# Testing of the OMERACT 8 Draft Validation Criteria for a Soluble Biomarker Reflecting Structural Damage in Rheumatoid Arthritis: A Systematic Literature Search on 5 Candidate Biomarkers

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*ABSTRACT*. *Objective*. To test the OMERACT 8 draft validation criteria for soluble biomarkers by assessing the strength of literature evidence in support of 5 candidate biomarkers.
*Methods*. A systematic literature search was conducted on the 5 soluble biomarkers RANKL, osteoprotegerin (OPG), matrix metalloprotease (MMP-3), urine C-telopeptide of types I and II collagen (U-CTX-I and U CTX-II), focusing on the 14 OMERACT 8 criteria. Two electronic voting exercises were conducted to address: (1) strength of evidence for each biomarker as reflecting structural damage according to each individual criterion and the importance of each individual criterion; (2) overall strength of evidence in support of each of the 5 candidate biomarkers as reflecting structural damage endpoints in rheumatoid arthritis (RA) and identification of omissions to the criteria set.
*Results*. The search identified 111 articles. The strength of evidence in support of these biomarkers reflecting structural damage was low for all biomarkers and was rated highest for U-CTX-II [score of 6.5 (numerical rating scale 0–10)]. The lowest scores for retention of specific criteria in the draft set went to criteria that refer to the importance of animal studies, correlations with other biomarkers reflecting damage, and an understanding of the metabolism of the biomarker.
*Conclusion*. Evidence in support of any of the 5 tested biomarkers (MMP-3, CTX-I, CTX-II, OPG, RANKL) was inadequate to allow their substitution for radiographic endpoints in RA. Three of the criteria in the draft criteria set might not be required, but few omissions were identified. (J Rheumatol 2009;36:1769–84; doi:10.3899/jrheum.090262)

Key Indexing Terms:
RHEUMATOID ARTHRITIS          BIOMARKERS          VALIDATION
RADIOGRAPHY                              STRUCTURAL DAMAGE

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Radiographic damage scoring systems are the gold standard for assessing structural damage outcomes in rheumatoid arthritis (RA), psoriatic arthritis (PsA), and spondyloarthritis (SpA). However, with the introduction of highly effective biological therapies, it is now desirable to identify patients at risk of joint damage prior to the appearance of radiographic change. Recent work has suggested that several soluble biomarkers (biomarkers measured in body fluids), primarily those reflecting tissue remodeling in joints, are independent predictors of joint damage in RA. As the level of a biomarker, or particularly the short-term change in the level, may predict radiographic progression, these markers may constitute indicators of early response to disease modifying agents in clinical trials, and may also be useful to the clinician managing individual patients.

At OMERACT 8 a special interest group (SIG) was assembled comprising individuals with a special interest in biomarkers and structural damage outcomes, to develop validation criteria for a soluble biomarker to substitute for radiographic outcome measures in clinical trials. A list of 14 validation criteria was generated (see Appendix 1) and structured according to the key requirements of the OMERACT filter for validation of an outcome measure: truth, discrimination, and feasibility[1,2]. The performance of the criteria was initially examined using the example of C-reactive protein (CRP)[3]. This exercise showed that some of the criteria, particularly those itemized under the category of truth, were regarded as comparatively less useful in the validation process. However, CRP is regarded as an indirect marker of joint inflammation rather than a marker of joint tissue remodeling, and its association with radiographic damage appears to be rather weak. The OMERACT 9 soluble biomarker working group, therefore, decided that the next step was to test the criteria using other biomarkers considered to be high priority candidates, particularly those that might reflect joint remodeling. The 5 biomarkers identified by the group were C-telopeptide of type I collagen (CTX-I), C-telopeptide of type II collagen (CTX-II), metalloproteinase 3 (MMP-3), the receptor activator of nuclear factor-κB ligand (RANKL), and osteoprotegerin (OPG), which were chosen for this exercise after meeting 2 criteria: (a) evidence that the biomarker is directly related to joint tissue remodeling; and (b) availability of published data evaluating the association between the biomarker and radiographic damage in RA.

The aims of this study were to test the performance of the OMERACT 8 validation criteria by assessing the strength of evidence (SOE) from the literature in support of 5 candidate biomarkers as reflecting structural damage in RA, to appraise the importance of inclusion of each criterion, and to identify omissions to the criteria set as a prelude to the drafting of revised criteria.

## METHODS

*Literature search.* A systematic literature search focusing on the 5 chosen biomarkers: serum RANKL, serum OPG, serum and urine CTX-I, urine CTX-II, and serum MMP-3, specifically directed towards each individual criterion, was conducted by the fellow in this working group (SWS). The MeSH terms used for the biomarkers were: RANK ligand/osteoclast differentiation factor/Receptor Activator of Nuclear Factor-kappa B, osteoprotegerin, matrix metalloproteinase 3. For CTX-I and CTX-II there were no specific MeSH terms. The search, therefore, included: CTX-I, collagen type I, C-terminal telopeptide, CTX-II, and collagen type II. Searches were performed in MEDLINE and EMBASE in April 2007 with no date restriction. The search was limited to English language and peer-reviewed journals. Eligible studies were regarded as all studies addressing the individual items of the criteria set (see Figure 1 for a description of article retrieval). References of articles read in full text were also examined. A survey was mailed to companies manufacturing assays for the 5 biomarkers to obtain information on unpublished literature. Study quality was not assessed by the fellow. However, the main study characteristics and findings (i.e., author, year, journal, study design, duration of study, study population, outcome measure, number of participants, and strength of the relevant effect measure) were presented to the group both orally (EULAR 2007) and as a written summary for evaluation of SOE.

*Rating the strength of evidence supporting the biomarkers as reflecting structural damage in RA and the strength of recommendation in support of including each criterion.* Two electronic voting exercises were conducted by Web survey. The primary aims of the first exercise were: (a) To examine the SOE for each biomarker as reflecting structural damage according to each individual criterion; and (b) To appraise the importance of individual criteria. The results of the literature search were therefore organized so that the evidence for all 5 biomarkers was compiled and presented according to individual criteria before the voting questions were presented. After each criterion had been reviewed, the members of the group (n = 19) were asked to rate SOE on a 0–10 numerical rating scale (NRS; 0 = no supporting evidence at all, 10 = unequivocal evidence) in response to the following question: "Please rate to what degree you consider the available data from the literature as supporting (biomarker) as reflecting structural damage in RA according to this specific criterion."

Participants were then asked to vote on the following question on a numeric rating scale of 0 (definitely exclude) to 10 (definitely include) to determine the strength of the recommendation in support of including each individual criterion in the draft criteria set: "Please rate to what degree you consider this a required criterion in the validation of a biomarker reflecting structural damage endpoints in RA."

