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Plasma proteomics to identify biomarkers – application to cardiovascular diseases

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ABSTRACT

There is an unmet need for new cardiovascular biomarkers. Despite this only few biomarkers for the diagnosis or screening of cardiovascular diseases have been implemented in the clinic. Thousands of proteins can be analysed in plasma by mass spectrometry-based proteomics technologies. Therefore, this technology may therefore identify new biomarkers that previously have not been associated with cardiovascular diseases. In this review, we summarize the key challenges and considerations, including strategies, recent discoveries and clinical applications in cardiovascular proteomics that may lead to the discovery of novel cardiovascular biomarkers.

1. Introduction

Cardiovascular disease (CVD) refers to any disease that affects the cardiovascular system such as cardiac disease, vascular diseases of the brain and kidney, and peripheral arterial disease. The underlying pathology is often atherosclerosis, but may also relate to other changes in the arterial systems (i.e. aneurysms, increased stiffness) and different pathologies in the heart as well (valvular alterations, cardiomyopathies etc.). CVD is the most common cause of mortality in the western countries and the prediction of cardiovascular events most often relies on the monitoring of prevalent risk factors such as smoking habits, diabetes, hyperlipidemia and hypertension [1]. Today, the cornerstone diagnosis and monitoring of CVD among individuals are clinical assessment of patients, imaging modalities together with electrocardiography (EGG). Moreover, the prevention of CVD is based on a strict management of the traditional risk factors of CVD; smoking, diabetes, hyperlipidemia, and hypertension. Although successful risk diminishing and effective treatment is available this strategy is not viable for all CVD. For example, the majority of individuals who actually develop manifest coronary heart disease have rarely more than one of the conventional risk factors and thus falls in to the low risk or intermediate risk group, which complicates the risk stratification before manifest disease occur [2]. Therefore new proteomic biomarkers are needed to support the information obtained from the conventional risk factors to improve the stratification of patients and to provide treatment at a personalized level. The recent advances in method development, bioinformatics and instrument speed, sensitivity, resolution and dynamic range of mass spectrometers used in proteomics hold the promise that the proteomics field will be a significant contributor of plasma markers enabling the prediction of early onset of CVD and treatment of the specific CVD at a personalized level.

In present work we summarize the proteomics platforms applied in cardiovascular plasma proteomics and also review the recent achievement in plasma proteomics related to cardiovascular diseases that may lead to new protein biomarkers with the potential for developing into a future clinical protein test.

2. Biomarkers for cardiovascular disease

A biomarker may serve a variety of functions when used in a clinical context. It has been defined as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention" [3] and serve various functions corresponding to different stages in disease evolution. Thus, biomarkers may be considered as indicators of disease trait (risk factor or risk marker), disease state (preclinical of clinical), or

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disease rate (progression) [4]. Therefore, biomarkers can be grouped into (1) risk stratification biomarkers (identifying the risk of developing a disease), (2) screening biomarkers (screening for subclinical diseases), (3) diagnostic biomarkers (recognizing overt disease), (4) staging biomarkers (categorizing disease severity), or (5) prognostic biomarkers (predicting future disease course, recurrence and therapy response) [3]. A biomarker for CVD is not limited to a specific molecule (e.g. protein, RNA, or a metabolite) measured in a biological sample such as body fluids (plasma, urine, cerebrospinal fluids) or tissue, but may also be a recording of physical parameters such as a person's blood pressure, electrocardiogram, echocardiogram or computerized tomography (CT) scan.

Ideally, a biomarker for a CVD should enhance the ability of the clinician to manage the patient in an optimal way. Although proteomics technologies can identify and quantify thousands of proteins and several studies have proposed a variety of protein biomarkers for CVD only a limited number of plasma or serum biomarkers have been implemented successfully into cardiology practice – most of them being of diagnostic markers (Table 1). These include, for example, the myocardium-specific structural proteins troponin I and troponin T for the diagnosis of myocardial injury [5] and B-type natriuretic peptide (BNP) that is a hormone released from the ventricles during myocardial stress and used for the diagnosis and management of acute congestive heart failure. They form the cornerstone for the diagnosis of myocardial infarction and myocardial stress. Moreover, it has been demonstrated that – when measured with high sensitive assays – these markers have the potential to be risk prediction markers. In fact, several studies have demonstrated that within the reference level there is a risk gradient corresponding to an increased risk of an adverse cardiac event with increased troponin levels [6–8]. However, none of the routinely used protein biomarkers for CVD have initially been found in proteomics discovery experiments.

