Evidence-based evaluation of proteomic biomarkers: from unmet medical needs to clinical application

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What is clinical proteomics?

1. The quantitative measurement of multiple proteins in a biological sample that is related to human disease.

2. The identification and relative quantification of proteins in a biological sample that is related to human disease.

3. The quantitative measurement of proteins in human samples ...that will ...lead to an improvement in the care of patients.

Clinical Chemistry 2014;60(10):1258–1266
Proteomics: “The Knight in Shining Armour”

- Overcomes problems with immunoassays:
  - analytical sensitivity
  - analytical specificity/selectivity
  - interferences, cross-reactivity
  - lot-to-lot variation
  - poor harmonization/standardization

- Multiplexing capacity

- More granular identification of one’s proteotype – “move disease-care to healthcare”

- Translation of novel protein biomarkers to clinically useful medical tests

Workflow for quantitative clinical proteomics

Imagine:
You are a specialist in a laboratory of a busy metropolitan hospital.
A scientist from HUPO comes to you and says:

“We have just discovered a panel of protein biomarkers (New Wonder®) that may help predict coronary artery disease (CAD).

We wonder if it could be a much better and earlier test than the test panel of TnT, NT-pro-BNP, lipids and CRP (Old Bore®) that you are currently using for risk prediction of CAD?
Protein signature predictive of a high risk plaque

Standardized mean protein levels

Protein signature predictive for CCTA-defined absence of coronary atherosclerosis

Standardized mean protein levels

- Presence of coronary plaque
- Absence of coronary plaque

RESULTS: In the 10-year period analyzed, 4257 articles cited the 107 diagnostic studies; 118 (2.8%) were diagnostic studies of the same test, and of these papers, 25 (21.2%) did not constitute progress toward validation of the test for use in clinical practice (potential research waste). Of the 107 molecular- or “omics”-based tests described in 2006, only 28 (26.2%) appeared to have made progress toward clinical application. Only 4 (9.1%) of 44 proteomics-based tests had made progress toward clinical application.
How would you evaluate this test before implementing it?
Special report

From biomarkers to medical tests: The changing landscape of test evaluation

Andrea R. Horvath a,b,*, Sarah J. Lord b,c,l, Andrew StJohn d, Sverre Sandberg e, Christa M. Cobbaert f, Stefan Lorenz g, Phillip J. Monaghan h, Wilma D.J. Verhagen-Kamerbeek i, Christoph Ebert j, Patrick M.M. Bossuyt k, For the Test Evaluation Working Group of the European Federation of Clinical Chemistry Laboratory Medicine
Cyclical framework for the evaluation of *in vitro* medical tests

Key components of the test evaluation process are driven by the clinical need of using a test in the clinical pathway.
SUMMARY: The current biomarker pipeline is too prone to failures. Consideration of clinical needs should become a starting point for the development of biomarkers.
The Test Evaluation Cycle

Is there an unmet clinical need and is there an effective intervention?

Unmet clinical need: any missing or inadequately performing component of a clinical pathway.
Unmet clinical need for CAD biomarker

- Traditional risk stratification markers have modest predictive value for CAD.
- Lipid lowering therapy has not provided the expected benefits of prevention of AMI.
- There is a need for novel biomarkers predictive of CAD.
The checklist is intended to achieve more efficient biomarker development and translation into practice [2].
Discussion

Biomarker development targeting unmet clinical needs

Phillip J. Monaghan a,*, Sarah J. Lord b,c, Andrew St John d, Sverre Sandberg e,f,g, Christa M. Cobbaert h, Lieselotte Lennartz i, Wilma D.J. Verhagen-Kamerbeek j, Christoph Ebert k, Patrick M.M. Bossuyt l, Andrea R. Horvath m,n, for the Test Evaluation Working Group of the European Federation of Clinical Chemistry and Laboratory Medicine

https://elearning.eflm.eu/
Clinical pathway mapping:
What is the purpose and role of the test?
Test role and purpose in the clinical pathway

