Meeting Report

Successes Achieved and Challenges Ahead in Translating Biomarkers into Clinical Applications

Greg Tesch,¹ Shashi Amur,² John T. Schousboe,^{3,4} Jeffrey N. Siegel,⁵ Lawrence J. Lesko,⁶ and Jane P. F. Bai^{6,7}

Received 5 November 2009; accepted 28 January 2010; published online 16 March 2010

Abstract. Biomarkers are important tools for identifying and stratifying diseases, predicting their progression and determining the effectiveness, safety, and doses of therapeutic interventions. This is important for common chronic diseases such as diabetic nephropathy, osteoporosis, and rheumatoid arthritis which affect large numbers of patients worldwide. This article summarizes the current knowledge of established and novel biomarkers for each of these diseases as presented at the 2008 AAPS/ACCP joint symposium “Success Achieved and Challenges Ahead in Translating Biomarkers into Clinical Applications,” in Atlanta, Georgia. The advantages and disadvantages of various proteomic, metabolomic, genomic, and imaging biomarkers are discussed in relation to disease diagnosis and stratification, prognosis, drug development, and potential clinical applications. The use of biomarkers as a means to determine therapeutic interventions is also considered. In addition, we show that biomarkers may be useful for adapting therapies for individual needs by allowing the selection of patients who are most likely to respond or react adversely to a particular treatment. They may also be used to determine whether the development of a novel therapy is worth pursuing by informing crucial go/no go decisions around safety and efficacy. Indeed, regulatory bodies now suggest that effective integration of biomarkers into clinical drug development programs is likely to promote the development of novel therapeutics and more personalized medicine.

KEY WORDS: biomarkers; bone; diabetic nephropathy; drug development; genetic; inflammation; osteoporosis; proteomics; rheumatoid arthritis.

INTRODUCTION

Biomarkers are frequently used for disease diagnosis and stratification, treatment selection, monitoring disease progression, and establishing the patients' responses to therapies (efficacy or adverse events). For example, low-density lipoprotein cholesterol levels and blood pressure are recognized surrogate endpoints in developing drugs for treating cardiovascular disease, fasting blood glucose and glycosylated hemoglobin levels measure diabetes control, and plasma thyroid levels are indicators of thyroid function. Recently, scientific and technological advancements have seen an

exponential growth of biomarkers derived from proteomics, metabolomics, and genomics. Efforts are being made to dovetail these scientific branches of biology into an integrated wealth of biomarker knowledge for providing the best evidence-based health care. Proteomic and metabolomic biomarkers, although diverse, are potentially related to the clinical manifestation of disease and to patient's responses to drug treatment. These categories of biomarkers have begun to be applied in drug discovery and development. Genomic biomarkers are adding another dimension to health care by enabling us to understand the germ line and somatic basis of disease development and of differential clinical responses to a specific medical treatment.

Despite current therapies, chronic diseases such as diabetic nephropathy, osteoporosis, and rheumatoid arthritis remain incurable and, in some cases, not effectively treated. The increasing size and longevity of our population makes these diseases a major clinical problem and a growing financial burden. The current review article summarizes the current knowledge of established and novel biomarkers for diseases presented at the 2008 AAPS/ACCP joint symposium “Success Achieved and Challenges Ahead in Translating Biomarkers into Clinical Applications,” in Atlanta, Georgia, and highlights the challenges that researchers and clinicians are facing in the development and application of biomarkers to manage and treat these diseases.

¹ Department of Nephrology, Monash Medical Center, Clayton, Victoria, Australia.

² Genomics group, OCP, OTS, CDER, FDA, Silver Spring, Maryland, USA.

³ Park Nicollet Health Services, Minneapolis, Minnesota, USA.

⁴ Division of Health Policy and Management, University of Minnesota, Minneapolis, Minnesota, USA.

⁵ Division of Anesthesia, Analgesia and Rheumatology Products (DAARP), OND, CDER, FDA, Silver Spring, Maryland, USA.

⁶ OCP, OTS, CDER, FDA, Silver Spring, Maryland, USA.

⁷ To whom correspondence should be addressed. (e-mail: jane.bai@fda.hhs.gov)

INTEGRATION OF BIOMARKERS IN DRUG DEVELOPMENT, REGULATION, AND CLINICAL PRACTICE

Use of biomarkers in drug development has been identified as a key prospect in the US FDA's Critical Path Initiative, to resolve the discrepancy between increasing resources going towards development and the decrease in the number of submissions for drug approval (1–3). Effective integration of biomarkers into clinical drug development programs is expected to promote the development of novel therapeutics and personalized medicine.

A wealth of information about novel biomarkers, including genomic and pharmacogenetic biomarkers, has become available since last decade. A similar but slower trend is observed in the incorporation of genomic/genetic information in regulatory submissions. The genomics group in the Office of Clinical Pharmacology/Center for Drug Evaluation and Research (CDER) is leading CDER's efforts on incorporating genomics into drug development and patient care. The core mission of the genomics group is to review genomic/genetic information in regulatory submissions and to participate in guiding drug developers in optimizing drug development.

