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What is the Role for Biomarkers for Lower Urinary Tract Disorders? ICI-RS 2013

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Abstract

Aims—A biomarker is an entity that measures a normal or pathological process, or the response to an intervention. A biomarker must measure exclusively and be sufficiently sensitive to the process of interest. Alternatively, a biomarker may give clues regarding the underlying pathology of the condition and be a useful research or specialist tool. If a biomarker is to be of practical benefit then it must also be economical and practical to use. This article will consider chemical moieties as biomarkers, although in principle physical markers (e.g., bladder wall thickness) could also be defined as such.

Results and Conclusions—The validation of a biomarker for detrusor overactivity (DO) must appreciate the fact that the condition is likely to multifactorial and thus no single entity may be sufficiently selective and sensitive. However, more specific conditions, such as bladder pain associated with DO, may make the biomarker search easier. Several prospective agents including antiproliferative factor (APF) and epidermal growth factors (EGF) are discussed. Several urinary biomarkers, including neurotrophins (NGF, BDNF) and cytokines, and a serum marker, C-reactive protein, are considered as reaching the above criteria. All suffer from relatively poor lack of discrimination, as they all change in response to other, often inflammatory, conditions; BDNF may offer the highest expectations. Urinary ATP has also been proposed as a DO/OAB biomarker but requires further evaluation. Finally genetic markers offer potential to understand more about the pathophysiology of DO/OAB. The increasing availability of genome-wide association studies and micro-RNA assays offer genetic markers as a new generation of biomarkers.

Keywords

biomarker; bladder pain syndrome; cytokine; genetic markers; nerve growth factor; overactive bladder

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INTRODUCTION

The Value of a Biomarker

An objective characteristic of a biomarker is that it may be accurately measured and evaluated to indicate: a normal biological process; a pathological condition; or a response to therapeutic interventions. Validation of a suitable biomarker is useful for several reasons:

- To reveal the underlying pathology of the clinical condition or the fundamental basis of a physiological function.
- To describe accurately the presence, severity or progression of a clinical condition, or physiological function.
- To act as a surrogate clinical endpoint to predict clinical benefit or harm; based on epidemiological, therapeutic, or pathophysiological evidence.

Identification of biomarkers for lower urinary tract disorders is difficult as such disorders are manifold (e.g., bladder pain; overactive bladder (OAB); detrusor overactivity (DO)), often based on subjective symptoms rather than objective clinical measures and are multifactorial in origin. Thus, identification and validation of a biomarker requires a hypothesis-driven approach, rather than the serendipitous identification of a suitable entity. Urinary urgency, chronic bladder pain, and even OAB are difficult to quantify as these are subjective, symptom-based conditions and with several underlying causes. Thus, to validate the level of a biomarker against the severity of the above conditions will be difficult. On the other hand, DO is an objective description of bladder function, and whilst it too may have several causes it has an objective end-point against which to compare a potential biomarker. The literature tends to emphasise the assessment of potential biomarkers for OAB or bladder pain and often it is only possible to measure an increase or decrease of a biomarker against the absence or presence of the condition. Validation of a biomarker against DO may be more quantitative but it must be remembered that DO does not equate to OAB; only a subset of OAB patients have DO, and subjects without OAB symptoms may exhibit DO under urodynamic investigation.

Criteria and Range of Biomarkers

Several basic and practical criteria are required:

- Does the biomarker measure exclusively the clinical condition or physiological function of interest?
- Can the biomarker be reliably and consistently measured and is it sensitive to treatment or progression of a condition.
- Does the biomarker assay advance the diagnosis/prognosis of a clinical condition or the evaluation of a physiological or pathophysiological activity?
- Is the biomarker affordable and are there practical conditions that allow it to be used in healthcare or experimental settings?

With these considerations biomarkers can be of several forms. The most familiar are chemical moieties in tissues, plasma or urine that may, for example, indicate a pathological

condition, or a pharmacological or physiological response (say drug metabolite or hormone). Physical measurements are also used, and include urodynamic parameters, bladder wall thickness or fMRI images. Qualitative indices can include validated questionnaires or clinical endpoints that may describe the mental or physical state of a patient. In this survey chemical biomarkers will be considered, in particular with respect to how specific and sensitive they are in relation to lower urinary tract disorders and if they yield information about the pathophysiology of these disorders. Cost-effectiveness and practicality will not be discussed.

Urinary biomarkers are potentially useful as it is relatively easy to collect urine samples. However, an increase or decrease of a marker in urine may not just be because of over- or underproduction from bladder wall tissues but may also result from changes to the barrier function of the urothelium. Thus, whilst validation of a urinary biomarker may be a useful heuristic approach, the underlying reasons for an altered urinary level might not represent changes to the tissues from which it originated.

