Diagnostic biomarkers in cardiovascular disease: the proteomics approach

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This editorial refers to ‘Unbiased plasma proteomics for novel diagnostic biomarkers in cardiovascular disease: identification of quiescin Q6 as a candidate biomarker of acutely decompensated heart failure’†, by A. Mebazaa et al., on page 2317

Identification of the correct diagnosis as early as possible after presentation at the Emergency Department is particularly important in patients with acute cardiovascular disease, as any delay in the appropriate therapeutic countermeasures may result in potentially life-threatening aggravation of the disease. The skills and analytical perception of the experienced clinician may doubtless be among the most important means to establish the right diagnosis and to initiate the correct therapy. While intuition is essential for any good doctor in the diagnostic work-up, objective and verifiable measures are increasingly required in the time of evidence-based guideline recommendations and of flow chart-guided decision trees. Thus, the era of biomarkers as qualitative, or—even better—quantitative indicators of the problem has emerged. Within less than two decades, biomarkers have been established as indispensable tools in acute cardiovascular diagnostics. Moreover, not only is the initial diagnostic decision increasingly based on biomarker evaluation, but also upstream (risk prediction) and downstream (prognostic estimation) characteristics of the disease and eventually therapeutic monitoring are increasingly relying on biomarker assessments. Technological advances in genomics, proteomics, and non-invasive imaging have led to increasing numbers of biochemical factors and variables being tested for their potential role as biomarkers, and hardly a week goes by without a novel biomarker being proposed for clinical use as diagnostic, prognostic, or therapeutic guidance.

The critical evaluation of the multitude of proposed biomarkers is increasingly important in order to identify those with the strongest, most accurate, and reliable information. Accordingly, benchmarks for the assessment of novel biomarkers have been proposed to prove clinical usefulness.1 These include the criteria of the quality of the assay (precision, availability, costs), the added value of the novel information on top of established methods, and the quantifiable value to support clinical decision-making. Characteristics of novel biomarkers were summarized in major categories of discrimination, calibration, and reclassification;2 each addressed by different analytical and statistical methods. A practical framework for the critical appraisal and stepwise evaluation of novel biomarkers from the proof of concept stage to clinical and commercial applicability has been outlined (Table 1).3

Acute decompensated heart failure (ADHF) is one of the prime examples for successful implementation of biomarkers in diagnostic and therapeutic concepts along this evaluation pipeline. Patients admitted to hospital with signs of acute fluid overload present with cardinal symptoms such as dyspnoea, congestion, elevated jugular venous pressure, pulmonary rales, and oedema. Accordingly, a number of biomarkers have been established in clinical practice that reflect the decompensated haemodynamic status, myocardial strain, or other organ-specific injury or acute global stress response.4 Natriuretic peptides (NPs) are the current reference biomarkers and gold standard for diagnostics and therapy monitoring of heart failure. Notably, NPs, like many other biomarkers in clinical practice (such as troponins, copeptin, C-reactive protein, neutrophil gelatinase-associated lipocalin, galectin-3, etc.), were identified based on pathophysiological considerations and hypothesis-driven efforts.

In contrast, Mebazaa et al.5 now present results on a novel candidate diagnostic biomarker for ADHF that has been derived by a hypothesis-free approach based on a novel clinical proteomics biomarker discovery platform. Out of 103 differential proteins, a selection process via several steps resulted in the identification of the protein quiescin Q6 sulfhydryl oxidase 1 (QSOX-1) as the most promising candidate to identify patients with ADHF. Notably, in a hypothesis-driven selection, this protein would not have been identified as a potential biomarker candidate for heart failure. In fact, the exact pathophysiological role of QSOX-1 in the setting of ADHF is far from well established. The QSOX1 protein is a...
FAD-linked quiescin/sulphhydryl oxidase that catalyses the oxidation of sulphhydryl groups in peptide and protein thiols to disulfide bonds with the reduction of oxygen to hydrogen peroxide. QSOX-1 RNA has been identified in a range of tissues including myocardium (left atrium and left ventricle), but also in smooth muscle cells, monocytes, placenta, lung tissue, testes, prostate, and thyroid, and, in low abundance, as well in several other tissues and organs. QSOX1 has been implicated in protein folding, production of extracellular matrix, redox regulation, protection from apoptosis, angiogenesis, and cell differentiation and, recently, in the progression of atherosclerosis.6 The kinetics of QSOX1 expression suggest an antioxidative protective role for QSOX1 rather than an involvement in apoptosis.7 While the potential link between QSOX1 and myocardial injury may thus be explained, the current literature did not link QSOX1 to heart disease or acute haemodynamic decompensation.

