Development of Draft Validation Criteria for a Soluble Biomarker to Be Regarded as a Valid Biomarker Reflecting Structural Damage Endpoints in Rheumatoid Arthritis and Spondyloarthritis Clinical Trials

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ABSTRACT. ***Objective.*** Recent work has shown that several soluble biomarkers, detectable in peripheral blood, synovial fluid, and/or urine, reflect remodeling of joint tissues and may therefore constitute outcome measures that reflect joint damage. Consequently, it is now desirable to begin the process of developing criteria for validation of a soluble biomarker as an outcome measure reflecting structural damage progression in trials of disease-modifying therapies for rheumatoid arthritis (RA) and spondyloarthritis (SpA). Our objective was to develop validation criteria for a soluble biomarker to be regarded as a valid biomarker reflecting radiological endpoints in RA and SpA clinical trials.

Methods. A special interest group was established comprising investigators with expertise in soluble biomarker assay development as well as in outcomes research. This project was initiated by means of a Delphi consensus exercise. A list of draft criteria was first generated following a review of a US National Institutes of Health (NIH) 2000 white paper (available at: http://www.niams.nih.gov/ne/oi/oabiomarwhipap.htm) that focused on biomarkers in OA, and these were organized under subject headings relevant to the OMERACT filter: truth, discrimination, and feasibility. Additional criteria were solicited from the working group. This was followed by 3 rounds of voting.

Results. A list of 31 criteria was generated prior to voting. The first 2 rounds of voting resulted in cumulative agreement that 19 criteria be retained and 4 discarded, while discrepancies were recorded for 8 criteria. In the third round of voting, cumulative agreement was achieved to retain 5 of the 8 discrepant criteria, so that the final list included 24 criteria.

Conclusion. A draft set of criteria for validation of a soluble biomarker to be regarded as reflecting radiological damage endpoints in clinical trials has been proposed on the basis of consensus. (J Rheumatol 2007;34:634–40)

Key Indexing Terms:

BIOMARKER | RHEUMATOID ARTHRITIS | SPONDYLOARTHRITIS
VALIDATION CRITERIA | STRUCTURAL DAMAGE | OMERACT

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The influence of therapies for chronic inflammatory arthritis in clinical trials is measured using clinical outcomes that remain difficult to quantify and whose ability to reflect changes in radiographic joint damage is limited. Similarly, the traditional laboratory markers of inflammation, erythrocyte sedimentation rate and C-reactive protein (CRP), lack specificity and correlate relatively poorly with radiographic joint damage[1-3]. Plain radiography therefore remains the gold standard for assessment of both rheumatoid arthritis (RA) and spondyloarthritis (SpA). In RA, it has been estimated that 3 months may be sufficient to show treatment group differences in plain radiograph scores in patients receiving disease-modifying therapies[4]. However, the benchmark for a successful outcome has risen substantially with the introduction of highly effective biological therapies and novel combinations of traditional antirheumatic drugs, and it is now desirable to reliably detect early joint damage prior to the appearance of plain radiographic damage. In ankylosing spondylitis (AS), plain radiography lacks sensitivity to change, and established clinical outcome measures do not appear to be associated with change in structural damage[5,6]. Baseline damage score on plain radiography is currently the only known predictor of structural damage in AS. This constitutes a severe limitation to the development of disease-controlling therapies in AS, which currently require placebo-controlled trials of at least 2 years' duration. A similar challenge exists in defining fracture risk in patients with osteoporosis, and this has been addressed by the development of bone densitometry and soluble biomarkers of bone turnover. They have been shown to predict rates of bone loss[7], to predict fractures[8], and to monitor treatment efficacy[9]. Prospective studies of up to 5 years' duration show that increased levels of some biomarkers are associated with a 2-fold increased fracture risk independent of bone mineral density[10]. Consequently, biomarkers of bone turnover are now being recommended by some regulatory bodies as validated primary variables in dose-finding Phase II clinical trials for osteoporosis, although not in Phase III trials, as it is considered that a causal link (surrogacy) between the markers and longer term clinical endpoints has not been proven unequivocally (European Medicines Evaluation Agency osteoporosis guidance document; available at: http://www.emea.eu.int/pdfs/human/ewp/055295en.pdf).

