THE JOURNEY OF THE BIOMARKER FROM BENCH TO BEDSIDE

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Toronto, ON
The promise of a healthy heart.

The Journey of the Biomarker from Bench to Bedside
HF Update 2018

Douglas S. Lee, MD, PhD
Ted Rogers Chair in HF Outcomes, Associate Professor, Univ. of Toronto
Peter Munk Cardiac Centre, University Health Network
Disclosures

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- **Consulting fees:** None
- **Speaker fees:** None
- **Other:** None

- I will **NOT** discuss off-label use
Objectives

- Discuss current biomarkers used in heart failure
- Discuss challenges in identifying pre-clinical biomarkers
- Discuss new approaches to biomarker discovery in cardiovascular disease

Clinical Course of HF

Onset of CHF → Sudden Death → Decompensations → Pump Failure

Quality of Life

Transition to Advanced Heart Failure:
- Oral therapies failing
- A time for many major decisions
- Consider MCS and/or transplantation, if eligible
- Consider inversion of care plan to one dominated by a palliative approach, which may involve formal hospice

Traditional Care
Including disease-modifying therapies

Palliative Care
Including symptom management


The promise of a healthy heart.
Association of Cardiovascular Biomarkers With Incident Heart Failure With Preserved and Reduced Ejection Fraction

Rudolf A. de Boer, MD, PhD; Matthew Nayor, MD, MPH; Christopher R. deFilippi, MD; Danielle Enserro, PhD; Vijeta Bhambhani, MS, MPH; Jorge R. Kizer, MD, MSC; Michael J. Blaha, MD, MPH; Frank P. Brouwers, MD, PhD; Mary Cushman, MD; Joao A. C. Lima, MD; Hossein Bahrami, MD, PhD, MPH; Pim van der Harst, MD, PhD; Thomas J. Wang, MD; Ron T. Gansevoort, MD, PhD; Caroline S. Fox, MD, MPH; Hanna K Gaggin, MD, MPH; Willem J. Kop, PhD; Kiang Liu, PhD; Ramachandran S. Vasan, MD; Bruce M. Psaty, MD, PhD; Douglas S. Lee, MD, PhD; Hans L. Hilleges, MD, PhD; Traci M. Bartz, MS; Emelia J. Benjamin, MD, ScM; Cheeling Chan, MS; Matthew Allison, MD, MPH; Julius M. Gardin, MD, MBA; James L. Januzzi Jr, MD; Sanjiv J. Shah, MD; Daniel Levy, MD; David M. Herrington, MD; Martin G. Larson, ScD; Wiek H. van Gilst, PhD; John S. Gottdiener, MD; Alain G. Berton, MD, MPH; Jennifer E. Ho, MD
Only BNP and hs-Troponin improved the c-statistic and NRI compared with clinical, echocardiographic and ECG variables.
Question # 1

What is the primary way that you use BNP or NT-proBNP in practice?

A) Diagnosis of HF  
B) Determine prognosis  
C) Guide medical management with HF drugs  
D) I do not have access to BNP  
E) A, B, and C
ELAN-HF NT-proBNP Discharge Model

Both disch BNP + %Δ BNP (disch - admit) incorporated into model

Model predictors: Age, peripheral edema, SBP, hyponatremia, serum urea, NYHA class, Discharge NT-proBNP and discharge – admission ΔBNP ≤30%

Salah, Heart 2014;100:115-25
Do changes in BNP predict HF hospitalization?

Study ID OR (95% CI) Weight
A-HEFT 0.61 (0.45, 0.82) 10.48
Abullul et al 1.00 (0.06, 16.82) 0.56
AREA IN-CHF 0.34 (0.13, 0.89) 3.71
ASTRONAUT 0.93 (0.74, 1.15) 11.66
Bielecka-Dabrowa 1.07 (0.31, 3.69) 2.46
CARE-HF 0.44 (0.31, 0.60) 10.15
COPERNICUS 0.88 (0.58, 1.32) 9.01
Krum et al 0.15 (0.01, 2.99) 0.50
NorthStar 1.04 (0.72, 1.49) 9.63
Paterna et al 0.03 (0.00, 0.50) 0.54
PROTECT 0.31 (0.14, 0.69) 4.74
RESOLVD 2.21 (0.96, 5.06) 4.47
RESOLVD 1.50 (0.64, 3.51) 4.33
TIME-CHF 0.63 (0.43, 0.92) 9.44
Tobill 0.07 (0.00, 1.34) 0.51
Troughton et al 0.32 (0.10, 1.02) 2.70
VAL-HEFT 0.72 (0.62, 0.84) 12.45
Wojnicz 0.31 (0.03, 3.06) 0.83
Wojnicz 0.20 (0.01, 4.31) 0.48
Yamada et al 0.25 (0.04, 1.48) 1.35
Overall (I-squared = 62.1%, p = 0.000) 0.68 (0.55, 0.84) 100.00