In order to encourage submission of biomarker data in regulatory submissions, a guidance document on pharmacogenomics data submissions was published by FDA in March 2005 (4). In addition, a voluntary genomic data submission process has been set up at FDA to facilitate scientific exchange including discussion on technologies, data analysis methods, and genomic/genetic biomarkers, outside the regulatory framework. This program was later expanded to voluntary exploratory data submissions (VXDSs) to include other exploratory data such as proteomics and metabolomics data. The VXDS program has received more than 50 VXDS submissions and has had a significant impact on science and on development of new policy (5). The VXDS program has also been used strategically to discuss the possible use of biomarkers in drug development studies. A pilot process for qualification of biomarkers has also been set up to facilitate translation of exploratory biomarkers to qualified biomarkers (6). Recently, the US FDA and EMEA concluded that seven kidney injury preclinical biomarkers submitted by Predictive Safety Testing Consortium (PSTC) are considered qualified biomarkers (7). These biomarkers can now be used in pre-clinical safety studies to detect drug-induced nephrotoxicity.

Pharmacogenomic biomarkers can be applied in targeted drug design to improve efficacy of drugs. The biomarkers can also be applied to identification and exclusion of biomarker positive/negative patients at risk of developing serious adverse events. In addition, pharmacogenomic biomarkers can be used in dose selection/adjustment of drugs to improve efficacy and safety of drugs. The analytical performance characteristics should be established before utilizing the biomarker in stratification, identification of responders, or as tests to avoid prescribing to either biomarker positive or biomarker negative subjects.

Drug Efficacy/Safety and Biomarkers

Biomarkers can be used in designing drugs that selectively target populations effectively and help select potential

responders. For example, maraviroc, an antiviral drug approved for treatment of human immunodeficiency virus (HIV) patients, is a targeted therapeutic. This drug was designed to block the cellular CCR5 receptor and prevent infection of the host cells by HIV-1. Thus, maraviroc is effective in patients who harbor CCR5-tropic HIV-1 (8). Another example is trastuzumab, a recombinant DNA-derived humanized monoclonal antibody that selectively binds to human epidermal growth factor receptor 2, HER2. This biologic was developed to treat a subpopulation of breast cancer patients (25–30% of primary breast cancers) who overexpress HER2. Trastuzumab has been shown to inhibit the proliferation of human tumor cells that overexpress HER2 in both *in vitro* assays and in animals before demonstrating efficacy in clinical trials (9).

Dose adjustment. Interindividual variability makes it challenging to find a dose of a drug that works for all patients. One of the strategies used to circumvent the situation is dose adjustment, increasing the dose if the efficacy is low or decreasing the dose if adverse events occurred. As the understanding of the association between polymorphisms in genes of drug metabolizing enzymes (DME) or drug transporter proteins (DTP) with systemic exposure (pharmacokinetics) and with adverse events increases, it may be possible to have a dosing strategy based on the genetic polymorphisms of DMEs and DTPs. Some of the drug labels have already been updated with new information. For example, azathioprine label was updated to recommend genotype or phenotype patients for thiopurine methyltransferase (TPMT) (10). This recommendation is based on the results of studies that patients with TPMT deficiency or with lower activity are at increased risk for myelotoxicity, and the absence or decrease in TPMT activity was caused by mutation and, thus, could be predicted by genotyping data.

Exclusion of patients at risk of developing adverse reactions. This strategy is applicable only when patients at risk can be identified before prescribing the drug. One example is HLA-B*5701 genotyping to identify HIV patients at risk of developing abacavir hypersensitivity. It was noticed in the clinical trials that about 5% of the patients treated with abacavir developed a hypersensitivity reaction that resolved with discontinuation of the drug. The drug manufacturer, Glaxo Smith Kline, carried out a prospective study and several retrospective studies and showed a strong association between HLA-B*5701 and abacavir-induced hypersensitivity reaction (11). Similar findings were also reported by many other groups (12–14). Recently, the abacavir label was updated to reflect the new findings, and genotyping HLA-B*5701 was recommended before prescribing abacavir. Prescreening of HIV-1-infected patients for HLA-B*5701 has shown to significantly reduce the number of abacavir hypersensitivity cases in various parts of the world (11).

Often, a panel of biomarkers is used in clinical practice, for example, to monitor/test liver function. The objective of using a panel of biomarkers is to get higher sensitivity and specificity that represent the association of the biomarker to relevant clinical events. For example, assessment of serum α -fetoprotein (AFP) used for diagnosis of liver cancer shows a sensitivity of 65% and a specificity of 89% at a cutoff of 30 ng/mL. However,

when a panel of biomarkers, AFP, vascular endothelial growth factor, and α -fucosidase, is used for diagnosis of liver cancer, the sensitivity is 100% and specificity is 95% (15). This is likely to be true for pharmacogenomic biomarkers, and a panel of pharmacogenomic biomarkers or a combination of pharmacogenomic and other biomarkers may provide higher predictive power than individual biomarkers.

Future Opportunities and Challenges

Development of biomarkers through analytical and clinical validation and by demonstrating evidence of clinical utility is time-consuming, labor-intensive, and financially challenging. Availability of sufficient number of good quality samples from which biomarkers can be measured is also a challenge. Thus, collaborations through consortia are a feasible path forward to qualify biomarkers in some cases. In the drug development scenario, the biomarkers that can be used for increasing the drug's efficacy or safety may be identified early in development. In addition, in the case of targeted therapeutics that may work in patient subpopulations, the biomarker to be tested can be qualified early in the development process also, e.g., in the proof-of-concept study. In other cases, particularly those related to adverse events that occur in a very small percentage of the patients or are idiosyncratic, discoveries and observations of association of biomarkers might be made either late in the development process or in the postmarketing stage. Based on the evidence, drug labels are updated with biomarker data to better inform health care professionals, thereby providing better patient care.

