Proteomics in Cardiovascular Disease: Clinical Considerations

Dr. Christian Delles
BHF Glasgow Cardiovascular Research Centre
Institute of Cardiovascular and Medical Sciences
University of Glasgow

Systems Biology and “Omics”

DNA
mRNA
Protein
Metabolites
small molecules

Genomics
Transcriptomics
Proteomics
Metabolomics

Maturity of research

Cardiovascular Continuum

Tissue injury
(heart, stroke, renal
insufficiency)

Atherothrombosis
and progressive CV disease

Endothelial dysfunction

Oxidative and mechanical stress

Inflammation

Pathological remodeling

Target organ damage

End-organ failure
(CHF, ESRD)

Death

Risk factors

Genome

Altered gene expression

Altered protein expression

The screen versions of these slides have full details of copyright and acknowledgements
Proteomics in Cardiovascular Disease: Clinical Considerations
Christian Delles

Proteomics

Proteomics (2)

The goal of proteomics is a comprehensive, quantitative description of protein expression and its changes under the influence of biological perturbations such as disease or drug treatment.

Publications on Proteomics

The screen versions of these slides have full details of copyright and acknowledgements
Rationale for Proteomic Studies

- Research
  - Unravelling the pathophysiology
  - Discovery of new pathways

- Clinical application: biomarker
  - Screening for disease
  - Prediction of risk
  - Diagnosis of (severity of) disease
  - Monitoring of treatment
Proteomics in Cardiovascular Disease: Clinical Considerations
Christian Delles

Platforms: LC-MS/MS

Biological samples (cases vs. control) → LC-MS/MS → Data analysis

Protein Mixtures
- Blood cells
- Tissue lysates

Separate and Analyze Peptides by LC-MS/MS
- Digest to peptides
- Fractionate peptides

LC-MS/MS
- Meas and intensity of peptides
- Fragnement ions from peptides

Search DB using peptide miz and sequence
- Peptide identity
- Protein identity
- Abundance

Technical Challenges

Genomics/transcriptomics
- All possible features known
- Sample is static during analysis
- All features measured
- Robust means to amplify low numbers DNA or RNA (PCR)
- Signal not detected means feature not present

Proteomics
- All possible features not known
- Sample is dynamic during analysis
- 10-50% of features measured
- No protein PCR (analytics have to deal with enormous dynamic range)
- Signal not detected means either that feature not present or feature present but not detected

Post Translational Modifications

Van Eyk JE, Circ Res 2011
Unravelling pathophysiology

Biomarker of disease
Pre-eclampsia
Organ damage
Biomarker of risk

Unravelling Pathophysiology: Epicardial Fat

Unravelling Pathophysiology

Epicardial adipose tissue
Subcutaneous adipose tissue

The screen versions of these slides have full details of copyright and acknowledgements.
Proteomics in Cardiovascular Disease: Clinical Considerations
Christian Delles

Western Blot and IHC

Function: NBT Assay of ROS

Unravelling pathophysiology
Biomarker of disease
Pre-eclampsia
Organ damage
Biomarker of risk
Urinary Proteomics: CE/MS Platform
Capillary electrophoresis coupled to mass spectrometry

- Separation and analysis of proteins and peptides (>1,000)
- Run time ~60 min

CE
- Fast
- Robust
- Inexpensive
- Reproducible

MS
- Resolution
- Scan speed

Data Storage and Evaluation
Report
Disease-specific biomarker patterns

Why Urine?
- Easily accessible
- Non-invasive sampling
- Available in large quantities
- Urinary polypeptides are stable, yielding comparable datasets
- Urinary polypeptides display the “status” of the kidney, bladder, prostate and vascular architecture, capable of depicting systemic diseases

De Hortus Sanitatis
Mainz, Germany, 1491

Urinary Proteomics: CE/MS Platform

The screen versions of these slides have full details of copyright and acknowledgements
### Patients