Biomarkers of Bladder Pain Syndrome/Interstitial Cystitis

The urothelium provides a barrier between urine and underlying tissues, and is metabolically active related to its ability to transduce external chemical or physical stimuli to reflex-mediated contractile function. Biomarkers would be useful to measure the extent of functional change as well as generate insight into the underlying pathways that mediate such functions.

Alteration of barrier function may underlie bladder pain syndrome/interstitial cystitis (BPS/IC). For example, a glycoprotein antiproliferative factor (APF) is secreted by bladder urothelial cells from BPS/IC patients and slows urothelial cell growth.¹⁻³ Other potential biomarkers associated with BPS/IC include: epidermal growth factor (EGF) and heparin-binding epidermal growth factor-like growth factor (HP-EGF) and reciprocal functions may exist between APF and EGF/HP-EGF.⁴ Evidence that markers may differentiate between patients with or without ulcerative BPS/IC varies, one study claims EGF may differentiate⁵ whereas another does not find differentiation for any marker.⁶ APF may mediate the pathological changes observed in BPS/IC, including suppression of cell growth, increase of barrier permeability, and reduction of expression of proteins, such as cadherins,⁷ contributing to the formation of intercellular complexes. Other examples include the urinary glycoprotein GP51, a bacterial defence molecule that is secreted from uroepithelial cells in reduced amounts from BPS/IC patients.⁸ Increased susceptibility to urothelial damage may be due to altered factors that regulate the development of structural elements. The matrix metalloproteinases degrade the extracellular matrix and altered activity has been linked to a number of disease processes including tumor growth and gastrointestinal disorders such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).^{9,10} Thus, these proteases have been proposed as potential biomarkers or to provide assessment of disease progression, but have yet to be validated in lower urinary tract disorders.

Alteration to ion channels and receptor function that transduce sensory functions of the urothelium may underlie lower urinary tract symptoms of urgency and pain. For example, excessive gene expression of the Na⁺ channels Nav1.7 or Nav1.8 is measured in tissue

biopsies from patients who exhibit exaggerated pain symptoms.¹¹ In addition, mediators released from the mucosa, including ATP or nitric oxide, may provide information about disease progression in addition to sub-typing groups of patients. For example, BPS/IC patients with Hunner's lesions can exhibit higher levels of luminal NO.¹² Thus, anylates may be used for investigating the pathophysiology of the dysfunction, for diagnostic purposes or to monitor treatment effects.

Nerve-Growth Factors, Prostaglandins, Cytokines, and C-Reactive Protein

Increased recent emphasis has been placed on the role of urinary neurotrophins, including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), as agents underlying LUT disorders and also as urine biomarkers of dysfunction.^{13,14} NGF is a member of a family of neurotrophic factors (including NGF, BDNF, NT3 NT4) and binds to trkA and p75 receptors. It is required for growth, survival and development of sensory, autonomic, and CNS neurones and modulates neuronal plasticity in micturition pathways. Amongst other functions it modulates neurotransmitter release and lowers the activation threshold in afferents and sensitises nociceptive fibres, so that its neutralisation attenuates hyperreflexia in spinal cord injured animals.¹⁵ A raised urinary NGF level has been proposed as a biomarker for OAB,^{16,17} with BDNF showing superiority.¹⁸ However, a significant issue is that NGF levels are also raised in other conditions, including BPS/IC, urinary tract infections, stones, and bladder tumors.^{19,20}

Moreover, NGF and BDNF are sensitive indicators of resolution of lower urinary tract disorders by therapeutic management. For example, in patients with OAB elevated levels of urinary NGF²¹ and BDNF,²² normalized to creatinine (Cr) concentrations, were diminished in those who responded to antimuscarinic agents with a decrease of urgency scores. In a similar way, urinary NGF and BDNF levels were reduced in patients with DO or BPS/IC who responded to Botulinum Toxin A treatment.^{16,23}

Elevation of urinary prostaglandins and cytokines have also been proposed as markers of OAB. Prostaglandin E2 (PGE₂) has been most extensively studied and has been claimed to be superior to NGF as a discriminatory marker,^{24,25} but this is not a universal observation.²⁶ Of interest is that in a group of patients with OAB syndrome urinary PGE₂ levels were lower in those with detrusor underactivity on urodynamic evaluation, compared to those without detrusor underactivity.²⁵ Similarly, different cytokines are raised in the urine of patients with OAB, including monocyte chemotactic protein-1 (MCP-1) and soluble fraction of the CD40 ligand (Scd40L).^{27,28} However, the exclusive association with OAB is lacking and thus may not yet be considered as suitable biomarkers.

