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## Review on use of biomarkers in small animal disease diagnosis

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### Abstract

Biomarkers are defined and classified in many ways and are considered as an indicators of normal biological or pathogenic processes or pharmacological responses to a therapeutic intervention. They are mainly used to diagnose various diseases and to monitor various organ dysfunction or injury or damage. The increased quantity of each biomarker in respective organ system indicate that the animal is diseased, thereby aids in ease of differentiation of healthy and unhealthy animal. This review gives a brief summary of biomarkers, their classification and use in different organ system (hepatic, urinary etc.) of small animals.

**Keywords:** Biomarker, biological marker, small animal, disease, condition

### Introduction

#### Biomarkers in brief

Biomarker, a biological marker is defined as a “cellular, biochemical or molecular alterations that are measurable in biological media such as tissues, cells or fluids”<sup>[1]</sup> (Hulka *et al.*, 1990). Later, the National Institutes of Health Biomarkers Definitions Working Group defined it as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”<sup>[2]</sup> (Biomarkers Definition Working Group, 2001). Also, WHO together with United nation and International labor organization defined biomarker as “any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease” in International Programme on Chemical Safety<sup>[3]</sup> (<http://www.inchem.org/documents/ehc/ehc/ehc222.htm>). It broadly included the biological features that act as an indicator of normal biological or pathogenic process or pharmacological response to a therapeutic intervention<sup>[4, 5, 6]</sup> (Naylor, 2003; Wells and Sleeper 2008; Strimbu and Tavel, 2010). It upholds various tools and technologies that aid in gaining a detail comprehension in the aspects of disease prediction, cause, diagnosis, progression, regression or consequence of treatment<sup>[7]</sup> (Mayeux, 2004). Characteristically a biomarker should be (a) accurate, sensitive and specific (b) able to differentiate diseased and non-diseased population (c) quantified reliably from accessible body fluids or tissues and (d) measured reproducibly under different conditions<sup>[8,9]</sup> (Aronson, 2005; LaBaer, 2005). They are widely used in clinical drug development and research including pharmacokinetic/pharmacodynamic modeling and clinical trial simulation<sup>[10]</sup> (Krejsa and Freeman, 2009). Further, they are appreciably used in the field of genetic disorders, cancer and diagnostic and management of cardiovascular disease, infections, immunological disorders<sup>[11, 12]</sup> (Hulka, 1990; Perera and Weinstein, 2000) and also as surrogate outcomes<sup>[13, 14, 15, 16]</sup> (Ellenberg and Hamilton, 1989; Wittes *et al.*, 1989; Aronson, 2005; Fleming and Powers, 2012). Biomarkers are broadly classified as:

- Type 0 (natural history marker), Type 1 (drug activity biomarker – in turn divided<sup>[14]</sup> (Jain, 2010) in to efficacy, mechanism and toxicity biomarkers and Type 2 (surrogate biomarker) - based on genetics and molecular biology methods<sup>[17]</sup> (Sahu *et al.*, 2011)
- Prognostic, Predictive and Pharmacodynamic biomarker<sup>[18]</sup> (Drucker and Krapfenbauer, 2013)
- Biomarkers of exposure (antecedent) and disease<sup>[7]</sup> (Mayeux, 2004)
- Biomarkers of risk, diagnosis/trait, state/acuity, stage, treatment response and prognosis<sup>[19]</sup> (Davis *et al.*, 2015)
- Diagnostic, prognostic and theranostic<sup>[20]</sup> (Weickert *et al.* 2013)

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- f. Imaging and molecular biomarkers (non-imaging) (can be classified based on different parameters, including their characteristics, such as imaging biomarkers (computed tomography, positron emission tomography, magnetic resonance imaging) or molecular biomarkers

### Some of the biomarkers involved in various systems of small animals are

#### Urinary Biomarkers

Preceding to renal failure, chronic progressive kidney disease (CKD) is considered as one of the cause for morbidity and mortality in dogs. This disease is often a result of primary glomerular diseases [21, 22] (Macedougall *et al.*, 1986; Müller-Peddinghaus and Trautwein, 1977). Some of the urine proteins indicators are retinol binding protein, b2-microglobulin, N-acetyl-b-D-glucosaminidase, neutrophil gelatinase-associated lipocalin and immunoglobulin G in dogs where retinol binding protein is considered as promising one for diagnosis and monitoring of tubular injury and dysfunction in dogs [23] (Nabity *et al.*, 2012). Transitional cell carcinoma or prostatic carcinomas, naturally occurring lower urinary tract malignancy in dogs [24, 25, 26] (Mutsaers *et al.*, 2003; Knapp *et al.*, 2014; Fulkerson *et al.*, 2015) can be identified by using potential biomarker S100A8/A9-to-S100A12 ratio [27] (Heilmann *et al.*, 2014). Similar markers were also used as a diagnostic tool in humans where the patients with urinary tract infections were identified [28, 29, 30] (Tolson *et al.*, 2006; Kim *et al.*, 2011; Ebbing *et al.*, 2014). Recently, a multiplex biomarker approach for the diagnosis of transitional cell carcinoma from canine urine was made where the combination of ultra-filtration and LC-MS/MS was found as a favorable one for characterizing the proteome of canine urine [31] (Bracha *et al.*, 2014). Also, urinary heat shock protein-72 to urinary creatinine ratio (uHSP72/uCr) was also used as a diagnostic and prognostic marker in acute kidney infection in dogs [32] (Bruchim *et al.*, 2017)

