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Editorial
Whither osteoarthritis biomarkers?

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Dramatic advances in our understanding of the biochemistry of cartilage and of the ability to image biochemical and structural changes within cartilage using magnetic resonance imaging techniques have led to a cascade of studies testing proteins and imaging findings as biomarkers of osteoarthritis (OA). Gene assay signatures and other signals of molecular changes within the joint promise other exciting future opportunities to characterize dynamic processes within the joint.

In parallel with this progress has come an attempt by a consortium of NIH investigators to define different categories of biomarkers in OA so that this growing field can use a common language and approach. The BIPED classification[1] subclassifies biomarkers according to whether they are intended to provide information on burden of disease, be investigative, predict prognosis, reflect efficacy of treatment, or diagnose disease.

Despite these advances in cartilage biochemistry and imaging, biomarkers have not emerged as accepted tools for characterizing the status of the disease or its prognosis, nor as measures of treatment response, areas where biomarker need is the greatest. It may be too early in the evolution of this field to expect such a change, and numerous obstacles have been discussed: the inherently slow rate of disease development, the lack of a gold standard for presence or absence of disease, the lack of standardized disease models and the absence of ways to predictably modify disease in these models[2,3]. We suggest that additional limitations in reporting results of biomarker studies and in conceptualizing the role of biomarkers in OA may be inhibiting advances. In the context of these scientific and conceptual challenges, we propose in this editorial to firstly reexamine the approach to reporting biomarker results and to secondly reconsider what insights our current OA biomarker experience might provide to the scientific community investigating OA. While most directly relevant to biochemical markers of disease, this editorial treats the topic of biomarkers broadly encompassing a range of approaches to measuring disease processes.

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Is it a biomarker or a risk factor?

The NIH Biomarker's Definitions Working Group in 2001 defined a biomarker as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes or pharmacological responses to a therapeutic intervention"[4]. Interestingly, there is nothing in this definition that requires a biomarker to measure disease or a disease process. Since a biomarker by definition must reflect normal biological processes or a response to a therapeutic intervention, validation of a marker thus defined requires some independent measure of that biological process or of treatment response.

If the biological process being measured by the biomarker is a disease process, then the biomarker should reflect disease occurrence. If, on the other hand, it is a biological process that may increase risk of disease (for example, the accumulation of advanced glycation end products in osteoarthritic cartilage) and not necessarily a measure of the disease or its change, then the parameter being measured should be tested as a risk factor and not as reflecting a disease process. A risk factor is something that predisposes to the occurrence of disease or protects against it. Well known risk factors include obesity for knee OA and hypercholesterolemia for cardiovascular disease. The distinction between a risk factor and a disease biomarker is sometimes not clear cut. For example, for advanced glycation end products, their presence as a constituent of molecules within cartilage may change the stiffness of cartilage, predisposing to disease (a risk factor) but, to the extent that their presence in assays represents the release of long standing molecules within cartilage and serves as a measure of cartilage turnover, they could also represent a disease process biomarker. Another example could be matrix molecule fragments, which by their presence in cartilage matrix could elicit adverse cellular responses and be regarded as a risk factor, while on the other hand they on their release may represent a process marker.

The relationship of a risk factor to disease is usually measured with relative risk or odds ratio measures, which test whether those with a high level of a biomarker risk factor have a higher risk of disease or its progression than those with a lower level. Identifying a risk factor for progression (e.g., obesity) creates the opportunity to develop a treatment that eliminates this risk factor (e.g., weight loss) to see if disease progression becomes less frequent. While risk factors influence whether disease occurs,

progresses or even improves, they are NOT measures of the disease process.

Assessing diagnostic biomarkers

On the other hand, if a putative biomarker is being tested as an indicator of disease or a disease process then it needs to be compared to an independent 'gold standard' measure of disease. This assessment should include either an evaluation of diagnostic test performance (e.g., sensitivity, specificity, positive and negative predictive value) or an agreement statistic such as a kappa statistic or intraclass correlation coefficient.