The conclusions of several recent studies support the concept that soluble biomarkers can predict structural joint damage in RA, independent of disease activity measures and baseline damage scores on plain imaging[11-16]. These typically reflect different facets of both synthesis and degradation of matrix components and include markers of bone formation and resorption, cartilage turnover and/or degradation, and synovial hyperplasia and/or inflammation. Examples of biomarkers that independently predict joint damage in RA include urinary C-terminal crosslinking telopeptide of type I collagen (CTX-I) and CTX-II[12], which reflect bone and cartilage turnover, respectively, serum cartilage oligomeric matrix protein (COMP)[16], which reflects cartilage remodeling and synovial hyperplasia, and serum metalloproteinase 3 (MMP-3)[17-19], which primarily reflects synovial inflammation. Some soluble biomarkers, e.g., COMP, appear to be highly specific for cartilage degradation processes[20-22]. They can be readily measured using simple commercially available ELISA-based assays well within the scope of most diagnostic laboratories, and generally demonstrate relatively little intra- and inter-day variation in healthy individuals. Their predictive validity has also been demonstrated in RA patients with early disease who did not yet have joint damage on plain imaging[12]. Although reductions in some of these biomarkers have been noted in trials of biologics for RA and AS, it has not been shown that changes in the level of any of these biomarkers parallel the degree of radiographic progression[23-28].

Other categories of biomarkers have also been shown to independently predict joint damage in patients with RA. These include cytokines that regulate the process of osteoclast activation and include the receptor activator of nuclear factor-κB (NF-κB) ligand (RANKL) and soluble osteoprotegerin (OPG)[29,30], and autoantibodies such as anti-cyclic citrullinated peptide (CCP) antibodies[31-33]. There has also been progress in defining the precise molecular events that occur during matrix turnover in chronic joint disorders. Several novel biomarkers targeting epitopes generated *de novo* following cleavage of type II collagen molecules by collagenases have now been shown to have predictive validity for structural damage in osteoarthritis (OA)[13,34-36]. Reduced serum concentrations of these latter markers have also been noted in one trial of etanercept for AS[20]. Finally, there are now examples of combinations of biomarkers that have substantially greater predictive validity for structural damage than observed with single biomarkers[13,37-39]. Again, it has not been shown that changes in the level of any of these biomarkers parallel the degree of radiographic progression.

Although evidence from prospective cohorts increasingly supports the predictive validity of several candidate biomarkers for joint damage, few studies have examined the relationship between changes in soluble biomarkers and changes in radiographic progression, either in longitudinal cohorts or in clinical trial settings, in a manner that would allow any conclusions regarding its validity as a biomarker reflecting change in radiological endpoints. However, the science has advanced to the point where it is now both realistic and desirable to begin the process of developing criteria for validation of soluble biomarkers as outcome measures reflecting structural damage in RA, and to focus on a more limited group of biomarkers in RA to determine the adequacy of their validation according to these criteria. The draft criteria could then be used to focus the research agenda on those aspects of the validation process that are lacking and to serve as a template for the development of new biomarkers in the future. Although reported data on soluble biomarkers in SpA are limited, defined and standardized clinical and radiographic outcome

instruments have now been validated, and it is therefore appropriate to develop a research agenda for the validation of biomarkers in SpA also. In principle, these criteria might also be appropriate for the validation of soluble biomarkers as outcome measures reflecting structural damage in OA despite the obvious pathophysiological differences from RA and SpA, because the most essential aspect of validation is the process, which is independent of the specific biomarker and damage endpoint that is evaluated. The latter concept is further discussed elsewhere in these proceedings in the report from the superworkshop on surrogate outcomes in rheumatology[40].