The primary aim of the second voting exercise was to examine the overall SOE in support of each of the 5 candidate biomarkers as valid biomark-
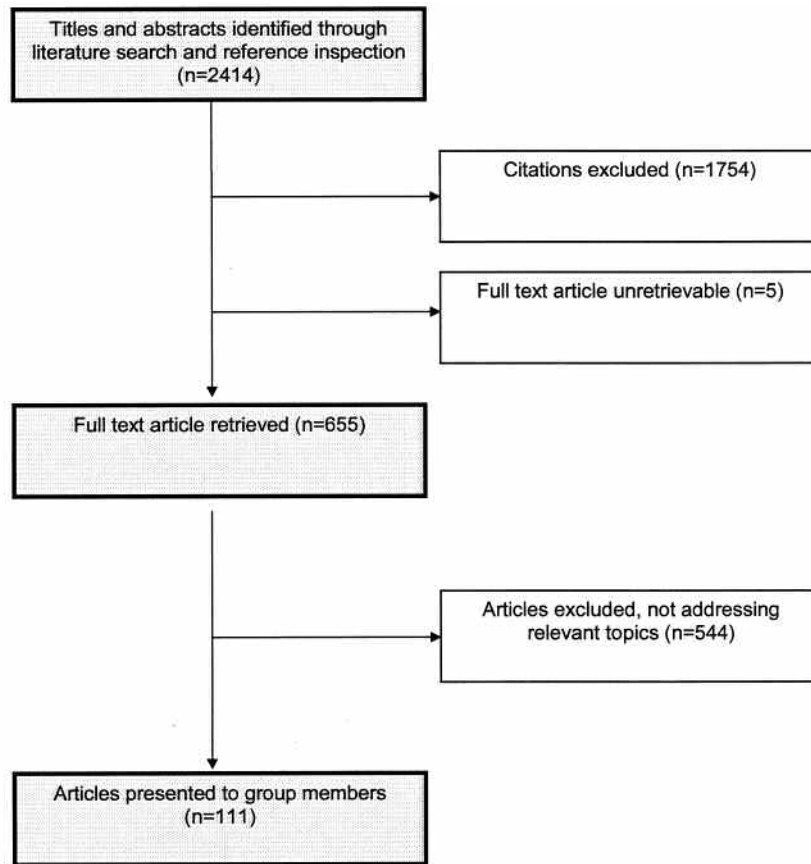
Figure 1. The process of article retrieval.

ers reflecting structural damage endpoints in RA and to identify omissions in the draft set as a prelude to the drafting of revised criteria. The group members were presented with the same literature review but the results were organized so that all the evidence was presented for each biomarker before the following voting question was presented: "Please rate on a scale of 0 (no supporting evidence) to 10 (unequivocal evidence) to what degree you consider (biomarker) as a valid biomarker reflecting structural damage in RA after a consideration of the entire literature addressing all 14 draft criteria." Group members were also asked to respond to the following question by providing written feedback: "What further information not addressed by the draft criteria is required to support (biomarker) as a valid biomarker reflecting structural damage in RA?"

Results of the voting exercises are provided as means (standard deviation).

## RESULTS

*Literature search and first voting exercise*. A summary of the findings has been organized under the 3 domains of the OMERACT filter. Table 1 shows the results of the voting exercise addressing the SOE for each biomarker as reflecting structural damage according to each specific criterion. Table 2 shows the results of the voting exercise addressing the strength of recommendation in support of including each individual criterion in the draft criteria set. MeSH terms used in the literature search for each criterion and more detailed findings of the search are reported in Appendix 2.

*Truth (Criteria 1–5)*. The systematic literature search focusing on the 5 criteria itemized under the category of truth revealed limited documentation and SOE was accordingly rated as low. Studies describing an association between the biomarker level and structural damage in established animal models of arthritis (Criterion 1) were only found for CTX-II[4,5]. All biomarkers were reported as being immunohistochemically localized to joint tissues (cartilage, bone, synovial tissue) (Criterion 2), although most are neither sensitive nor specific for target of joint tissue origin (Criterion 3), with the exception of CTX-II, which is a specific marker for type II collagen in hyaline cartilage[6,7]. The relation of the biomarker to synthesis, degradation, and turnover of joint tissue components (Criterion 4) has been well characterized for OPG, RANKL, and MMP-3, while the relation of CTX-I and CTX-II to joint degradation is not as well documented. With the exception of one cross-sectional study, which found that MMP-3 levels were strongly correlated with synovitis on magnetic resonance imaging (MRI) of the knee in RA[8], there were no other studies that reported correlations between biomarker levels and scores for other surrogates that have been shown to have predictive validity for structural damage (Criterion 5). The group vote for the strength of recommendation in support of including each

*Table 1.* Group rating (0–10 numerical rating scale) of the strength of evidence (SOE) in support of each biomarker as reflecting structural damage according to individual validation criteria.

| Criterion | RANKL (SOE) mean (SD) | OPG (SOE) mean (SD) | CTX-I (SOE) mean (SD) | CTX-II (SOE) mean (SD) | MMP 3 (SOE) mean (SD) |
|---|---|---|---|---|---|
| Truth | | | | | |
| 1. (Data from animal models) | 2.3 (1.9) | 1.4 (1.3) | 2.3 (1.9) | 2.3 (1.9) | 0.3 (0.8) |
| 2. (Localization to joints) | 8.1 (1.0) | 4.9 (3.1) | 2.5 (3.3) | 2.5 (3.3) | 7.4 (2.0) |
| 3. (Sensitivity/specificity for joints) | 1.9 (2.0) | 1.8 (2.1) | 3.1 (2.5) | 3.1 (2.5) | 2.2 (2.1) |
| 4. (Known relation to joint turnover) | 6.8 (1.9) | 6.5 (1.9) | 5.2 (2.9) | 5.2 (2.9) | 6.9 (2.0) |
| 5. (Correlation with other surrogates) | 2.7 (1.6) | 2.6 (1.4) | 3.2 (1.8) | 3.2 (1.8) | 5.3 (2.1) |
| Discrimination | | | | | |
| 6. (Assay reproducibility) | 7.6 (2.5) | 7.8 (2.1) | 8.4 (1.7) | 8.4 (1.7) | 7.0 (1.9) |
| 7. (Effect of sources of variability) | 4.8 (1.8) | 5.8 (1.8) | 5.8 (1.5) | 5.8 (1.5) | 5.7 (1.9) |
| 8. (Known metabolism of biomarker) | 0.1 (0.3) | 0.1 (0.3) | 0.1 (0.3) | 0.1 (0.3) | 0.1 (0.3) |
| 9. (Sensitivity/specificity in disease vs controls) | 2.9 (1.7) | 2.9 (1.7) | 4.0 (1.5) | 4.0 (1.5) | 5.9 (1.5) |
| 10. (Association with damage in prospective studies) | 3.8 (2.0) | 4.2 (1.8) | 4.3 (1.7) | 4.3 (1.7) | 4.7 (1.7) |
| 11. (Association with damage in RCT) | 0.3 (0.4) | 0.3 (1.4) | 1.4 (2.2) | 1.4 (2.2) | 0.2 (0.5) |
| 12. (Association with damage in pre-radiographic disease) | 0.0 (0.0) | 0.0 (0.0) | 3.9 (2.3) | 3.9 (2.3) | 0.0 (0.0) |
| Feasibility | | | | | |
| 13. (Well characterized assay) | 3.7 (2.2) | 3.8 (2.0) | 4.1 (2.1) | 4.1 (2.1) | 4.1 (2.1) |
| 14. (Biomarker stability) | 4.3 (2.7) | 1.7 (1.8) | 6.3 (2.8) | 6.3 (2.8) | 1.3 (1.8) |