Comparative accuracy: assessing new tests against existing diagnostic pathways
Patrick M Bossuyt, Les Irwig, Jonathan Craig, Paul Glasziou

- **Existing situation**
  - Population
    - Initial tests
      - Existing test
        - +
        - -

- **Replacement**
  - Population
    - Initial tests
      - New test
        - +
        - -

- **Triage**
  - Population
    - New test
      - +
      - -
      - Existing test
        - +
        - -

- **Add-on**
  - Population
    - Initial tests
      - Existing test
        - +
        - -
        - New test
          - +
          - -

Bossuyt et al. BMJ 2006
Key messages

Before a new test is fully evaluated, the
- unmet clinical needs,
- intended purpose (screening, diagnosis, monitoring, etc.),
- role (add on, replacement, triage),
- population,
- healthcare setting in which the test is intended to be used,
- condition that is intended to be managed with the use of the test,
- procedures for evaluating these, and
- potential final outcomes of testing
must be clearly defined.

All the above are best mapped out by drawing the clinical pathway

Bossuyt, 2010
The Test Evaluation Cycle

- The ability of an assay to correctly detect or measure a particular analyte/measurand.
- Preanalytical considerations
- Analytical sensitivity/specificity
- Limit of detection/quantitation
- Measurement range
- Linearity
- Metrological traceability
- Imprecision and trueness
CLSI C62-A:
A Best Standard for Clinical Mass Spectrometry
Kara L. Lynch¹*

News & views
Quality requirements for quantitative proteomics

Nico P.M. Smit¹, Irene van den Broek¹, Fred P.H.T.M. Romijn¹, Martin van den Heuvel², André M. Deelder³, Yuri E.M. van der Burgt³, Arnoud van der Laarse¹, Christa M. Cobaert²,³,*
Increasing recognition of the importance of pre-analytical steps in the bottom-up approach to measuring protein biomarkers by LC-MS/MS

Pre-analytical considerations:
- patient preparation
- specimen collection
- transport
- sample handling and stability
BACKGROUND: Several plasma proteins have been suggested as markers for a variety of cardiovascular conditions but fail to qualify in independent patient cohorts. This may relate to interference of medication on plasma protein concentrations.

METHODS:
• proteomic approach to quantify several hundred proteins in a discovery study using individual plasma samples after an AMI before and after heparin administration
• validated findings in 500 patients with suspected STEMI at admission, of whom 363 were treated with heparin before admission.

RESULTS: In the discovery study, 25 of 653 identified plasma proteins displayed a changed concentration after heparin administration; 14 proteins changed significantly among heparin-treated patients in the validation study.

CONCLUSIONS: Medications such as heparin administration given before blood sampling may confound biomarker discovery and should be carefully considered in such studies.
CTAD tubes to avoid platelet activation

RST tubes for rapid clotting

Protease inhibitor tubes
HUPO Proteomics Standards Initiative

HUPOST: Where does your interest in quality control come from?

DT: I’ve been in bioinformatics for twenty years now, and I have come to realize that black boxes harm users as well as bioinformaticists. I have therefore started to dedicate quite a bit of time to bioinformatics education. Many researchers in proteomics and metabolomics simply do not know the extent to which technical variation may be hiding biological variation in their experiments, so quality control represents a look inside the black box of these important methods.

HUPOST: Can you tell us a bit more about the HUPO PSI Quality Control Working Group?

DT: The Quality Control Working Group (QC WG) is the first new Working Group of the HUPO Proteomics Standards Initiative in over a
Summary

This chapter contains sections titled:

- Introduction
- Materials and methods
  - HUPO reference sample collection protocol
  - Differential peptide display
  - Stability studies and SELDI analysis
Key messages

Analytical performance specifications

- should reflect clinical needs

- can be based on 3 different models:
  1/ outcomes
  2/ biological variation
  3/ state-of-the art;

- should be set at a level that achieves net health benefit for patients at reasonable costs;

- should be tailored to the purpose and role of the test in a well defined clinical pathway;

- should be commensurate with the impact of the laboratory test on subsequent medical decisions and actions

High quality analytical performance does not guarantee high quality clinical action or patient compliance or that the chosen treatment will be effective.