Use of diagnostic biomarker tests in clinical disease management is not new. In fact, it has been reported that about 70% of the decisions made by physicians for managing diseases in USA are based on results of diagnostic tests (16). Novel tests, like MammaPrint for prediction of disease outcome for breast cancer patients and AlloMap® test for prediction of acceptance or rejection of heart transplants, will provide additional help to health care professionals in better disease management.

Summary

Pharmacogenomic biomarkers have allowed updates of some drug labels to better patient care. Genomic biomarkers are evolving to allow prediction of disease onset and individualized medicine. Proteomic and metabonomic biomarkers make it possible to diagnose diseases earlier and more accurately and to implement precise plans for management of disease treatment. However, there are a tremendous amount of challenges facing health professionals and regulatory agencies in treating diseases such as diabetes, osteoporosis, rheumatoid arthritis, and many other diseases. As science advances, better mechanism-based and evidenced-based biomarkers will be available for diagnosis of disease, treatment and management of disease, and prediction of disease onset. A concerted effort between clinicians, regulatory agencies, drug developers, and research scientists in development of biomarkers for clinical utility will importantly revolutionize health care.

DIABETIC NEPHROPATHY

Standard Biomarkers Used for the Diagnosis of Diabetic Nephropathy

Diabetic nephropathy is usually first diagnosed by the onset of microalbuminuria (a urine albumin excretion rate of 20–200 $\mu\text{g}/\text{min}$) which may steadily progress to overt albuminuria ($>200 \mu\text{g}/\text{min}$) if left unmanaged. Conventional methods for measuring albuminuria are based on immuno-detection, which include immunonephelometry, immunoturbidimetry, and radioimmunoassay. However, these techniques underestimate the true level of urine albumin, because the detection antibody used does not recognize some forms of albumin, which are modified in the circulation by ongoing processes such as glycation, lipidation, or oxidative stress. This problem has been overcome by the development of a high-performance liquid chromatography-based screening assay (Accumin test) which detects both immunoreactive and nonimmunoreactive albumin (17). The increased sensitivity of this technique can predict the onset of overt albuminuria in diabetic patients 2 to 4 years earlier than conventional methods. However, the accuracy of Accumin test in quantifying albumin has been shown to be dependent on the quality of the calibrators used and may be influenced by contaminants which co-elute with albumin (18).

Renal function in diabetic patients is commonly estimated by measuring serum creatinine or the rate of creatinine clearance using the Jaffe assay. However, this technique has some limitations such as being affected by muscle mass, age, and gender, and is most sensitive when renal injury is advanced and the patient glomerular filtration rate (GFR) is relatively poor (19). In comparison, when GFR is near normal, renal function can be measured with greater accuracy and sensitivity in diabetic patients using cystatin-C (20). This allows earlier detection of declining renal function earlier when albumin excretion is in the microalbuminuria range.

Novel Biomarkers which Predict the Clinical Progression of Diabetic Nephropathy

Significant efforts have been made to identify novel serum and urine biomarkers which can clinically predict and evaluate diabetic nephropathy. Two promising serum biomarkers are uric acid and sTNF-R1, both of which are independently associated with variations in renal function in type 1 diabetic patients (21). These markers correlate with an early decline in renal function, prior to the onset of overt albuminuria, suggesting that they may play a causal role in renal function impairment, although no definitive mechanisms have yet been established.

A number of urine biomarkers are known to reflect different components of diabetic renal damage, including hemodynamic changes, injury to specific cell types, inflammation, and fibrosis. Elevated intraglomerular hydraulic pressure is induced by hyperglycemia and is thought to play a pivotal role in the development of diabetic glomerulosclerosis. This hemodynamic change is accompanied by increased urine excretion of immunoglobulin G, transferrin, and ceruloplasmin in diabetic patients, which precedes the development of microalbuminuria, indicating that these proteins are biomarkers of

early diabetic glomerular injury (22). Cell specific proteins produced by glomerular podocytes (nephrin) and proximal tubules (kidney injury molecule-1) are detected in patient urine during the development of diabetic nephropathy and are sensitive markers of glomerular injury and tubulointerstitial damage, respectively (23,24). Resident kidney cells produce chemokines in response to stimulation by the diabetic milieu, which subsequently promotes the development of renal inflammation and tissue injury. Some of these chemokines (monocyte chemoattractant protein-1, interleukin (IL)-8, IP-10, and macrophage inflammatory protein-1 δ) are elevated in the urine of diabetic patients and correlate with a decline in renal function (25). Urine chemokine levels appear to reflect the level of diabetic renal inflammation, which contributes to disease progression (26). Renal fibrosis in diabetic kidneys is known to be dependent on transforming growth factor (TGF)- β 1; however, urine levels of this cytokine are low in diabetic patients, indicating that it is not an analytically sensitive marker of injury. In contrast, TGF- β inducible gene-h3 (β ig-h3) is 1,000 times more abundant in urine and serves as an excellent measurable marker of TGF- β bioactivity. β ig-h3 is elevated in diabetic patients before the onset of microalbuminuria, indicating that it is an early marker of fibrosis and renal injury (27).

