New diagnostic markers for acute coronary syndromes
- Nye diagnostiske markører for akutt iskemisk hjertesykdom

Bertil Lindahl, Professor Cardiology, Uppsala University and Uppsala Clinical Research Center
Are there any new, clinically useful, diagnostic markers for ACS?

No, not really!
• What is needed for the implementation of new biomarkers?

• Unmet needs
  • Early diagnosis - New approaches to rule-out and rule-in of AMI using hs-cTn assays
  • Subclassification of myocardial injury
  • Risk stratification
• What is needed for the implementation of new biomarkers?
  • Unmet needs
    • Early diagnosis - New approaches to rule-out and rule-in of AMI using hs-cTn assays
    • Subclassification of myocardial injury
    • Risk stratification (?)
Possible applications

Diagnosis

cTn and NT-proBNP/BNP are the only "new" biomarkers that have got widespread use in clinical practice – and they are useful for prognosis.
Possible applications

Choice / monitoring of treatment

- Even quite "bad" markers can be widely used if they affect the choice of treatment, e.g. CHA2DS2VASC
- Few examples of useful biomarkers for choice/monitoring if treatment in cardiology, i.e. cTn and NT-proBNP
- Genetic markers in Oncology
Possible applications

Prognosis

• Even very "good" general prognostic markers (e.g. for death), but without affecting the choice of treatment, have not gain widespread, e.g. GDF-15

• Somewhat greater chance if the biomaker gives more disease-specific information – e.g. reinfarction or rehosp. for CHF (ST2)
Other prerequisites

Strong evidence base – high quality studies have shown added value of the biomarker

Reasonable balance between PPV and NPV (e.g. Copeptin)

High quality assay (precision, speed, possible to automate)

Acceptable costs

Education of the users
<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Prognostic impact</th>
<th>Diagnostic impact</th>
<th>Therapeutic impact</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Markers of necrosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine phosphokinase MB</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Troponin</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
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<tr>
<td><strong>Markers of myocardial dysfunction or stress</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Atrial natriuretic peptides</td>
<td>+++</td>
<td>+++*</td>
<td>?</td>
</tr>
<tr>
<td>Brain natriuretic peptides</td>
<td>+++</td>
<td>+++*</td>
<td>+</td>
</tr>
<tr>
<td>Copeptin</td>
<td>++</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Proadrenomedullin</td>
<td>++</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td><strong>Markers of inflammation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin</td>
<td>++</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>++</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Growth differentiation factor 15</td>
<td>+++</td>
<td>?</td>
<td>+</td>
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<tr>
<td>Interleukin 6</td>
<td>+++</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Soluble ST2</td>
<td>+</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Tumor necrosis factor α</td>
<td>+</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Myeloid-related protein 8/14</td>
<td>+</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td><strong>Markers of ischemia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline</td>
<td>+</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Heart-type fatty acid-binding protein</td>
<td>++</td>
<td>++</td>
<td>?</td>
</tr>
<tr>
<td>Ischemia modified albumin</td>
<td>+</td>
<td>?</td>
<td>?</td>
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<tr>
<td><strong>Markers of plaque destabilization/rupture</strong></td>
<td></td>
<td></td>
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<tr>
<td>Lipoprotein-associated phospholipase A2</td>
<td>+++</td>
<td>?</td>
<td>?</td>
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<tr>
<td>Matrix metalloproteinase-9</td>
<td>++</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>+++</td>
<td>++</td>
<td>?</td>
</tr>
<tr>
<td>Placental growth factor</td>
<td>++</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Pregnancy-associated plasma protein A</td>
<td>+++</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Secretory phospholipase A2</td>
<td>+</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Soluble fms-like tyrosine kinase 1</td>
<td>+</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Soluble intercellular adhesion molecule 1</td>
<td>+++</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td><strong>Markers of platelet activation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble CD40 ligand</td>
<td>++/?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Soluble P-selectin</td>
<td>++</td>
<td>?</td>
<td>?</td>
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</tbody>
</table>

• What is needed for the implementation of new biomarkers?

