% reduction in CTX-II (p=0.016), while established RA patients (n=20) showed no change (p=0.421). Patients in remission (DAS28 <2.6, n=12) had CTX-II levels comparable to controls (p=0.005). High baseline CTX-II (≥479 ng/mmol, n=8) predicted a 46% reduction post-therapy (p=0.017).

Conclusion: Urinary CTX-II is a dynamic biomarker of cartilage turnover and anti-TNF response, particularly in early RA. Its association with RF positivity and remission suggests a role in personalized RA management. Larger studies are needed to validate standardized cut-offs for clinical use.

Keywords: rheumatoid arthritis, urinary CTX-II, anti-TNF therapy, cartilage turnover, early RA, rheumatoid factor, biomarker

ÖZET

Giriş: Bu çalışma, romatoid artrit (RA) hastalarında idrar C-telopeptit tip II kollajen (CTX-II) düzeylerinin kıkırdak döngüsü, hastalık aktivitesi ve anti-TNF tedavi yanıtı açısından bir biyobelirteç olarak rolünü, özellikle erken hastalıkta değerlendirmeyi amaçladı.

Yöntemler: Haziran - Ekim 2010 tarihleri arasında gerçekleştirilen prospektif kohort çalışmasında, anti-TNF tedaviye (adalimumab, etanercept veya infliksimab) yeni başlayan 39 RA hastası ve yaş-cinsiyet uyumlu 36 sağlıklı kontrol dahil edildi. Başlangıç ve 3. ay değerlendirmelerinde idrar CTX-II (kreatinine göre düzeltilmiş), 28 eklem Hastalık Aktivite Skoru (DAS28), Sağlık Değerlendirme Anketi (HAQ) ve van der Heijde modifiye Toplam Sharp Skoru (mTSS) ölçüldü. CTX-II ile hastalık aktivitesi ve radyografik hasar arasındaki ilişkiler Spearman rank korelasyonu ile analiz edildi. CTX-II değişiklikleri Wilcoxon işaretli sıralar testi ile değerlendirildi; erken (<3 yıl) ve yerleşik RA alt grup analizleri yapıldı.

Bulgular: Başlangıçta RA hastalarında idrar CTX-II düzeyleri kontrollere göre anlamlı derecede yüksekti (447.8 ± 359.3 vs. 233.8 ± 122.4 ng/mmol, p<0.005). CTX-II, DAS28 (ρ=0.609, p<0.001), HAQ (ρ=0.493, p=0.001) ve eklem sayıları ile pozitif korelasyon gösterdi; romatoid faktör (RF) pozitif hastalarda iki kat yüksekti (557.0 ± 395.0 vs. 252.8 ± 159.6 ng/mmol, p=0.018). Üç ay sonra erken RA hastaları (n=11) %45'lik anlamlı CTX-II azalması gösterirken (p=0.016), yerleşik RA hastalarında (n=20) değişiklik olmadı (p=0.421). Remisyona ulaşan hastalar (DAS28 <2.6, n=12) kontrollere benzer CTX-II düzeylerine sahipti (p=0.005). Yüksek başlangıç CTX-II'si olanlar (≥479 ng/mmol, n=8) %46 azalma gösterdi (p=0.017).

Sonuç: İdrar CTX-II, özellikle erken RA'da kıkırdak döngüsü ve anti-TNF yanıtını izleyen dinamik bir biyobelirteçtir. RF pozitifliği ve remisyon ile ilişkisi, kişiselleştirilmiş RA yönetiminde potansiyelini vurgular. Klinik kullanım için standardize kesim değerlerinin validasyonu gereklidir.

Anahtar Kelimeler: romatoid artrit, idrar CTX-II, anti-TNF tedavi, kıkırdak turnover, erken RA, romatoid faktör, biyobelirteç

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INTRODUCTION

Stiff Rheumatoid arthritis (RA) is a chronic autoimmune disease marked by inflammation that primarily targets the joints, leading to subsequent cartilage degradation and subchondral bone erosion (1). While conventional radiography has been a long-standing modality for tracking joint damage in RA, its significant limitations in detecting early bone erosions and its inability to directly assess cartilage integrity are well-recognized (2). Consequently, advanced imaging techniques such as MRI and ultrasound have become increasingly important for visualizing inflammation and early structural lesions (3, 4). However, despite their enhanced sensitivity, these modalities may still not fully capture the initial, subtle biochemical alterations and dynamic changes occurring within the cartilage matrix at the earliest stages of the disease process, often identifying damage only once it has progressed beyond these initial molecular events (5).

Traditional predictors for future joint damage in rheumatoid arthritis (RA) encompass clinical measures of disease activity, such as the erythrocyte sedimentation rate (ESR) and disease activity score (DAS), alongside serological markers like C-reactive protein (CRP), rheumatoid factor (RF), and the extent of baseline radiographic damage (1). However, the prognostic landscape is evolving with the identification of novel autoantibodies, notably anti-carbamylated protein (anti-CarP) antibodies, which can predict more severe joint damage independently of established markers and even in ACPA-negative patients (1, 6). One of the central challenges in treating RA lies in the recurrent mismatch between measurable systemic inflammation and the pathobiological forces responsible for ongoing structural damage (7). The dissociation between systemic inflammation and tissue destruction, where inflammation may appear controlled yet joint damage progresses, or joint destruction continues despite reduced systemic inflammation, underscores the critical need for biomarkers that accurately reflect active tissue catabolism (7). This divergence has shifted focus toward turnover markers, with urinary C-telopeptide of type II collagen (CTX-II) emerging as a reliable indicator of cartilage degradation, offering valuable insights into disease progression and therapeutic response (8).