NOTE: Weights are from random effects analysis.
GUIDE-IT: Guiding Evidence Based Therapy Using Biomarker Intensified Treatment in HFrEF

HF Hospitalization or CV Death

All-Cause Mortality

Felker GM, et al. JAMA 2017; 318:713-20
Effect of cTn+ on IHD Readmission


Days After Hospital Discharge
Expressed genome: analytic approaches

DNA (genetics, genomics)

Epigenetic modification (epigenomics)

RNA (transcriptomics)


protein (proteomics)

metabolite (metabolomics)

cellular phenotypes (peripheral cell analysis, molecular imaging)

The promise of a healthy heart.
CnA: calcineurin transgenic mouse model

R9C: PLN R9C HF mouse model

Gene expression omnibus microarrays:
GSE18801 Isoproterenol induced CMP
GSE2459 Pressure overload CMP
GSE4678 Alpha tropomyosin familial CMP
**CnA:** calcineurin transgenic mouse model

**R9C:** PLN R9C HF mouse model

**Gene expression omnibus microarrays:**
- GSE18801 Isoproterenol induced CMP
- GSE2459 Pressure overload CMP
- GSE4678 Alpha tropomyosin familial CMP

Correlation with human urine or plasma proteomes

Additional notes:
- IGFBP7

IGFBP-7 and diastolic dysfunction in HFpEF

RELAX Trial: 216 pts with LVEF >50% and stable HF, elevated NT-proBNP >400 pg/mL or elevated filling pressures

Prognostic Value of Insulin-Like Growth Factor-Binding Protein 7 in Patients with Heart Failure and Preserved Ejection Fraction

PARUL U. GANDHI, MD,1,2 SHERYL L. CHOW, PharmD,3 THOMAS S. RECTOR, PhD,4,5 HENRY KRUM, PhD FRACP,6 HANNA K. GAGGIN, MD, MPH,7 JOHN J. MCMURRAY, MD,8 MICHAEL R. ZILE, MD,9 MICHEL KOMAJDA, MD,10 ROBERT S. MCKELVIE, MD, PhD,11 PETER E. CARSON, MD,12 JAMES L. JANUZZI Jr., MD,7,* AND INDER S. ANAND, MD, DPhil4,5,*

I-PRESERVE
N= 4128

With baseline IGFBP7 measurements
N=302

Without baseline IGFBP7 measurements
N=3826

With baseline and 6 month IGFBP7 measurements
N=293

Irbesartan
N=147

Placebo
N=146

Primary Event
All-Cause Death
Heart Failure Event

Hazard Ratio

100 150 200 250 300 350 400 450
IGFBP7 ng/ml
<table>
<thead>
<tr>
<th>Utility</th>
<th>Low-density lipoprotein</th>
<th>C-reactive protein</th>
<th>Creatine kinase-MB</th>
<th>NT-pro BNP</th>
<th>High-sensitivity troponin</th>
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<tbody>
<tr>
<td>Normal concentration</td>
<td>~10 μg/ml</td>
<td>&lt;10 μg/ml</td>
<td>16 - 87 ng/ml</td>
<td>10 - 300 pg/ml</td>
<td>10 - 17 pg/ml</td>
</tr>
<tr>
<td>Changes in patient/at-risk individuals</td>
<td>~1.5 - 2x baseline</td>
<td>&gt;3x baseline</td>
<td>&gt;20x baseline</td>
<td>&gt;20x baseline</td>
<td>Up to 1,000x baseline</td>
</tr>
</tbody>
</table>
Platforms for protein biomarker discovery

