## Application form for the Biospecimens from the Doxycycline Trial Repository

| | |
|---|---|
| **INVESTIGATOR NAME AND CONTACT INFORMATION** | |
| **Address** | |
| **Telephone** | |
| **Fax** | |
| **Email** | |
| **BIOMARKER NAME** | |
| **BACKGROUND & SIGNIFICANCE** | |
| **Current BIPED classification of biomarker** (to what category would you assign this marker) | |
| **Biological process reflected by biomarker** | |
| **Summary of studies establishing an association with OA** | |
| **Variation with race, gender, BMI, age, menopause, comorbidities, drugs (if known)** | |
| **Strengths and unique features of this/these biomarker** | |
| **Gaps in knowledge to be filled by these analyses** | |
| **Significance** | |
| **APPROACHES** | |
| **Biomarker Performance Characteristics** | |
| **Assay format (ELISA, RIA, HPLC etc)** | |
| **Intra- and Inter- assay precision -** calculation of coefficient of variation within and between assays. | |
| **Sensitivity** - minimal detectable concentration | |
| **Specificity** - cross-reactivity to contaminants | |
| **Effect of freeze thaw of biomarker concentrations** (if known) | |
| **Stability** (At various temperatures including room temp, 4C, -20C, -80C for various lengths of time) | |
| **Intrinsic t1/2 of the marker in circulation** (if known) | |
| **Diurnal Variation** | |
| **Sample Request** | |
| **Cohort requested** (Doxy study; longitudinal sample no Doxy; both; and) | |
| **Patient sample number requested** (include number of treated and placebo samples for each biomarker) | |
| **Gender** (male, female or both) | |
| **Body fluid requested** (plasma, urine) | |
| **Sample volume requested for duplicate analyses** (list amount for each biomarker requested) | |
| **Laboratory Methods** | |
| **Quality control procedures** | |
| **Statistical Analyses** | |
| **Study design** | |
| **Power calculations for each** | |

| | |
|---|---|
| **biomarker** | |
| **Other** | |
| **Method of funding analyses** | |
| **Timeline for analyses** | |
| **Plan for submission of results to Biomarkers Network Database** | |
| **Plan for reporting biospecimen remainder and providing back to the repository if requested** | |
| **Date by which you anticipate making the details of the findings public** | |
| **Investigator Qualifications** | |
| **Resources and Environment** | |
| **Seminal References** | |

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**Osteoarthritis and Cartilage**

International Cartilage Repair Society

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## Review

# Classification of osteoarthritis biomarkers: a proposed approach[1]

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## Summary

*Objective*: Osteoarthritis (OA) biomarkers are needed by researchers and clinicians to assist in disease diagnosis and assessment of disease severity, risk of onset, and progression. As effective agents for OA are developed and tested in clinical studies, biomarkers that reliably mirror or predict the progression or amelioration of OA will also be needed.

*Methods*: The NIH-funded OA Biomarkers Network is a multidisciplinary group interested in the development and validation of OA biomarkers. This review summarizes our efforts to characterize and classify OA biomarkers.

*Results*: We propose the "BIPED" biomarker classification (which stands for Burden of Disease, Investigative, Prognostic, Efficacy of Intervention and Diagnostic), and offer suggestions on optimal study design and analytic methods for use in OA investigations.

*Conclusion*: The BIPED classification provides specific biomarker definitions with the goal of improving our ability to develop and analyze OA biomarkers, and to communicate these advances within a common framework.


*Key words*: Osteoarthritis, Biomarkers, Study design.

## Introduction

Osteoarthritis (OA) is one of the leading causes of chronic disability. Recent estimates suggest that symptomatic knee OA occurs in 13% of persons age 60 and over[1], and the prevalence is expected to increase further as the population ages. There are a multitude of ways in which outcomes in OA may be measured, including patient-relevant measures (measures of pain and function), structural measures (such as plain radiographs and magnetic resonance imaging), and biomarkers in the form of molecules or molecular fragments that are released as a result of joint tissue metabolism. Although a full understanding of OA requires consideration of a range of biopsychosocial factors[2], our traditional method of defining clinical OA has relied upon plain radiography[3].