The therapeutic landscape of rheumatoid arthritis has evolved significantly over the past two decades with the advent of biologics, particularly anti-tumor necrosis factor agents. Pivotal trials demonstrate that these agents halt radiographic progression more effectively than conventional synthetic DMARDs when initiated early (9). Notably, they protect joint structure even when clinical symptom relief is incomplete (7). However, despite their efficacy in preserving joint integrity, the impact of these biologics on urinary CTX-II, an established marker of cartilage degradation, remains poorly understood. This knowledge gap persists, underscoring the need to further explore cartilage-specific biomarkers to complement advances in deep remission and personalized treatment strategies (9).

This study aimed to evaluate the association between urinary CTX-II levels and radiological damage, disease activity, and anti-TNF therapy response in RA patients, with a focus on early disease.

METHODS

Study Design and Ethical Oversight

This prospective cohort study was conducted between June and October 2010. The investigation received ethical approval from the Ondokuz Mayıs University Local Ethics Committee (Approval Date/Number 2009/34) and was performed in accordance with the ethical principles outlined in the Declaration of Helsinki. The study was financially supported by the Ondokuz Mayıs University (OMU) Scientific Research Projects Commission (code no: PYO.TIP.1904.10.012). All participants provided written informed consent prior to their inclusion in the study.

Study Population

Patient Recruitment and Eligibility

A total of 39 consecutive patients with Rheumatoid Arthritis (RA), who were naïve to anti-tumor necrosis factor (anti-TNF) therapy and had a new prescription for such an agent, were enrolled. Eligible patients met the American College of Rheumatology (ACR) 1987 revised criteria (10) for RA and were aged between 18 and 75 years.

Control Group

Thirty-six healthy individuals, age- and sex-matched to the patient group, were recruited as controls for baseline comparisons of specific parameters. Healthy controls had no history of joint disease or systemic conditions affecting cartilage metabolism.

Inclusion and Exclusion Criteria for RA Patients

Anti-TNF therapy was initiated in patients adhering to the Turkish consensus statement on the use of TNF-blocking agents. This guideline recommended anti-TNF therapy for patients with active disease, defined by a DAS in 28 joints (DAS28) greater than 5.1, who had demonstrated an inadequate response to at least three conventional synthetic disease-modifying antirheumatic drugs (DMARDs).

Exclusion criteria for patients encompassed serious systemic or metabolic diseases, a history of active infectious diseases, latent tuberculosis (unless appropriate prophylaxis was administered), current or recent malignancy, use of medications known to affect joint or bone metabolism (other than those permitted as stable concomitant RA therapy), and significant hepatic or renal insufficiency.

Treatment Regimen and Concomitant Therapy

Patients initiated one of three anti-TNF agents based on clinical decision: adalimumab (40 mg subcutaneous injection every two weeks), etanercept (50 mg subcutaneous injection weekly), or infliximab (3 mg/kg intravenous infusion at weeks 0, 2, and 6, followed by maintenance infusions every 8 weeks in the clinic). Throughout the 3-month study period,

Table 2. Baseline Clinical, Laboratory, and Radiographic Characteristics of Rheumatoid Arthritis Patients

Parameter	All Patients (n=39)	Patients with Complete Follow-Up (n=31)
Morning stiffness, min (mean ± SD, median)	94.2 ± 85.3 (60.0)	92.7 ± 73.0 (60.0)
DAS28 score (mean ± SD, median)	5.5 ± 1.2 (5.31)	5.5 ± 1.2 (5.2)
VAS pain, 0–10 cm (mean ± SD, median)	5.5 ± 2.1 (5.0)	5.5 ± 2.1 (5.0)
Tender joint count, 0–28 (mean ± SD, median)	11.8 ± 8.3 (11.0)	12.0 ± 7.8 (11.0)
Swollen joint count, 0–28 (mean ± SD, median)	4.7 ± 4.1 (4.0)	4.4 ± 4.0 (3.7)
HAQ score (mean ± SD, median)	0.6 ± 0.4 (0.6)	0.6 ± 0.4 (0.6)
Physician global VAS, 0–10 cm (mean ± SD, median)	6.6 ± 1.3 (6.0)	6.6 ± 1.3 (6.0)
Patient global VAS, 0–10 cm (mean ± SD, median)	6.6 ± 1.7 (6.0)	6.9 ± 1.6 (6.0)
ESR, mm/h (mean ± SD, median)	45.3 ± 25.2 (44.0)	44.5 ± 26.1 (44.0)
CRP, mg/L (mean ± SD, median)	30.9 ± 31.3 (19.6)	35.4 ± 33.0 (29.5)
Urinary CTX-II, ng/mmol (mean ± SD, median)	447.8 ± 359.3 (326.5)	398.3 ± 318.1 (326.5)
mTSS (mean ± SD, median)	56.5 ± 74.7 (37.5)	56.5 ± 74.7 (37.5)
Erosion score (mean ± SD, median)	29.3 ± 45.5 (17.0)	29.3 ± 45.5 (17.0)
Joint narrowing score (mean ± SD, median)	27.3 ± 30.7 (16.5)	27.3 ± 30.7 (16.5)

Abb. DAS28: 28-joint Disease Activity Score; VAS: Visual Analogue Scale; HAQ: Health Assessment Questionnaire; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; CTX-II: C-telopeptide of type II collagen; mTSS: modified Total Sharp Score. Data presented as mean ± SD (median where applicable) SD: standard deviation.

the type and dosage of pre-existing conventional DMARD therapy were maintained constant. Concomitant oral prednisolone (≤10 mg/day) and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) were permitted as needed, with stable doses maintained throughout the study. Prior to anti-TNF initiation, all patients underwent purified protein derivative (PPD) skin testing and chest radiography to screen for latent tuberculosis; patients with positive findings received prophylactic isoniazid (300 mg/day) as appropriate.