RANKL: receptor activator of NF-κB ligand; OPG: osteoprotegerin; CTX-I: C-telopeptide of type I collagen I; CTX-II: C-telopeptide of type II collagen; MMP-3: metalloproteinase 3; RCT: randomized controlled trial.

*Table 2.* Rating (0–10 numerical scale) for the strength of recommendation in support of the retention of each criterion in the OMERACT 8 draft set of validation criteria for a biomarker reflecting structural damage endpoints in RA.

| Criterion | Mean (SD) |
|---|---|
| 1 | 5.8 (2.7) |
| 2 | 7.5 (2.7) |
| 3 | 6.4 (2.8) |
| 4 | 7.7 (1.7) |
| 5 | 5.8 (2.8) |
| 6 | 9.1 (1.7) |
| 7 | 8.3 (1.7) |
| 8 | 4.4 (2.9) |
| 9 | 7.0 (2.4) |
| 10 | 8.9 (1.4) |
| 11 | 8.2 (1.5) |
| 12 | 7.6 (1.6) |
| 13 | 7.2 (2.7) |
| 14 | 7.1 (2.5) |

individual criterion under the category of truth in the draft criteria ranged from 5.8 for Criteria 1 and 5 to 7.7 for Criterion 4 (see Table 2).

*Discrimination (Criteria 6–12).* Assay reproducibility data are largely based on the manufacturer's studies (package inserts); kits with intra-assay coefficient of variation (CV) less than 10% and inter-assay CV less than 15% (Criterion 6) are commercially available for all the biomarkers. Several studies have clarified the influence of potential sources of variability on biomarker levels including: age, sex, menopause, circadian rhythms, body mass index, physical activity, nonsteroidal antiinflammatory drugs (NSAID),

renal and hepatic disease, and contribution of different affected joints, although studies have primarily been cross-sectional (Criterion 7). Variation with age seems to occur especially for OPG, while sex appears to have a particular influence on MMP-3 levels[9-12]. Menopausal status influences levels of all the biomarkers, while diurnal variation is most pronounced for S-CTX-I[13-15]. Hepatic disease influences levels of OPG, RANKL, and MMP-3[11,16], while renal failure influences S-CTX-I and OPG levels[17,18]. Nevertheless, the literature is still somewhat contradictory and often neglects potential confounders. Metabolism of the biomarkers has not been studied in either normal individuals or in patients with RA (Criterion 8). Some cross-sectional studies have compared biomarker levels in RA patients with healthy, but not age and gender matched, controls (Criterion 9). One study revealed higher levels of RANKL and OPG in RA patients[19], U-CTX I was slightly elevated in RA patients compared to healthy controls in 2 small studies[6,20], and S-CTX-I was elevated in destructive, but not in non-destructive RA in another study[21]. Three studies showed increased U-CTX-II in RA patients compared to controls[6,22,23], and 7 reported higher levels of MMP-3 in RA[9,10,23-27].

Several prospective cohort studies have examined the independent association between the baseline level of a biomarker and the structural damage endpoint (Criterion 10). Studies from the COBRA cohort concluded that the OPG/RANKL ratio, U-CTX-I, and U-CTX-II are independent predictors of radiographic progression[23,28,29]. In a recent study both baseline levels of U-CTX-II and the longitudinal values (area under the curve, AUC) independently predicted

radiographic progression[30]. Evidence supporting MMP-3 as a predictor of radiographic progression is conflicting, as some studies examined only baseline levels, several did not address potential confounders through multivariate analysis, and sample sizes were typically small[10,30-37]. MMP-3 decreased after initiation of MTX in one study, but there was no association between this change and subsequent change in the structural damage endpoint[30].

Only one randomized controlled trial examined the association between biomarker levels and radiographic progression (Criterion 11) and this showed that the change in U-CTX-II, but not U-CTX-I, was an independent predictor of subsequent radiographic progression[38]. Associations have not been specifically studied in pre-radiographic cohorts (Criterion 12), but subgroup analysis of pre-radiographic patients in the COBRA study showed that both U-CTX-I and U-CTX-II levels were strongly associated with radiographic progression[23]. The group vote for the strength of recommendation in support of including each individual criterion under the category of discrimination was generally high and ranged from 7 for Criterion 9 to 9.1 for Criterion 6 (Table 2). The only exception was the low score of 4.4 for the criterion that addressed the metabolism, clearance, and half-life of the biomarker (Criterion 8).

*Feasibility (Criteria 13 and 14).* Evidence addressing the 2 criteria listed under the category of feasibility is based largely on unpublished data obtained from the manufacturers of the assays. There is no international standardization of the assays for any of the markers. According to the manufacturers, the assays are quite well characterized and methodologically simple (Criterion 13). There is limited documentation on stability of the biomarkers at room temperature and in frozen specimens (Criterion 14). Degradation after longterm storage seems to be a particular problem with RANKL[39], while CTX-I and CTX-II remain stable after repeat freeze-thaw[6,40,41], but documentation of the effect of longterm storage was not found. There is very limited documentation for this criterion as regards MMP-3 and OPG. The group vote for the strength of recommendation in support of including Criteria 13 and 14 was 7.2 and 7.1, respectively.