Radiographic measures have been the traditional outcomes in studies involving diagnosis and progression of OA. Radiographic measures, however, are less than adequate for diagnosing and assessing the actual progress of this disease for several reasons. First, radiographs indicate changes in bone, and only indirectly measure alterations in cartilage. Second, the measurement of articular cartilage change, namely joint space narrowing, is itself confounded by meniscal cartilage lesions and meniscal extrusion[4]. Third, bone marrow perturbations and synovial abnormalities may go undetected. Radiographic features

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characteristic of OA appear only after significant deterioration has occurred both in the hard and soft tissues within and around the joint and the change may occur relatively slowly. Finally, radiographic features are usually poorly correlated with joint function. The interest in developing remittive therapy has stimulated the search and development for more sensitive indicators of OA for use in conjunction with, or possibly as a substitute for, the traditional radiographic outcomes. Preliminary studies suggest that both biomarkers and magnetic resonance imaging (MRI) measurements are sensitive to change. Biomarkers are defined as objective indicators of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic interventions[5], and have the potential to decrease the length and cost of trials and enrich our understanding of the pathogenesis of OA.

## Methods

The Osteoarthritis Biomarkers Network is a consortium of five sites, funded by the National Institutes of Health/National Institute of Arthritis, Musculoskeletal, and Skin Disease (NIH/NIAMS) to develop and characterize new biomarkers and refine existing OA biomarkers. This consortium has adopted a mandate to draft a classification scheme for biomarkers that could be employed in clinical trials and other studies of OA. This classification scheme is intended to capture information in the early stages of biomarker development and to facilitate the design of future validation studies with radiographic or patient-centered outcomes. Such an approach would be helpful in the appropriate allocation of potentially scarce sample resources. Another advantage to a well-developed classification scheme is the application of a common biomarker vocabulary among investigators, their laboratories, and potentially, across fields. A useful classification scheme would facilitate research (both independent and collaborative), decrease redundancy, and expedite validation of potential biomarkers.

Although biomarkers are classically thought of as biochemical substances, it is also possible to consider RNA, DNA, their fragments, or a combination or multiplicity of these, as biomarkers. Although imaging techniques may themselves be considered biomarkers for the pathologic joint abnormalities that define OA, this paper addresses only protein and nucleic acid based biomarkers. Similarly, traditional clinical risk factors (such as body mass index and gender) may be considered biomarkers by some, but clinical risk factors will not be considered biomarkers for purposes of this classification scheme as they typically assess factors that increase the likelihood of disease change but do not themselves reflect the disease process.

The process of validation of any biomarker depends upon the availability of a gold standard method for defining disease. The approach to biomarker development and validation, typically a stepwise progression of studies that ultimately demonstrate an association with the clinical outcome of interest, is beyond the scope of this paper. An outline of such procedures for markers in rheumatoid arthritis is provided in a recent review[6]. For the purposes of elucidating the categories proposed here, we provide examples based upon imaging outcome criteria against which OA biomarkers may be validated. It is valuable to appreciate, however, that other clinically relevant outcomes, such as pain, joint inflammation, and function, could provide alternatives to structural modification as endpoints against which biomarkers can be validated. The process of biomarker validation should also include more patient-centered outcomes to complement measures of structure. However, until our currently limited understanding of the biopsychosocial determinants of pain and disability improves, the use of these clinical outcomes will prove difficult[7].

Uniform guidelines for the technical specifications of *in vitro* diagnostic immunoassays already exist[8]. These published guidelines encompass clinical performance of the assay, including precision and variability (which must be met), but not clinical utility, and are not discussed further here.

The proposed biomarker classification scheme includes five categories: diagnostic, burden of disease, prognostic, efficacy of intervention, and investigative. These classification categories are developed to assist OA researchers with ongoing biomarker work, and in most instances, will be achieved in a progressive validation strategy (Fig. 1). Thus, a biomarker may fall into more than one category. Efforts were made to maintain a clinical functionality to the classification scheme while minimizing redundancies.

## Results

Based upon the considerations noted above, we propose the following classification of OA biomarkers.

### DIAGNOSTIC MARKER

Diagnostic markers are defined by the ability to classify individuals as either diseased or non-diseased. New diagnostic tests should be evaluated by comparison against an established gold standard in an appropriate spectrum of subjects. For OA, an accepted "gold standard" diagnostic test is the radiograph, and typically a Kellgren–Lawrence (K-L) grade $\geq 2$ is required for a diagnosis of OA[9].