#### Cardiac Biomarkers

Most frequently used cardiac biomarkers are N-terminal pro-brain natriuretic peptide (NT-pro BNP) and cardiac troponins I and T (cTnI and cTnT) besides atrial natriuretic peptide, endothelin, tumor necrosis factor  $\alpha$  (TNF  $\alpha$ ) and C-reactin protein [33,34,35,36] (Shaw *et al.*, 2004; Gu *et al.*, 2006; Saunders *et al.*, 2009; Fonfara *et al.*, 2010). Of which, cardiac troponins are cardiomyocyte injury specific bio-marker, while natriuretic peptides are used to assess myocardial stress. The natriuretic peptides are a class of hormones controlling body fluid homeostasis (via natriuretic and diuretic effects) and influencing the renin-angiotensin-aldosterone mechanism. Endothelin and Big endothelin-1, used to diagnose dogs with dilated cardiomyopathy, congestive heart failure and myocardial ischemia [37, 38] (O'Sullivan *et al.*, 2007; Prosek *et al.*, 2007). This assay ease in differentiating cardiac from non-cardiac cause of respiratory signs and in detection of preclinical cardiomyopathy [39] (Oyama, 2013).

#### Hepatic Biomarker

Many biochemical indicators (plasma alanine aminotransferase activity, alkaline phosphatase activity and bile acid concentration) were used to diagnose liver disease in dogs. However, they cannot differentiate parenchymal, biliary, vascular, and neoplastic hepatobiliary diseases. Some of the serum biomarkers are TGF $\beta$ -1, tumor necrosis factor- $\alpha$ , angiotensin II, hyaluronic acid, procollagen peptides, MMPs,

TIMPs, chitinase-3-like protein 1. The dogs with hepatic diseases such as cirrhosis/ fibrosis exhibited increased concentration of serum hyaluronic acid [40, 41] (Kanemoto *et al.*, 2009; Glinska-Suchocka *et al.*, 2015), TGF $\beta$ -1 and procollagen type III N-terminal peptide [42, 43] (Spee *et al.*, 2006; Neumann *et al.*, 2008) unlike in study by [44] Lidbury *et al.* (2016). Recently, in dogs, serum microRNA was used as a non-invasive biomarker to detect hepatobiliary diseases where considerable amount of microRNAs were noticed in bile [45, 46, 47] (Dirksen *et al.*, 2016; Verhoeven *et al.*, 2016; Eulenberg and Lidbury, 2018).

#### Neurobiomarkers

Biomarkers causing nervous system damage are present in cerebrospinal fluid (CSF) or blood serum where in the CSF, they are derived from central nervous system (CNS), parenchyma or systemic circulation. [48] Ptezold *et al.* (2007) classified biomarkers present in the CSF as (i) pigments (ii) metabolic biomarkers (iii) cell-type specific biomarkers (including neurofilaments, glial fibrillary acidic protein, myelin proteins, myelin basic protein (MBP), and circulating nucleic acids) (iv) CNS specific biomarkers (v) free radicals (vi) inflammatory and (vii) immunological biomarkers. Cell specific biomarkers are Glial fibrillary acidic protein, phosphorylated axonal form of the neurofilament subunit NF-H and Myelin basic protein [49, 50, 51] (Usui *et al.*, 1994; Oji *et al.*, 2007; Weiss *et al.*, 2009) and Central nervous system-specific biomarkers are S100 $\beta$ , Neuron specific enolase (NSE), Tau protein, Alpha II-spectrin, Ubiquitin carboxy-terminal hydrolase L1 and Creatine kinase BB [52] (Płonek *et al.*, 2016). These biomarkers are studied in experimental dogs by induction of neurological disorders. However, there is a wide scope of their usage in developmental, auto-immunological and neoplastic CNS and PNS disorders.

#### Enterobiomarkers

Ca<sup>2+</sup> binding proteins of S100/calgranulin family such as Canine calprotectin, the S100A8/A9 protein complex and S100A12 are mainly associated with acute and chronic enteritis with malignant transformation [53] (Heilmann and Allenspach, 2017). An 11-fold increased expression of mucosal S100-mRNA was found in a dog with chronic inflammatory enteropathy [54] (Wilke *et al.*, 2012) and the fecal calprotectin and S100A12 were correlated with the clinical disease activity [27, 55] (Otoni *et al.*, 2012; Heilmann *et al.*, 2014). However, they are not in current use [56,57,58,59,60] (Mc Cann *et al.*, 2007; Anfinson *et al.*, 2014; Berghoff *et al.*, 2014; Heilmann *et al.*, 2014; Sattasathuchana *et al.*, 2015; Heilmann *et al.*, 2016).

#### Summary

Biomarkers are used as surrogate outcomes and are potently used in basic and clinical research. Their use as surrogate endpoint need thorough re-evaluation. They provide information of cascade of events happening and on molecular mechanism involved in normal and abnormal pathophysiology. They play critical role in processes of development of drug and in the field of biomedical research. In order to deepen our knowledge in the aspects of normal physiology and disease therapy, the relationship between biological process and clinical outcome is a prerequisite.

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