Two-dimensional gel electrophoresis

Scope (# proteins) ~10²
Throughput (# samples) ~10²
Dynamic Range ~10³
Sensitivity μg/ml

Gel-free tandem mass spectrometry

untargeted
Scope (# proteins) ~10²
Throughput (# samples) ~10²
Dynamic Range ~10³
Sensitivity ng/ml

targeted
Scope (# proteins) 10³ - 10⁴
Throughput (# samples) ~10²
Dynamic Range ~10⁷
Sensitivity pg/ml

Aptamer-based protein array

Scope (# proteins) ~10²
Throughput (# samples) up to ~1,000
Dynamic Range ~10⁸
Sensitivity pg/ml

Unbiased, systems-based
Low-abundance proteins underdetected

Capture target proteins by using conjugated DNA aptamers
High sensitivity


The promise of a healthy heart.
## Proteins Predicting Acute MI (selected from 753 proteins; analysis using Mass Spec)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein Name</th>
<th>Function / Role in MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPIA</td>
<td>Cyclophilin A</td>
<td>Protein folding; Secreted in response to inflamm stimuli &amp; oxid stress; ↑ in HF</td>
</tr>
<tr>
<td>CD5L</td>
<td>CD5 antigen-like</td>
<td>Cell surface ligand on activated lymphocytes; Fn in atherosclerosis unknown</td>
</tr>
<tr>
<td>MCAM</td>
<td>Cell-surface GP MUC18</td>
<td>Role in cell adhesion and cohesion of vascular endothelial monolayer</td>
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<tr>
<td>AMY1A</td>
<td>α-amylase 1 (salivary)</td>
<td>Cleaves glycosidic linkages; Modulate glycemic response after glucose intake</td>
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<tr>
<td>COL18A1</td>
<td>Collagen α-1 (XVIII) chain</td>
<td>Encodes for endostatin → inhibits angiogenesis; Plaque neovascularization</td>
</tr>
<tr>
<td>MMRN2</td>
<td>Multimerin-2</td>
<td>Extracellular matrix glycoprotein of elastin family</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
<td>Acute phase reactant; Risk factor for CAD</td>
</tr>
</tbody>
</table>

7 proteins increased c-statistic of prediction model for AMI to 0.84 from 0.71 (of base clinical model that includes known clinical factors: age, sex, smoking, total & HDL cholest, statin use, other lipid-lowering Rx, SBP, HTN Rx, DM, and BMI)
Human Dilated Cardiomyopathy (4263 proteins)


78% of all changed genes were membrane proteins


The promise of a healthy heart.
Myocardial Infarct Border Zone (Swine)

Inflammation

Angiogenesis

Electron transport chain & high-energy phosphate metabolism

Yang L, J Proteome Res 2017; 16:2101-12
Aptamer Protein Arrays

- DNA aptamers:
  - Single-stranded deoxyribonucleotides with 3D-folded structures
  - Affinity to specific epitopes on target proteins
  - Sequence library for specific ligand targets

- Modified into SOMAmers (slow off-rate modified aptamers):
  - Chemical modifications of nucleotides to enhance stability or hydrophobic interactions
Prognostic Index

Lower score: ANGPT2 (Angiopoietin-2), C7 (Complement 7), CCL18 (Chemokine ligand 18), ANGPTL4 (Angiopoietin-related protein 4), SERPINA3 (α1-antichymotrypsin complex), MMP12 (Matrix metalloproteinase 12), TNN13 (Troponin I)

Higher score: GDF8/11 (Growth differentiation factor 11/8), SERPINF2 (α2-antiplasmin)

Prognostic Index

Lower score: ANGPT2 (Angiopoietin-2), C7 (Complement 7), CCL18 (Chemokine ligand 18), ANGPTL4 (Angiopoietin-related protein 4), SERPINA3 (α1-antichymotrypsin complex), MMP12 (Matrix metalloproteinase 12), TNN13 (Troponin I)

Higher score: GDF8/11 (Growth differentiation factor 11/8), SERPINF2 (α2-antiplasmin)