Studies of Diagnostic markers for OA must include individuals with and without OA, and need to include a spectrum of subjects tested in terms of age, sex, disease severity, and specific eligibility criteria. Initially, the test should be verified on a population from a cross-sectional dataset that includes mild and severe disease, treated and untreated

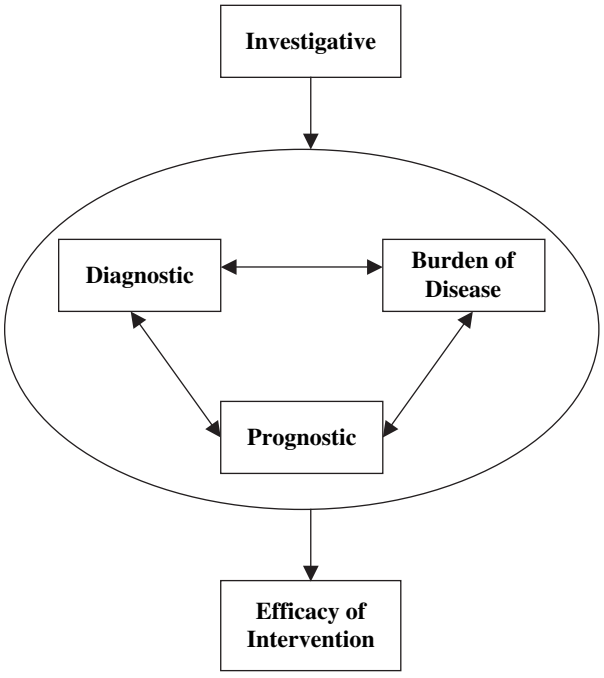


Fig. 1. Hypothetical development of OA biomarkers.

subjects, and those with conditions sometimes confused with OA, such as rheumatoid arthritis. Case–control designs, where subjects with and without documented OA are studied, are also suitable for the evaluation of Diagnostic markers.

Diagnostic tests are seldom 100% accurate (false positives and false negatives will occur). A test is valid if it detects most people with the target disorder (high sensitivity) and excludes most people without the disorder (high specificity), and if a positive test usually indicates that the disorder is present (high positive predictive value). In addition to sensitivity and specificity, which are independent of disease prevalence but are not useful to determine the probability that a positive or negative test indicate those with or without OA, other parameters are often used to assess the usefulness of a Diagnostic marker. The positive likelihood ratio (LR) indicates how much more likely a positive test is to be found in someone with, as opposed to without, the disorder[10]. Another useful diagnostic test parameter derived from receiver operator curve (ROC) analyses is the area under the curve (AUC), which quantifies the overall ability of a diagnostic test to classify diseased and non-diseased individuals correctly[11]. For example, Jung *et al.*[12] recently found that among 88 subjects with hip or knee OA and 48 age matched healthy controls, the mean level of urinary CTXII was 527 ng/mmol for OA subjects and 190 ng/mmol for the controls. The AUC for CTX-II was 0.92 (95% confidence interval [CI]: 0.87, 0.99) for hip OA and 0.82 (95% CI: 0.73, 0.92) for knee OA. Although risk and odds ratios (OR) are often reported in studies of Diagnostic markers, unless extremely large, they provide little information about the clinical utility of a Diagnostic marker[13].

## BURDEN OF DISEASE MARKER

Burden of disease markers assess the severity or extent of disease, typically at a single point in time, among individuals with OA. This can be thought of as severity within a particular joint, and/or severity in terms of number of joints involved. The establishment of such a marker classification is often based on cross-sectional data of individuals with OA from cohorts from the community or baseline assessments of subjects enrolled in a clinical trial. This terminology is not to be confused with the characterization of the economic and social impact of the disease often also referred to as ''burden.'' Studies of Burden of disease markers require comparison with one or more gold standard methods of determining disease severity, such as radiographic criteria. The parameters used to assess Burden of disease markers are similar to those described for Diagnostic markers: sensitivity, specificity, LRs, and AUC estimates derived from ROC analyses. For example, studies of Burden of disease markers might use AUC or LRs to describe how a test differentiates severity of disease (e.g., delineating persons with K-L grade 2 radiographic OA from those with K-L grade 3; or alternatively differentiating persons with two joints involved from those with three joints involved using a threshold criterion such as K-L grade $\geq 2$ to define disease).

Examples of Burden of disease marker exist in the OA biomarker literature. These include serum cartilage oligomeric matrix protein (COMP)[14], serum hyaluronan[15], and urinary CTXII[16], to name a few.