• Unmet needs
  • Early diagnosis - New approaches to rule-out and rule-in of AMI using hs-cTn assays
Symptoms suggestive of cardiac origin (e.g., acute chest pain)

Clinical history

- cTn
- ECG

ACS (working diagnosis)

Coronary angiography & other imaging techniques

No ACS

- Serious condition
- Nonserious condition

AMI (final diagnosis)

UA

UCR UPPSALA CLINICAL RESEARCH CENTER
High sensitivity cTn assays
- CV% < 10 % att 99th perc
- >50 % of healthy individuals with detectable levels

RAPID RULE-OUT Biomarker-based strategies

3h: ESC 2011 algorithm
2h: 2h-Advanced diagnostic protocol and 2h-algorithm
1h: 1h-algorithm
0h: dual-marker Strategy (cTn+ copeptin)
0h: undetectable Hs cTn
Single sample at presentation for rule-out

High sensitivity cTn assays - using a very low cut-off level
– LoD i.e. 20-55 % ruled-out with NPV 98-100.

Sample at presentation

T0 < LoD

RULE-OUT
20-55 %; NPV 98-100%

T0 ≥ LoD

Observational zone
45-80 %

Rule-out of AMI ≠ possible to discharge directly from ED
CENTRAL ILLUSTRATION: A 1-h Algorithm for Chest Pain Evaluation: Patient Presenting to the ED

Patients presenting to emergency department with chest pain

“The extended algorithm”

**Rule out (60% of cohort)**
- At admission (0h) high sensitivity cardiac Troponin T (hs-cTnT) < 12 ng/L and
- 1h post admission hs-cTnT delta < 3 ng/L and
- Non-ischemic ECG and
- Patient history not high risk

**Observational zone (26% of cohort)**

**Rule in (14% of cohort)**
- At admission (0h) hs-cTnT ≥ 52 ng/L or
- 1h post admission hs-cTnT delta ≥ 5 ng/L or
- At 0h or 1h hs-cTnT > 14 ng/L combined with either:
  - Ischemic ECG or
  - High risk patient history

**MACE event rate:**
- 30-day MACE 0.5%
- 30-day MACE without unstable angina (UA) 0%

**Management:**
- Consider differential diagnosis
- Discharge

**MACE event rate:**
- 30-day MACE 10.1%
- 30-day MACE without UA 2.6%

**Management:**
- Additional troponin testing
- Stress testing/cardiac imaging if diagnosis unclear

**MACE event rate:**
- 30-day MACE 62.3%
- 30-day MACE without UA 52.7%

**Management:**
- Cardiology consult
- Admit

Table 5. Diagnostic Accuracy: Accuracy of cTnl, ECG, TIMI, and ADP for Prediction of MACE

<table>
<thead>
<tr>
<th></th>
<th>ECG</th>
<th>Troponin†</th>
<th>Troponin and ECG‡</th>
<th>TIMI and ECG§</th>
<th>ADP (ECG+ TIMI + Troponin)¶</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>24.5 (20.0–29.7)</td>
<td>87.4 (83.2–90.7)</td>
<td>89.1 (85.1–92.1)</td>
<td>98.3 (96.2–99.3)</td>
<td>99.7 (98.1–99.9)</td>
</tr>
<tr>
<td><strong>Negative predictive value</strong></td>
<td>86.7 (85.0–88.2)</td>
<td>97.6 (96.7–98.3)</td>
<td>97.7 (96.7–98.3)</td>
<td>98.7 (97.1–99.5)</td>
<td>99.7 (98.6–100.0)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>88.5 (86.8–89.9)</td>
<td>92.6 (91.2–93.7)</td>
<td>82.6 (80.7–84.3)</td>
<td>23.5 (21.5–25.6)</td>
<td>23.4 (21.4–25.5)</td>
</tr>
<tr>
<td><strong>Positive predictive value</strong></td>
<td>27.7 (22.7–33.4)</td>
<td>68.0 (63.2–72.5)</td>
<td>48.0 (43.9–52.2)</td>
<td>18.8 (17.0–20.8)</td>
<td>19.0 (17.2–21.0)</td>
</tr>
</tbody>
</table>

20 % of the study population

Cullen L et al. JACC, 2012; 59: 2091-2098
Copeptin

The antidiuretic hormone arginin-vasopressin (AVP) is secreted neurohypophyseal and controls osmotic homeostasis. The glycosylated peptide copeptin is part of the uncleaved pro-AVP and emerges equimolar to AVP.