COMP levels may reflect cartilage degradation. If COMP represented a 'risk factor' for OA (e.g., if COMP caused cartilage loss), then one might envision developing interventions that lowered the level of COMP so as to prevent cartilage loss. One example might be to develop a drug which metabolized COMP in the blood to lower its levels. Obviously, this is not an appropriate intervention to reduce OA progression, suggesting COMP should not be conceptualized or tested as a risk factor. COMP is instead a measure of cartilage matrix turnover and should be evaluated by determining its accuracy in assessing that turnover. Using a gold standard measure (e.g., cartilage loss over time), a study might test whether high COMP levels predict those likely to experience cartilage loss (positive predictive value for high COMP level) and whether low COMP levels identify persons who experience little if any cartilage loss (a high negative predictive value for a low COMP level).

Biomarkers as measures of treatment response

Biomarkers could be valuable as measures of response in OA, especially since traditional or morphological measures of response have been challenging to measure and may not change rapidly over time. Successful biomarkers have been developed as response measures in other diseases including blood pressure and viral load in HIV/AIDS. Yet others such as bone mineral density in osteoporosis have been advocated as good biomarkers but may not necessarily reflect the efficacy of treatment on the major clinical outcome, fracture. This was the case, for example, in fluoride trials in which bone density measurements suggested improvements on fluoride treatment, yet the trial outcome of fracture showed worsening in some cases.

Ultimately, to be a valid measure of treatment response, effects of the intervention on the biomarker 'must reliably predict the overall effect on the clinical outcome'[5]. As noted by Fleming and DeMets, this requirement that the biomarker predict the overall effect of the clinical outcome frequently underlies its failure. Reasons include the possibility that the disease process can have several different ways of affecting clinical outcome, only one of which is captured by biomarker measurement. The most common misconception is that a biomarker just needs to correlate with the outcome (for example in the case of OA, this could translate into a stabilization of COMP levels correlating with an absence of cartilage loss over time). The biomarker must not only correlate with the clinical outcome but also must capture the net effect of treatment on the clinical outcome. This latter criterion is often very difficult for a biomarker to satisfy. Testing this latter requirement involves carrying out a clinical trial or multiple trials that include measurements of the clinical outcome and of the biomarker and testing whether the

treatment effect on the clinical outcome can be explained either fully or in part by the biomarker.

Many studies of OA biomarkers have focused on measures of association such as relative risks or odds ratios and few have adopted the more appropriate standard of diagnostic test evaluation to evaluate an indicator of disease process. Recent studies[6,7] have reported impressive odds ratios associating a particular biochemical biomarker with disease status but unimpressive diagnostic test performance. Such biomarkers would not serve as valuable diagnostic tests for OA.

Validity and reliability of OA biomarkers

Concerns about OA biomarkers can be divided into concerns about validity and reliability. In clinical epidemiology, reliability means repeatability—whether a given measurement yields the same result when repeated at different times of the day and in different circumstances and even using the same assay in different laboratories. Kraus[2] extensively reviewed sources of variability in the measurement of biomarkers including whether food intake affects their measurement, whether they fluctuate diurnally, whether they are affected by different activity states and whether they vary based on drugs ingested. As Kraus noted, we need better characterization of each biomarker with respect to sources of variability so that these variations can be minimized. Only when a biomarker is reliably assessed can its true relation to the underlying disease parameter of interest be gauged.

Although focused only on diagnostic markers, the STARD initiative[8] has provided a checklist of specific information about biomarker measurement and the subjects tested that should be provided in any study validating a biomarker regardless of its intended use. These include the following specific requirements: to blind those measuring the marker as to disease status (in a study of prognosis, this would mean blinding to prognosis status); to define the rationale for and selection of cutoffs differentiating 'normal' from 'abnormal' marker levels and importantly, to note the source of subjects in a study, reporting whether they were selected because of their biomarker status or unique clinical findings (results in such preselected subjects would not generalize to other persons).