*Second voting exercise.* After consideration of the entire literature search addressing all 14 criteria (Table 3), the group

*Table 3.* Rating (0–10 numerical scale) for the strength of evidence in support of each biomarker as reflecting structural damage based on a systematic literature search.

|  | Mean (SD) | Range |
|---|---|---|
| CTX-II | 6.5 (1.2) | 4–8 |
| MMP3 | 4.5 (1.2) | 2–6 |
| RANKL | 3.2 (1.2) | 1–5 |
| OPG | 2.8 (1.3) | 0–5 |
| CTX-I | 3.8 (1.3) | 2–7 |

For definitions, see Table 1.

rated the SOE in support of the biomarkers as reflecting structural damage outcomes in RA highest for U-CTX-II [6.5 (NRS 0–10)]. Key omissions identified in this second voting exercise were the desirability of demonstrating associations between changes in the biomarker and radiographic progression for all drug classes and in individual patients.

## DISCUSSION

Our literature search and the succeeding voting exercises show that the evidence in support of any of the 5 tested biomarkers as reflecting structural damage endpoints in RA is insufficient to justify their substitution of radiographic changes, with the highest score being only 6.5 for U-CTX-II. Moreover, some criteria, particularly those categorized under truth (Criteria 1 and 5), were regarded as being of lesser importance for inclusion in the draft set. As noted in a companion report of the soluble biomarker workshop at OMERACT 9, in retrospect it was recognized that in the setting of validation of predictive biomarkers, the OMERACT filter criteria of truth and discrimination largely overlap[42]. The importance of demonstrating associations between biomarkers and structural damage in several drug classes as well as in individual patients was also newly raised.

As with the example of CRP[3], the criteria itemized under the category of truth were poorly supported by the literature for all the tested biomarkers. The relation of the biomarkers to joint remodeling is well described for all the markers, but there are very few animal studies or studies comparing these biomarkers to other surrogates of structural damage. Voting exercise 2, however, showed that the group questioned both the relevance of animal studies in this context and the importance of studies proving that the marker is associated with a surrogate endpoint, even if it has been previously shown that the surrogate is associated with the damage endpoint. Even if animal models are easy to replicate and allow for evaluation of the biomarker's performance throughout the course of the disease, including the influence of pharmacologic manipulations, in a spectrum of models with correlations to "gold standards" such as histopathology and imaging, animal models do not necessarily reflect the pathophysiological process evident in humans. Moreover, negative animal data do not exclude an association in humans and, therefore, might not be considered an essential requirement. All the biomarkers have been immunohistochemically localized to joint tissue, but the presence of the marker in the joint does not prove its relevance to the destructive process. Only CTX-II is specific for joint tissue (hyaline cartilage), as the other markers are also involved in other physiological and pathological processes in the body. It is conversely questionable if a marker should be excluded if it is not totally specific for joint tissue, as in the example of the CRP.

Under the subheading of discrimination, a major concern is the variability in the biomarker level due to sex, age,

menopausal status, and time of day, not to mention the possible variation due to meals, which has been poorly studied. The studies focusing on this criterion were many, but insufficient for all the markers and sometimes flawed by inadequate study design (e.g., few patients, no adjustments for confounders, cross-sectional design). There were only a few studies comparing biomarker levels in RA patients and controls, and in most studies the controls were not matched for age and gender. The metabolism and half-life were not described for any of the biomarkers (Criterion 8). The group, however, did not consider this criterion as particularly desirable in the validation process. Criteria requiring demonstration of an independent association between biomarker and radiographic damage in clinical studies were rated highest by the group for retention in the draft set. Although the strongest independent association with damage was observed for U-CTX-II, associations were only moderate. Frequently noted limitations in study design were: low sample size, analysis of biomarker limited to baseline samples, failure to address known confounders in multivariate analyses, and use of different radiographic endpoints.

Some of the lowest scores for SOE were found under the category of feasibility. In clinical trials, analyses are typically done simultaneously on samples frozen for different lengths of time. This might lead to alterations in measured serum protein concentrations, as indicated for RANKL[41]. Information on stability of the biomarker in frozen specimen and the effect of repeated freeze-thaw is not readily available from assay manufacturers and is rarely indicated in the inserts that come with the assay kits. International standardization or reference values for commercially available assays are not available. This makes comparisons of levels across studies and analyses at different laboratories difficult.

This study has some limitations. The literature search was performed by one single reviewer, but the same search strategy with some additions as in the previous testing of the criteria with CRP was used[3]. The heterogeneous and limited study selection identified by the literature search allowed only descriptive data synthesis. Study quality was not formally assessed by the reviewer, although group members were provided with a study description that included the principal features of the study design. A survey was sent to the kit manufacturers to obtain unpublished data, but we cannot exclude publication bias.

This literature search, and the subsequent voting exercises, has guided the further discussions and development of the validation criteria set within the working group at OMERACT 9. In conclusion, more documentation is needed for any of the 5 tested biomarkers to be regarded as reflecting structural damage outcomes in clinical trials. In particular, there is a paucity of data under the categories of truth and feasibility. This testing exercise of the OMERACT 8 preliminary validation criteria for soluble biomarkers

using RANKL, OPG, CTX-I, CTX-II, and MMP-3 revealed that some of the criteria might not be essential in the validation process, and some omissions to the set were highlighted.

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*Appendix 1.* OMERACT 8 Draft validation criteria.

| | | |
|---|---|---|
| *Truth* | 1 | Evidence that the biomarker reflects tissue remodelling in established animal models of disease (e.g., collagen arthritis for RA) |
| | 2 | The biomarker has been immunohistochemically localised to joint tissues |
| | 3 | The biomarker demonstrates sensitivity and specificity for target of joint tissue origin |
| | 4 | Relation of biomarker to synthesis, degradation, turnover of joint tissue components has been characterized |
| | 5 | Levels of the biomarker correlate with scores for other surrogates that have been established as possessing predictive validity for structural damage (e.g., MRI for erosive RA) |
| *Discrimination* | 6 | The assay for measurement of the biomarker is reproducible (coefficient of variation: intra-assay less than 10%, interassay less than 15%) |
| | 7 | The effects of the following sources of variability on levels of the biomarker in normal individuals are known: age, sex, menopause, circadian rhythms, body mass index, physical activity, NSAID, renal and hepatic disease, contribution of different affected joints |
| | 8 | The metabolism, clearance, and half-life of the biomarker have been characterized in normal individuals and in patients with arthritis |
| | 9 | The biomarker demonstrates high sensitivity and specificity in comparisons of the disease population with age- and sex-matched healthy controls |
| | 10 | The biomarker demonstrates independent association with the structural damage endpoint (van der Heijde modification of Sharp score for RA, mSASSS for AS, joint space narrowing score for OA) at the level of both absolute and relative change in a clinically well-defined prospective cohort of adequate sample size and followed for a sufficient duration to detect change in x-ray damage score |
| | 11 | The biomarker demonstrates independent association with the structural damage endpoint (van der Heijde modification of Sharp score for RA, mSASSS for AS, joint space narrowing score for OA) at the level of both absolute and relative change in a randomized controlled trial of adequate sample size and of sufficient duration to detect change in structural damage score |
| | 12 | The biomarker demonstrates predictive validity for the structural damage endpoint (van der Heijde modification of Sharp score for RA, mSASSS for AS, joint space narrowing score for OA) in a clinically well-defined prospective cohort of patients with pre-radiographic disease of adequate sample size and followed for a sufficient duration to detect structural damage |
| *Feasibility* | 13 | The assay for measurement of the biomarker has been well-characterized, is internationally standardized (availability of reference standards), and is methodologically simple |
| | 14 | Stability of the biomarker at room temperature and in frozen specimen has been documented |