## PROGNOSTIC MARKER

The key feature of a Prognostic marker is the ability to predict the future onset of OA among those without OA at baseline or the progression of OA among those with existing disease. The optimal validation or application of a Prognostic marker generally excludes individuals treated with disease-modifying agents, but population-based samples may include a small number of such individuals.

The evaluation of Prognostic markers requires longitudinal studies (prospective or retrospective) showing an association of the marker at baseline with the risk of development of new OA (for example, new K-L grade $> 1$) or progression (for example, at least one level increase in K-L grade). It is valuable to recognize that some of today's examples of incident OA may in fact later prove to be cases of progressive OA as we become more sophisticated in our methods of early OA detection. As measurements of biochemical substances, molecules or fragments of protein, RNA or DNA, and genotypes are potential Prognostic markers, they may be analyzed as continuous, dichotomous, or categorical variables. Relevant outcomes in Prognostic marker studies will usually be dichotomous (onset or progression: yes vs no), but both categorical and continuous outcomes from imaging assessments are acceptable, such as degree of joint space narrowing or change in cartilage volume by MRI.

The analytic approach to a Prognostic marker differs from either Diagnostic or Burden of disease markers, as the intent is to predict future conditions and not classify individuals by disease or severity. Thus, for dichotomous or categorical outcomes, such as worsening K-L score, the preferred methods of analyses are relative risk (RR) or odds ratio (OR) given the presence or absence of the marker. For Prognostic markers that are continuous, the RR or OR per SD increase or decrease in the marker, and/or the AUC estimated from ROC are accepted analytic approaches. Genetic biomarkers are possible in this category if they predict, for instance, a better or worse prognosis. For example, certain HLA-DRB1 gene polymorphisms predict a more aggressive clinical course of rheumatoid arthritis[17]. Currently, a comparable genetic Prognostic marker for OA has not been described. As an example of a variant Prognostic marker, Vilim *et al.*[18] found that among individuals with symptomatic knee OA, those with elevated levels of serum COMP at baseline were more likely to show knee OA progression defined as an increase in one K-L grade or joint space narrowing over 3 years.

Prognostic markers may also encompass some assessment of disease activity. Our deliberations on the subject of biomarker classification revealed nuances of meaning associated with markers that reflect disease activity. The term ''disease activity marker'' is most often used to denote a biomarker demonstrating meaningful variation in concert with the varying phases of the disease, ranging from periods of structural or symptomatic progression to periods of disease quiescence, and is therefore best considered as Prognostic markers.

Two recent biomarker studies provide illustrative examples of Prognostic markers that appear to assess ''disease activity.'' In the first, Sharif *et al.*[19] showed that mean serum COMP levels (measured every 6 months in a cohort with knee OA), were higher during periods of radiographic progression and that on average, a 1-unit increase in serum COMP levels increased the probability of radiographic progression by 15%. In the second example, Garnero *et al.*[20] recently found that the prevalence of bone marrow abnormalities on MRI varied in 30% of the patients over a 3-month interval among individuals with painful knee OA. Those with elevated levels of urinary CTX-II were more likely to develop worsening bone marrow abnormalities on

serial MRI measurements over this 3-month interval. In these two examples, the phasic nature of the disease reflected meaningful variation of the marker, and marker levels were predictive of OA progression over intervals as short as 3 months (urinary CTXII) and as long as several years (COMP). A ''disease activity marker'' might also indicate severity of disease within a joint or total extent of disease within a person. In this case, the marker is more appropriately designated a Burden of disease marker as described earlier.

#### EFFICACY OF INTERVENTION MARKER

An Efficacy of intervention biomarker chiefly provides information about the efficacy of treatment among those with OA or those at high risk of developing OA. Efficacy of intervention markers may be measured prior to therapy to predict treatment efficacy, or may be measured more than once to assess short-term changes that occur as a result of pharmacologic or other interventions. Candidate efficacy of intervention markers must be tested in a clinical trial with appropriate OA outcomes, such as symptoms and/or function, or progression on imaging studies. Pre-treatment Efficacy of intervention markers may be variant or invariant, but only variant markers may be used as serial determinants for monitoring treatment efficacy.