Proteomic analysis of urine has been used as an unbiased method for identifying (1) individual proteins that are selectively increased or decreased in diabetic nephropathy, (2) protein patterns which are specific indicators of diabetic nephropathy, and (3) protein patterns which can predict the progression of diabetic nephropathy. A recent study has identified the ubiquitin fusion protein (UbA52) as a urine biomarker that is increased in patients with diabetic nephropathy but is not elevated in otherwise normal patients, diabetics without nephropathy, or patients with other proteinuric renal diseases (28). Interestingly, this study also identified a 6.2-kDa fragment of degraded ubiquitin as a urine protein that was specifically absent from patients with diabetic nephropathy. Clinical analysis has also found urine proteomic patterns can predict the progression of diabetic nephropathy with high specificity. One report has identified a 12-peak proteomic mass spectrometer signature that could predict cases of diabetic nephropathy with 76% specificity (29). Similarly, a more complex panel of 65 biomarkers has been shown to predict cases of diabetic nephropathy with 97% specificity and differentiate from other renal diseases with 91% specificity (30). In this latter study, it was noted that many of the urine biomarkers identified were fragments of collagen type I that were reduced in diabetic patients.

Biomarkers for Assessing Therapeutic Efficacy in Diabetic Nephropathy

Currently, most new therapies for diabetic nephropathy are evaluated by their ability to reduce albuminuria as a “surrogate” endpoint; however, this approach may not properly define the therapeutic potential of some treatments. Diabetic microalbuminuria is not reliable at predicting the progression to end stage renal disease, and up to 60% of diabetic patients will show some regression in albuminuria levels during the course of disease (31). Furthermore, once overt albuminuria is established, it can be difficult to reverse,

indicating that the processes responsible for albuminuria are complex. Indeed, albuminuria appears to be influenced by multiple mechanisms, involving glomerular filtration and tubular reabsorption, which may differ during the progression from micro- to overt albuminuria (32). An alternative approach to evaluating diabetic nephropathy therapies may be to determine their effectiveness in targeting specific disease mechanisms such as inflammation, cell injury and fibrosis, as well as the impact on albuminuria and renal function. This might be achievable using specific urine biomarkers (described above) and may determine how and when the therapy could be best applied; however, this approach is currently unproven. It is also noteworthy that biopsy studies have identified accrual of interstitial myofibroblasts as one of the best predictors of diabetic nephropathy progression (33), suggesting that more emphasis should be placed on developing biomarkers that assess interstitial fibrosis.

Genetic Biomarkers for Determining Susceptibility to Diabetic Nephropathy

Familial clustering of diabetic nephropathy and ethnic variations in its prevalence indicates that genetic factors influence the susceptibility to this disease. Genome-wide linkage scans have suggested several loci (e.g., 1q43, 7q36, 8q21, and 18q23) are candidates for susceptibility (34–36); however, none of these appear to be a singularly important determinant of diabetic nephropathy. Similarly, the analysis of associations between disease traits and single nucleotide polymorphisms (SNPs) has so far only identified polymorphisms in candidate genes (e.g., angiotensin converting enzyme, aldose reductase, glucose transporter 1, and carnosinase) with small effects (36,37). The current inability to identify genetic biomarkers, which can significantly predict diabetic nephropathy in the general population, may be due to the large variations in racial and genetic backgrounds and the heterogeneity of disease phenotypes in the analysis groups. It is hoped that future developments in molecular biology and advances in the knowledge of disease mechanisms will provide us with genetic screening tools which can identify those patients with greatest risk of developing diabetic nephropathy and those who will respond best to specific treatments. This may lead to earlier therapeutic intervention and more customized strategies for patient management.

Summary

Recent research has seen significant developments in biomarkers for diabetic nephropathy, particularly in urine analysis. This has been made possible by advances in the methods of proteomic analysis and a greater understanding of the disease mechanisms involved. Current evidence from small clinical studies indicates that the probability of development of diabetic nephropathy may be predicted by evaluating a combination of serum and urine markers together with other risk factors such as age, the presence of retinopathy, and the need for insulin (in type 2 diabetes). However, clinical studies with greater number of patients are required to compare sets of biomarkers/risk factors and achieve agreement on which combination offers the most useful and

cost effective clinical information. Urine proteomic patterns and genetic biomarkers may also become part of this analysis in the future. In addition, future evaluation of therapeutic drugs is likely to involve selection of biomarkers which specifically reflect the disease mechanism being targeted and may serve as pharmacodynamic endpoints.

BIOMARKERS IN RHEUMATOID ARTHRITIS: OVERVIEW AND REGULATORY PERSPECTIVE

Rheumatoid arthritis (RA) is a chronic, destructive, inflammatory polyarthritis often accompanied by systemic features. In patients with RA, the disease process results in impairment in carrying out activities of daily living due to inflammation and damage to joints. Eventually, accumulated damage may lead to the need for joint replacement. Furthermore, there is a growing recognition that patients with RA are at increased risk of cardiovascular disease.

