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Review

Urine as a source for clinical proteome analysis: From discovery to clinical application ☆

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ABSTRACT

The success of clinical proteome analysis should be assessed based on the clinical impact following implementation of findings. Although there have been several technological advancements in mass spectrometry in the last years, these have not resulted in similar advancements in clinical proteomics. In addition, application of proteomic biomarkers in clinical diagnostics and practical improvement in the disease management is extremely rare. In this review, we discuss the relevant issues associated with identification of robust biomarkers of clinical value. Urine appears to be an ideal source of biomarkers, for theoretical, methodological, and practical reasons. Therefore, this review is focused on the search for biomarkers in urine within the last decade. Urine can be used for non-invasive assessment of a variety of diseases including those affecting the urogenital tract and also other pathologies such as cardiovascular disease or appendicitis. We also discuss the importance of data validation, an essential step in translating biomarkers into the clinical practice. Furthermore, we examine several examples of apparently successful proteomic biomarker discovery studies and their implications for disease diagnosis, prognosis, and therapy evaluation. We also discuss some current challenges in this field and reflect on future research prospects. This article is part of a Special Issue entitled: Biomarkers: A Proteomic Challenge.

1. Introduction

Extensive efforts to improve sample preparation, performance of the state-of-the-art mass spectrometers for peptide identification and quantification, and release of new software have helped to establish high throughput protein analyses of multiple samples [60,86,129]. The aim of most clinical proteomic studies is the identification of biomarkers for a specific disease. Biomarkers are defined as a laboratory measurement that reflects the activity of a disease process [72]. The process of biomarker discovery and validation requires a stringent workflow from the collection of well-defined clinical samples to the validation of the markers found in large-scale multicentre studies. In general, proteomic biomarker studies consist of 4 main steps: 1) sample preparation (e.g. protein extraction), 2) (semi-)quantitative analysis, 3) data analysis, and 4) validation. Fig. 1 presents a typical biomarker discovery workflow: after protein extraction and preparation of the samples, the analysis is performed using the chosen approach. Several tools can be used to interpret the data; in the last step, markers are validated, in the past using immunoassay techniques such as Enzyme-linked Immunosorbent Assay (ELISA) or Western blotting (WB), lately also by (targeted) mass spectrometric analysis like capillary electrophoresis coupled to mass spectrometry (CE–MS) or selected reaction monitoring (SRM). However, following these essential steps does not guarantee success in biomarker discovery. In fact, despite the technological progress in mass spectrometry instrumentation, the contribution of proteomics to the clinical practice is still modest. [2]. Issues that appear to be responsible in part for a lack of translation have been described in detail in [111], these include poorly defined clinical need, moderate sample sizes, and also economical considerations.

Abbreviations: 2DE, two dimensional electrophoresis; AKI, acute kidney injury; ANN, artificial neural networks; ATN, acute tubular necrosis; BPN, brain natriuretic peptide; CAD, coronary artery disease; CE–MS, capillary electrophoresis coupled to mass spectrometry; CKD, chronic kidney diseases; CT, computed tomography; DIGE, difference gel electrophoresis; DN, diabetic nephropathy; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; EMA, European Medicines Agency; EuroKUP, European Kidney and Urine Proteomics; FDA, Food and Drug Administration; GvHD, graft-versus-host-disease; HF, heart failure; HPLC, high pressure liquid chromatography; HSCT, hematopoietic stem cell transplantation; IgAN, immunoglobulin A nephropathy; iTRAQ, isobaric tags for relative and absolute quantitation; LC, liquid chromatography; LRG, leucine-rich-2-glycoprotein; LTQ-FT, linear ion trap quadrupole and Fourier transform; LV, left ventricular; MALDI, matrix-assisted laser desorption/ionization; MI, myocardial infarction; MS/MS, tandem mass spectrometry; MS, mass spectrometry; NIHSS, National Institutes of Health Stroke Scale; PC, prostate cancer; PSA, prostate-specific antigen; PRA, perenal azotemia; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SCr, serum creatinine; SRM, selected reaction monitoring; UPJ, ureteropelvic junction obstruction; WB, western blot

