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# Toward Imaging Biomarkers for Glycosaminoglycans

By Martha L. Gray, PhD

Advances in the diagnosis and treatment of cartilage degeneration will be accelerated with the availability of validated biomarkers that reveal the features relevant to the health of cartilage. Using the delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC) technique for evaluating tissue glycosaminoglycan as a case study, I review the types of evidence needed to validate imaging (or other) biomarkers. In addition, I present discussions about face validity and technical validity and offer a review of emerging data that provide pathophysiologic validity. Examples of such data include evidence that glycosaminoglycan content is restored after an injury-induced loss and evidence suggesting that dGEMRIC can indicate when it is too late for protective (load-modifying) surgery. These and other data suggest that new imaging biomarkers may indeed be able to provide a state-of-cartilage proxy that can be of use in the diagnosis and staging of disease.

Many new therapeutic strategies have been and are being developed to correct, prevent, or slow the progression of osteoarthritis. The ability to evaluate the efficacy of these techniques, or to determine the situations for which they might provide the most benefit, critically depends on diagnostic measures that can serve as proxies or “biomarkers” for the present or predicted state of the cartilage. Establishing valid biomarkers has proved challenging for several reasons. Cartilage degeneration occurs over the course of several years or even decades, yielding a comparably long time frame required for strong validation. The widely used standard for evaluating cartilage degeneration is radiographic measures of joint-space narrowing, so the focus has been on late-stage degeneration after substantial tissue loss has occurred; we have a very limited understanding of the etiopathology of cartilage degeneration. Although this knowledge would certainly be enhanced with information made available by appropriate biomarkers, this lack of understanding makes it difficult to know which biomarkers will turn out to be appropriate and meaningful. Nevertheless, the harsh reality is that, without biomarker development, future advances in the diagnosis and treatment of cartilage degeneration will be delayed, if not stymied.

Accordingly, much research over the past decade has been devoted to the development of measurements that will provide insight into cartilage degeneration and repair, including considerable efforts in developing imaging-based measurements. Efforts going forward should focus on establishing these methods (and others) as validated biomarkers. A biomarker, as defined by the National Institutes of Health

Biomarkers Definitions Working Group, is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or responses to a therapeutic intervention<sup>1</sup>. Some biomarkers are predictive of clinical benefit or harm (or lack of benefit), in which case the marker is referred to as a surrogate end point. Biomarkers (including surrogate end points) require rigorous scientific validation.

The biomarker development process (Fig. 1) involves three phases: (1) face validity (Based on what is known, would the putative biomarker be generally viewed as relevant and useful?); (2) technical validity (Is the marker measuring what it aims to measure?); and (3) pathophysiologic validity (Is the information provided by the marker of pathophysiologic or clinical use?). Many of the magnetic resonance imaging techniques that have been emerging over the past decades appear promising in that they have shown face and technical validity in measuring the morphologic and molecular state of cartilage. In addition, with emerging clinical studies, efforts are beginning to offer evidence regarding pathophysiologic validity.

As a case study, this article focuses on biomarkers for glycosaminoglycan. Delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC), sodium magnetic resonance, and T1rho are three magnetic resonance-based imaging methods that have been studied as potential measures of tissue glycosaminoglycan.

## Face Validity

With respect to face validity, glycosaminoglycan has long been viewed as a critical macromolecule for cartilage

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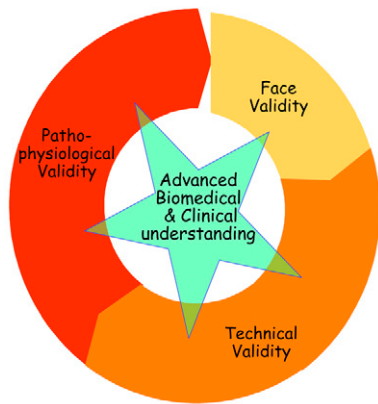


Fig. 1  
The biomarker development process. The broad stages of biomarker development include establishment of face validity, technical validity, and pathophysiological validity. Development generally involves iterating among these, as additional pathophysiological insights are gained.

