New Biomarkers in Heart Failure

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University Medical Center Groningen
The Netherlands
Potential Conflicts of Interest

- AAV received consultancy fees and/or research grants from: Alere, Bayer, Cardio3Biosciences, Celladon, Ceva, European Committee, Dutch Heart Foundation, Novartis, Servier, Torrent, Vifor,
Explosion of Biomarker Publications

Pubmed Search; [heart failure] AND [biomarkers]
<table>
<thead>
<tr>
<th>Inflammation</th>
<th>Neurohormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Renin</td>
</tr>
<tr>
<td>TWEAK (TNF-like weak inducer of apoptosis)</td>
<td>Angiotensin II</td>
</tr>
<tr>
<td>IL-1, −6, −10, and −18</td>
<td>Aldosterone</td>
</tr>
<tr>
<td>LP-PLA2 (Lipoprotein-associated phospholipase A2)</td>
<td>Arginine vasopressin, copeptin</td>
</tr>
<tr>
<td>Soluble TNF receptors 1 and 2</td>
<td>Endothelin-1</td>
</tr>
<tr>
<td>YKL-40</td>
<td>Urocorin</td>
</tr>
<tr>
<td>IL-1 receptor antagonist</td>
<td>Chromogranin A and B</td>
</tr>
<tr>
<td>Midkine</td>
<td>MR-proADM</td>
</tr>
<tr>
<td>Leucine-rich 2-glycoprotein</td>
<td><strong>Myocyte injury and apoptosis</strong></td>
</tr>
<tr>
<td>PTX3</td>
<td>Troponins I and T</td>
</tr>
<tr>
<td>CA-125</td>
<td>Myosin light-chain kinase I</td>
</tr>
<tr>
<td>S100A8/A9 complex</td>
<td>Heart-type fatty-acid-binding protein</td>
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<tr>
<td>Osteoprotegerin</td>
<td>Creatine kinase MB fraction</td>
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<tr>
<td>Serine protease PR3</td>
<td>sFAS (soluble apoptosis-stimulating fragment)</td>
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<tr>
<td>Soluble endoglin</td>
<td>Heat shock protein 60</td>
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<tr>
<td>Adiponectin</td>
<td>sTRAIL (soluble TNF-related apoptosis-inducing ligand)</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td><strong>Myocyte stress</strong></td>
</tr>
<tr>
<td>Oxidized LDLs</td>
<td>BNP, NT-proBNP, MR-proANP</td>
</tr>
<tr>
<td>MPO</td>
<td>sST2</td>
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<tr>
<td>Urinary biopyrrins</td>
<td>GDF-15</td>
</tr>
<tr>
<td>Urinary and plasma isoprostanes</td>
<td><strong>Extracardiac involvement</strong></td>
</tr>
<tr>
<td>Urinary 8-hydroxy-2′-deoxyguanosine</td>
<td>RDW</td>
</tr>
<tr>
<td>Plasma malondialdehyde</td>
<td>Cystatin-C, β-trace protein</td>
</tr>
<tr>
<td><strong>Extracellular-matrix remodelling</strong></td>
<td>NGAL, NAG [N-acetyl-β-(D)-glucosaminidase], KIM-1 (kidney injury molecule-1)</td>
</tr>
<tr>
<td>MMPs (MMP2, MMP3, MMP9)</td>
<td>β2-microglobulin</td>
</tr>
<tr>
<td>TIMP1</td>
<td>Urinary albuminin-to-creatinine ratio</td>
</tr>
<tr>
<td>IL-6</td>
<td>Triiodothyronine</td>
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<tr>
<td>Collagen propeptides</td>
<td></td>
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<tr>
<td>N-terminal collagen type III peptide</td>
<td></td>
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<tr>
<td>Myostatin</td>
<td></td>
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<tr>
<td>Syndecan-4</td>
<td></td>
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<tr>
<td>Galectin-3</td>
<td></td>
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</table>
New Biomarkers in Heart Failure