Development of the research agenda

A special interest group has been established comprising investigators with expertise in soluble biomarker assay development and in outcomes research. The preliminary activities of the group have been to achieve consensus (1) on the primary objective(s) of this OMERACT initiative, (2) on methodological approaches to validation followed by testing of the criteria based on soluble biomarkers that are currently available, e.g., CRP in RA, and (3) on a prioritized research agenda and compilation of the required resources. Considerable work on these themes has already been undertaken by investigators participating in the Osteoarthritis Biomarkers Network, a consortium of 5 sites, supported by the NIH/NIAMS to develop and characterize new biomarkers and refine existing OA biomarkers. This consortium will draft a classification scheme for biomarkers that would be useful for research in OA. Since the primary mandate of the OMERACT superworkshop on surrogate outcomes in rheumatology is to develop consensus on a methodology to validate candidate biomarkers that will predict response in phase 3 trials in rheumatology, with a focus on RA as working example, there was agreement among the special interest group members that the initial objective of the soluble biomarker initiative would be development of draft validation criteria for soluble biomarkers to be regarded as valid biomarkers reflecting key clinical and radiological endpoints in RA and SpA, especially structural damage/tissue remodeling. The latter was chosen as the primary focus of the exercise, rather than a patient-centered clinical outcome, in recognition of the limitations in the use of radiological endpoints as one of the high priority areas for further research in outcome assessment. The research agenda would proceed on a parallel, complementary path with the OA Biomarker Network with investigator participation in both working groups.

To begin the process of generating consensus on a research agenda for validation of soluble biomarkers, it was decided that this would first be accomplished by means of a Delphi consensus exercise followed by testing of the criteria using the CRP in RA as an example to identify limitations in the criteria and then to modify them[41]. The solicitation of criteria for the exercise was structured on the key requirements of the OMERACT filter for validation of an outcome measure although focusing on issues of truth and discrimination. Although feasibility is clearly important, there was agreement that this necessitated technical considerations such as assay methodology that are less germane to the process of validation established by OMERACT. The solicitation of criteria was followed by 3 rounds of voting in which the relative importance of each criterion was rated on a 5-point Likert scale ranging from definitely not important (score 1) to essential (score 5). Criteria were selected on the basis of having achieved cumulative agreement as defined by a score of 4 or 5 by ≥ 70% of working group participants in the first 2 rounds of voting. The third round of voting was necessary to resolve discrepancies in cumulative agreement between the first and second rounds of voting.

Proposal for validation criteria

A list of draft criteria was generated from a US National Institutes of Health white paper published in 2000 that focused on biomarkers in OA (available at: http://www.niams.nih.gov/ne/oi/oabiomarwhipap.htm), and these were organized under subject headings that reflect the key requirements of the OMERACT filter: truth, discrimination, and feasibility. Additional criteria were solicited from the working group so that the preliminary list included 31 candidate criteria organized under the headings of truth, discrimination, and feasibility (Table 1). The first 2 rounds of voting resulted in cumulative agreement that 19 criteria be retained and 4 discarded, while discrepancies were recorded for 8 criteria. In the third round of voting cumulative agreement was achieved to retain 5 of the 8 discrepant criteria so that the final list included 24 criteria. As several of these criteria focused on the individual effects of different sources of variability on biomarker measurements, the final list has been condensed into 14 criteria (Table 2).

Discussion

Consideration of aspects of truth led to the proposal for criteria that focus on the localization and specificity of the biomarker for tissues of joint origin, the demonstration that it reflects some aspect of joint tissue remodeling, construct validity in relation to biomarkers that have themselves been validated as reflecting structural damage, e.g., disease activity score (DAS), magnetic resonance imaging (MRI)[42], and the experience with the biomarker in animal models of arthritis. Experimental animal models offer the opportunity to study the biomarker under more carefully controlled conditions and particularly to analyze tissue levels at the site of disease in relation to disease onset, disease activity, stage of disease, and treatment. However, the findings in animal studies may not be replicated in human disease.

The draft criteria highlight the importance of demonstrating an association between change in biomarker levels and change in radiological endpoints in longitudinal studies and randomized controlled trials. This reflects important concep-

Table 1. Draft validation criteria for a soluble biomarker to be regarded as a valid biomarker reflecting structural damage in RA/SpA generated prior to consensus voting by Delphi technique.