MeSH terms for the biomarkers used for the entire literature search: RANK ligand or osteoclast differentiation factor or Receptor Activator of Nuclear Factor-Kappa B or osteoprotegerin or CTX-I or collagen type I C-terminal telopeptide or CTX-II or collagen type II C-terminal telopeptide or Matrix metalloproteinase 3 or biomarker.

urinary type I collagen crosslinked C-telopeptide and free and peptide-bound forms of pyridinium crosslinks. Bone 1998;22:73-8.

16. Moschen AR, Kaser A, Stadlmann S, et al. The RANKL/OPG system and bone mineral density in patients with chronic liver disease. J Hepatol 2005;43:973-83.

17. Kazama JJ. Osteoprotegerin and bone mineral metabolism in renal failure. Curr Opin Nephrol Hypertens 2004;13:411-5.

18. Pagani F, Bonetti G, Stefini F, Panteghini M. Evaluation of a fully automated assay to measure C-telopeptide of type I collagen in serum. Clin Chem Lab Med 2000;38:1111-3.

19. Ziolkowska M, Kurowska M, Radzikowska A, et al. High levels of osteoprotegerin and soluble receptor activator of nuclear factor kappa B ligand in serum of rheumatoid arthritis patients and their normalization after anti-tumor necrosis factor alpha treatment. Arthritis Rheum 2002;46:1744-53.

20. Molenaar ET, Lems WF, Dijkmans BA, de Koning MH, van de Stadt RJ, Voskuyl AE. Levels of markers of bone resorption are moderately increased in patients with inactive rheumatoid arthritis. Rheumatology 2000;39:742-4.

21. Garnero P, Jouvenne P, Buchs N, Delmas PD, Miossec P.

*Appendix 2A.* **Truth Criteria**
*1. Evidence that the biomarker reflects tissue remodelling in established animal models of disease (e.g., collagen arthritis for RA)*
*MeSH: Arthritis experimental, Disease model and arthritis*
Only a few studies have examined biomarker levels in animal models of arthritis. Ostergaard, et al, in 2 studies, showed a strong correlation between S-CTX-II levels after induction of arthritis and subsequent microscopic severity score in arthritic joints in rats[4,5] Two studies have also demonstrated increased levels of U-CTX-II in rats directly after induction of arthritis[43,44], but associations between the biomarker levels and the grade of structural damage were not described. The CTX-I, OPG, and RANKL levels in serum were increased after induction of arthritis in mice[4,43,45,46], but associations with structural damage were not examined. The RANKL and OPG levels increased together with signs of inflammation. Although joint degradation started immediately, S-CTX-I did not increase until 2 weeks after induction of arthritis in rats[4]. The authors concluded that the amount of CTX-I from the joints was too scarce to be detected, and that the CTX-I levels in serum merely reflects the ongoing general bone loss. No studies were found for MMP-3.

*2. The biomarker has been immunohistochemically localized to joint tissues*
*MeSH: immunohistochemistry (arthritis or joint) and (pathogenesis or histology)*
Several studies have localized RANKL to synovia and bone both in diseased humans and in animal models of arthritis[47-53]. The staining was most abundant at the bone-pannus interface and at erosion sites[47,50,53]. Two studies have localized OPG to synovia in arthritis[47,48]. OPG staining was found remote from bone and RANKL expression[47], but was not found in patients with very active disease[48]. Garnero, et al have demonstrated fragments from degradation of type I collagen C-telopeptide (CTX-I) in bone from both Paget patients and healthy controls[54]. CTX-II has been localized by immunohistochemistry to cartilage in rats, bovine animals, and humans[4,55]. The staining was localized to areas with proteoglycan loss[4], but only after stimulation with proinflammatory cytokines[55]. Several studies have described the localization of MMP-3 in synovial tissue and cartilage in RA patients and in animal models of arthritis[56-63]. Remission was associated with a decrease in MMP-3 staining[61].

*3. The biomarker demonstrates sensitivity and specificity for target of joint tissue origin*
*Only a selection of studies describing the presence of the biomarkers in other diseases is presented to illustrate that the markers are neither sensitive nor specific for joint tissue*
RANKL and OPG are neither sensitive nor specific markers of processes in joint tissue, but are also found in other conditions, i.e., in periodontitis[64], cholesteatoma[65], bronchitis[66], tumor angiogenesis[67] and in cancer[68], cirrhosis[69], and atherosclerosis[70]. CTX-I is a marker of collagen type I degradation. Collagen type I is neither sensitive nor specific for joint tissue, as type I collagen is the main component of bone. CTX-I levels are, for example, also elevated in Paget's disease[40,54]. CTX-II is a specific marker for the collagen type II C-terminal teleopeptide[6]. Collagen type II is specific for cartilage[7], thus CTX-II can be said to be relatively sensitive and specific for joint tissue. MMP-3 is neither specific nor sensitive for joint tissue. It may be elevated in several settings such as cancer invasion, pregnancy, inflammatory bowel disease, graft vs host reactions, asthma, hepatitis, and in the menstruation cycle[71-76]. High serum levels have been measured in hypercholesterolemia[77], coronary disease[78,79], multiple sclerosis[80], and systemic sclerosis[81], among others.

Uncoupling of bone metabolism in rheumatoid arthritis patients with or without joint destruction: assessment with serum type I collagen breakdown products. Bone 1999;24:381-5.

22. Olsen AK, Sondergaard BC, Byrjalsen I, et al. Anabolic and catabolic function of chondrocyte ex vivo is reflected by the metabolic processing of type II collagen. Osteoarthritis Cartilage 2007;15:335-42.