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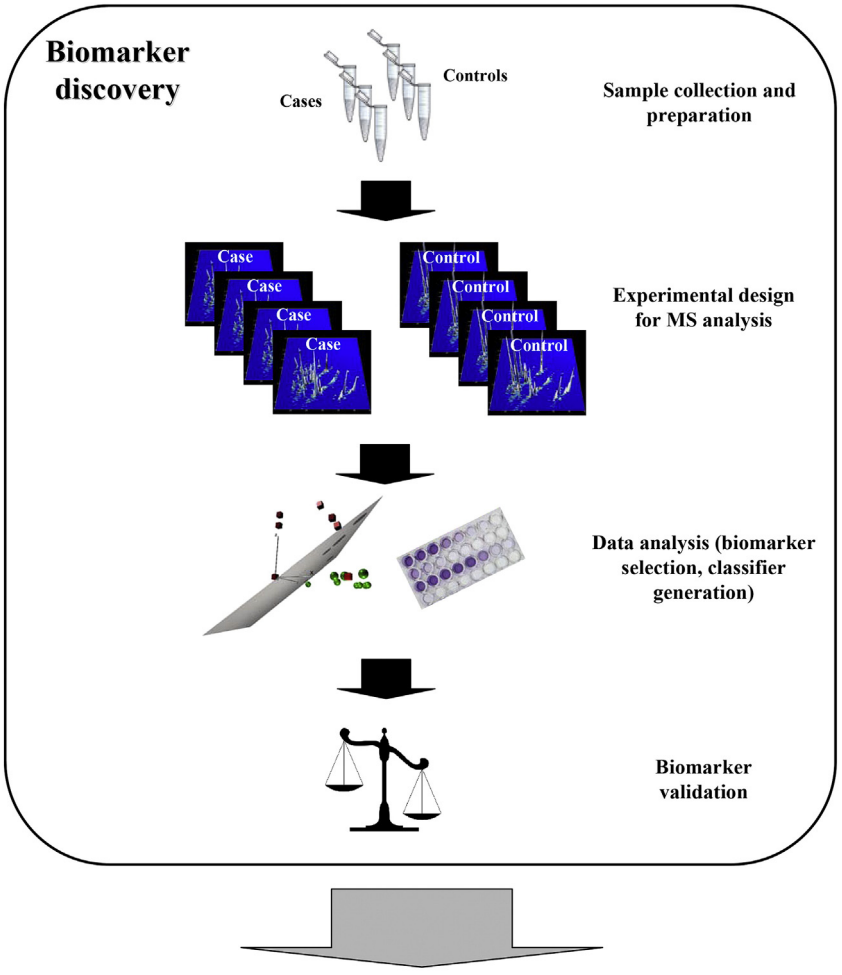

Fig. 1. Overview of the biomarker discovery experiment workflow. Proteomic experiments can be divided in four main steps: protein extraction and preparation, experimental design for MS analysis, data analysis (including biomarker selection and classifier generation), and data validation. The next step after the biomarker discovery will be the clinical application of the biomarkers.

This review will focus on the search for biomarkers in urine, representing a very well suited non-invasive source of proteome biomarkers. The use of this source allows extensive mining of the biological variability, avoiding the instability of other human body fluids such as plasma or blood. We will also examine the currently available screening approaches and discuss their drawbacks and advantages.

2. Urine as a source of biomarkers

In the past, visual appearance or smell of the urine was used by physicians to diagnose renal pathologies. For example, ancient physicians knew that the urine from patients with diabetes tastes sweet [84]. Investigation of urine in clinical diagnosis is second to that of plasma. Urine generated as a result of filtration of plasma by glomeruli in the kidneys, retaining the majority of the proteins. The excreted urine contains water, glucose, salt and proteins derived from plasma or the urogenital tract [32]. Hence, urine can be viewed as modified ultrafiltrate of plasma and proteins derived from the organs involved in its production and excretion (kidney and urinary tract), with protein concentration approximately 1000-fold lower than in plasma itself [56]. Some molecules are detectable in urine only when their plasma levels are high enough to exceed the capacity of tubular cells to reabsorb them [64]. Of note: the concentration of the urinary proteome is highly variable. Hence, not the absolute, but relative concentration, in reference to e.g. creatinine or other internal standards as describe in detail in Jantos-Siwy et al. [62] are best assessed. Changes in urinary proteome can reflect disease-related changes in the damaged tissue, not just related to urogenital diseases but also to some systemic diseases.

An international urine collection protocol created as a result of joint consensus of European Kidney and Urine Proteomics (EuroKUP) [173] and the Human Kidney and Urine Proteome Project (http://eurokup.org) [164] and the availability of a standard human urine sample [114] allows comparing samples from different hospitals and institutions, making the conclusions of the urine proteomic studies more relevant and independent of the sample source. These tools and protocols are generic for special cases/diseases (e.g. associated with high proteinuria, hematuria, etc) the application of specialized protocols may be recommended. For example, for the development of biomarkers for prostate cancer, the first voided urine was used, because here the prostatic fluid is preferentially present [155]. On the other hand, in the case of urine-based biomarker studies for acute inflammatory/infectious diseases, the time point of sampling (morning, afternoon, etc.) cannot be specified. Furthermore, study of exosomal or other compartmented urine proteins requires the employment of specialized protocols. An important point is that urine must be collected avoiding containers with coating which may interfere in further mass spectrometric analysis.

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