Clinical and Functional Assessments

Clinical assessments were performed by the same trained investigator for each patient at baseline (prior to anti-TNF initiation) and at 3 months post-treatment initiation.

Disease Activity Assessment

Disease activity was assessed using DAS28 (based on 28-tender/swollen joint counts and CRP), individual joint counts, patient/physician global VAS (0-10 cm), pain VAS (0-10 cm), CRP, and ESR. At 3 months, EULAR response criteria defined good response as DAS28 <3.2 with >1.2 improvement, moderate response as DAS28 <5.1 with >0.6 to ≤1.2 improvement or DAS28 <3.2 with ≤1.2 improvement or DAS28 3.2–5.1 with >0.6 improvement, and remission as DAS28 <2.6 (11).

Functional Disability Assessment

Functional disability was assessed at baseline and 3 months using the Health Assessment Questionnaire (HAQ). The HAQ evaluates a patient's ability to perform daily activities across eight categories (dressing, rising, eating, walking, hygiene, reach, grip, and usual activities) with 20 questions. Scores for each category range from 0 (no difficulty) to 3 (unable to do). The overall HAQ score was calculated as the mean of the eight category scores (12).

Radiographic Assessment

Radiographs of the hands and feet were obtained at baseline and evaluated using the van der Heijde modified Total Sharp Score (mTSS) (13). This score quantifies joint damage by assessing erosions (erosion score, ES; range 0-280 for combined hands and feet) and joint space narrowing (JSN score; range 0-168 for combined hands and feet). For hands, the maximum ES was 160 and JSN score was 120; for feet, the maximum ES was 120 and JSN score was 48. The total mTSS is the sum of the ES and JSN scores, with a maximum possible score of 448.

All radiographs were assessed and reported centrally by a single experienced reader who was blinded to clinical data and the temporal sequence of images (if applicable, though only baseline is mentioned for scoring). To ensure reliability, each radiograph was scored twice by the same reader at different times, and the mean of the two readings was used for analysis. Intra-observer reliability for mTSS, ES, and JSN scores was high (Cronbach's alpha values: 0.988, 0.992, and 0.966, respectively) (13).

Biochemical Measurements

Sample Collection and Processing

Venous blood and second morning void urine samples were collected from patients at baseline (prior to anti-TNF initiation) and at the 3-month follow-up visit, after an 8 to 12-hour overnight fast for blood samples. Urine samples were aliquoted and stored frozen at -20°C until analysis.

Urinary C-telopeptide of Type II Collagen (CTX-II) Measurement

Urinary levels of CTX-II were quantified using a competitive enzyme-linked immunosorbent assay (Urine

Table 3. Correlations Between Baseline Urinary CTX-II Levels and Disease Activity Parameters

Parameter	Correlation Coefficient (r)	P-value	n
DAS28 score	0.609	<0.001*	39
Tender joint count, 0–28	0.465	0.003*	39
Swollen joint count, 0–28	0.490	0.002*	39
HAQ score	0.493	0.001*	39
Physician global VAS, 0–10 cm	0.426	0.007*	39
Patient global VAS, 0–10 cm	0.424	0.007*	39
IgM-RF (positive vs. negative)	N/A	0.018†	25/14

Spearman's rank correlation coefficient for continuous variables; †Independent t-test for comparison of urinary CTX-II levels between IgM-RF positive (n=25, mean ± SD: 557.0 ± 395.0 ng/mmol) and negative (n=14, 252.8 ± 159.6 ng/mmol) groups, Cohen's d = 0.87. DAS28: 28-joint Disease Activity Score; HAQ: Health Assessment Questionnaire; VAS: Visual Analogue Scale (0–10 cm); IgM-RF: Immunoglobulin M rheumatoid factor; N/A: Not applicable. * p < 0.05; † p < 0.05

Table 4. Partial Correlations Between Baseline Urinary CTX-II Levels and Radiographic Scores, Adjusted for Disease Duration

Parameter	Correlation Coefficient (r)	P-value
mTSS	0.483	0.238*
Erosion score	0.429	0.406*
Joint narrowing score	0.536	0.101*

mTSS: modified Total Sharp Score; CTX-II: C-telopeptide of type II collagen. Pearson r

CartiLaps® ELISA, Immunodiagnostic Systems Ltd., Boldon, UK). To account for variations in urine dilution, urinary CTX-II concentrations were corrected for urinary creatinine levels, which were determined using a standard colorimetric method. The ELISA kit had a minimum sensitivity of 0.20 ng/mmol for corrected CTX-II, with an intra-assay coefficient of variation (CV) of <8% and an inter-assay CV of <10%. The corrected CTX-II value (ng/mmol creatinine) was calculated using the formula: [urinary CTX-II (µg/L) / urinary creatinine (mmol/L)] × 1000. A cut-off value of 479 ng/mmol creatinine was used to define increased cartilage degradation, with higher scores indicating greater cartilage damage.

Other Laboratory Investigations

Baseline and 3-month blood samples were analyzed for complete blood count, liver function tests, and kidney function tests. ESR (0-20 mm/h), CRP (0-3.5 mg/L), and rheumatoid factor (RF; 0-15 IU/mL) were measured using standard laboratory techniques.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows, Version 15.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as mean ± standard deviation (SD) or median (interquartile range, IQR) as appropriate for data distribution. Categorical data were summarized as frequencies and percentages.