The opposite is also true; poor analytical performance of a test that plays a small part in a complex clinical pathway may not necessarily lead to adverse or unfavourable outcomes.
The Test Evaluation Cycle

the ability of a biomarker to detect patients with a particular clinical condition or in a physiological state

- How well does it work in practice?
- In what subset of patients?
- Is it really better than Old Bore®?
- How alternative tests compare?
Comparative diagnostic accuracy

Is New Wonder® better than Old Bore® to diagnose CAD?

Consecutive patient series suspected for target condition

Index test

Comparator index test

Reference standard

Blinded cross-classification

Out-patients with stable angina & suspected CAD

New Wonder®

Old Bore®

Coronary CT angiography (CCTA)

CAD

Graphics adapted from Patrick Bossuyt, Amsterdam
A subset of 35 proteins using a machine learning model was predictive of the presence of high-risk plaques (AUC of 0.79 ± 0.01), and outperformed prediction with generally available clinical characteristics (AUC = 0.65 ± 0.04, p<0.05).
A subset of 34 proteins using a machine learning model was predictive for the absence of CAD (AUC=0.85±0.05), and outperformed prediction with generally available clinical characteristics (AUC = 0.70 ± 0.04, p<0.05).
The Test Evaluation Cycle

The ability of a test to improve outcomes relevant to the individual patient or patient population.

- How does the test result affect patient’s health and quality of life?
- What are the benefits and harms and risks of testing?
Limitations of the findings needing further validations

dataset. Although we believe these techniques corroborate the validity of our findings, **external validation in larger cohorts is mandatory to confirm the predictive value of the identified biomarker subsets. Similarly, validation with coronary events was not possible in this cohort due to the relatively small sample size. Instead, the presence of high-risk plaque was used as a surrogate for coronary events. It must however be noted that although high-risk plaque has been extensively associated with adverse outcome, not all high-risk plaques cause events [1,2,14]. Validation with coronary events in an external cohort is therefore crucial to validate our findings. Third, our study population consisted of symptomatic patients with intermediate risk of CAD. Therefore, our prediction model may be less suitable for subjects in a low-risk, primary prevention cohort. Fourth, our predictive methodology focused on the performance of the developed machine learning model involving**
Diagnostic RCT to assess clinical effectiveness

Do patients who undergo the new test fare better (in terms of health outcomes) than those who have the old test?

Patients with suspected target condition

New Wonder®

Old Bore®

Outcome

Outcome
The link between testing and health outcomes is indirect and is dictated by the clinical pathway.

Improved diagnostic or prognostic accuracy of a test or the effects of the test on medical decisions are not necessarily indicative of patient benefit.

Direct evidence of clinical effectiveness would be ideal but, under specific circumstances, indirect evidence is sufficient for regulatory approval of a new biomarker.
The Test Evaluation Cycle

- What does it cost?
- Can we afford it?
- Could it save money?
- Does this require investment into new technology and staff skills?
- How will this impact on work-flow and budget?
- Is it good value for money?

Assessment of changes of costs in relation to changes in outcomes
The Test Evaluation Cycle

Consequences of testing beyond clinical effectiveness and cost-effectiveness

- organisational
- social
- psychological
- ethical
- legal consequences
Conclusions

After initial discovery of potential biomarkers, careful consideration should be given to its intended use and its consequences/outcomes in clinical practice.

No new biomarker should be subjected to tedious evaluation and released to the market if it is unlikely that it will result in improved clinical actions and measurable outcomes.

Test evaluation should focus on the impact of variations in analytical performance on clinical performance, and thereby clinical effectiveness, through the testing-management-outcomes pathway.
The wheels are rolling on...

Clinicians
Laboratory professionals
Regulators & Policy makers

Epidemiologists and methodologists

Researchers Industry &
The working group is supported by EFLM through an educational grant sponsored by Roche, Abbott Diagnostics and Thermo Fisher.