Perhaps an even more serious concern in OA is the issue of validity which is defined as whether a biomarker measures what it is purported to measure. In the case of OA, a diagnostic biomarker should measure whether disease exists or not and a prognostic biomarker should predict the trajectory of disease in individuals. The validity of biomarkers depend on, among other things, whether a biomarker is close enough to the biological process so that its level reflects that process. This is a special concern for OA biomarkers derived from urine or blood and may be less of a concern for an imaging biomarker from an affected joint. For serum or urine, a cartilage degradation marker originating in a single joint must be released from cartilage into the synovial fluid where it may be diluted by a large volume of fluid. It must then be cleared through the synovial membrane, and the rapidity of clearance is dependent probably on perfusion of the synovium and perhaps by the gradients of levels of the biomarker in synovial fluid vs blood[9]. And then, in leaving the joint cavity, the circulating biomarker may be metabolized in synovial cells, lymph nodes or liver to the point where assays of it no longer detect the epitopes that originally identified it. It may then be further

metabolized in the kidney and cleared through urinary excretion. Its handling by the kidney will affect its levels in urine. The farther away the specimen is from its origin in the joint, the more challenging it is to tie a biomarker level to ongoing processes in the individual joint.

The validity issue also relates to whether the process in one joint is not so diluted by similar processes in other joints as to be drowned out by the noise of biomarker release from multiple joints or other cartilages in the body. It is no wonder that COMP levels which may be diagnostic for OA are much more strongly correlated with the occurrence of disease in multiple joints than with the occurrence of disease in one[10].

There are many formidable challenges to identifying a joint specific biomarker, a molecule whose fluctuation within one or more joints is reflected by levels in the serum, plasma or urine.

In addition, there is the conceptual issue of defining the location of the biological process that the biomarker is expected to assess. Since OA often occurs in joints that are asymptomatic and may not have been imaged, the disease process may be far more widespread than indicated by clinical evaluation. This may compromise an investigator's ability to detect a relation of the putative biomarker with disease in a single target joint since the involvement of other joints through the body is unknown and may vary from person to person. Also, OA is not a disease relegated to cartilage alone, so that a marker may need to be specified as reflecting cartilage turnover and not necessarily disease *per se*. To the extent that some markers measured or proposed originate not just in hyaline cartilage but also in fibrocartilage (meniscus) and even synovium, the biological process being assessed needs to be characterized accurately. The recognition that bone turnover is involved in OA has created an opportunity for the development of biomarkers of bone as reflectors of the dynamic process of OA but, just as levels of cartilage biomarkers are affected by the biological dynamism of multiple joints, systemic biomarkers of bone will reflect systemic processes within bone in general and not just turnover within a small area of one joint.

Biomarkers that reflect cartilage turnover may fail to show strong correlations with the underlying biological parameter, cartilage turnover, not because the biomarkers do not reflect their intended target well but because we have been unable to measure cartilage turnover successfully. First, cartilage turnover or enhanced synthesis and degradation of matrix is not the same as the cartilage loss which is the focus of clinical studies. Second, cartilage loss is challenging to assess. Progressive joint space narrowing on X-ray over time is probably an inadequate measure of such loss because, at least in the knee, it can reflect change in meniscal position and is insensitive to cartilage loss[11]. For knee MRI, cartilage loss may be painfully slow. Morphological tissue loss may actually not be the biological parameter of interest but rather change in the composition of cartilage.

Interesting lessons were recently provided by what was felt to be a well characterized biomarker of hyaline cartilage degradation, CTX2, representing cross-linked fragments of the C-telopeptide of type II collagen[12]. This biomarker, assumed to result only from the proteolytic degradation of type II collagen in hyaline cartilage matrix was shown to reflect both the presence of OA, and to predict its development[13]. Animal model data and results from a pilot human study using risedronate, a bisphosphonate, showed that urine levels of CTX2 were dramatically decreased by risedronate treatment, together with expected decreases on bone