A. Truth
1. A preclinical body of evidence that the soluble biomarker reflects tissue remodeling in established animal models of disease (e.g., collagen arthritis).
2. Evidence that the biomarker reflects tissue remodeling in human *ex vivo* models of tissue remodeling (e.g., cartilage, bone, synovial explant).
3. The biomarker has been immunohistochemically localized to joint tissues.
4. The molecular target and/or proteolytic cleavage site has been well characterized.
5. The biomarker demonstrates sensitivity and specificity for target of joint tissue origin.
6. Relation of biomarker to synthesis, degradation, turnover of joint tissue components has been characterized.
7. Levels of the biomarker correlate with scores for other biomarkers that have been established as possessing predictive validity for structural damage (e.g., MRI for erosive RA).

B. Discrimination
1. The assay for measurement of the biomarker is reproducible (coefficient of variation: intraassay ≤ 10%, interassay ≤ 15%).
2. The effects of the following sources of variability on levels of the biomarker in normal individuals are known (rate each item independently): age, sex, menopause, circadian rhythms, body mass index, ethnicity, physical activity, meals, seasonal variation (9 criteria).
3. The effects of the following sources of variability on levels of the biomarker in patients are known: NSAID, renal and hepatic disease, the contribution of different affected joints (3 criteria).
4. The metabolism, clearance, and half-life of the biomarker have been characterized in (A) normal individuals and (B) patients with arthritis (2 criteria).
5. The biomarker demonstrates high sensitivity and specificity in comparisons of the disease population with age and sex matched healthy controls.
6. The biomarker demonstrates independent association with the structural damage endpoint (van der Heijde modification of Sharp Score for RA, mSASSS for AS) at the level of both absolute and relative change in (A) a clinically well defined prospective cohort, (B) a randomized controlled trial, and (C) a clinically well defined prospective cohort of patients with preradiographic disease. These should be of adequate sample size, and followup should be of sufficient duration to detect change (3 criteria).
7. The biomarker demonstrates increased responsiveness (i.e., magnitude of change) compared to the structural damage endpoint (e.g., erosion score) in patients receiving disease-modifying therapies.

C. Feasibility
1. The assay for measurement of the biomarker has been well characterized and is internationally standardized (availability of reference standards).
2. The assay for measurement of the biomarker is (A) methodologically simple and (B) commercially available (2 criteria).
3. The biomarker assay should demonstrate adequate analytical dilution recovery, sensitivity, and quantification limit.
4. Stability of the biomarker at room temperature and in frozen specimen has been documented.
5. Analytical performance of the biomarker assay has been documented in several body fluids (serum, synovial fluid, urine).

tual considerations that are distinct from most studies of biomarkers undertaken to date. Longitudinal studies have mainly shown that certain biomarkers measured at baseline are predictive of radiological change. This information may eventually lead to development of a useful prognostic tool but is insufficient for its development as a biomarker that can substitute for radiological endpoints in clinical trials. The latter requires demonstration of an independent association between change in biomarker levels and change in radiological progression. Moreover, it requires that the biomarker is considerably more responsive to change, e.g., disease activity, DMARD, compared to the radiographic instrument. A major priority is therefore to validate biomarkers in longitudinal clinical studies over a sufficiently long period where clinical change, especially structural damage, can be clearly defined. Although this may require as little as 3 months to show plain radiographic change as documented by the van der Heijde-modified Sharp Score in patients with RA, it is recommended that initial studies evaluate patients over at least one year, since the magnitude and consistency over time of the association and consequently the required sample size will be unknown without evaluation for this duration. For AS, a minimum period of 2 years' followup will be required to demonstrate significant change in plain radiographic scores as documented by the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS)[5]. This approach will also require that patients are clinically well characterized according to predefined criteria that at a minimum should include age, sex, disease duration, disease-modifying therapy (i.e., specific drug(s) and duration of treatment), Health Assessment Questionnaire, DAS, rheumatoid factor (RF), anti-CCP, CRP, and baseline plain radiographic score for RA. This level of characterization will allow regression analysis to determine the validity of biomarker levels independently of known confounders such as disease activity, RF and anti-CCP status, and baseline radiographic damage. Longitudinal analyses using generalized estimating equations with sequential clinical, laboratory, and biomarker assessments every 3 months for RA and every 6 months for AS rather than baseline assessments alone are also recommended to address the effects of changes in disease activity and changes in disease-modifying therapy that might be anticipated during followup[43]. All analyses should be stratified by the presence or absence of radiographic damage at baseline, since there is particular interest in identifying patients at risk of structural damage prior to any discernible changes on plain radiographs.