Power Analysis

The sample size was determined using PASS 2008 (NCSS, LLC, Kaysville, Utah, USA). Sample-size estimation anchored on the absolute change in CTX-II excretion, selected as the primary endpoint. Earlier research reported baseline urinary CTX-II ~600 ng/mmol in rheumatoid arthritis and roughly 300 ng/mmol in healthy controls (14-16). These data led us to expect post-treatment values to approach normal levels, thus a 250 ng/mmol reduction with a standard deviation near 300 ng/mmol. Such parameters, entered into PASS 2008 for a two-sided paired t-test with $\alpha = 0.05$ and power = 0.80, indicated that 30 patients would suffice. Sufficiency was adjusted for anticipated attrition, a 15 % loss seen in three-month biologic trials plus a 5 % safety margin, so 39 patients were enrolled.

Inferential Statistics

The normality of data distribution was assessed using the Kolmogorov-Smirnov test. For demographic characteristics and comparisons of categorical variables between groups (e.g., patients vs. controls at baseline), the Chi-square test or Fisher's exact test was used as appropriate. For comparisons of continuous variables between two independent groups (e.g., patient vs. control baseline CTX-II), the Mann-Whitney U test was employed for non-normally distributed data. To assess changes in continuous variables within the patient group from baseline to 3 months (e.g., DAS28, CTX-II), the Wilcoxon signed-rank test was used. Intra-observer agreement for radiographic scores was assessed using reliability analyses (Cronbach's alpha). Relationships between variables were investigated using Spearman's rank correlation analysis for non-normally distributed data. Correlations between CTX-II and disease activity parameters (DAS28, HAQ, joint counts) and radiographic scores (mTSS, ES, JNS) were assessed. A p-value of <0.05 was considered statistically significant.

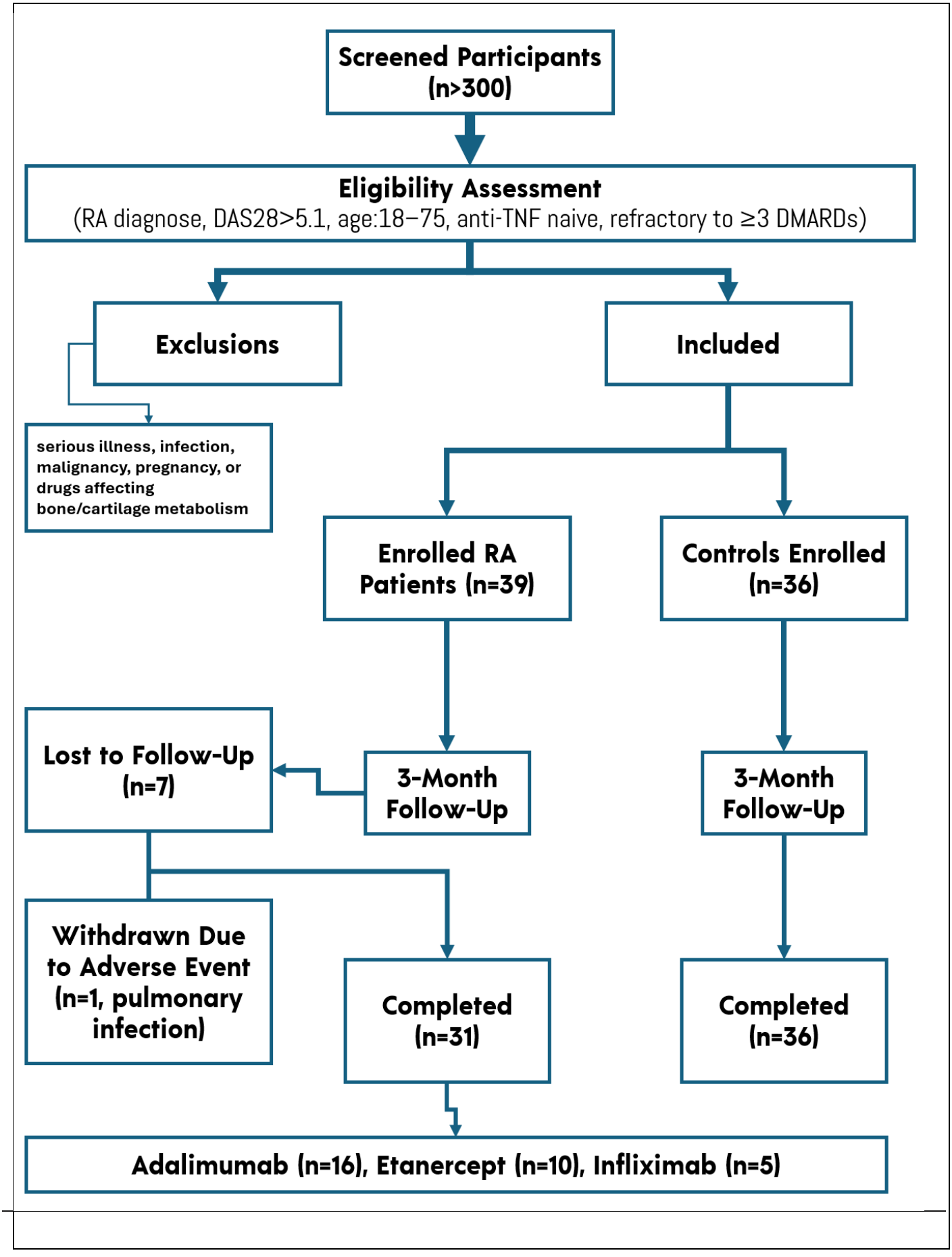
RESULTS

This prospective, observational study enrolled 39 patients with rheumatoid arthritis (RA) and 36 age- and sex-matched healthy controls between June and October 2010. Of the 39 RA patients, 31 (79.5%) completed the 3-month follow-up and were included in the statistical analyses. Eight patients were excluded from analyses due to withdrawal: one discontinued anti-TNF therapy in the first month due to a serious adverse event (pulmonary infection), and seven were lost to follow-up. The study flowchart is presented in Figure 1. Anti-TNF therapy was initiated as follows: adalimumab (n=22), etanercept (n=11), and infliximab (n=6).