Mebazaa et al. show in a well-designed series of animal model and human studies, that the expression of QSOX1 specifically in the left ventricle and left atrium corresponds to the degree of pressure overload, subsequent hypertrophy, and development of ADHF. Validation of the identified protein was performed in a multicentre cohort of patients with acute dyspnoea of cardiac origin vs. non-cardiac origin (true control group), patients with stable heart failure, and healthy controls (secondary control groups). Up-regulation of myocardial QSOX1 expression was seen in ADHF, but remained low in both patients with acute dyspnoea of non-cardiac origin and in patients with stable compensated heart failure. The diagnostic performance of QSOX1 on its own [area under the curve (AUC) 0.86, 95% confidence interval (CI) 0.79–0.92] was in the range of brain natriuretic protein (BNP) and N-terminal proBNP (NT-proBNP) in the entire study population, but showed a significant added value when combined with BNP (AUC 0.95, 95% CI 0.92–0.98).

There seem to be even some advantages of QSOX-1 over the performance of NPs as QSOX-1 was largely unaffected by impaired renal function. Moreover, the discriminatory strength of QSOX-1 was particularly present in the grey zone of mildly elevated NP levels. Indeed, while BNP lost its predictive power in this borderline zone (BNP 100–400 pg/mL: AUC 0.63, 95% CI 0.45–0.82), the diagnostic value of QSOX-1 was not different from that in the overall cohort (AUC 0.91, 95% CI 0.83–1).

If these characteristics of QSOX1 can be confirmed, it might be a promising addition to the NPs in the diagnostic work-up of ADHF that circumvents some of the limitations seen with NPs. According to the American Heart Association criteria for evaluation of novel markers,2 the study addresses the early phases of evaluation (Table 1).

A number of questions emerged from the promising results on this novel biomarker. It needs to be established whether other confounding factors such as age, adiposity, or other co-morbidities may interfere with QSOX1 expression just as with NPs. The kinetics of QSOX1 after injury need to be established in order to identify the time course of the plasma profile. In the study of Mebazaa et al., blood samples were taken at enrolment but it would be interesting to know the time span from onset of symptoms or from admission to hospital. Identifying the pathophysiological role of QSOX1 in the event of the acute failing heart is warranted to gain insight into hitherto unrecognized mechanistic concepts of ADHF pathophysiology. Ultimately, the remaining steps in the evaluation of novel biomarkers7 need to prove the clinical applicability of QSOX1.

While ADHF and acute myocardial ischaemia are well established diseases for the use of biomarkers in early diagnostic work-up, another acute vascular condition, namely stroke, is running alarmingly behind in the successful implementation of biomarkers for clinical use. A number of reasons could be addressed why stroke and transient ischaemic attack are more challenging for the application of biomarkers, including the complexity of the ischaemic cascade and presence of the blood–brain barrier. Despite increasing research in this field and promising early reports on prognostic prediction,8–10 current stroke diagnosis remains strongly reliant on clinical assessment and cerebral imaging. Implementing of biomarkers for stroke diagnostics would be a major step forward to catch up with myocardial ischaemia and ADHF.

**Conflict of interest:** none declared.

### Table 1 Phases of evaluation of a novel marker

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<th>Phase</th>
<th>Applicable for QSOX1&lt;sup&gt;3&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>1 Proof of concept—do novel marker levels differ between subjects with and without disease?</td>
<td>Yes</td>
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<td>2 Prospective validation—does the novel marker predict development of disease in a prospective cohort or nested case–cohort study?</td>
<td>Yes</td>
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<td>3 Incremental value—does the novel marker add predictive information to the established risk marker?</td>
<td>Yes</td>
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<td>4 Clinical utility—does the novel marker change predicted risk sufficiently to change recommended therapy?</td>
<td>To be studied</td>
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<tr>
<td>5 Clinical outcome—does the use of the novel marker improve clinical outcomes, especially when tested in randomized clinical trial?</td>
<td>To be studied</td>
</tr>
<tr>
<td>6 Cost-effectiveness—does the novel marker improve clinical outcomes sufficiently to justify the additional costs of testing and treatment?</td>
<td>To be studied</td>
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Adapted from Hlatky et al., with kind permission from the American Heart Association.3
References