23. Garnero P, Landewe R, Boers M, et al. Association of baseline levels of markers of bone and cartilage degradation with long-term progression of joint damage in patients with early rheumatoid

*4. Relation of biomarker to synthesis, degradation, turnover of joint tissue components has been characterized*
*As this topic has been extensively studied and the relation well known , the literature search was limited to review papers and references of review papers and other papers in this search according to this criterion for RANKL, OPG and MMP-3. MeSH for CTX I and CTX II: (joint or cartilage or bone) and (degradation or turnover or synthesis)*

This topic has been extensively studied and reviewed in several papers[82-86]. CTX-I have been found in supernatants of stimulated osteoclasts on bone slices[6,87-89], and the amount is correlated to the pit area[6]. CTX-I is found to be a result of cathepsin K stimulation of osteoclasts, and not of MMP stimulation[87]. The degradation of collagen II has been extensively studied and reviewed in several papers[56,90]. Cartilage is composed primarily of water, proteoglycan and collagen II. Degradation of proteoglycan is considered reversible, but degradation of type II collagen is irreversible and, therefore, represents a key point in the pathogenesis of RA. This degradation is mainly due to MMP[56,90]. CTX-II is a collagen type II C-telopeptide degradation product[6]. When stimulating cartilage explants with pro-inflammatory cytokines, CTX-II was released into the supernatant[6,22]. The relation of MMP-3 to degradation and turnover of joint tissue components has also been reviewed in detail elsewhere[56,91]. MMP-3 is considered to have a pivotal role in cartilage degradation and is induced by IL-1 beta and TNF alpha and secreted by synovial fibroblasts and other synovial cells. The transduction pathway and transcription is well described. MMP-3 degrades non-collagen matrix components of the joints, especially proteoglycans. It is also an activator of pro MMP 1 and other MMP. It is present in normal joints but is up regulated in RA[56,91]. MMP-3 knock-out mice did not show cartilage degradation during arthritis[92,93], although the evidence is conflicting[94]. Exposure of cartilage to MMP-3 caused proteoglycan loss, swelling, and deterioration in physical properties[95].

*5. Levels of the biomarker correlate with scores for other surrogates that have been established as possessing predictive validity for structural damage (e.g., MRI for erosive RA)*
*MeSH: Magnetic resonance imaging or ultrasonography*

No studies were found according to this criterion. However, a cross-sectional study found MMP-3 levels to be strongly correlated to signs of synovitis on MRI of the knee in 16 RA patients[8]. For the other biomarkers no studies were found on correlations with erosions on MRI or other suggested surrogates for structural damage.

**Discrimination Criteria**
*6. The assay for measurement of the biomarker is reproducible (coefficient of variation: intra-assay less than 10%, inter-assay less than 15%)*
*Based on the manufacturer's studies (package inserts), kits with intra-assay CV less than 10% and inter-assay CV less than 15% are commercially available for all the biomarkers*

*7. The effects of the following sources of variability on levels of the biomarker in normal individuals are known: age, sex, menopause, circadian rhythms, body mass index, physical activity, NSAID, renal and hepatic disease, contribution of different affected joints*
*MeSH: (age or age factors or age distribution), sex, menopause, circadian rhythms, body mass index, obesity, (physical activity or motor activity), nonsteroidal antiinflammatory drugs, kidney diseases, liver diseases, joint distribution*

Age: There is conflicting evidence regarding the effect of age on RANKL levels. One study of 566 healthy subjects found no correlation with age[96]. Two other studies on healthy (n = 283 / n = 504) subjects found a weak negative[97] and a weak positive[98] correlation, respectively. Several studies have established a positive association between OPG levels and age[96-109]. Mouritzen, et al and Kawana, et al found urinary and serum CTX-I to be high in young healthy adults, which decrease to the menopause, and then increase again[103,104]. Bordiere, et al, however, found no

correlation with age in premenopausal women[105], and Minisola, et al found a linear increase with age in women[106]. CTX-II is higher in adults younger than 45 years than in older adults[6,103]. One study has found MMP-3 to increase with age[12]. None of these cross-sectional studies assessing the effect of age on biomarker levels or the other sources of variability covered by criterion 7 have adjusted for possible confounders.

Sex: One study of 566 healthy subjects found a slightly lower RANKL level in men[96]. Khosla, et al (n = 650) found lower OPG levels in pre menopausal women than in men below 50 years[99]. Two other studies (n = 1134 / 566) did not find any gender differences in OPG levels[96,100]. CTX-I levels are increased in women, but not in men after 45 years[103,106]. For U-CTX-II no sex differences were found[6,32,103]. MMP-3 levels are found to be higher in healthy men than in women[9-12]. This gender effect is not found in RA patients[32].

Menopause: One study in 504 pre- and postmenopausal Chinese women found decreased RANKL levels after menopause[97]. Levels of OPG[97,99,101] and CTX have been found increased after menopause[40,104]. U-CTX-II levels are found to be lower in post-menopausal[6,103] rather than premenopausal women. However, the sample size in both studies was small. No studies were found on the effect of menopause on MMP-3 levels.

Circadian rhythms: No studies were found on the effect of circadian rhythms on RANKL. A 12-h component was found to characterize serum OPG concentrations with peak concentrations around noon and midnight[107]. Both serum- and urine CTX-I have circadian variation of 36-54%, which is reduced by fasting[13-15]. Two small studies have found U-CTX-II to have a diurnal variation of 15-34%[6,108]. No diurnal variation has been shown for MMP-3[109].

Body mass index: One study showed no correlation between U-CTX-I and BMI in 130 healthy women[110]. U-CTX-II levels were higher in healthy adults with BMI > 25[103]. No studies on the relationship between OPG, RANKL or MMP-3 and BMI were found.

Physical Activity: In regards to MMP-3, there are conflicting conclusions on this issue. Manicourt, et al found that the serum MMP-3 level rose significantly after getting out of bed[109]. MMP-3 levels in SF from the knee, however, did not increase after training in 33 athletes[111]. No studies were found on RANKL, OPG, CTX-I and CTX-II according to this criterion.

NSAID: No studies addressing this criterion were found.

Renal Disease: The effect of kidney failure on OPG levels is reviewed by Kazama, et al who concluded that OPG in serum is elevated along with deterioration in GFR[17]. An inverse relationship between serum creatinine and serum CTX-I have been found in 73 patients with renal failure[18]. No studies were found on RANKL, CTX-II, and MMP-3 according to this criterion.

Hepatic disease: One study found elevated RANKL and OPG levels in chronic liver disease[16]. The levels correlated with laboratory parameters of liver disease. MMP-3 concentration in serum was 50% lower in patients with chronic liver disease than controls[11]. No studies were found on CTX-I and CTX-II according to this criterion.