<table>
<thead>
<tr>
<th>Study cohort</th>
<th>Samples</th>
<th>CAD</th>
<th>Control</th>
<th>Primary Usage</th>
<th>Secondary Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variation discovery</td>
<td>360</td>
<td>204</td>
<td>156</td>
<td>CAD markers</td>
<td>SVM modeling</td>
</tr>
<tr>
<td>CAD (N=120)[7]</td>
<td>183</td>
<td>151</td>
<td>32</td>
<td>CAD markers</td>
<td>SVM modeling</td>
</tr>
<tr>
<td>AAT (N=40)[6]</td>
<td>24</td>
<td>24</td>
<td>8</td>
<td>SVM modeling</td>
<td>n.a.</td>
</tr>
<tr>
<td>Additional controls (N=153)[6]</td>
<td>239</td>
<td>0</td>
<td>239</td>
<td>SVM modeling</td>
<td>n.a.</td>
</tr>
<tr>
<td>TRENDS baseline (N=17)[11]</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>Medication markers</td>
<td>SVM modeling</td>
</tr>
<tr>
<td>TRENDY follow-up (N=12)</td>
<td>26</td>
<td>26</td>
<td>0</td>
<td>Medication markers</td>
<td>SVM modeling</td>
</tr>
<tr>
<td>Fenofibrate baseline (N=26)[16]</td>
<td>26</td>
<td>0</td>
<td>26</td>
<td>Therapy monitoring</td>
<td>SVM modeling</td>
</tr>
<tr>
<td>Fenofibrate follow-up</td>
<td>26</td>
<td>0</td>
<td>26</td>
<td>Therapy monitoring</td>
<td>SVM modeling</td>
</tr>
<tr>
<td>Blinded cohort (N=138)</td>
<td>138</td>
<td>71</td>
<td>67</td>
<td></td>
<td>Validation</td>
</tr>
<tr>
<td>HIB 0 mg (N=55)[10]</td>
<td>55</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Drug interference</td>
<td>n.a.</td>
</tr>
<tr>
<td>HIB 300 mg</td>
<td>48</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Drug interference</td>
<td>n.a.</td>
</tr>
<tr>
<td>HIB 600 mg</td>
<td>45</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Drug interference</td>
<td>n.a.</td>
</tr>
<tr>
<td>HIB 900 mg</td>
<td>45</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Drug interference</td>
<td>n.a.</td>
</tr>
<tr>
<td>Long-term treatment effects [16]</td>
<td>44</td>
<td>n.a.</td>
<td>n.a.</td>
<td></td>
<td>Validation</td>
</tr>
<tr>
<td>IRMA -2 Irbesartan follow-up</td>
<td>11</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Therapy monitoring</td>
<td>n.a.</td>
</tr>
<tr>
<td>IRMA -2 Placebo baseline (N=11)</td>
<td>11</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Therapy monitoring</td>
<td>n.a.</td>
</tr>
<tr>
<td>IRMA -2 Placebo follow-up</td>
<td>11</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Therapy monitoring</td>
<td>n.a.</td>
</tr>
<tr>
<td>Total (N=623)</td>
<td>961</td>
<td>n.a.</td>
<td>n.a.</td>
<td></td>
<td>Validation</td>
</tr>
</tbody>
</table>

*Methods (2)*

<table>
<thead>
<tr>
<th>Methods</th>
<th>Cases</th>
<th>Controls</th>
<th>Diagnostic pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE/MS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Methods*

<table>
<thead>
<tr>
<th>Methods</th>
<th>Cases</th>
<th>Controls</th>
<th>Diagnostic pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE/MS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*References*

Kolch et al., RCM 2004; 18: 2635-2636
Neuhoff et al., RCM 2004; 18: 149-156
Mischak et al., PROTEOMICS - Clinical Applications 2007; 1: 148-156

*Notes*

- The screen versions of these slides have full details of copyright and acknowledgements.
Proteomics in Cardiovascular Disease: Clinical Considerations
Christian Delles