Drug therapies for RA include symptomatic therapies and disease modifying antirheumatic drugs (DMARDs). Symptomatic therapies include nonsteroidal anti-inflammatory drugs (NSAIDs), hydroxychloroquine, and sulfasalazine. Low dose corticosteroids are often used to control disease activity, but their use is limited by well-known adverse effects. The mechanisms of action of DMARDs are varied and include antimetabolites (such as methotrexate and leflunomide), tumor necrosis factor (TNF) blockers (such as infliximab, etanercept, and adalimumab and, more recently, golimumab and certolizumab), IL-1 blockade (with anakinra), T cell co-stimulation blockade (with abatacept), and B cell depletion (with rituximab). The American College of Rheumatology (ACR) published treatment guidelines in 2008 (38), recommending that the choice of RA treatment should be based on the presence or absence of features of poor prognosis, the level of disease activity, and the duration of disease. The ACR guidelines recommend nonbiologic DMARDs for patients with low or moderate disease activity. Biologic DMARDs are recommended for patients with persistent high disease activity lasting for 3–6 months or longer, for patients with high disease activity for a shorter time when they have features of poor prognosis, or for patients with high disease activity who are unresponsive to nonbiologic DMARDs. The cost of treatment and the level of insurance coverage are recognized to impact the choice of RA treatment regimens as well.

What Are Biomarkers and How Can They Contribute to Addressing Unresolved Questions in RA Therapy?

Biomarkers are objectively measured biological characteristics that reflect predisposition to disease, disease status, or response to pharmacologic intervention. They have the potential to contribute in several important ways to the treatment of patients with RA. Given the wide array of therapeutic options, biomarkers could be developed to help select the optimal treatment for individual patients. This could represent an important advance over the empiric method for choosing therapies used today. Biomarkers could help distinguish those patients most likely to respond to a particular therapy from those unlikely to respond. For example, a biomarker could be valuable if it could identify patients particularly likely to respond to a TNF blocker or to

a B cell depleting agent. There is also precedent for developing biomarkers to identify patients at particularly high risk for developing severe adverse events in response to a particular product to allow clinicians to avoid use of that product or to proceed with extra caution.

Currently, there are a variety of biomarkers in widespread use in routine care of patients with RA and in clinical trials. Useful biomarkers generally reflect some important feature of the pathophysiology of disease. For RA, although the cause of the disease is unknown, there is a growing knowledge of the underlying predisposing factors and the mechanisms that lead to the clinical manifestations. Predisposition to RA is believed to derive from a combination of genetic predisposition and environmental factors. A variety of immunologic and inflammatory mechanisms is known to perpetuate the disease process and to damage tissues. Currently used biomarkers and ones being explored fall into the general categories of genetic biomarkers, imaging biomarkers, and biomarkers related to the immune system and inflammation.

Genetic Biomarkers

Polymorphisms at several genetic loci have been shown to be associated with RA. Classical genetic studies showed an important association between RA and certain structurally related alleles of the DRB complex of the major histocompatibility complex (MHC) termed the “Shared Epitope.” More recently, genome-wide association studies have confirmed association with the MHC and have identified several new loci based on SNPs. The new genetic loci implicated in predisposition to RA include (39) the following:

- *PTPN22* encoding the protein lymphoid tyrosine phosphatase that has been shown to inhibit T cell activation,
- *TRAF1/C5*, a susceptibility locus close to the gene encoding complement component C5 and the gene encoding the signaling molecule TNF receptor-associated factor 1
- *CD40*, encoding a member of the TNF receptor superfamily
- *TNFAIP3*, encoding TNF, alpha-induced protein 3
- *STAT4*, encoding signal transducer and activator of transcription 4
- *IRF5*, encoding interferon regulatory factor 5
- *CTLA4*, encoding cytotoxic T-lymphocyte antigen 4

Interestingly, a number of these susceptibility loci are not associated with RA only but have also been implicated in other autoimmune diseases. Clearly, the preponderance of genetic loci related to signal transduction reflects something important about the cause of RA and other autoimmune diseases. However, it is important to be aware that the newly identified genetic loci, individually and in the aggregate, only explain a small portion of the heritability of RA, so there remains a great deal more to be learned about genetic susceptibility to RA (39).

Genetic biomarkers do not vary over time for an individual; therefore, they would not be useful for assessing the level of disease activity or response to therapy. Rather, their usefulness would likely be as markers of an individual's predisposition to developing disease or as prognostic

indicators. They may also permit subsetting of patient populations into patients who respond differentially to different therapies.

Imaging Biomarkers

Joint imaging is useful for diagnosing RA, in understanding disease status/progression and in predicting prognosis. Plain films (x-rays) of hands and feet are examined to assess whether the disease process has led to erosions of the joint surface and narrowing of the joint space. Clinical trials utilize imaging as a biomarker to assess damage to joints. Erosions and joint space narrowing are assessed by blinded readers and combined in a single score (the total Sharp score) that is used to assess joint damage at baseline and over the course of the trial. Agents active at slowing or halting radiographic progression reduce the increase in Sharp score that occurs during the course of the trial compared to untreated controls. Another imaging modality that may prove useful as a biomarker in clinical trials is magnetic resonance imaging (MRI). MRI is a highly sensitive technique that reveals erosions and inflammation. Efforts are underway to standardize MRI using the RAMRIS scoring system. MRI has shown promise not only in assessing the status of a joint at one point in time but also in determining response to treatments. For example, MRI has been able to demonstrate reduction in inflammation in response to TNF blockade in a very short timeframe (40). Ultrasound is another imaging modality that may prove utility as a biomarker of RA disease activity and progression.