health<sup>2,3</sup>. Loss or absence of histological stains such as toluidine blue or safranin O is interpreted as a loss or absence of glycosaminoglycan and is pathognomonic for arthritis. Furthermore, changes in tissue glycosaminoglycan are associated with changes in tissue mechanical properties, consistent with the view that glycosaminoglycan is among the macromolecules that strongly influence the functional properties of tissue. It is also worth noting that, in general, macromolecular composi-

tion (i.e., macromolecules in addition to glycosaminoglycan) is known to be affected by disease processes and strongly influences the material properties of tissue. In short, macromolecules, including glycosaminoglycan, are important to tissue function and are affected by disease, suggesting that biomarkers for glycosaminoglycan and other macromolecules will provide important information. It is important to note that there is not as yet good face validity that such biomarkers would serve as surrogate end points, since there is no evidence that there is a direct relationship between macromolecular composition and the usual clinical end points of pain and function.

### Technical Validity

There are many *in vitro* and *in vivo* studies that support the technical validity of putative glycosaminoglycan imaging methods; that is, these studies support the notion that the resulting images can be used as an indicator of glycosaminoglycan concentration<sup>4-17</sup>. In recent reviews<sup>11,13,14</sup>, these putative measures have included sodium (Na) magnetic resonance imaging and the dGEMRIC method (designed to measure fixed charge as a surrogate for glycosaminoglycan<sup>4-9</sup>) as well as the T1rho imaging method (for which contrast is influenced by macromolecular composition<sup>10,12,15-17</sup>). It is important to realize, however, that none of the existing techniques have fully demonstrated their technical validity. To do so would require demonstration that the measurement is accurate (“true”) and precise (repeatable). Challenges in achieving this goal include the lack of a “gold standard” and insufficient knowledge to determine the level of sensitivity and precision that would be needed. Normally, the latter issues are resolved through an

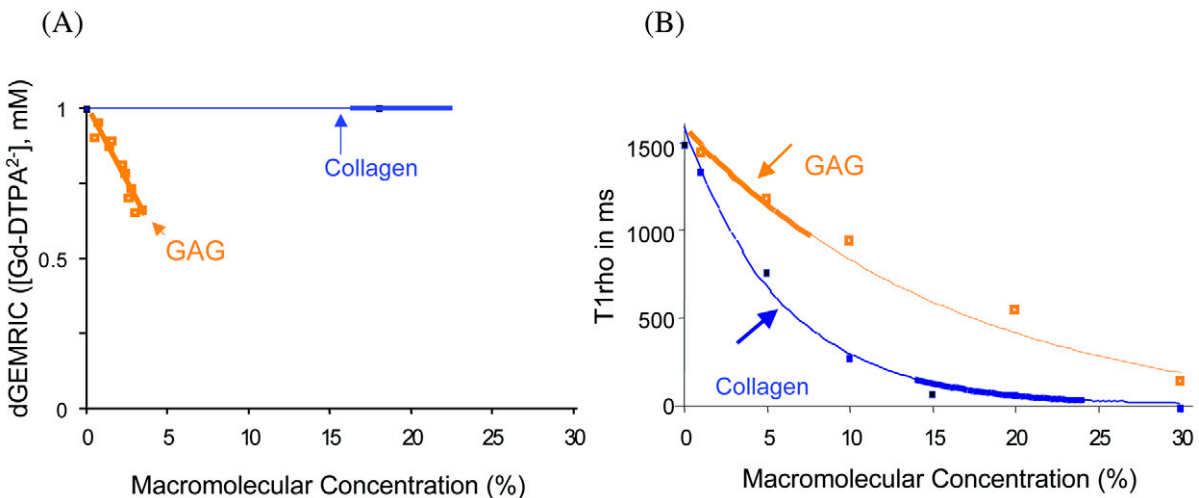


Fig. 2  
dGEMRIC (A) and T1rho (B) versus macromolecular concentration<sup>4</sup> for glycosaminoglycan (GAG) and collagen suspensions. dGEMRIC shows an approximately linear dependence on GAG, with no dependence on collagen; T1rho shows approximately exponential dependencies on both GAG and collagen. The thicker regions of the trend lines indicate the regions corresponding with the range of values found in native cartilage: GAG ranges from approximately 7% at the high end of normal to 0% in disease, and collagen ranges from approximately 15% to 25%. Gd(DTPA)<sup>2-</sup> = gadolinium diethylenetriamine pentaacetic acid. (Reproduced, with modification, from: Menezes NM, Gray ML, Hartke JR, Burstein D. T2 and T1rho MRI in articular cartilage systems. *Magn Reson Med*. 2004;51:503-9. Reprinted with permission.)