Baseline Demographic and Clinical Characteristics

The RA cohort comprised 27 women (69.2%) and 12 men (30.8%), with a mean age of 50.7 ± 10.7 years and a mean disease duration of 115.9 ± 100.4 months (median: 96.0 months). Thirteen patients (33.3%) with disease duration <3 years were classified as early RA (mean duration: 24.4 ± 11.8 months), while 26 patients (66.7%) with duration ≥3 years were classified as established RA (mean duration: 161.6 ± 93.4 months). Most patients were on combination therapy at baseline, with 92.3% receiving prednisone (mean dose: 7.9 ± 4.4 mg/day), 82.1% methotrexate (mean dose: 12.8 ± 5.0 mg/week), 38.5% hydroxychloroquine, 23.1% sulfasalazine, and 12.8% leflunomide. Five percent of patients received isoniazid prophylaxis before anti-TNF therapy. Baseline demographic and clinical characteristics are summarized in Table 1.

Figure 1. Study flowchart. Study flowchart illustrating participant recruitment, enrollment, 3-month follow-up, and reasons for withdrawal for 39 rheumatoid arthritis patients and 36 healthy controls between June and October 2010. Withdrawals included 1 patient due to a serious adverse event (pulmonary infection) and 7 patients lost to follow-up.



Baseline Disease Activity and Radiographic Features

At baseline, RA patients exhibited significant disease activity and structural damage. The mean DAS28 score was 5.5 ± 1.2, indicating high disease activity, and the mean Health Assessment Questionnaire (HAQ) score was 0.6 ± 0.4, reflecting moderate functional impairment. Radiographic damage, assessed by the van der Heijde modified Total Sharp Score (mTSS), averaged 56.5 ± 74.7, with erosion score (ES) of 29.3 ± 45.5 and joint narrowing score (JNS) of 27.3 ± 30.7. Urinary CTX-II levels were significantly elevated in RA patients compared to controls (447.8 ± 359.3 vs. 233.8 ± 122.4 ng/mmol, p<0.005). Baseline clinical, laboratory, and radiographic characteristics for the 31 patients with complete follow-up data were similar to the full cohort (Table 2).

Baseline Associations of Urinary CTX-II with Disease Activity

At baseline, urinary C-telopeptide of type II collagen (CTX-II) levels showed significant positive correlations with multiple disease activity parameters in the 39 rheumatoid; arthritis patients, including the 28-joint DAS (DAS28; ρ=0.609, p<0.001), tender joint count (ρ=0.465, p=0.003), swollen joint count (ρ=0.490, p=0.002), Health Assessment Questionnaire (HAQ) score (ρ=0.493, p=0.001), physician global Visual Analogue Scale (VAS; ρ=0.426, p=0.007), and patient global VAS (ρ=0.424, p=0.007), as determined by Spearman's rank correlation analysis due to non-normal data distribution (Table 3). Additionally, patients with positive immunoglobulin M rheumatoid factor (IgM-RF; n=25) had significantly higher urinary CTX-II levels compared to those with negative IgM-RF (n=14; 557.0 ± 395.0 vs. 252.8 ± 159.6 ng/mmol, p=0.018, independent t-test, Cohen's d=0.87) (Table 3).

Baseline Associations with Radiographic Damage

After adjusting for disease duration using partial correlation analysis, baseline urinary CTX-II levels showed non-significant positive trends with mTSS (r=0.483, p=0.238), ES (r=0.429, p=0.406) and JNS (r=0.536, p=0.101) (Table 4). Disease duration was included as a covariate in a linear regression model to control its confounding effect on cartilage degradation and radiographic progression.

Figure 2. Graph showing significant CTX-II reduction in early RA (p=0.016) compared to no change in established RA