Immune Mechanisms

Since RA is an autoimmune disease characterized by inflammation, markers of immunity and inflammation offer another potential source of useful biomarkers. Biomarkers of immunity include immune cells (circulating levels of B and T cells and specific subsets), autoantibodies, and cytokines. A variety of immune cells is involved in RA progression and initiation, including T cells, B cells, and macrophages. The autoantibodies implicated in RA include rheumatoid factor (RF), which is an antibody to immunoglobulin and anti-citrullinated protein antibodies (ACPA). A variety of cytokines is involved in RA pathogenesis, including TNF- α , IL-1, IL-6, and others. RF and ACPA are important for diagnosing RA and as predictors of poor prognosis; however, they may not be useful for assessing disease activity or response to therapy since there is no established correlation between the levels of these autoantibodies with disease activity. An emerging area for assessing the state of the immune system in RA and other autoimmune diseases is measurement of gene expression in peripheral blood by microarray analysis. Markers of inflammation include acute phase reactants such as erythrocyte sedimentation rate as well as C-reactive protein, which are well-established biomarkers both in clinical practice and in clinical trials. Other soluble biomarkers that reflect inflammation and tissue damage in joint and bone include collagen degradation products, matrix metalloproteinases, receptor activator of nuclear factor kappa B ligand, and osteoprotegerin. Another marker of inflammation is the measurement of circulating levels of soluble receptors, such as soluble TNF receptor.

Use of Biomarkers in RA Drug Development

Qualification of RA biomarkers. Biomarkers can be used in a variety of different ways in clinical trials. They can be used as an early measure of clinical activity or as a guide in drug development to make go/no go decisions. They can be used to determine optimal dosing of a new drug. They can be used to select patients most likely to respond to therapy or to select patients at risk of toxicity. Finally, in select situations, they can be used as surrogate markers to assess efficacy. Whether a particular use of a biomarker in a clinical trial is appropriate depends on whether its level of qualification is adequate for that specific purpose (termed “fitness for use”). For example, use in early decision-making in drug development, such as go–no go decisions, may require only a relatively low level of qualification. Selecting patients for phase 3 clinical trials requires a higher level of evidentiary data. Use of biomarkers as surrogate markers of efficacy in phase 3 trials necessitates the highest level of validation.

RA Biomarkers in Clinical Trials: Some Considerations

For biomarkers to be used in clinical trials, biomarkers should have been standardized and qualified in multicenter experience and shown to reliably correlate with disease status. If a biomarker is intended to be used in prescribing the drug when it is approved, the developer should consult the appropriate FDA center (CDRH or CBER) during development.

Surrogate markers are a subset of biomarkers that have been shown to predict clinical benefit; for example, blood pressure for antihypertensives as a surrogate for strokes and myocardial infarction or viral titers in HIV disease as a surrogate for progression to AIDS or development of opportunistic infections. Therapeutic effects on validated surrogate endpoints can substitute in many situations for clinical endpoints in clinical trials of efficacy. Surrogate endpoints are particularly valuable when (1) they are clearly qualified as outcome measures, such as blood pressure or hypertension trials and viral load for HIV, and when (2) relevant clinical outcomes are not measurable in the time-frame of clinical trials, for example, complete remission/partial remission *versus* survival for oncology trials and long-term functional outcomes in RA. In the case of serious or life-threatening disease, if a surrogate marker is not fully validated but is considered reasonably likely to predict clinical benefit, then it may be used to assess efficacy under the provisions of accelerated approval. In this case, there is a requirement for studies to validate the surrogate marker postapproval. In the case of RA drug development, the usefulness of biomarkers as surrogate markers of efficacy is not clear since clinical outcome measures are sensitive to drug effects in the time frame of a clinical trial.

Biomarkers may be particularly useful in clinical trials as part of an enrichment design. For example, rather than enrolling all comers, a clinical trial may select patients based on those most likely to respond or those most likely to tolerate the drug and can target therapy to those most likely to benefit. Enrichment clinical trial designs could include randomizing patients with a gene signature on microarray

that has been demonstrated to be predictive of drug responsiveness or randomizing patients based on a predictive biomarker such as anti-citrullinated protein antibodies (ACPA) status. However, there are some caveats to such enrichment designs. Efficacy observed in enrichment designs may not be generalizable to the whole patient population. If efficacy is only shown in a subset of patients, that might need to be reflected in the drug label. Generally, there should be good evidence that the criteria used for selection represent a clinically meaningful way to categorize patients. Furthermore, if it is likely that patients not belonging to a selected subpopulation will also take the drug, it is important to study the efficacy and safety of the drug in these patients as well. One approach to address issues of generalizability would be to conduct one trial in the enriched population and one in the general population. If qualitatively similar results are seen in the enriched and unenriched populations, this would suggest that the efficacy is not restricted to the selected population.