• Selection of biomarkers in this presentation is based on:
  • Personal preference/interest
  • (Potential) clinical applicability
Promising HF biomarkers: a personal selection

- Galactin-3
- Renal tubular markers
- MRproANP
- Advanced Glycation Endproducts
- Micro RNAs
Galectin-3 in CHF - Scientific Discovery

Galectin-3 was discovered in a rat experiment where high levels predicted heart failure development and progression (TGRmRen2-27 rats)

Hypothesis — Galectin-3 is responsible for cardiac fibro genesis resulting in HF development and progression

GAL-3 adds to NT-proBNP KM curves for Mortality in HF (VaLHeFT)

Anand I, presented at the ACC, Chicago, IL, 2012
New onset HF by Galectin-3 (75th percentile)

N=7968
378 incident cases of HF
FU 10.5 years

de Boer RA, et al. Data from PREVEND, unpublished data
Galectin-3: A target for therapy?
Galectin-3 inhibition attenuates heart failure development

• Mechanisms involved:
  • ↓ Fibroblast-myofibroblast differentiation
  • Downregulation of pro-fibrotic genes

Yu et al. Circulation: Heart Failure (under revision)
Promising HF biomarkers: a personal selection

- Galactin-3
- Renal tubular markers
- MRproANP
- Advanced Glycation Endproducts
- Micro RNAs
Tubular markers in CHF

- **NAG (U/gCr)**
  - Controls: 5
  - CHF: 25

- **NGAL (µg/g Cr)**
  - Controls: 100
  - CHF: 400

- **KIM-1 (ng/gCr)**
  - Controls: 200
  - CHF: 800

**Significance Levels:**
- NAG: $P < 0.0001$
- NGAL: $P < 0.0001$
- KIM-1: $P = 0.0001$

*Damman, EJHF 2008*
New Biomarkers in HF

- University Medical Center Groningen

Tubular markers in GISSI-HF

- 2130 patients participating in the GISSI-HF trial
- Measurements of UACR, eGFR, and NAG, KIM-1, NGAL
- Combined endpoint of all-cause mortality and HF hospitalizations.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate HR</th>
<th>P-value</th>
<th>Multivariate HR*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>logNAG (per SD)</td>
<td>1.62 (1.47 - 1.78)</td>
<td>&lt; 0.001</td>
<td>1.22 (1.10 – 1.36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>logKIM-1 (per SD)</td>
<td>1.42 (1.30 - 1.55)</td>
<td>&lt; 0.001</td>
<td>1.13 (1.02 – 1.24)</td>
<td>0.018</td>
</tr>
<tr>
<td>logNGAL (per SD)</td>
<td>1.28 (1.18 – 1.39)</td>
<td>&lt; 0.001</td>
<td>1.10 (1.00 – 1.20)</td>
<td>0.04</td>
</tr>
<tr>
<td>eGFR (per 10 mL/min/1.73m² decrease)</td>
<td>1.19 (1.14 – 1.23)</td>
<td>&lt; 0.001</td>
<td>1.06 (1.02 – 1.10)</td>
<td>0.007</td>
</tr>
<tr>
<td>logUACR (per SD)</td>
<td>1.41 (1.32 – 1.51)</td>
<td>&lt; 0.001</td>
<td>1.20 (1.10 – 1.31)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Tubular markers in GISSI-HF

- No Albuminuria, no CKD and low NAG
- Only high NGAL
- Only high KIM-1
- Only high NAG
- Albuminuria and high NAG
- CKD and high NAG
- Albuminuria, CKD and high NAG

Follow up time (days)
Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery

Jaya Mishra*, Catherine Dent*, Ridwan Tarabishi*, Mark M Mitsnefes, Qing Ma, Caitlin Kelly, Stacey M Ruff, Kamyar Zahedi, Mingyuan Shao, Judy Bean, Kiyoshi Mori, Jonathan Barasch, Prasad Devarajan