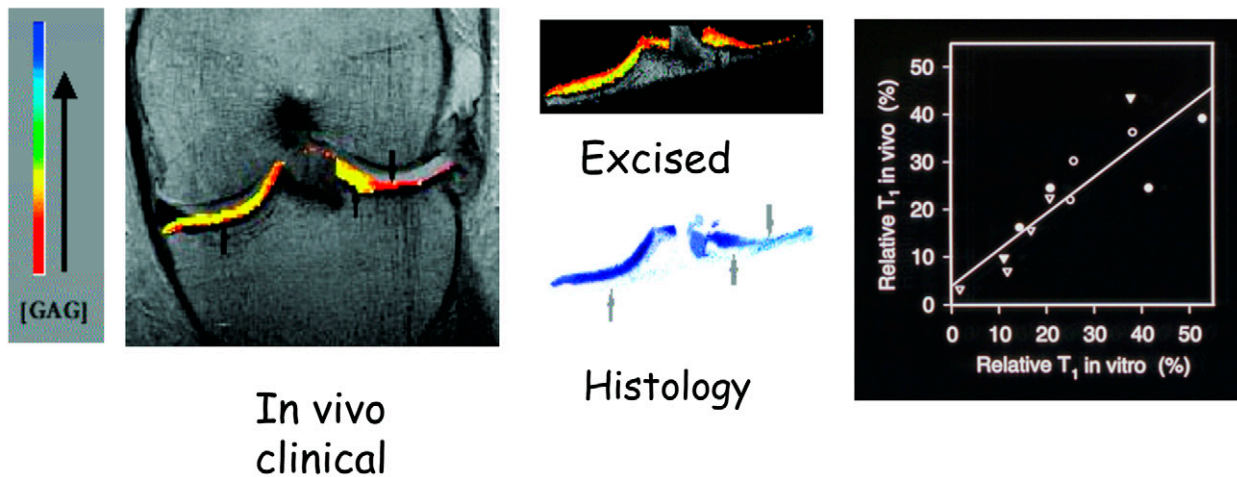


Fig. 3

The spatial distribution of T<sub>1</sub> in the presence of gadolinium diethylenetriamine pentaacetic acid (Gd[DTPA]<sup>2-</sup>) in a preoperative clinical image is similar to that seen for the same tissue excised and imaged postoperatively, and both provide a similar qualitative impression of glycosaminoglycan (GAG) distribution as the corresponding toluidine-blue stained histological section. The arrows indicate regions for comparison. The plot on the right-hand side compares dGEMRIC T<sub>1</sub> values measured clinically (preoperatively) with those measured in vitro (postoperatively) for the same regions of interest in the same joint in four patients. Each patient is shown with a different symbol. To allow comparison, values for each patient are shown normalized to one region of interest in each joint. The high correlation suggests that in vivo T<sub>1</sub> images in the presence of Gd(DTPA)<sup>2-</sup> provide an accurate assessment of the concentration of GAG relative to other regions in the same tissue. (Reproduced, with modification, from: Bashir A, Gray ML, Hartke J, Burstein D. Nondestructive imaging of human cartilage glycosaminoglycan concentration by MRI. *Magn Reson Med*. 1999;41:857-65. Reprinted with permission.)

iterative process involving studies of technical and pathophysiological validity.