3. The plasma proteome – the never-ending challenge

Circulating blood is a convenient source for sampling and can be collected without any interventional procedures. This is probably the main reason that most proteomic biomarker discovery studies have been done on circulating biomarkers and not on site-specific blood samples. Although advantageous, this has also some serious drawbacks; the discovered biomarkers – unless organ or disease-specific like troponin T for myocardial damage – may reflect the disease state of any organ in the body. Moreover, the circulating biomarker will also suffer from a poor signal-to-noise ratio. This may lead to poor predictive value and limited clinical use of the identified biomarker. Signal-to-noise ratio can be increased by sampling the blood distal to the diseased tissue, where the biomarker concentration most probably will be highest [9]. This strategy for blood sampling requires an interventional procedure and will not be feasible in most plasma biomarker discovery projects.

The most challenging feature of the plasma proteome with respect to proteomics biomarker discovery, however, is the presence of an exceptional wide concentration range of the proteins comprising this proteome. Today, more than 10,000 proteins have been identified in human plasma (http://www.plasmaproteomedatabase.org). The extreme concentration range of proteins (12 orders of magnitude) ranging from >600 μM to the low femtomolar level per litre of blood [10] and the set of extremely high abundant proteins (such as serum albumin, immunoglobulins and complement factors) that constitute more than 99% of the total protein amount makes the plasma proteome to the most heterogeneous and complex sub-proteome of the human body [11,12]. As a consequence, discovering and validating novel protein biomarkers for CVD such as atherosclerosis in plasma is very challenging. This dramatically impacts the results that can be obtained by proteomic discovery experiments on plasma samples that should not be compared with the results from proteomic discovery studies on tissue or cell models with the respect to number of identified and quantified proteins. These facts have driven the evolvement of several proteomic experimental approaches that have been applied for the discovery of new plasma biomarkers for CVD diseases. Many of these have applied highly advanced analytical strategies to overcome the two major obstacles in plasma discovery proteomics; the complexity of plasma (number of proteins and the presence of many post-translational modified proteins) and the extreme dynamic range of plasma proteins confining the number of identifiable and quantifiable proteins. Another important issue is the number of

Table 1
Commonly used diagnostic and prognostic plasma protein biomarkers for cardiovascular diseases.

Protein biomarker	Origin	Pathology	Use	References
Brian natriuretic peptide (BNP)	Secreted from membrane granules in the cardiac ventricles – cardiac specific	Congestive heart failure, acute coronary syndrome	Diagnostic, prognostic	[61,62–64]
Troponin T (TnT)	Cardiac-specific isoform, released upon myocardial injury	AMI, myocardial injury	Diagnostic	[65,66]
Troponin I (TnI)	Cardiac-specific isoform, released upon myocardial injury	AMI, myocardial injury	Diagnostic	[65,66]
Creatinine kinase/CK-MB	Primarily released from the cardiac muscle-not tissue specific	AMI, myocardial necrosis	Diagnostic	[67]
C-reactive protein	Liver-produced non-specific acute-phase reactant	Atheromatic plaque vulnerability, coronary artery disease, coronary vasospasm, left ventricular dysfunction, angina pectoris, myocardial infarction.	Screen for risk of CVD	[68–70]
Myoglobin	Cardiac muscle – tissue unspecific	Tissue necrosis, AMI, MI	Diagnostic	[71]
Apolipoprotein (A)	Liver, intestine	None	Prognostic, screen for risk of CVD	[72–74]
Apolipoprotein (a)	Liver, intestine	None	Prognostic, screen for risk of CVD	[72–74]
Apolipoprotein-B100	Liver	None	Prognostic, screen for risk of CVD	[75,76]