Serial assessments of Efficacy of intervention markers typically focus on the intervention group in a randomized controlled trial. Most Efficacy of intervention biomarkers will be continuous. For studies of Efficacy of intervention markers with dichotomous outcomes, such as progression vs no progression on imaging studies, logistic or hazard models reporting the relative hazard or OR per unit or standard deviation of change in biomarker, or AUC from ROC analyses are appropriate. For continuous outcomes, regression models relating change in biomarker (per unit or SD) to change in the outcome variable are suggested.

To qualify for the Efficacy of intervention category, a marker must demonstrate a statistically significant relationship between treatment-related changes in a biomarker and the relevant clinical or radiographic OA outcomes. Additional analyses, such as the proportion of treatment effect explained[21], may be estimated by comparing the treatment effect with and without the change in biomarker in the statistical model.

A hypothetical example of an Efficacy of intervention marker is the case where concentrations of a biomarker of cartilage degradation, measured serially, are associated with an improved or beneficial clinical or radiographic outcome (dichotomous or continuous) among subjects who receive an effective OA intervention.

#### INVESTIGATIVE MARKER

An Investigative marker is one for which there is insufficient information to allow inclusion into one of the existing categories. It can be represented by a genotype or an assay of a molecule or fragment released into the synovial fluid or systemic circulation, where its relationship to various normal and abnormal parameters of cartilage extracellular matrix turnover has not yet been established in human subjects. The purpose of creating this category in the classification scheme is to facilitate and encourage codification of potential OA biomarkers, and thereby engender further research development aimed at establishing a role for the biomarker in one or more of the other categories in the scheme.

## Discussion

We propose a new classification scheme for OA biomarkers, which can be represented by the acronym BIPED to connote the five categories of markers: ***B***urden of disease, ***I***nvestigative, ***P***rognostic, ***E***fficacy of intervention, and ***D***iagnostic. As outlined above and in Table I, we have summarized the definitions, characterized the subjects and typical study design, and suggested preferred analytic approaches for each marker category. For each biomarker, classification can be further defined as variant (proteomic, RNA), or invariant/less variant (DNA). Moreover, for each biomarker of interest, one would want to know as much as possible about the tissue(s) of origin, and biological processes reflected by the biomarker, as well as a clear understanding of the nature of the association of the biomarker with OA. The examples presented here readily demonstrate that a biomarker may fit simultaneously into more than one category based upon the weight of evidence at hand.

Table I
*Summary of "BIPED" biomarker classification for OA*

| | Burden of disease | Investigative | Prognosis | Efficacy of intervention | Diagnostic |
|---|---|---|---|---|---|
| Definition | Biomarker associated with extent or severity of OA | Biomarker not yet meeting criteria for another category | Predicts onset or progression | Indicative or predictive of treatment efficacy | Differentiates diseased from non-diseased |
| Type of biomarker | Variant only | Variant or invariant | Variant or invariant | Variant or invariant | Variant or invariant |
| Subjects | Must have OA | NA | With and/or without OA | With OA | With and/or without OA |
| Design | Cross-sectional, case–control | NA | Longitudinal | Controlled trial | Cross-sectional or case–control |
| Outcomes | Extent or severity of OA | NA | New or worsening OA | New or ameliorated OA | OA vs no OA |
| Analysis | Sensitivity, specificity, LR, AUC from ROC | NA | Risk or odds ratio with 95% CI | Risk or odds ratio with 95% CI among treated | Sensitivity, specificity, LR, AUC from ROC |
| Criteria | Significant association between marker and extent or severity of OA | NA | Significant association between marker and onset or progression of OA | Significant association between marker and treatment effect | Significant association between marker and OA diagnosis |

NA, not applicable.

The ultimate marker for clinical research purposes, a surrogate endpoint, substitutes for a clinical outcome of how a patient feels, functions, or survives[5]. This definition cuts across all the classification schemes and may apply to any marker and is dependent on the proper validation study to establish this relationship. However, markers that change with a disease state (albeit Burden of disease markers or Prognostic markers indicative of disease activity among untreated subjects, or serial Efficacy of intervention markers among treated subjects) are more readily accepted as surrogate endpoints when they have proven dynamic modulation with disease state. Surrogate markers have particular value when resource constraints limit the extent to which more costly outcomes can be conducted. Nevertheless, as illustrated by this classification scheme, markers in any of the categories can provide useful information for clinical and research applications. It is hoped that the development of this classification scheme will help to provide a common language and structure with which to communicate knowledge and advances related to OA biomarkers for both clinical and research applications.

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