W13: Biomarkers for detrusor overactivity

Workshop Chair: Christopher Fry, United Kingdom

27 August 2013 09:00 - 12:00

Start	End	Topic	Speakers
09:00	09:05	Introduction to the workshop	• Christopher Fry
09:05	09:30	The use of biomarkers in evaluating detrusor overactivity	• Karl-Erik Andersson
09:30	09:55	Imaging techniques as markers of detrusor overactivity	• Vikram Khullar
09:55	10:20	Neurotrophins as biomarkers lower urinary tract disorders	• Celia Cruz
10:20	10:30	Questions	All
10:30	11:00	Break	None
11:00	11:30	Emerging biomarkers for bladder dysfunction	• Arun Sahai
11:30	11:50	Using biomarkers to understand bladder pathology	• Christopher Fry
11:50	12:00	Discussion	All

Aims of course/workshop

The workshop aims to highlight the role of biomarkers in defining detrusor overactivity in patients and also to provide insight into potential causes of the condition. Many markers of detrusor overactivity have been proposed, including chemical markers in the urine, physical properties of the lower urinary tract or subjective symptom scores. Their relative merits will be discussed as well as their ability to point to particular changes of lower urinary tract pathology causing detrusor overactivity.

Biomarkers for detrusor overactivity

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"The bladder is an unreliable witness" [1,2]. Discuss

This statement was posed in response to the observation that patient symptoms have a poor correlation with voiding dysfunction in the lower urinary tract. If this is true then new ways to determine the incidence and even severity of lower urinary tract symptoms need to be devised – the search for reliable biomarkers is initiated.

This workshop will consider:

i. the value of identifying and validating different biomarkers with respect to the overactive bladder and detrusor overactivity and
ii. the pros and cons of different biomarkers.

The Faculty

Karl-Erik Andersson (Wake Forest Institute of Regenerative Medicine, USA) will discuss the desirability for novel biomarker development in the diagnosis and development of overactive bladder or detrusor overactivity.

Célia Duarte Cruz (University of Porto, Portugal) will describe the development of nerve growth factor as a particular biomarker for bladder dysfunction

Christopher Fry (University of Surrey, UK) will consider the value of small molecule markers in the urine (e.g. ATP, prostaglandins, cytokines) and plasma (e.g. C-reactive protein)

Arun Sahai (Guy's Hospital and King's College London, UK) will describe the place of genetic markers and how they may provide insight into the pathogenesis of overactive bladder or detrusor overactivity.

Vikram Khullar (St Mary's Hospital and Imperial College London, UK) will discuss the role of physical methods, such as measurement of bladder wall thickness

What is a biomarker and what do they offer?

The term biomarker means different things to different people but perhaps the widest definition with respect to biological processes is "An objective characteristic that may be accurately measured and evaluated to indicate a normal biological processes, a pathological condition or a response to a therapeutic intervention". This allows us to step back from the idea that a biomarker can only be a chemical moiety, but includes physical measurements and even validated qualitative measures.

The advantages of a validated biomarker are several-fold:

To provide an improved diagnosis of a clinical condition and, if possible, information on its progression and prognosis.

To identify subgroups of eth population who may be of increased risk to develop a particular condition

To yield information about the fundamental cause of a clinical condition

To evaluate the effect of drug, or other therapeutic intervention, on the management of a clinical condition.

In the case of detrusor overactivity or overactive bladder there may be several fundamental causes of these conditions and so a single biomarker may be inadequate. The evaluation of success of a particular biomarker may therefore yield information about the pathophysiology of the condition. For this reason several biomarkers are being evaluated for their value in diagnosing and understanding these conditions [3].

Criteria for the successful development of a biomarker

The development and use of a biomarker requires several objective criteria to be met other than the fact that the test may offer some association with the condition that requires evaluation. For example does the development of a new biomarker advance the diagnosis and prognosis of a condition and is it economically and practically feasible in practise. With respect to overactive bladder or detrusor overactivity we can demand several such criteria are met when developing and new biomarker.

- Is there a biomarker that is associated with these clinical conditions?
- Is the biomarker reliably and consistently measured?
- Does the biomarker measure exclusively the clinical condition of interest?
- Is the biomarker assay sensitive enough to measure changes to the clinical state?

- Is the biomarker assay advancing diagnosis/prognosis of the clinical condition?
- Is the biomarker assay affordable?
- What are the practical conditions for a biomarker to be used in healthcare settings?

Neurotrophins as urinary biomarkers: are we there yet?

The detection and quantification of peptides and low molecular weight proteins in the urine is possibly the most popular approach to biomarker development as urine is a stable biofluid and can be obtained in sizeable amounts without inconvenience to the patient [4,5]. Among the several elements detected in the urine, neurotrophins (NTs) have attracted considerable attention in recent years.

NTs are tissue-derived trophic factors that exert pro-survival and plastic effects on neurons [6,7]. The most well-known NT is Nerve Growth Factor (NGF) that is abundantly produced by the urothelium and detrusor [8]. NGF is a major modulator of bladder function and is upregulated both in experimental models of lower urinary tract (LUT) dysfunction and in human biopsies.