Summary

Biomarkers have the potential to facilitate drug development in RA and other rheumatic diseases. Biomarkers may be useful at various stages of drug development; for example, dose selection, assessment of clinical benefits, and selection of the target population. There are a variety of promising biomarkers that may prove to have utility in RA clinical trials. However, prior to their implementation in clinical trials, biomarkers should undergo an appropriate process to demonstrate that they have been appropriately qualified with regard to their role in clinical trials ("fitness for use").

CLINICAL USE OF MARKERS OF BONE TURNOVER IN FRACTURE RISK ASSESSMENT AND MANAGEMENT

Fractures related to osteoporosis continue to be a substantial and growing public health problem. At the age of 60, Caucasian men and women, respectively, have a 26% and 44% chance of suffering a fracture related to osteopo-

rosis during their remaining lifetime (41). Only one third of those who have a hip fracture regain their prefracture ability to walk. Clinical vertebral fractures are a common cause of physical disfigurement, often result in chronic back pain (42), and can reduce pulmonary function. Fractures related to osteoporosis were estimated to have a direct medical cost of \$16 billion in 2005 in the USA, and that cost is projected to rise to \$25 billion by 2025 (43).

A variety of medications have become available over the past 13 years to treat patients who are at high risk of fracture, beginning with the market release of alendronate in November 1995 (44). These medications prevent fractures in part by reducing bone metabolic activity called bone turnover. Markers that reflect both aspects of bone turnover, bone formation, and bone resorption are now commercially available for use in clinical practice. However, use of markers of bone turnover has not become widespread in the management of osteoporosis.

What are Markers of Bone Turnover?

The two main classes of bone turnover markers are protein products of bone type I collagen degradation or synthesis and enzymes produced with the activities of osteoblasts and osteoclasts (45). Markers of bone formation reflect osteoblastic activity, and markers of bone resorption reflect osteoclast activation.

Markers of Bone Resorption

When bone is resorbed, the N-terminal and C-terminal products of bone type 1 collagen peptide chains, called N-telopeptides (NTX) and C-telopeptides (CTX), are released and can be measured in either serum or urine (Table I) (46). Cross-links between lysine and hydroxylysine on separate peptide chains are also present in mature type 1 bone collagen, remain present in the fragments of type 1 bone collagen degradation, and can be measured in either serum or urine (47). The enzyme tartrate resistant acid phosphatase (TRACP) is synthesized and released with osteoclastic activation. Serum levels and urinary excretion of these markers show circadian rhythm and are highest in the early

Table I. Major Biomarkers of Bone Turnover

Biomarker	Type	Source	Measurement	Precision
C-Telopeptide (CTX)	Resorption	Type I collagen degradation	ELISA (urine, serum) RIA (urine)	3–5.8% (serum) 19–23% (urine)
N-Telopeptide (NTX)	Resorption	Type I collagen degradation	ELISA (urine, serum)	13% (serum) 16–23% (urine)
Deoxypyridinoline (DPD)	Resorption	Type I collagen degradation	ELISA (urine) HPLC (serum)	23% (urine)
Tartrate resistant acid phosphatase (TRACP5b)	Resorption	Enzyme secreted by osteoclasts	ELISA (serum)	2.2%
Bone alkaline phosphatase	Formation	Enzyme secreted by osteoblasts	ELISA (serum)	4.4%
Procollagen type I N-terminal propeptide (PINP)	Formation	Type I collagen synthesis	ELISA (urine) HPLC (serum)	7.2% 4.4%
Osteocalcin	Formation	Bone matrix component synthesized by osteoblasts	ELISA (serum)	8–63%

Adapted from reference (46) by Seibel *et al.*

morning (3 to 7:00 a.m.) and fall to a nadir in the early afternoon hours (48). The difference between the nadir and peak levels is often as much as 50% of their 24-h mean level. Bone marker levels need to be assessed in a fasting state, as food intake tends to decrease bone resorption markers. The clinical assays of bone resorption markers have become increasingly automated and efficient with coefficients of variation now below 10% (49).

Markers of Bone Formation

Procollagen 1 N-terminal propeptide and procollagen 1-C-terminal propeptide directly reflect bone formation activity (50). Bone alkaline phosphatase is an enzyme produced by activated osteoblasts that appears to have a role in calcium hydroxyapatite deposition on bone. Osteocalcin is a bone matrix component manufactured by osteoblasts but also released from bone during bone resorption and, hence, reflects both osteoblastic activation and bone resorption activity.

Markers of bone formation have less diurnal variation than markers of bone resorption (51). The diurnal variation of serum osteocalcin is higher perhaps due to the fact it reflects both bone formation and bone resorption. Food intake has a lower effect on bone formation markers than on bone resorption markers (52).

Changes in Bone Marker Levels Over the Lifespan

Bone metabolism during childhood and adolescence is dominated by bone formation, and bone formation markers are higher than in adult premenopausal women (53). In middle-aged women during the time of transition to menopause, bone resorption activity begins to increase, and the balance of resorption *versus* formation tilts in favor of resorption. This often results in rapid bone loss during the early postmenopausal period (50).

Bone turnover marker levels increase substantially within a couple of weeks after acute fractures and can stay elevated up to a year or more following a fracture (54). Exercise will increase bone formation markers acutely to a slight degree but has a variable effect on bone resorption markers (55). Conversely, immobilization significantly increases bone resorption (56). Systemic glucocorticoid medications initially raise but subsequently depress markers of bone formation (57).