Identification of Proteins

- Collagen type 1
- Collagen type 3
- Alpha-1-antitrypsin (AAT)
- Granin-like neuroendocrine peptide precursor (ProSAAS)
- Membrane associated progesterone receptor component 1
- Sodium/potassium-transporting ATPase gamma chain
- Fibrinogen-alpha-chain

Single Marker

The screen versions of these slides have full details of copyright and acknowledgements
Proteomics in Cardiovascular Disease: Clinical Considerations
Christian Delles

Two Markers

Three Markers

Better Discrimination with More Markers
Effect of Drug Therapy

Delles C et al., J Hypertens 2010

Unravelling pathophysiology
Biomarker of disease
Pre-eclampsia
Organ damage
Biomarker of risk

Pre-eclampsia
Hypertension
Proteinuria
> 20 weeks gestation
Without pre-existing hypertension

Maternal
• Oedema
• Headaches/blurred vision/seizures
• Renal failure
• Coagulation problems
• HELLP syndrome

Foetal
• Intra-uterine growth restriction
• Preterm delivery
• Death
Proteomics in Cardiovascular Disease: Clinical Considerations
Christian Delles

Normal Pregnancy

Maternal Risk Factors

- Antiphospholipid syndrome, previous pre-eclampsia, pre-existing diabetes, twins vs. singleton, nulliparity, family history, raised BMI, age > 40 yrs, raised DBP

Study Design

Booking (Week 12-14)
2407 pregnant women
Blood/urine/risk factors

“Risk-factor” subgroup
n = 132
Weeks 16 and 28

Delivery
n = 2385

Outcome
PE - yes/no
Proteomics in Cardiovascular Disease: Clinical Considerations
Christian Delles

Clinical Characteristics

<table>
<thead>
<tr>
<th>Material characteristics</th>
<th>Pre-eclampsia n=45</th>
<th>Controls n=86</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29±5</td>
<td>29±5</td>
<td>0.69</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>42 (93%)</td>
<td>61 (74%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Other</td>
<td>3 (7%)</td>
<td>5 (6%)</td>
<td>0.76</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>55 (97%)</td>
<td>55 (64%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Parous</td>
<td>4 (9%)</td>
<td>21 (24%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Previous pre-eclampsia</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>0.42</td>
</tr>
<tr>
<td>Family history of pre-eclampsia</td>
<td>8 (17%)</td>
<td>7 (8%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Atbooking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>121±10</td>
<td>114±12</td>
<td>0.002</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>76±9</td>
<td>69±10</td>
<td>0.001</td>
</tr>
<tr>
<td>Proteinuria (³ + on dipstick)</td>
<td>1 (2%)</td>
<td>3 (2%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Gestation at sampling (wks)</td>
<td>13±1.6</td>
<td>13±1.3</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Pregnancy/Outcome

| Highest systolic blood pressure (mmHg) | 165±11 | 125±12 | <0.001 |
| Highest diastolic blood pressure (mmHg) | 104±8 | 71±8 | <0.001 |
| Caesarean section | 20 (44%) | 10 (12%) | <0.001 |
| Gestation at delivery (wks) | 38±3 | 40±1.4 | <0.001 |
| Birthweight (g) | 3155±621 | 3558±416 | <0.001 |
| Small for gestational age | 12 (27%) | 3 (2%) | <0.001 |

Pregnancy Specific Proteomic Biomarkers

Week 28: Pre-eclampsia Specific Biomarkers

The screen versions of these slides have full details of copyright and acknowledgements.
**Week 28: Pre-eclampsia Specific Biomarkers (2)**

![Graph showing comparison between controls and cases](image)

- Controls: n = 17
- Cases: n = 18

**Change in Classification Factor**

![Graph showing change in classification factor](image)

- Week 12-16 vs Week 28:
  - Controls: P = 0.031
  - Cases: P < 0.001

**Sequencing**

- Collagen alpha-1 (I) chain
- Collagen alpha-1 (III) chain
- Collagen alpha-2 (I) chain
- Collagen alpha-2 (I) chain
- Collagen alpha-3 (IX) chain
- Fibrinogen alpha chain
- Ig kappa chain V-IV region B17
- Retinol-binding protein 4
- Uromodulin
Proteomics in Cardiovascular Disease: Clinical Considerations
Christian Delles