The contribution of different joints: No studies were found that addressed this criterion.


*8. The metabolism, clearance, and half-life of the biomarker have been characterized in normal individuals and in patients with arthritis*
*MeSH: Metabolism or clearance or half-life*
No studies were found that addressed this criterion.


*9. The biomarker demonstrates high sensitivity and specificity in comparisons of the disease population with age and sex matched healthy controls*
*MeSH terms for the below criteria (9-12): Rheumatoid arthritis*
A small study has compared RANKL levels in RA patients (n = 43) with controls (n = 30), finding higher levels in the RA patients[19]. The controls were not age and sex matched. The same study also found higher OPG levels in the RA patients. However, the OPG/RANKL ratio was similar to the controls[19]. Skoumal, et al found lower RANKL levels and higher OPG levels in 44

RA patients than the normal levels suggested by the manufacturer, but controls were not included[112]. Feuerherm, et al examined serum OPG levels in 78 RA patients[113]. They found similar levels in seronegative RA patients as in controls (n = 35), but the seropositive RA patients had elevated OPG levels. U-CTX I have been found slightly elevated in RA patients versus healthy controls in 2 small studies[6,20]. The controls were not age and gender matched. Garnero, et al found serum CTX-I to be elevated in destructive, but not in non-destructive RA (n = 318) compared to non-matched controls (n = 319)[21]. A small study by Wong, et al did not show higher s-CTX-I levels in RA patients (n = 28) than controls (n = 19)[114]. Garnero, et al compared 116 RA patients with very active disease with 76 age and sex matched controls. In the RA patients 32% had increased U-CTX-II values. Two small studies comparing U-CTX-II in RA patients with non-matched controls also revealed higher levels in the patients[6,22]. Several cross-sectional cohort comparisons, all showing higher MMP-3 levels in RA patients than non-matched healthy controls, have been published[9,10,23-27]. The elevations, however, are rather non-specific as patients with other rheumatic diseases also had elevated levels[9,25].

*10. The biomarker demonstrates an independent association with the structural damage endpoint (van der Heijde modification of Sharp score for RA, mSASSS for AS, joint space narrowing score for OA) at the level of both absolute and relative change in a clinically well-defined prospective cohort of adequate sample size and followed for a sufficient duration to detect change in x-ray damage score*

Several prospective cohort studies (See Appendix 2) have examined the independent association between the baseline biomarker level and the structural damage endpoint. One study has examined the association between baseline OPG and RANKL levels and the structural damage endpoint. Geusens, et al found that the OPG/RANKL ratio in the first-year independently predicted radiographic progression in a 5-year prospective study (COBRA study) (Appendix 2)[28]. RANKL levels correlated weakly with radiographic progression (p = 0.17) while the OPG/RANKL ratio correlated negatively (p = -0.38). Results from the logistic regression showed that a high RANKL level gave an OR of 4.4 (1.5-13.0) for progression and high OPG levels an OR of 0.29 (0.10-0.85). Garnero, et al (COBRA study) found baseline levels of U-CTX-I to be a strong independent predictor of radiographic progression (Appendix 2)[23]. In a multivariate logistic regression U-CTX-I (highest tertile) had an OR of 7.9 (2.1-29.4) for progression. High U-CTX-I levels were a stronger predictor than RF, ESR, or CRP. When stratifying for baseline damage, this was only evident in patients with no joint damage at baseline. However, Jansen, et al did not find S-CTX-I to be independently associated with radiographic progression (Appendix 2)[29]. In this cohort of early arthritis patients, 78% of the patients were non-erosive after 2 years. Two studies by Garnero, et al have found a univariate association between baseline U-CTX-II level and radiographic progression (Appendix 2)[23,32]. In the COBRA study, U-CTX-II level in the highest tertile was a stronger independent predictor of radiographic progression than RF, ESR, or CRP with an OR of 11.2 (2.3-55.3)[23]. In the other study by Garnero, et al, the association was not significant when corrected for CRP[32]. In a recent study by Young-Min, et al both baseline levels of U-CTX-II and the longitudinal values (AUC) independently predicted radiographic progression[30]. Estimate of the strength of the association was not given in the paper. The evidence for MMP-3 levels at baseline being a predictor of subsequent radiographic progression is conflicting (See Appendix 2) as the longitudinal cohort studies on this issue have revealed opposite conclusions (see Appendix 2). Two studies did not find any association[31,36]. In these studies, Broeder, et al examined the relationship in 47 patients on adalimumab therapy, and Jensen, et al studied 75 patients with early arthritis. An association between baseline level MMP-3 and radiographic progression is found in several other studies (Appendix 2)[10,32-35,37]. Some studies finding an association did not use multivariate analyses adjusting for confounders[10,32,35]. Tchetverikov, et al, Roux-Lombard, et al, Green, et al and Young-Min, et al all showed that MMP-3 independently predicted progression (See Appendix 2 for study

details)[30,33,34,37]. MMP-3 decreased after MTX initiation, but there was no association between this change and the structural damage endpoint[30].

*11. The biomarker demonstrates independent association with the structural damage endpoint (van der Heijde modification of Sharp score for RA, mSASSS for AS, joint space narrowing score for OA) at the level of both absolute and relative change in a randomized controlled trial of adequate sample size and of sufficient duration to detect change in structural damage score*
Only one randomized controlled trial examining the association between biomarker levels and radiographic progression was found, the COBRA study in which 110 patients with disease duration of less than 2 years were followed for 1 to 6 years. Landewé, et al. did not find any association between the change in U-CTX-I after treatment initiation and grade of radiographic progression[38]. U-CTX-II levels correlated with radiographic progression at all time points and the change in U-CTX-II from baseline to 3 and 6 months was significantly associated with radiographic progression at 5 years (b = 0.48)[38]. In a multivariate model ΔCTX-II was an independent predictor of radiographic progression. In patients with high CTX-II that did not decrease, 82% progressed as compared to 50% in the total study population.

*12. The biomarker demonstrates predictive validity for the structural damage endpoint (van der Heijde modification of Sharp score for RA, mSASSS for AS, joint space narrowing score for OA) in a clinically well-defined prospective cohort of patients with pre-radiographic disease of adequate sample size and followed for a sufficient duration to detect structural damage*
In a subgroup of patients in the COBRA study with no baseline joint damage, U-CTX-I and U-CTX-II levels were strongly associated with radiographic progression (Appendix 2)[23].