To illustrate the process, a few examples are drawn from in vitro and in vivo studies designed to explore technical validity. Consider first the “pure” macromolecular solutions containing glycosaminoglycan or collagen, which are the dominant macromolecules in cartilage. Both dGEMRIC and T<sub>1</sub>rho measurements are sensitive to glycosaminoglycan, although only T<sub>1</sub>rho is sensitive to collagen (Fig. 2, A and B). Glycosaminoglycan can vary from ~7% in healthy tissue to ~0% in degenerated tissue; this range of glycosaminoglycan concentration is associated with a relatively large range of dGEMRIC and T<sub>1</sub>rho values. While T<sub>1</sub>rho is clearly not specific for glycosaminoglycan, the degree to which this is a confounding issue is not clear from these data. Collagen concentration is expected to vary by a small percentage from its nominal level of ~20%, and over this range, T<sub>1</sub>rho varies by only a small percentage. What may be more confounding is the sensitivity of T<sub>1</sub>rho to both collagen (macromolecular) concentration and structure. A comparison of polarized light and toluidine blue histological images with corresponding dGEMRIC and T<sub>1</sub>rho images shows that T<sub>1</sub>rho images do not reveal purely collagen organization or purely glycosaminoglycan concentration, but rather some combination of the two<sup>18</sup>.

As an example of technical validity in an in vivo study, consider Figure 3. The dGEMRIC images were acquired prior to total knee arthroplasty and then again after retrieval of the tibial plateaus, and both images were compared with toluidine blue histology. Qualitative comparison of these (and similarly

derived) image sets suggests that dGEMRIC is providing information comparable with the present “gold standard” of toluidine blue histology. A comparable study of T<sub>1</sub>rho and toluidine blue histology would be very valuable in evaluating the promise of T<sub>1</sub>rho.

Collectively, these and other data provide evidence of technical validity by showing that dGEMRIC and T<sub>1</sub>rho are sensitive to differences in glycosaminoglycan, that the measure changes monotonically with glycosaminoglycan and with interventions that modify tissue glycosaminoglycan, and that the in vivo measures correspond with in vitro measures. These imaging methods do differ with regard to their underlying basis. The dGEMRIC (and sodium magnetic resonance) methods are designed to measure fixed charge, so they are sensitive to sulfated glycosaminoglycans and any other charged macromolecule but insensitive to neutral macromolecules like collagen. T<sub>1</sub>rho, by contrast, depends on differential relaxation among different macromolecules and, to the extent that predominant macromolecules such as collagen exhibit T<sub>1</sub>rho relaxation for the measurement parameters, is not specific for glycosaminoglycan. Although these measures differ substantially in their biophysical basis and their degree of specificity for glycosaminoglycan, much work remains to be done before it can be determined whether any one of them or all of them will provide valuable pathophysiological information.

#### Pathophysiological Validity

The ultimate utility—and thus the ultimate validation—of any biomarker is dependent on evidence of pathophysio-