turnover marker levels. These results were confirmed in a large randomized clinical trial of knee OA. However, neither knee joint structure as monitored by standardized radiographs nor symptoms were affected by risedronate treatment over 2 years[7]. We may conclude based on other studies, that CTX2 might be a diagnostic or even a predictive biomarker: however, it failed as a biomarker to reflect efficacy of response in this trial. As noted earlier in this review, one reason for this paradoxical result could be that the standard radiographic assessment used in this trial was not sensitive enough to detect the effects of risedronate on osteoarthritic cartilage. Were this true, it would still leave us concerned as to why the putative beneficial effects of risedronate on cartilage did not translate into a patient-reported benefit over 2 years. Beyond the challenges in proving structural change or its absence by imaging, we may consider other reasons why the earlier CTX2 data might have been misleading in this instance. While hyaline cartilage is the dominant source of type II collagen, contributions to circulating levels of CTX2 coming from calcified cartilage in the tidemark, or even from bone, cannot be excluded. Since the calcified cartilage in the tidemark is affected in OA, and both bone and calcified cartilage turnover are powerfully affected by risedronate[14], the systemic CTX2 signal resulting from risedronate treatment may originate, in part, from calcified cartilage or even bone[3,15]. Unless we understand the details of how the biomarker is generated, and where, we risk being misled.

To advance the science of OA biomarkers, we may need to skip much of what would be the usual validation work because our biological parameter of interest, change in OA, is not readily measured. The lack of treatments which prevent morphological deterioration in the joint, presents great difficulties for biomarker development. Without such treatments and without measures of morphological change in OA, the development and validation of biomarkers in OA will continue to be a huge challenge.

It is our view that biomarkers will likely not be successful as diagnostic tests for OA. For success to be achieved in this area, we must be able to identify persons without OA of a similar age to those with disease. Without a comprehensive imaging survey of both cases and controls, it will be impossible to determine whether older controls actually have the disease. Further, the difficulty in comprehensively imaging joints of supposed cases with OA will make assessing the burden of OA in 'cases' similarly difficult. Most of the markers being tested are not markers of disease status but rather markers which reflect cartilage turnover and turnover in other joint structures. Just as in osteoporosis where markers reflect synthesis and degradation of bone and not bone density, an assessment of the anabolic and catabolic activities of joint structures will not be reflected in static imaging measures such as conventional Magnetic Resonance Imaging (MRI) or X-ray. It is possible that imaging techniques focusing on biochemical composition of cartilage such as delayed Gadolinium Enhanced MRI of Cartilage (dGEMRIC) and T1RHO will be more helpful than images of joint anatomy.

In conclusion, the barriers for validating and using OA biomarkers are formidable. One barrier relates to failures in imaging osteoarthritic changes so as to facilitate validation of biomarkers. Another relates to the complexity of OA, which is not a homogeneous disease. Not only does it affect more than just one joint, but different persons with OA have different regions and structures within the joint affected, each of which may produce its own characteristic biomarker profile. Yet other barriers to discovery are posed

by the failure to comprehensively evaluate all joints that may be affected by disease and thus contribute to the biomarker signal detected. This barrier is further heightened by selecting serum or urine markers which are distant from the pathologic process within a single joint. Since biomarkers may ideally measure the dynamic processes within affected joints, validating them against the disease state of the joint and not its change may be inappropriate.

Given these challenges, what questions can biomarkers successfully address? We suggest that there are opportunities in the development of biomarkers which relate first to the use of biomarkers as a tool to understand the pathophysiologic process of cartilage loss and change in other joint structures. We suggest further that, unlike in some other diseases, the development of biomarker science in OA may have to jump over and even ignore limitations in morphological assessment of the joint. Conventional approaches using risk factor analyses and X-rays to evaluate the relation of putative markers with these disease parameters are not likely to be as revealing as studies of new imaging biomarkers, in part because such imaging may produce insights into pathophysiology and is proximate to the site of pathology. Changes in biomarkers with treatment may reflect real effects of treatment and may signal that biomarkers can detect these changes even though we may not yet have sensitive enough measures of morphologic change to assess them or understand their significance. Any proposed diagnostic biomarker needs to be tested not as a risk factor but as a diagnostic test. Validation of other biomarkers (e.g., treatment response) needs to be purpose specific. The final proof of utility will probably require a large and long-term prospective study with clear *a priori* hypotheses.

Conflict of interest

The authors have no conflict of interest for the submitted manuscript entitled: Whither osteoarthritis biomarkers.

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