NT exerts its effect via its high affinity receptor TrkA, which is expressed by bladder sensory afferents and urothelial cells [7,8]. Another important NT is Brain Derived Neurotrophic Factor (BNDF) [6]. Like NGF, BDNF is also produced in the bladder where the urothelium is possibly the major source [9]. TrkB, the high affinity receptor for BDNF, is also present on urothelial cells and also on bladder sensory afferents [7].

Both NGF and BDNF can be found in the urine. In this workshop, the current state-of-the-art about the putative use of urinary NGF and BDNF as biomarkers for LUT dysfunction will be reviewed. Several studies have demonstrated the presence of NGF in the urine of patients with overactive bladder syndrome (OAB) and a decrease after successful treatment. Hence, NGF has been proposed as a biomarker for OAB [10] (Liu and Kuo, 2008).

However, raised levels of urinary NGF have also been described in a number of lower urinary react syndromes including not only idiopathic and neurogenic detrusor overactivity bladder but also bladder pain syndrome/interstitial cystitis (BPS/IC), bladder oversensitivity and bladder outflow obstruction. There are considerably fewer studies

addressing a role for urinary BDNF as a biomarker. This NT can also be found in the urine of OAB and BPS/IC patients and its concentration also decreases with treatment.

However, the use of urinary NTs as biomarkers is still debatable. Advantages and pitfalls of their use will also be addressed in the workshop.

Other molecular markers for OAB

Cytokines. These are a family of secretory proteins produced by leucocytes as well as other tissues, including the bladder. They have homeostatic and inflammatory functions and include classes such as the interleukins and interferons as well as TNF-α. Altered urinary levels of cytokines, chemokines, and growth factors have been measured that are involved in bladder inflammation and tissue repair associated with OAB. This suggests not only an association between bladder inflammation and OAB but could also provide suitable diagnostic tests for OAB [11].

Alternatively OAB itself may produce inflammatory cytokines due to the plasticity of afferent nerves or urothelial dysfunction. Analysis of urinary urinary proteins using an antibody-based array chips for an array of cytokines found some to up-regulated whereas others were depressed [12]. Apart from inflammation, other co-morbidities occur with OAB and it is important to determine if biomarkers associated with these co-morbidities are causal of OAB and/or are sufficiently sensitive to diagnose it. For example, other cytokines are increased in urinary tract infections, which itself can be associated with OAB symptoms [11,12].

Prostaglandins. Prostaglandins (PG) are synthesised in the muscle and urothelium layers of the bladder and are released, with a fraction appearing in the urine, upon bladder wall stretch, nerve stimulation and when the bladder is inflamed [13]. Prostaglandins have multiple effects including: an increase of detrusor contraction and lowering the threshold for afferent activation [14]. Intravesical instillation of PGE_2 for example increases detrusor contractions [15]. Several studies have revealed an association between lower urinary tract disorders and altered PG levels in the urine. Increased concentrations of PGE_2 and $PGF_{2\alpha}$, as well as nerve growth factor, are measured in OAB patients [13,16], but this is not a universal finding [17]. In boys with bladder outflow obstruction as a result of urethral stricture raised PGE_2 levels are associated with the incidence of OAB [18]. Non-steroidal

anti-inflammatory agents, such as aspirin, and prostaglandin receptor antagonists may have a role in the management of OAB [19] because of the strong association between PG release and OAB. However, it also highlights the question of whether inflammatory conditions and OAB are only associated observations or whether there is a causal relationship.

ATP and nitric oxide (NO). ATP and NO are released from the bladder urothelium during several physical and chemical stresses, and the amount released is increased in tissue from neuropathic and idiopathic DO patients and analogous animal models [20]. Thus, a quantifiable relationship between their urinary levels and the presence of detrusor overactivity may be available. The increase of these moieties in urine may represent an increased permeability of the urothelium or an enhanced release so that the overspill into the bladder lumen is greater.

Urinary ATP levels have been measured in patients with or without detrusor overactivity and it was not possible to distinguish between the two groups. However, there were significant negative relationships between urodynamic filling variables (maximum cystometric capacity, voided volume) and ATP in both groups [21]. Furthermore, urinary ATP levels were reduced in patients with OAB or BPH who had improved symptoms having been treated with a1-adrenoceptor antagonists [22]. It is suggested that the urinary ATP/creatinine ratio was a useful index of lower urinary tract symptoms in these patients.

The urinary ATP/NO ratio has also proposed as a clinically relevant and perhaps more sensitive biomarker to measure the extent of bladder dysfunction. ATP release positively correlates, whilst NO release negatively correlates with bladder contraction frequency [23].

C-reactive protein (CRP). The inter-relationship between chronic inflammation and OAB has been further explored in relation to CRP. An elevation of serum CRP has been associated with chronic inflammation and LUTS [24,25].