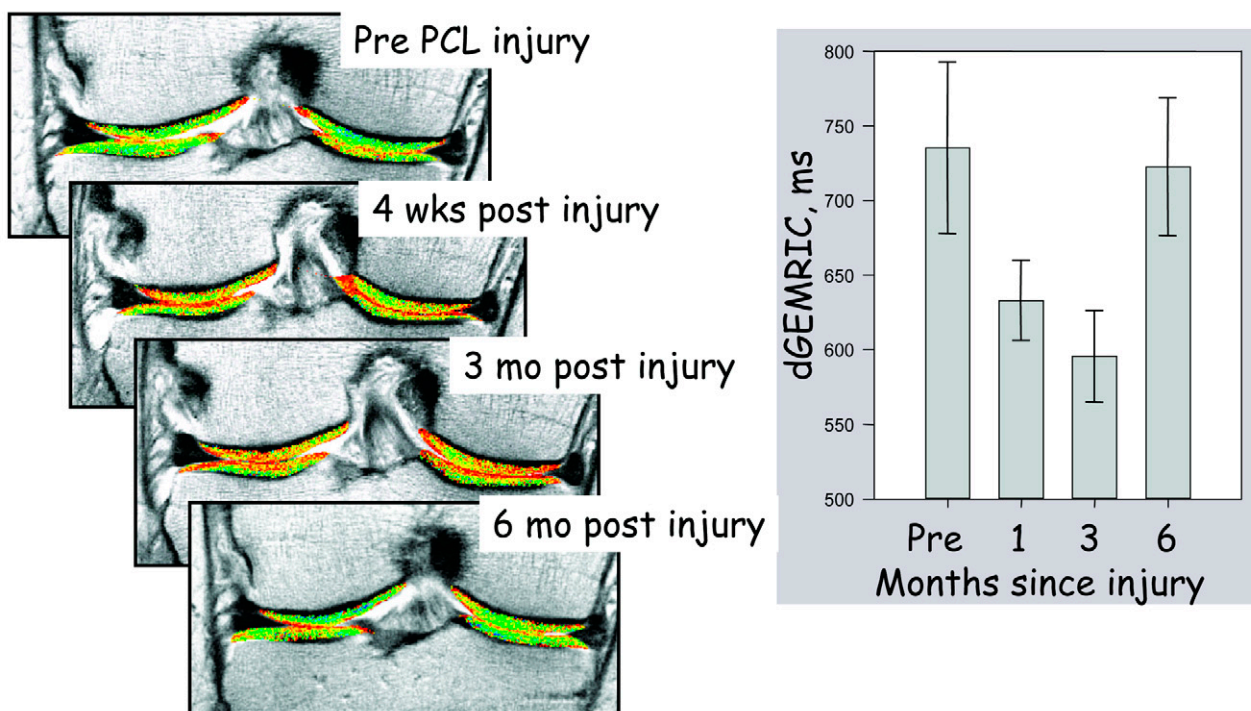


Fig. 4

dGEMRIC images of the right medial compartment from before and after a posterior cruciate ligament (PCL) tear showing a drop from the baseline dGEMRIC index at one and three months after the injury and a return to baseline values by six months. (Reproduced, with modification, from: Young AA, Stanwell P, Williams A, Rohrsheim JA, Parker DA, Giuffre B, Ellis AM. Glycosaminoglycan content of knee cartilage following posterior cruciate ligament rupture demonstrated by delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC). A case report. *J Bone Joint Surg Am.* 2005;87:2765.)

logic validity. Cross-sectional and longitudinal studies provide insight into the nature of differences or changes, respectively, in glycosaminoglycan (or the biomarker). Data from such studies are just emerging and should begin to provide the foundation for further work to test hypotheses about disease etiology and/or the relationship of these measurements to therapeutic and clinical outcomes.

One example of a longitudinal study is a recent case report of a patient who had experienced a traumatic posterior cruciate ligament tear. Immediately following the tear, the dGEMRIC index dropped appreciably, where it stayed for a few months before returning at six months to its pretrauma value (Fig. 4)<sup>19</sup>. More work and longer time frames are needed to determine if these changes are representative, sustained, or predictive in any way of clinical outcome. Nevertheless, this study is encouraging in suggesting that dGEMRIC may be a sensitive indicator of posttraumatic changes in cartilage<sup>19</sup>.

A second example derives from a body of work focusing on dGEMRIC in individuals with hip dysplasia<sup>20-23</sup>. One treatment strategy is to perform a periacetabular osteotomy to modify the loading environment of the hip in an effort to slow degeneration and thereby delay the time at which total hip replacement is necessary. In addition to other considerations, an orthopaedist must consider the best timing for surgery: the most effective timing might be when there are