Clinical Uses of Bone Marker Turnover Measurement

Assess Risks of Bone Loss and Fractures

Baseline levels of serum and urine levels of osteocalcin (58,59), TRACP (59), urine CTX, and bone alkaline phosphatase (58) are modestly correlated with subsequent femoral neck bone loss. Three large prospective epidemiologic studies reported that baseline urine CTX was associated with subsequent fractures independent of bone mineral density. Garnero *et al.* showed that among elderly women with a femoral neck T-score of -2.5 or lower, hip fracture incidence was doubled in those with a urine CTX or urine deoxypyridinoline more than two standard deviations above the premenopausal mean value (60). In the Hawaii Osteoporosis Study, baseline urine CTX

and bone alkaline phosphatase were both associated with subsequent clinical fractures among postmenopausal women and with clinical and radiographic vertebral fractures among older postmenopausal participants (61). Gerdhem *et al.* found that baseline serum TRACP and urinary long osteocalcin fragments were associated with subsequent vertebral fracture but that baseline serum CTX were not associated with subsequent vertebral or nonvertebral fracture, after adjustment for bone mineral density (62).

Predict Response to Drug Therapy

The standard way to assess the effectiveness of pharmacologic fracture prevention therapy has been to measure bone mineral density one and/or 2 years after the start of therapy. This has been unsatisfactory because of the long delay between initiation of therapy and assessment of the effectiveness of that therapy and because changes in bone mineral density on fracture prevention therapies are only mildly correlated with fracture reduction efficacy (63,64). Short-term changes in bone turnover marker levels appear to predict response to fracture prevention agents. Greenspan *et al.* noted that decreases in serum NTX of $>30\%$ and of serum CTX $>50\%$, respectively, during the first 6 months of alendronate therapy were associated with a significantly greater improvement in bone mineral density (65). Reductions of serum osteocalcin are similarly predictive of bone mineral density changes on alendronate (66). The magnitude of procollagen 1N-terminal propeptide increase seems to be strongly associated with the bone mineral density increases (67).

In two large randomized controlled trials, the vertebral fracture risk reduction was correlated in a linear fashion with decreases of urine NTX down to 35% below baseline and with decreases of urine CTX down to 60% below baseline. Further reductions of either NTX or CTX were not associated with any additional vertebral fracture prevention (68). Moreover, a single level of bone resorption measured after 3 to 6 months of drug therapy was significantly associated with vertebral fracture risk reduction on risedronate over 3 years (68). In contrast, in randomized controlled trials of alendronate (69), risedronate (70), raloxifene, or teriparatide (71) *versus* placebo, baseline bone turnover marker levels were not associated with the magnitude of fracture risk reduction.

Importantly, because of how bone resorption and bone formation are coupled, markers of bone resorption or formation can be used to monitor response to either antiresorptive or anabolic fracture prevention medications. While markers of bone resorption decrease the fastest after commencement of an antiresorptive agent, decreases of bone formation follow within a month or two (51). Similarly, while markers of bone formation rise first with teriparatide therapy, markers of bone resorption start to increase a couple of months later (72). Among the bone formation markers, procollagen 1 N-terminal propeptide increases more dramatically on teriparatide therapy than bone alkaline phosphatase and is the preferred test with which to monitor response to teriparatide therapy (72).

Detect and Improve Compliance with Drug Therapy

A major impediment to reduction of the burden of fractures related to osteoporosis is noncompliance with

fracture prevention medications (73). Significant changes in bone markers are expected after commencement of these agents if the patients comply with the prescription, and the lack of such a change within approximately 3 months of initiating fracture prevention therapy can aid identification of those who are not complying with therapy (74). That may be a good entry point for the practitioners to engage those patients to improve their compliance by addressing the patients' concerns regarding the long-term safety of those medications and by assessing whether or not the patient's perceptions of the risk and benefit of drug therapy are appropriate and realistic.

Summary

The measurement of markers of bone turnover has improved substantially over the past decade and is increasingly important in routine clinical practice in the management of osteoporosis and treatment of those at high risk of fracture. In particular, measurement of baseline and follow-up bone turnover markers can accurately assess whether or not the patient is experiencing the expected biologic response to the drug. Moreover, this may help in the identification of those who are nonpersistent or noncompliant with their prescribed fracture prevention medication. Bone turnover markers may have a modest role to play in fracture risk assessment. However, further studies of the associations of baseline bone markers drawn under appropriate conditions (fasting state and in the early morning) are needed to better establish what, if any, additional information bone marker measurements may give regarding fracture risk beyond what can be gleaned from bone densitometry and other clinical risk factors. At this time, there are insufficient data to support the use of bone marker measurements in individual patients to predict future bone loss.

ACKNOWLEDGEMENT

The authors would like to thank Drs. Issam Zineh (Associate Director of Genomics Group, Office of Clinical Pharmacology, CDER, FDA) and Federico Goodsaid (Associate Director of Operation of Genomics Group, Office of Clinical Pharmacology, CDER, FDA) for their comments on the manuscript. The authors would like to thank Dr. Jean Lee at Amgen for her assistance in co-moderating the symposium.

Disclaimer The views expressed in this article do not necessarily represent the views of the US Food and Drug Administration.

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