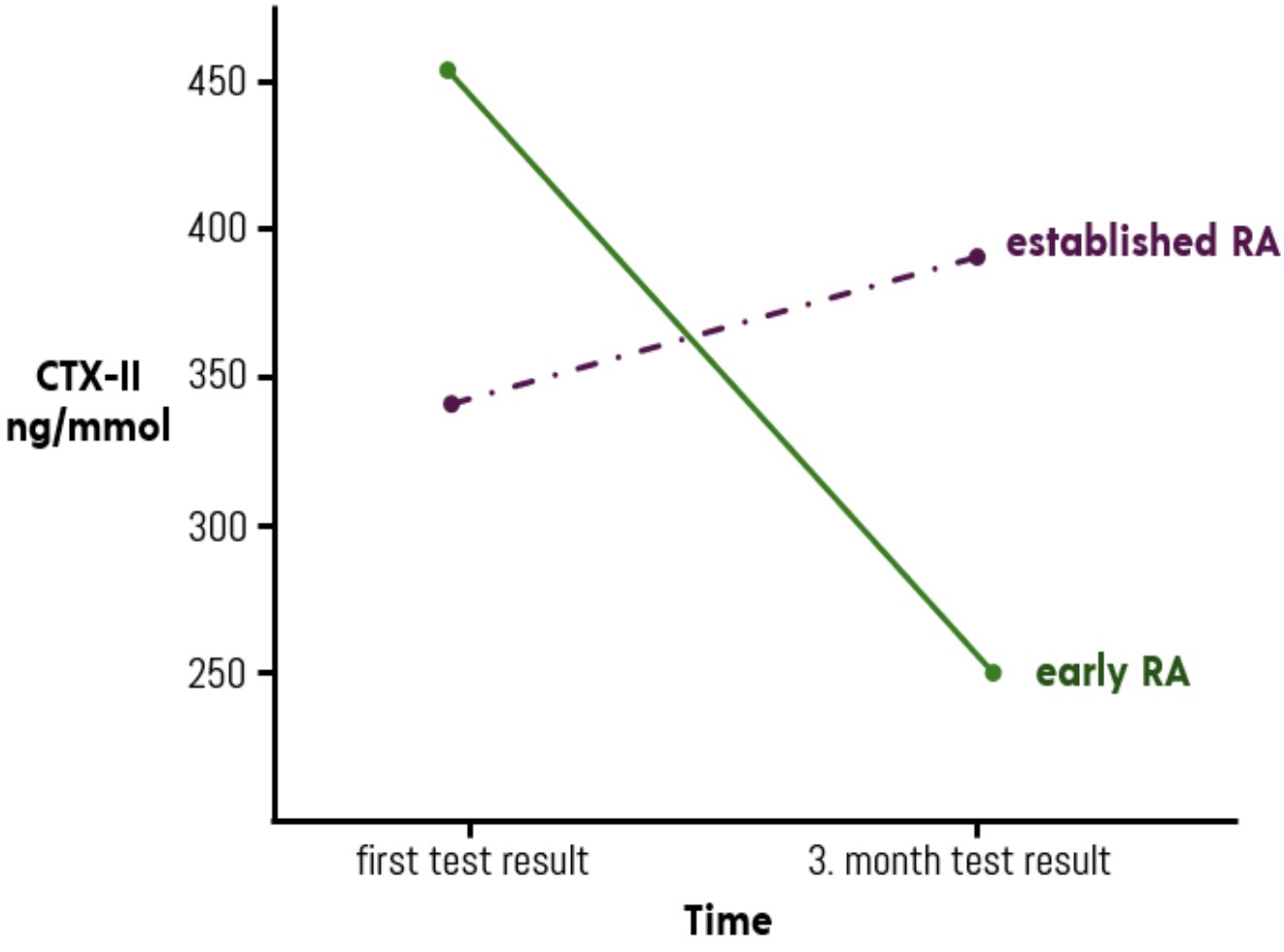


Table 1. Baseline Demographic and Clinical Characteristics of Rheumatoid Arthritis Patients and Healthy Controls

Characteristic	RA Patients (n=39)	Controls (n=36)	P-value
Age, years (mean ± SD)	50.7 ± 10.7	49.2 ± 9.8	0.523*
Sex, n (%)			0.876†
Female	27 (69.2)	24 (66.7)	
Male	12 (30.8)	12 (33.3)	
Body mass index, kg/m² (mean ± SD)	28.8 ± 4.9	27.5 ± 4.2	0.214*
Disease duration, months (mean ± SD, median)	115.9 ± 100.4 (96.0)	–	–
Early RA (<3 years), n (%)	13 (33.3)	–	
Established RA (≥3 years), n (%)	26 (66.7)	–	
IgM-RF* positive, n (%)	25 (64.1)	–	–
ESR, mm/h (mean ± SD)	45.3 ± 25.2	12.4 ± 5.6	<0.001*
CRP, mg/L (mean ± SD)	30.9 ± 31.3	3.2 ± 1.8	<0.001*
Nodular disease, n (%)	4 (10.3)	–	–
Medications, n (%)			
Prednisone	36 (92.3)	–	
Methotrexate	32 (82.1)	–	
Sulfasalazine	9 (23.1)	–	
Leflunomide	5 (12.8)	–	
Hydroxychloroquine	15 (38.5)	–	
Urinary CTX-II, ng/mmol (mean ± SD)	447.8 ± 359.3	233.8 ± 122.4	<0.005

*IgM-RF: Immunoglobulin M rheumatoid factor; ESR: Erythrocyte sedimentation rate; RA: rheumatoid arthritis, CRP: C-reactive protein; CTX-II: C-telopeptide of type II collagen. P-values calculated using independent t-test or †chi-square test n: number

Effects of Anti-TNF Therapy

After 3 months of anti-TNF therapy, the 31 RA patients who completed follow-up showed significant improvements in all clinical and laboratory parameters except urinary CTX-II levels. DAS28 decreased from 5.5 ± 1.2 to 3.3 ± 1.6 ($p<0.001$, Wilcoxon signed-rank test), HAQ from 0.6 ± 0.4 to 0.2 ± 0.3 ($p<0.001$), VAS pain from 5.5 ± 2.1 to 2.0 ± 2.5 cm ($p<0.001$), tender joint count from 12.0 ± 7.8 to 4.0 ± 6.3 ($p<0.001$), swollen joint count from 4.4 ± 4.0 to 1.5 ± 1.9 ($p<0.001$), ESR from 44.5 ± 26.1 to 27.9 ± 24.0 mm/h ($p=0.003$), CRP from 35.4 ± 33.0 to 11.2 ± 16.9 mg/L ($p<0.001$), physician global VAS from 6.7 ± 1.3 to 3.6 ± 2.0 cm ($p<0.001$), and patient global VAS from 6.9 ± 1.6 to 3.8 ± 2.0 cm ($p<0.001$). Overall, urinary CTX-II levels decreased non-significantly by 14% ($p=0.582$), but a significant 45% reduction was observed in early RA patients ($p=0.016$).