![Graph showing the change in urine NGAL levels over time in patients with and without acute renal failure. The graph indicates a significant rise in urine NGAL levels in patients with acute renal failure compared to those without.](image)
Volume Status and Diuretic Therapy in Systolic Heart Failure and the Detection of Early Abnormalities in Renal and Tubular Function

Kevin Damman, MD, PhD,* Marie J. Ng Kam Chuen, MD,§ Robert J. MacFadyen, MD, PhD,§ Gregory Y. H. Lip, MD,§ David Gaze, PhD,|| Paul O. Collinson, MD,†|| Hans L. Hillege, MD, PhD,*†|| Wim van Oeveren, PhD,‡ Adriaan A. Voors, MD, PhD,* Dirk J. van Veldhuisen, MD, PhD*

Groningen, the Netherlands; and Birmingham and London, United Kingdom
Increase in Renal Tubular Damage after stopping diuretics in CHF

Damman et al. JACC 2011
Improvement in renal function in CHF patients after decongestion

A

Furosemide 50 mg i.v.

Normal oral furosemide dose

KIM-1 (ng/gCr)

NAG (U/gCr)

Day 4, 0 hours
Day 4, 4 hours
Day 4, 8 hours
Day 7

* indicates statistical significance.

Damman et al. JACC 2011
Promising HF biomarkers: a personal selection

- Galactin-3
- Renal tubular markers
- MRproANP
- Advanced Glycation Endproducts
- Micro RNAs
2012 ESC HF Guidelines: Diagnosis

Suspected heart failure

Acute onset

- ECG
- Chest x-ray

Non-acute onset

- ECG
- Possibly chest x-ray

Echocardiography

BNP/NT-pro BNP

- ECG normal and NT-proBNP <300 pg/mL or BNP <100 pg/mL
  - Heart failure unlikely

- ECG abnormal or NT-proBNP ≥300 pg/mL or BNP ≥100 pg/mL

BNP/NT-pro BNP

- ECG abnormal or NT-proBNP ≥125 pg/mL or BNP ≥35 pg/mL

- ECG normal and NT-proBNP <125 pg/mL or BNP <35 pg/mL
  - Heart failure unlikely

Echocardiography
New Biomarkers in HF

Suspected heart failure

- Acute onset
  - ECG
  - Chest x-ray
- Non-acute onset
  - ECG
  - Possibly chest x-ray

- Echocardiography
- BNP/NT-pro BNP
- BNP/NT-pro BNP
- Echocardiography

*In the acute setting, MR-proANP may also be used*

- ECG normal and NT-proBNP <300 pg/mL or
- ECG abnormal or NT-proBNP ≥300 pg/mL
- ECG abnormal or NT-proBNP ≥125 pg/mL or
- ECG normal and NT-proBNP <125 pg/mL

(cut-off point 120 pmol/L, i.e. <120 pmol/L = heart failure unlikely).

- Heart failure unlikely
- Heart failure unlikely

Echocardiography
Diagnostic performance of MR-proANP

Maisel et al. JACC 2010

<table>
<thead>
<tr>
<th>Measurement</th>
<th>AUC</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNP</td>
<td>0.912</td>
<td>0.898-0.926</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MR-proANP</td>
<td>0.897</td>
<td>0.882-0.912</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>0.896</td>
<td>0.881-0.911</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Promising HF biomarkers: A personal selection

- Galactin-3
- Renal tubular markers
- MRproANP
- Advanced Glycation Endproducts
- Micro RNAs
Advanced Glycation Endproducts

1: GLYCAEMIC CONTROL
2: ANTI-OXIDANTS
3: AGE FORMATION INHIBITORS
4: AGE CROSS-LINK BREAKERS

5: EXOGEOUS AGES: Smoking cessation / Low AGE diet
6: AGE SCAVENGING: Lysozyme / Soluble RAGE
7: AGE-RECEPTOR INHIBITION: anti-RAGE antibodies
8: AGE SIGNAL TRANSDUCTION INHIBITION