Genetic biomarkers for OAB

Because the aetiology of LUT dysfunction is multifactorial it is likely to involve interactions between multiple genes. The EPINCONT study in Norway, a population-based cross-sectional study, suggested that women are more likely to develop urinary incontinence if

their mothers or older sisters have urinary incontinence. Twin studies give a unique insight into the genetic influence of disease when comparing monozygotic and dizygotic twins. Well-designed studies are able to give a view on genetics or environment as aetiological factors for causation of a particular disease. If monozygotic twins are more concordant than dizygotic a genetic influence is likely, however, discordant monozygotic twins points to environmental factors when compared to dizygotic twins (26).

A recent study has confirmed the hypothesis that a single nucleotide polymorphism of the β3 receptor gene is more common in women with OAB (27). This mis-sense mutation in codon 64 of the β3-AR gene, which changes the tryptophan to arginine, is particularly relevant now as these receptors mediate bladder relaxation and a selective drug agonist has shown efficacy in treating OAB patients (28-29).

Genetic factors contribute about half of the total risk for urgency incontinence. Functional polymorphisms of the cytochrome P450 IID6 gene significantly alter the metabolism of some commonly used antimuscarinic drugs, but no genetic loci that influence the risk of OAB have been definitively identified (30).

Genome wide association studies (GWAS) have helped identify genetic contributions to common multifactorial diseases but no data is present currently with regards to urinary incontinence or OAB. This approach involves rapidly scanning markers across complete genomes of people to find genetic variations associated with a particular disease. Once new genetic associations are identified, researchers can use the information to develop better strategies to detect, treat and prevent the disease. Such studies are particularly useful in finding genetic variations that contribute to common and complex diseases. In the future, after improvements are made in the cost and efficiency of genome-wide scans and other innovative technologies, health professionals will be able to use such tools to provide patients with individualized information about their risks of developing certain diseases. The information will enable health professionals to tailor prevention programmes to each person's unique genetic makeup. 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 (31).

Peripheral blood mononuclear cells (PBMCs) have been assessed for potential biomarkers of OAB (32). Twenty-one patients (16 female) were used in the study and PBMCs were obtained from whole blood samples and the subsequent RNA subjected to microarray gene-chip analysis. Sixteen genes were differentially regulated (8 up- and 8 down-regulated) when compared to healthy controls (n=6; 3 female). Cartwright et al., in an abstract recently presented at the ICS assessed gene expression from bladder biopsies in women with OAB (33). Cases consisted of those with OAB symptoms and DO on urodynamics and controls were those with urodynamically proven stress incontinence without DO or symptoms of OAB. Of 5 cases and 5 controls that passed all stages of quality assurance, 29 genes were differentially expressed with at least 2 fold changes, and 55 genes were differentially expressed with at least 1.5 fold change. The most significant pathways involved from the analysis of the differential gene expression were in cytoskeleton remodelling; cell adhesion; smooth muscle contraction; and cholinergic, G-protein coupled, and Ca^{2+} dependent signalling (33).

Certainly gene expression studies appear a very appealing approach to identify possible biomarkers for OAB as well as leading to potential insight into the pathophysiology of this largely still unknown condition.

Physical biomarkers: ultrasonography and near infra-red spectroscopy

Ultrasonography and bladder wall thickness. Because patients with OAB may have frequent detrusor contractions during the storage phase, sustained isometric detrusor contractions could result in increased muscle bulk and hence, increased detrusor wall thickness (DWT) or bladder wall thickness (BWT). It has therefore been hypothesized that DWT increases in patients with DO [34]. The thickened bladder wall might decrease in response to antimuscarinic treatment, and measurement of DWT might also be a potentially useful biomarker for evaluation of disease progression and effectiveness of treatment for OAB.

Values of BWT in patients with outflow obstruction, DO or increased bladder sensation did not indicate much difference, with respect to patients with stable bladders [35]. In more definitive groups some predictive value has been suggested. Measurements in men with outflow obstruction, children with enuresis and women with DO have indicated changes with respect to control. The reliability and reproducibility of these observations will be discussed, as well as whether they are likely to replace conventional urodynamics. In

addition, variations in patients' BMI etc may also confound accurate measurement of BWT, thus further limiting the potential usefulness of the technique.

Methodological factors can impact on the interpretation of data obtained from ultrasound measurements of bladder structure. Thus, different frequency probes and the placement of the probe call all impact on the particular measure and thus should be standardised. For example, a comparison of vaginal, perineal and abdominal ultrasound has been made to determine BWT values are comparable and significant differences were observed [36].

The technique as used to characterise LUTS is promising, especially as it is a non-invasive tool to assess the lower urinary tract. Bladder wall hypertrophy may be useful to assess LUT dysfunction, prediction of treatment outcomes, and for longitudinal studies investigating disease development and progression. However, data on asymptomatic subjects is still required to generate a reliable base-line of measurements.

Near infra-red spectroscopy (NIRS). This is an optical technology and detects haemodynamic changes in tissues via non-invasive measurement of changes to the concentration of tissue chromophores such as oxyhemoglobin and deoxyhemoglobin. Because many organ functional changes, including those of the bladder, are associated with tissue ischaemia the technique may be useful to investigate potential causes of lower urinary tract dysfunction. The technique requires careful validation at present but offers an alternative non-invasive method to investigate the functional properties of the human lower urinary tract. [37].

Reading

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Notes