Randomized controlled trials permit detailed biomarker analyses of construct validity and associations with structural

Table 2. Draft OMERACT validation criteria for a soluble biomarker to be regarded as a valid biomarker reflecting structural damage in RA and SpA.

A. Truth
1. Evidence that the biomarker reflects tissue remodeling in animal models of disease (e.g., collagen arthritis for RA).
2. The biomarker has been immunohistochemically localized 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 biomarkers that have been established as possessing predictive validity for structural damage (e.g., MRI for erosive RA).

B. Discrimination
6. The assay for measurement of the biomarker is reproducible (coefficient of variation: intraassay < 10%, interassay < 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) a clinically well defined prospective cohort, (B) a randomized controlled trial, (C) a clinically well defined prospective cohort of patients with preradiographic disease of adequate sample size and followed for a sufficient duration to detect change in radiographic damage score (3 criteria).

C. Feasibility
11. The assay for measurement of the biomarker has been well characterized, is internationally standardized (availability of reference standards), and is methodologically simple.
12. Stability of the biomarker at room temperature and in frozen specimen has been documented.

damage in highly controlled settings that also allow the evaluation of the effects of treatment on biomarker levels and whether the resultant change is associated with change in radiographic score in multivariate regression analyses. As for longitudinal studies, it is recommended that patients be evaluated over at least one year in RA clinical trials. This is also consistent with US Food and Drug Administration recommendations for the development of agents with structure-modifying properties.

The proposed criteria also reflect additional observations that bear on discrimination, particularly the findings that factors unrelated to disease may affect biomarker levels. The presence of synovitis may accelerate clearance rates of the biomarker from the joint, and the effects of inflammation on clearance of the biomarker should be investigated, as this may be an important confounder in comparisons with control populations. The timing of sample collection should be standardized in view of the effects of circadian rhythms as documented with osteocalcin (nocturnal peak) and collagen crosslinks (about 8:00 AM)[44,45]. Physical activity has been shown to affect circulating hyaluronan, MMP-3, and keratan sulfate epitope, and this may be more pronounced in those with RA[46,47]. Since the clearance of many biomarkers occurs predominantly via the liver and/or kidneys, any disease involving these tissues will influence biomarker levels in serum and urine. Hepatic disease (e.g., cirrhosis) causes marked elevations in serum hyaluronan, and would likely also influence the removal of glycosaminoglycan-rich molecules such as proteoglycans[48]. Renal disease would influence osteocalcin concentrations. Liver and renal function should be carefully documented in all cross-sectional comparisons.

Age- and sex-related changes in biomarkers are commonly seen[49]. For example, significantly higher values for cartilage and bone biomarkers are found in adult men compared to women[50]. Growth is accompanied by elevated serum skeletal biomarkers as a consequence of growth plate activity[51,52]. This is clearly reflected in the peripheral circulation and in urine for aggrecan and type II collagen biomarkers[53]. Changes may also occur at the menopause. Hence populations must be carefully matched and defined with respect to age and sex.

The NIH Osteoarthritis Initiative serves as a model for how the research agenda for the investigation of soluble biomarkers in inflammatory forms of arthritis ought to be pursued. This initiative integrates public and private scientific expertise with funding to collect, analyze, and make widely available the largest research resource to date of clinical data, radiological information, and a repository of biospecimens from individuals with early and progressing OA. It includes clinical centers, where patient cohorts are being compiled prospectively using standardized protocols, and a data coordinating center. The consortium recently reported a classification scheme for OA biomarkers that described 5 categories of markers: diagnostic, burden of disease, investigative, prognostic, and efficacy of intervention[54]. The draft validation criteria described in this report reflect the efficacy of intervention category of biomarker, and the conditions for inclusion in this category resemble those developed by this OMERACT group, which includes members contributing to the OA initiative. Collaboration with this consortium also constitutes an opportunity for OMERACT to exert its international leadership in the field of validation of outcome assessment.

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