Subgroup Analysis: Early vs. Established RA

Among the 31 patients who completed follow-up, 11 had early RA and 20 had established RA. At baseline, urinary CTX-II levels did not differ significantly between early (451.6 ± 279.7 ng/mmol) and established RA (369.0 ± 340.0 ng/mmol, $p=0.451$, Mann-Whitney U test). Established RA

patients had significantly higher mTSS ($p=0.042$) and erosion scores ($p=0.008$) than early RA patients, with no difference in joint narrowing scores (33.0 ± 35.4 vs. 15.8 ± 12.4, $p=0.126$). After 3 months, early RA patients showed a significant reduction in urinary CTX-II levels (from 451.6 ± 279.7 to 249.2 ± 109.9 ng/mmol, $p=0.016$, Wilcoxon signed-rank test), a 45% decrease, while established RA patients showed a non-significant increase (from 369.0 ± 340.0 to 392.8 ± 285.0 ng/mmol, $p=0.421$). At 3 months, early RA patients had significantly lower CTX-II levels than established RA patients ($p=0.048$, Mann-Whitney U test) (Figure 2).

Treatment Response and Urinary CTX-II

Per European League Against Rheumatism (EULAR) response criteria, 68% of patients (n=21) achieved a good response, 22% (n=7) a moderate response, and 10% (n=3) no response at 3 months. Remission (DAS28 <2.6) was achieved in 39% (n=12). Patients in remission had significantly lower urinary CTX-II levels at 3 months compared to non-remitters (217.1 ± 90.2 vs. 420.7 ± 281.2 ng/mmol, $p=0.005$, Mann-Whitney U test). Among eight patients with baseline urinary CTX-II levels ≥479 ng/mmol (five early RA, three established RA), levels decreased significantly from 838.0 ± 291.7 to 449.8 ± 206.0 ng/mmol ($p=0.017$, Wilcoxon signed-rank test), a 46% reduction. Early RA patients with high baseline CTX-II (n=5) showed a 64% reduction and were all good responders per EULAR criteria.

DISCUSSION

The advent of biologic therapies, particularly anti-tumor necrosis factor (anti-TNF) agents, has revolutionized the management of rheumatoid arthritis (RA), improving clinical outcomes and quality of life for many patients (17). However, a significant proportion of patients either fail to respond or experience waning efficacy over time, underscoring the need for biomarkers to predict treatment response and guide personalized therapy (17, 18). This study demonstrates that baseline urinary C-telopeptide of type II collagen (CTX-II) levels are associated with anti-TNF therapy response, particularly in early RA, and provides novel insights into the relationship between CTX-II and rheumatoid factor (RF) positivity.

Our findings indicate that patients with early RA (disease duration <3 years) exhibited a significant 45% reduction in urinary CTX-II levels after 3 months of anti-TNF therapy ($p=0.016$), whereas those with established RA showed a non-significant increase ($p=0.421$). This aligns with the concept of a “window of opportunity” in early RA, where aggressive intervention can mitigate cartilage degradation and alter disease trajectory (19). The significant reduction in CTX-II among early RA patients, coupled with 68% achieving a good European League Against Rheumatism (EULAR) response, suggests that CTX-II may serve as a dynamic biomarker of treatment response. Notably, patients achieving remission (DAS28 <2.6) at 3 months had CTX-II levels comparable to healthy controls (217.1 ± 90.2 vs. 233.8 ± 122.4 ng/mmol, $p=0.005$), reinforcing CTX-II’s potential as a marker of disease activity suppression.

The association between baseline CTX-II and clinical disease activity has been reaffirmed in contemporary cohorts. In a biomarker study of women with established RA, urinary CTX-II levels were approximately two-fold higher than in healthy controls and showed moderate-to-strong correlations with DAS28 ($r\approx0.58$), swollen joint count, and HAQ, while falling significantly after 24 weeks of anti-TNF treatment (20). Complementary evidence suggests that type II collagen metabolites, including CTX-II, reflect ongoing synovial inflammation and cartilage degradation, supporting their potential as dynamic biomarkers for personalized, biomarker-guided therapy in RA (21).

A novel contribution of this study is the observation that RF-positive patients (n=25) had twofold higher baseline CTX-II levels than RF-negative patients (557.0 ± 395.0 vs. 252.8 ± 159.6 ng/mmol, $p=0.018$, Cohen’s $d=0.87$). This is the first study to report such an association, suggesting that autoimmunity, in addition to inflammation, may exacerbate cartilage damage in RF-positive RA. RF positivity is a known risk factor for radiological progression (22) and our findings propose CTX-II as a potential mediator of this relationship. Recent studies have linked RF with increased synovial inflammation and bone erosion, potentially via immune

complex formation (23, 24), which may explain the elevated CTX-II levels observed.