Highest levels on admission – levels decreasing thereafter

Keller et al, JACC 2010
Copeptin – the C-terminal end of the prohormone of vasopressin

A useful rule-out marker?

n= 1967 (156 AMI:s)

CONCLUSION:
Adding copeptin to conventional cTnI allowed safe rule out of AMI with a NPV >99% in patients presenting with suspected ACS.
It has the potential to rule out AMI in 58% of patients without serial blood draws.
Study design

Patients with suspected ACS

Standard process
Standard CPU: Serial cTn, ECG, further care according to current guidelines

Randomization

Experimental process with Copeptin (from initial blood sample)

Copeptin positive (≥ 10 pmol/l)
Standard care

Copeptin negative (< 10 pmol/l)
Discharge

Follow-up 30 and 90 days: MACE
Efficacy: Discharge from the ED in the two process groups

Discharge from ED

p<0.001

Discharge from ED (%)

Standard group

Copeptin group
• What is needed for the implementation of new biomarkers?

• **Unmet needs**
  • Early diagnosis - New approaches to rule-out and rule-in of AMI using hs-cTn assays
  • Subclassification of myocardial injury
**Table 2** Universal classification of myocardial infarction

<table>
<thead>
<tr>
<th>Type 1: Spontaneous myocardial infarction</th>
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<tbody>
<tr>
<td>Spontaneous myocardial infarction related to atherosclerotic plaque rupture, ulceration, fissuring, erosion, or dissection with resulting intraluminal thrombus in one or more of the coronary arteries leading to decreased myocardial blood flow or distal platelet emboli with ensuing myocyte necrosis. The patient may have underlying severe CAD but on occasion non-obstructive or no CAD.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type 2: Myocardial infarction secondary to an ischaemic imbalance</th>
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</thead>
<tbody>
<tr>
<td>In instances of myocardial injury with necrosis where a condition other than CAD contributes to an imbalance between myocardial oxygen supply and/or demand, e.g. coronary endothelial dysfunction, coronary artery spasm, coronary embolism, tachy-brady-arrhythmias, anaemia, respiratory failure, hypotension, and hypertension with or without LVH.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type 5: Myocardial infarction related to coronary artery bypass grafting (CABG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarction associated with CABG is arbitrarily defined by elevation of cardiac biomarker values &gt; 10 x 99th percentile URL in patients with normal baseline cTn values (&lt;99th percentile URL). In addition, either (i) new pathological Q waves or new LBBB, or (ii) angiographic documented new graft or new native coronary artery occlusion, or (iii) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.</td>
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</tbody>
</table>

**Thygesen, K. et al.**
**Eur H J 2012; 33, 2551–2567**
<table>
<thead>
<tr>
<th>Injury not related to myocardial ischaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac contusion, surgery, ablation, pacing, or defibrillator shocks</td>
</tr>
<tr>
<td>Rhabdomyolysis with cardiac involvement</td>
</tr>
<tr>
<td>Myocarditis</td>
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<tr>
<td>Cardiotoxic agents, e.g. anthracyclines, herceptin</td>
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</table>

<table>
<thead>
<tr>
<th>Multifactorial or indeterminate myocardial injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart failure</td>
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<tr>
<td>Stress (Takotsubo) cardiomyopathy</td>
</tr>
<tr>
<td>Severe pulmonary embolism or pulmonary hypertension</td>
</tr>
<tr>
<td>Sepsis and critically ill patients</td>
</tr>
<tr>
<td>Renal failure</td>
</tr>
<tr>
<td>Severe acute neurological diseases, e.g. stroke, subarachnoid haemorrhage</td>
</tr>
<tr>
<td>Infiltrative diseases, e.g. amyloidosis, sarcoidosis</td>
</tr>
<tr>
<td>Strenuous exercise</td>
</tr>
</tbody>
</table>