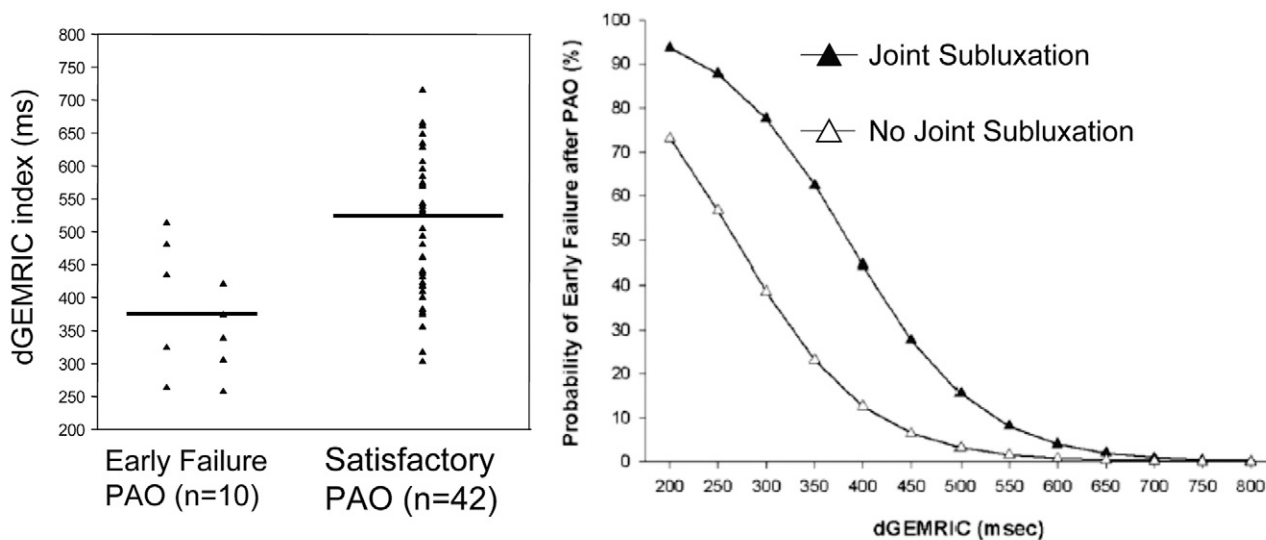
minimal symptoms; if surgery is too late, tissue destruction will already be too advanced to allow repair. A recent cross-sectional study indicated that the dGEMRIC index, together with subluxation, were strong predictors of the outcome of osteotomy (Fig. 5). Follow-on studies are needed to investigate whether dGEMRIC can be used as an indication of when to treat.

### Discussion

Considerable progress has been made in developing candidate biomarkers that could ultimately have a transformative influence on our ability to evaluate cartilage glycosaminoglycan in vitro and in vivo. Working to realize that potential, one must understand the many ways in which these techniques can fail.

The most obvious failures can arise when assumptions or conditions that underlie the technical validity fail. Were protocols implemented properly? Have quality assurance methods been developed and adhered to? Can underlying assumptions be generalized across the population (or sample set)?

Even with full technical validity, a biomarker can fail because it does not have adequate sensitivity or it does not measure the relevant pathophysiological pathways. Despite considerable face validity to the link between cartilage glycosaminoglycan loss and clinical manifestations, it is not known



(A)

(B)

Fig. 5

The dGEMRIC index, together with subluxation, is a strong predictor of the outcome of osteotomy. A: dGEMRIC index for subjects with hip dysplasia who have undergone periacetabular osteotomy (PAO), grouped by those who had an unsatisfactory result (defined as secondary arthroplasty, increased pain, and/or decreased joint-space width) ( $n = 10$ ) and those who did not ( $n = 42$ ). B: Statistical analysis indicated that the preoperative dGEMRIC index and subluxation were significant predictors of early postoperative failure. (Reproduced, with modification, from: Cunningham T, Jessel R, Zurakowski D, Millis MB, Kim YJ. Delayed gadolinium-enhanced magnetic resonance imaging of cartilage to predict early failure of Bernese periacetabular osteotomy for hip dysplasia. *J Bone Joint Surg Am.* 2006;88:1543-4.)

whether glycosaminoglycan loss is a central player in disease etiology, nor is it known exactly how much glycosaminoglycan loss is clinically relevant. The issue is complicated by the multifactorial nature of joint failure and the inadequacy of viewing osteoarthritis as a disease of any single joint structure, even articular cartilage. It is also complicated by the prolonged time frame over which cartilage destruction develops; several decades likely separate the first molecular changes from overt radiographic changes.

Nevertheless, cartilage glycosaminoglycan loss is the most broadly accepted histological measurement of progression and is a focus of most putative therapies for the disease. These unresolved issues notwithstanding, early data suggest that new imaging biomarkers may provide sorely needed information that is essential to enhancing our understanding of cartilage degeneration, to evaluating therapeutic efficacy, and to provid-

ing a state-of-cartilage proxy that can be of use in diagnosis and staging. Indeed, these techniques are part of a paradigm shift in which therapeutic strategies are developed hand in hand with diagnostic approaches—a shift that offers the promise of speeding the development of efficacious therapies and focusing their use in arenas where they can be most effective. ■

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