The relationship between baseline cartilage–breakdown markers and structural damage in RA remains equivocal. A long-term follow-up of the COBRA early-RA cohort showed that baseline urinary CTX-II, together with an elevated RANKL : OPG ratio, was an independent predictor of annual Sharp/van der Heijde progression over an 11-year horizon ($\beta \approx 0.28$ per SD, $p = 0.002$) (25). In contrast, a 2024 systematic review of synovial-fluid and urine biomarkers concluded that high u-CTX-II is one of only a handful of soluble markers that repeatedly forecast radiographic progression across several inception cohorts, although the strength of the association varies with sampling matrix and study design (8). A recent clinical-biomarker work in the biologic era illustrates this heterogeneity: a 15-month prospective study of women treated with etanercept or adalimumab documented marked falls in serum C2C/PIICP ratios (a collagen-II degradation-to-formation index) in parallel with clinical improvement, yet baseline values were not predictive of individual radiographic outcomes, suggesting treatment effects may obscure prognostic signals (26). In our cohort, the positive but non-significant correlations between CTX-II and mTSS ($r = 0.48$), and the numerically stronger link with joint-space-narrowing, are therefore compatible with the current literature: CTX-II preferentially reflects cartilage loss rather than cortical erosion, and its prognostic value appears greatest in untreated, early-disease populations before aggressive DMARD or biologic therapy is introduced.

Contemporary cohort data confirm that TNF-α blockade can down-regulate cartilage-degradation pathways. In a 15-month prospective study of women with RA, Szeremeta et al. showed significant falls in type II collagen neo-epitope (C2C) and COMP, with the C2C/PIICP ratio dropping by 27 % and tracking closely with DAS28 improvement, underscoring a disease-modifying effect on cartilage turnover (26). Conversely, a 2024 scoping review of synovial-fluid and extracellular-vesicle biomarkers highlighted that CTX-II responses to anti-TNF agents remain heterogeneous several recent cohorts reported minimal or no change despite clinical improvement, suggesting baseline turnover rate and disease chronicity modulate biochemical responses (8). In our series we likewise saw a modest overall 14 % fall in CTX-II, but a marked 46 % reduction among patients with the highest baseline values, especially in early RA, pointing to a “high-turnover” subgroup that derives the greatest cartilage benefit.

Modern reviews place CTX-II among the handful of soluble markers that capture active joint damage in real time. Sahin et al. (2025) emphasize that radiography, while indispensable for cumulative damage scoring, lags weeks to months behind tissue events, whereas urinary or serum CTX-II levels respond within days to therapeutic perturbation and correlate with both DAS28 and MRI erosive burden, giving clinicians a dynamic window on cartilage integrity (27). When integrated with established prognosticators such as anti-CCP, CTX-II can refine early-risk stratification “*particularly in seropositive, treatment-naive patients*” by signaling cartilage-specific damage before it becomes radiographically evident. Nonetheless, the 2025 review reiterates the need for harmonized assays and validated cut-points before CTX-II can be adopted alongside composite indices or multi-biomarker panels such as the 12-analyte Vectra and the recent seven-protein signature predicting tocilizumab response (28).

Our study has several limitations that should temper interpretation. First, the modest sample size (n=39) and the 3-month observation window restrict power and prevent conclusions about long-term structural outcomes. Second, concomitant conventional synthetic DMARDs, particularly methotrexate and hydroxychloroquine, may have blunted or amplified changes in cartilage biomarkers. Recent tissue-level analyses show that methotrexate alone produces only modest, short-lived suppression of collagen-degradation fragments such as C2M, yet still alters the extracellular-matrix signature enough to confound biologic-response read-outs (8, 29). Third, the three anti-TNF agents used (adalimumab, etanercept, infliximab) are not interchangeable: monoclonal antibodies and the receptor-fusion protein differ in structure, half-life, immunogenicity, and their ability to neutralize membrane-bound TNF. Real-world pharmacokinetic data show agent-specific drug levels and discontinuation risks that track with these molecular differences (30, 31). The use of three different anti-TNF agents may introduce variability in CTX-II response due to their distinct pharmacokinetics. Fourth, our healthy controls were characterized only by age, sex, and routine laboratory data, precluding full adjustment for subclinical joint pathology or osteoporosis, which could influence cartilage and bone metabolism. Finally, the non-significant correlations between baseline CTX-II and radiographic scores probably reflect limited statistical power and the inclusion of patients with long-standing disease in whom active cartilage degradation is less prominent.

Despite these constraints, the present work reinforces the value of urinary CTX-II as a dynamic marker of early cartilage turnover. Contemporary cohorts confirm that TNF-blockade lowers type-II-collagen degradation products within months and that this biochemical response parallels clinical improvement and reduced radiographic progression, particularly in biologic-naive, early RA patients (26). Large reviews of emerging RA biomarkers further highlight that CTX-II captures a dimension of joint damage *“ongoing cartilage breakdown”* that is not reflected by autoantibodies or acute-phase reactants, making it complementary to anti-CCP in early disease stratification (32). Moreover, multi-omic approaches are beginning to integrate CTX-II into multiplex

panels that successfully predict therapeutic response (e.g., an eight-protein signature tat discriminates tocilizumab responders with 86 % accuracy) (28).

To translate these insights into practice, larger multicenter studies with longer follow-up should determine CTX-II cut-offs that forecast radiographic progression, compare its performance head-to-head with other emerging tissue-remodeling markers and test CTX-II-guided treatment algorithms in randomized designs. Such work could pave the way for incorporating CTX-II into precision-medicine dashboards that match individual patients to the biologic (or small-molecule) most likely to halt their unique pattern of joint destruction. In conclusion, urinary CTX-II offers a dynamic, cartilage-specific biomarker to guide early RA management, paving the way for precision medicine in biologic therapy.

CONCLUSION

Urinary CTX-II appears to be a promising, dynamic biomarker reflecting cartilage degradation and treatment response in early RA. Its association with disease activity, RF positivity, and clinical remission supports its potential use in personalized treatment strategies. Further large- scale studies are needed to establish standardized thresholds for clinical application.

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Informed Consent: All participants provided written informed consent prior to their inclusion in the study.

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