Plasma Proteome: DIGE and LC-MS/MS
Samples from 20 weeks gestation

Pre-eclampsia and Cardiovascular Risk

Serum Proteomics: 2D-LC-MS/MS

The screen versions of these slides have full details of copyright and acknowledgements
Serum Proteomics: 2D-LC-MS/MS (2)

Rasanen J et al., J Proteome Res 2010

Urinary Proteomics: SELDI

Buhimschi IA et al., AJOG 2008

Urinary Proteomics: SELDI (2)

Buhimschi IA et al., AJOG 2008

Samples at time of severe disease
Some longitudinal samples
Unravelling pathophysiology

Biomarker of disease

Pre-eclampsia

Organ damage

Biomarker of risk

Stroke

<table>
<thead>
<tr>
<th>Protein</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWF</td>
<td>0.047</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>0.055</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>0.068</td>
</tr>
<tr>
<td>Apolipoprotein A</td>
<td>0.074</td>
</tr>
</tbody>
</table>


Stroke (2)

Diagnostic accuracy

Stoke severity
Unravelling pathophysiology

Biomarker of disease
- Heart Failure
- Pre-eclampsia
- Organ damage
- Biomarker of risk

Chronic Kidney Disease Pattern

<table>
<thead>
<tr>
<th>CKD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 230</td>
<td>n = 379 C</td>
</tr>
<tr>
<td>20ANCA, 31MGSA, 22MCID, 44qG 25FSGS, 58DI, 21SL</td>
<td>379 C</td>
</tr>
</tbody>
</table>

Fragments of:
- Various collagens
- Plasma proteins (serum albumin, transthyretin, alpha-1-antitrypsin, alpha-1B-glycoprotein, alpha-2-HS-glycoprotein; antithrombin III, apolipoprotein A-I, beta-2-microglobulin, fibrinogen alpha)
- CAST
- Uromodulin
- Nephropathy-related candidate genes
- Proinflammatory cytokine receptors
- Prostaglandin-H2 D-isomerase
- Proteinase-activated receptor 1
- Prostaglandin-endoperoxide synthase inhibitor
- Caspase
- Interleukin 1β
- Nephronectin
- Wnt-2 (Wingless-related MMTV integration site family, member 2)
- Matrilin
- Osteopontin
- Neurosecretory protein VGF
- Membrane associated glycoprotein receptor component 11
- COX-2
- Ig lambda chain C regions

Diabetic Nephropathy
Increase in ECM and collagen in diabetes and diabetic nephropathy

Normal
Diabetic

Diabetic Nephropathy (2)

early detection?

Study Flow Chart
Proteomic prediction and renin angiotensin aldosterone system inhibition prevention of early diabetic nephropathy in type 2 diabetic patients with normoalbuminuria

Screened population
N = 7000

Type 2 Diabetes
Normoalbuminuria
N = 3250

Positive (at risk) n = 656 (approx)
Negative (low risk) n = 2624 (approx)

Spironolactone
Placebo
Standard

Randomization
156 weeks
End of trial

The screen versions of these slides have full details of copyright and acknowledgements
Integrating "omics" data

Systems Biology and "Omics"

Summary and Conclusions

• Proteomics has a potential to elucidate pathophysiological mechanisms and as a clinical biomarker of disease and risk
• A range of different proteomic techniques is available; Results from experiments using different platforms do not necessarily overlap
• Rigorous standards for phenotypic characterisation and replication of results have to be applied to clinical proteomic studies
• Integration of data across "omics" modalities remains challenging
Proteomics in Cardiovascular Disease:
Clinical Considerations
Christian Delles

BHF Glasgow Cardiovascular Research Centre

Henry Stewart Talks

The screen versions of these slides have full details of copyright and acknowledgements