European Heart Journal (2012) 33, 2551–2567
ACS – atherothrombotic type (type 1 MI)
ACS – secondary type (type 2 MI)

With atherosclerosis

Increased oxygen demand and/or decreased supply

Without atherosclerosis
Clinical classification, coronary status and prognosis

Type 2 MI-CAD vs. Type 1 MI-CAD:

HR 1.72 (95% CI 1.45-2.03)
Adj. HR* 0.76 (95% CI 0.61-0.94)

Type 2 MINOCA vs. Type 1 MINOCA:

HR 1.14 (95% CI 0.84-1.55)
Adj. HR* 0.82 (95% CI 0.52-1.29)

* after adjustment for age, sex, comorbidities, treatments and triggering mechanisms

1. Is it possible to separate acute from chronic myocardial injury?

2. Is it possible to separate type 2 from type 1 MI by the use of biomarkers?

3. Is it possible to separate AMI patients with and without significant CAD?

To answer these important questions we need a much better understanding of the underlying pathophysiological mechanisms.
Proteomic approach

• Proseek® Multiplex CVD I, II & III are high-throughput, multiplex immunoassays, each allowing analysis of 92 CVD-related protein biomarkers across 96 samples simultaneously.

1. INCUBATION
   Proseek probes (DNA oligo labeled antibodies) bind in proximity to target proteins

2. EXTENSION
   Extension and creation of real-time PCR amplicons

3. DETECTION
   Proseek Amplification and Detection by real-time PCR on the BioMark™ HD System
• What is needed for the implementation of new biomarkers?

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Prognostic marker in ACS

- Myocardial cell biomarkers
- Inflammation biomarkers (pro- and anti-)
- Biomarkers of (coronary) atherosclerosis
- Biomarkers arising from (damaged) coronary endothelium
- Biomarkers of platelet activation
- Biomarkers of coagulation system and fibrinolytic system
- Biomarkers of renal function etc.
- Cardiac-derived micro-RNAs
- Cardiac–derived microvesicles (=exosomes)
- Gene biomarkers
Prognosis of what?

• Mortality – a large number of different markers have shown to be associated to mortality; few have shown added value over cTn and NT-proBNP

• A small number of markers have been associated with the risk of developing heart failure

• Very few markers have been consistently been associated to the risk of re-infarction (or stroke) (i.e cTn)

• Statistical vs clinical significance
Growth Differentiation Factor 15 (GDF-15)

Member of the transforming growth factor β superfamily

The exact biological functions are poorly understood

Up-regulated in many different pathological conditions including inflammation, cancer, CV-diseases, pulmonary and renal disease

• owing to the lack of tissue specificity, no useful role as a diagnostic marker

• strong and independent marker of mortality after ACS, less clear of future AMI
1-year mortality in tertiles of GDF-15

Wollert et al Circulation 2007;115:962-971
Combination of GDF-15, NT-proBNP and TnT
(1-year mortality in GUSTO-IV)
GDF-15 interaction with invasive vs. noninvasive strategy - results from FRISC-II (n=2079)

GDF-15 <1200 ng/L
GDF-15 1200-1800 ng/L: $\chi^2=2.18$, $P=0.14$
GDF-15 >1800 ng/L: $\chi^2=6.12$, $P=0.014$

Death or recurrent MI

Time (Months)

-51% ($P=0.001$)
-32% ($P=0.048$)
(P=0.81)
Biomarkers play a crucial role in the management of ACS
• cTn is the marker of choice for diagnosis of AMI, and very hard to beat
• Many biomarkers are associated with an adverse prognosis in ACS, but few have shown added value
• Only cTn (and to some extent BNP/NTproBNP) is widely used in clinical practice for risk assessment
• Among new markers, a few markers are potential candidates to be implemented in clinical practice, e.g. Copeptin and GDF-15
• New approaches (proteomics, microRNA etc) have the potential to give us better understanding of the pathophysiology
Thank you for your attention!