Cross-linking

Hartog et al. EJHF 2007
New Biomarkers in HF

University Medical Center Groningen

Hartog et al. EJHF 2007
CML: independent predictor of death or HF-hospitalization in 580 HF patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariable</th>
<th>Multivariable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age, per SD</td>
<td>1.37 (1.19-1.58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.92 (0.65-1.28)</td>
<td>0.60</td>
</tr>
<tr>
<td>Sex</td>
<td>0.84 (0.64-1.09)</td>
<td>0.18</td>
</tr>
<tr>
<td>eGFR, per SD</td>
<td>0.63 (0.55-0.73)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log NT-proBNP, per SD</td>
<td>1.58 (1.38-1.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hb, per SD</td>
<td>0.75 (0.63-0.90)</td>
<td>0.002</td>
</tr>
<tr>
<td>CML, per SD</td>
<td>1.41 (1.26-1.57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACE-i/ARB</td>
<td>0.80 (0.59-1.09)</td>
<td>0.16</td>
</tr>
<tr>
<td>LVEF, per SD</td>
<td>1.02 (0.89-1.18)</td>
<td>0.77</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.87 (1.45-2.41)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Willemsen et al. Heart 2012 In press
Effects of alagebrium, an advanced glycation endproduct breaker, on exercise tolerance and cardiac function in patients with chronic heart failure

Jasper W.L. Hartog¹, Suzan Willemsen¹, Dirk J. van Veldhuisen¹, Jan L. Posma², Leen M. van Wijk³, Yoran M. Hummel¹, Hans L. Hillege¹, Adriaan A. Voors¹* for the BENEFICIAL investigators

![Graph showing peak VO₂ (ml/min/kg) over baseline and 36 weeks for Placebo and Alagebrium]
PEACH-F

• Aim: to study the safety and efficacy of different doses of TRC4186 in 300 stable heart failure patients with HbA1c ≥ 6.5% or type 2 diabetes

• Design: randomised, double-blind, multinational, multi-centre, placebo-controlled, parallel group study

• Primary efficacy endpoints
  • Physical dimension of Minnesota Living with Heart Failure Questionnaire (MLHFQ)
  • Oxygen uptake efficiency slope (OUES)
Promising HF biomarkers: a personal selection

- Galactin-3
- Renal tubular markers
- MRproANP
- Advanced Glycation Endproducts
- Micro RNAs
What are miRNAs?

miRNAs are short (~ 22 nucleotides), noncoding RNA molecules.

miRNAs act as negative regulators of gene expression by:
- inhibiting mRNA translation or
- promoting mRNA degradation

Perfectly base pairing miRNAs lead to mRNA degradation of their target mRNA.

Imperfectly binding of the targeted transcript lead to translational repression.

So, miRNAs can regulate protein expression.
Serum levels of microRNAs in patients with heart failure

Yaron Goren¹, Michal Kushnir¹, Barak Zafrir², Sarit Tabak¹, Basil S. Lewis², and Offer Amir²*

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*Corresponding author.
MicroRNAs as diagnostic marker

Receiver operating characteristic (ROC) curve for miRNA-score discrimination between the HF (n=30) and control group (n=30).

AUC 0.90
Anti-miRNAs as therapeutic modalities?

Time line indicating seminal discoveries in miRNA Biology with a special focus on the cardiovascular field.

Van Rooij; Circ Res 2011
Conclusions (1)

• Explosion of biomarkers: mainly predictors of clinical events
• Galactin-3: fibrosis marker, predictive value for new onset HF and progression of HF; potential target for therapy
• Renal tubular markers: different from glomerular markers (creatinine), potential value as early and subtle renal damage in HF
• MR-proANP: alternative to (NT-pro)BNP in diagnosis of HF (acute setting)
Conclusions (2)

- Advanced Glycation Endproducts: related to prognosis in HF and a potential target for therapy in HF; studies ongoing
- MicroRNAs: interesting regulators of gene transcription, diagnostic marker in HF and a future potential target for therapy