Inactivates plasmin
Reduces fibrinolysis

Suppresses cardiac hypertrophy and HFpEF

Proteomics for incident HF

**Discovery cohort:** Prospective Investigation of Vasculature in Uppsala Seniors (n = 901)

**Validation cohort:** Uppsala Longitudinal Study of Adult Men (n = 685)

GDF-15: growth differentiation factor 15  
AM: adrenomedullin  
FGF-23: fibroblast growth factor 23  
TIM-1: T-cell immunoglobulin and mucin domain 1  
TRAIL-R2: TNF-related apoptosis-inducing ligand receptor 2  
SPON1: spondin-1  
MMP-12: matrix metalloproteinase-12  
FS: follistatin  
PIGF: placenta growth factor  
U-PAR: urokinase-type plasm activator surface receptor  
CSF-1: colony-stimulating factor-1  
FABP4: fatty acid-binding protein 4  
TNF-R1: TNF receptor 1  
OPG: osteoprotegerin  
ST2: suppression of tumorigenicity 2  
PAR1: proteinase-activated receptor 1  
CHI3LI: chitinase-3-like protein 1  
TNF-R2: TNF receptor 2

Stenemo M, Eur J Heart Failure 2018; 20:55-62
Proteomics for incident HF prediction

Baseline model
BMI
Lipids
Glycemic status
Blood pressure
Smoking
Left ventricular hypertrophy
Myocardial infarction prior/during study
Atrial fibrillation prior/during study
Kidney function
Fully adjusted

GDF-15  AM  FGF-23  TIM-1  SPON1  MMP-12  FS  U-PAR  PIGF  FABP4  CSF-1  TNF-R1  OPG  ST2  PAR-1  TNF-R2  CHI3L1
Incident HF prediction: adjusted for NT-proBNP
Non-Ischemic HF

Significant for incident non-ischemic HF

Baseline model
BMI
Lipids
Glycemic status
Blood pressure
Smoking
Left ventricular hypertrophy
Myocardial infarction prior/during study
Atrial fibrillation prior/during study
Kidney function
Fully adjusted
Question #2

The primary future need for a biomarker test in those diagnosed with HF is:

A) Determining who has HFP EF vs. HFr EF
B) Determining who has HF due to coronary disease
C) Determining who needs an implantable cardiac defibrillator for sudden death
D) Predicting who will be readmitted to hospital
To show this poll

1. Install the app from pollev.com/app
2. Start the presentation

Still not working? Get help at pollev.com/app/help
or
Open poll in your web browser
High Dimensional Data

- Multiple factors may contribute to disease
- Key regulatory components may appear relatively small quantitatively
- Interactions between proteins
- Effects identifiable by regression-based methods?
### Complexity: Proteins with Positive Association with Framingham Risk Score


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<thead>
<tr>
<th>Protein</th>
<th>FRS</th>
<th>Age</th>
<th>Female</th>
<th>TC</th>
<th>HDL</th>
<th>SBP</th>
<th>Diabetes</th>
<th>Smoking</th>
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</table>

**Estimated Beta Coefficient**

-0.976 to 1.162
Complexity: Proteins with Negative Association with Framingham Risk Score

Similarity Network Fusion
Integrated Toronto Analytic Model (i-TAM)

“Next Gen” ADHF Prediction

- Physician perspective
- Patient perspective (PROMs, Text)
- Clinical Data
- MEDLY (Remote Monitoring)

Precision Proteomics

- Proteomics
- Agnostic Clustering of Molecular – Clinical metadata
- Days until Readmission

Custom Code (AI, Machine learning)

- Predict HF readmissions
- Predict HF readmissions
- Predict HF readmissions

Toronto Analytic Model

Standard of Care

INTEGRATED Toronto Analytic Model

Effectiveness and Economic Impact Analyses

The promise of a healthy heart.
Conclusions

- Predicting HF events before they happen with greater precision
- BNP and Tp are important biomarkers at present time
- But there is need for new approaches to predict risk:
  - New biomarkers and ‘-omics’ approaches
  - New technologies, new types of data
  - New approaches to analyzing and integrating complex & deep data from multiple data sources
- Better care and outcomes & reduce costs of HF care
TRCHR Strategic Innovation Research Team

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Dr. Heather Ross
Dr. Anthony Gramolini
Dr. Patrick Lawler
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