**Feasibility Criteria**
*13. The assay for measurement of the biomarker has been well-characterized, is internationally standardized (availability of reference standards), and is methodologically simple.*
*Information from manufacturer:*
There is no international standardization for measurement of any of the biomarkers. According to the manufacturers, the assays are quite well characterized and methodologically simple. Sample volumes are between 40 and 100μl, incubation times vary between 60 minutes (Urine CartiLaps® (CTX-II), Nordic Bioscience®) and 24 h (OPG, Biomedica®).

*14. Stability of the biomarker at room temperature and in frozen specimen has been documented.*
*MeSH: Stability or storage or analytic sample preparation methods or frozen or freeze-thaw and information from manufacturers*
Chan, et al showed significant (> 50%) alterations in serum concentration of RANKL after storage for 6 months at both -20°C and -70°C[39]. Hawa, et al showed no alterations in s-RANKL after 4 freeze-thaw cycles[115]. According to the kit manufacturer (Biomedica®) samples should be stored at -20C and at -70°C for longterm storage. All samples should undergo only 4 freeze-thaw cycles. No published literature was found in regards to OPG. CTX-I and CTX-II remain stable after freeze-thaw cycles in 3 studies[6,40,41]. No studies were found in regards to CTX-I or CTX-II and longterm storage. No studies addressing MMP-3 and this criterion were found. According to the manufacturer (Amersham®) samples should be stored at -15 to -30 °C and freeze-thaw cycles should be avoided.

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26. Ichikawa Y, Yamada C, Horiki T, Hoshina Y, Uchiyama M. Serum matrix metalloproteinase-3 and fibrin degradation product levels correlate with clinical disease activity in rheumatoid arthritis. Clin

*Appendix 2B.* Prospective observational studies on the association between the biomarkers and structural damage endpoint.

| Biomarker | Study, Ref | No. of Patients | Disease Duration | Study Duration yrs | Radiogx Scoring System | Main Findings |
|---|---|---|---|---|---|---|
| RANKL and OPG | Geusens (28) | 92 | < 2 years | 5 | vdHmSs | OPG:RANKL ratio, independently predicted 5-yr radiographic progression of joint damage. Multivariate log regression: High RANKL OR 4.4 (1.5-13.0), high OPG OR 0.29 (0.10-0.85). |
| CTX-I | Jansen (29) | 279 | < 2 yrs early polyarthritis (73% RA). | 2 | vdHmSs | Multivariate log regression did not show association with radiographic progression (S-CTX-I) |
| | Garnero (23) | 110 | < 2 yrs | 1-6 | vdHmSs | Multivariate log regression: U-CTX-I (highest tertile) OR 7.9 (2.1-29.4) for progression. |
| CTX-II | Garnero (32) | 116 | < 3 yrs Very active disease | 1 | vdHmSs | Univariate RR for progression with high U-CTX-II 2.5, NS when adjusted for CRP |
| | Garnero (23) | 110 | < 2yrs | 1-6 | vdHmSs | Multivariate log regression: Independent predictor of progression. U-CTX-II (highest tertile) OR 11.2 (2.3-55.3). |
| | Young-Min (30) | 118 | < 2 yrs | 2 | Larsen | Both baseline value and AUC independently predicted progression. Estimate of the strength of the association was not given in the reference |
| MMP 3 | Broeder (31) | 47 Mab therapy | Long, DAS > 3.2 | 2 | vdHmSs | MMP-3 levels not higher in progressors vs non progressors and did not predict radiographic progression |
| | Garnero (32) | 116 | < 3 yrs | 1 | vdHmSs | RR (univariate) for progression with high MMP-3 2.5 (1.1-5.7). Association did not remain when correcting for CRP. |
| | Tchetverikov (33) | 109 | < 2 yrs | 2 | vdHmSs | ProMMP- 3 levels independent predictor of radiographic progression [multivariate linear regression, b=0.7 (0.3-1.1)] |
| | Green (34) | 98 | < 1year | 1 | Larsen | Baseline MMP-3 independent predictor of progression in multivariate linear regression. Beta not given |
| | Yamanaka (10) | 26 | variable | 3 | Larsen | Higher baseline levels in the progressors vs non progressors. Increase or decrease in MMP 3 did not influence progression. No multivariate analyses |
| | Posthumus (35) | 78 | < 1 year | 2 | vdHmSs | Higher baseline levels in patients with progression. Significant correlation between baseline levels and progression 0.28. No multivariate analyses |
| | Jensen (36) | 75 early arthritis | < 2 yrs | 2 | Larsen | No differences, progressors vs non progressors. No correlation between baseline level and progression |
| | Roux-Lombard (37) | 24 | < 2yrs | 5 | Larsen | Multivariate cox regression, pro MMP-3 only significant variable with HR 1.01 (1.00-0.02) |
| | Young-Min (30) | 118 | < 2 yrs | 2 | Larsen | Both baseline value and delta value independent predictor of progression. Estimate of strength of association not given |

vdHmSs: van der Heijde modified Sharp score; NS not significant

Exp Rheumatol 1998;16:533-40.

27. Yoshihara Y, Obata K, Fujimoto N, Yamashita K, Hayakawa T, Shimmei M. Increased levels of stromelysin-1 and tissue inhibitor of metalloproteinases-1 in sera from patients with rheumatoid arthritis. Arthritis Rheum 1995;38:969-75.

28. Geusens PP, Landewe RB, Garnero P, et al. The ratio of circulating osteoprotegerin to RANKL in early rheumatoid arthritis predicts later joint destruction. Arthritis Rheum 2006;54:1772-7.

29. Jansen LM, van der Horst-Bruinsma I, Lems WF, et al. Serological bone markers and joint damage in early polyarthritis. J Rheumatol 2004;31:1491-6.

30. Young-Min S, Cawston T, Marshall N, et al. Biomarkers predict radiographic progression in early rheumatoid arthritis and perform well compared with traditional markers. Arthritis Rheum 2007;56:3236-47.

31. den Broeder AA, Joosten LAB, Saxne T, et al. Long term anti-tumour necrosis factor a monotherapy in rheumatoid arthritis: Effect on radiological course and prognostic value of markers of cartilage turnover and endothelial activation. Ann Rheum Dis 2002;61:311-8.

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42. Maksymowych WP, Landewe R, Tak PP, et al. Re-appraisal of OMERACT 8 draft validation criteria for a soluble biomarker reflecting structural damage endpoints in rheumatoid arthritis,

43. Ishikawa T, Nishigaki F, Christgau S, et al. Cartilage destruction in collagen induced arthritis assessed with a new biochemical marker for collagen type II C-telopeptide fragments. J Rheumatol 2004;31:1174-9.

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