C-reactive protein (CRP) is an acute phase protein and serum levels rise in response to inflammation. It is synthesized by the liver in response to factors released by macrophages and adipocytes and activates the complement system. An increase of serum CRP levels has been recorded in patients with OAB, but also in those with BPS/IC.²⁹ However, the marker shows poor sensitivity and selectivity as a biomarker for OAB.³⁰

Overall, these urine and serum moieties as biomarkers of OAB suffer from the problem of poor discrimination between other, in particular inflammatory, conditions.^{27,29} Thus,

deviation from normal levels may reflect a fundamental pathology that may or may not also be associated with a lower urinary tract disorder.

Urinary ATP

ATP is released from urothelium when stretched and has been proposed as a mediator to transduce external stresses of the bladder wall. Stretch-activated ATP release is enhanced in tissues from patients with OAB or BPS/IC,^{31,32} as well as by a reduction of extracellular pH³³ as will occur in bladder hypoxia. The leakage of ATP into urine has thus been proposed as a biomarker for LUT disorders. Raised ATP in bladder washings is associated with reduced first desire to void and a weaker association with lower urinary pH in OAB patients.³⁴ A similar finding was made in OAB-dry and -wet patients when ATP was normalized to Cr levels.³¹ Moreover, when OAB activity was reduced with antimuscarinic agents or when men with BPH were treated with alpha-blockers normalized ATP levels were reduced.^{35,36} A practical note is that ATP tends to hydrolyze in solution, especially if samples are not stored on ice: therefore papers should state carefully how samples are stored prior to analysis.

Receiver operating characteristic (ROC) curves of a variable, such as a biomarker, for a given condition (i.e., OAB) plot the true positive rate (sensitivity) as a function of the false-positive rate (1-specificity).³⁷ Values of the area-under-the-curve generally range from 0.5 (no discrimination) to 1 (perfect discrimination). All too few studies use ROC curves but values of 0.78 for BDNF/Cr; 0.74 for ATP/Cr; 0.68–0.54 for NGF/Cr and 0.55 for CRP against OAB have been published.^{18,30,31}

Genetic Markers for LUT Disorders

The aetiology of LUT dysfunction is multifactorial and is likely to involve interactions between multiple genes. Twin studies give unique insights when comparing monozygotic and dizygotic twins and help decide whether genetics or the environment are aetiological factors for causation of a particular disease. If monozygotic are more concordant than dizygotic twins a genetic influence is likely; however, discordant monozygotic twins point to environmental factors when compared to dizygotic twins.³⁸ The EPINCONT study, a population-based cross-sectional study, suggested that women are more likely to develop urinary incontinence if their mothers or older sisters also have the condition.³⁹ Results from the Danish twin registry suggested genetic factors were important for urge and mixed urinary incontinence but the picture was less clear with regards to stress incontinence.⁴⁰ Finally recent data from the Swedish twin registry suggested genetic factors were important for the development of frequency, nocturia, and urinary incontinence and the lowest genetic effects were seen for OAB where environmental factors predominated.⁴¹

A recent study showed that a single nucleotide polymorphism of the β 3-adrenergic receptor (β 3-AR) gene, a mis-sense tryptophan to arginine mutation in codon 64, is more common in women with OAB.^{42,43} Although the mutation does not influence the action of β 3-agonists such as mirabegron⁴⁴ it may act as a useful marker for OAB.

Genome-wide association studies (GWAS) have helped identify genetic contributions to common multifactorial diseases, but no comprehensive data are yet available with respect to

urinary incontinence or OAB. This approach involves rapidly scanning markers across complete genomes to find genetic variations associated with a particular disease. Such studies are particularly useful in finding genetic variations that contribute to common and complex diseases. After improvements in the cost and efficiency of genome-wide scans, researchers as well as health professionals can use such tools to provide patients with individualised information about their risks of developing certain diseases and tailor prevention programmes. In type-2 diabetes mellitus, GWAS studies have identified 40 genetic variants to the already well-recognised risk factors for development of the disease such as lifestyle characteristics.⁴⁵ Peripheral blood mononuclear cells (PBMCs) have been assessed for potential biomarkers of OAB and the expression of several genes were shown to increased or decreased.⁴⁶ The main limitation was the small sample size, making firm conclusions difficult.

Micro-RNAs are an emerging class of agents that may be used as potential biomarkers for diseases. These small non-coding nucleotides are evolutionally well-conserved and their dysregulation is associated with some disease states, including bladder cancers.⁴⁷ Several disease areas are exploiting such changes to investigate their pathogenesis and LUT disorders offer a further useful area for study.

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Research Questions

- Define more precisely the clinical condition and phenotype for which a biomarker is required.
- Define the pathophysiological state towards which a biomarker will be useful.
- Improve the discriminatory power of a biomarker towards the required clinical state.
- Improve bioinformatics programs to facilitate the development of genetic biomarkers.