The Role of Biomarkers in Cardiovascular Disease

Shweta R. Motiwala, MD
Massachusetts General Hospital
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Scope of the Problem: Mortality

• CVD is the leading cause of death among women in the United States
  – Regardless of race or ethnicity

• In 2009, > 400,000 deaths
  – More deaths than stroke, COPD, lung cancer, and breast cancer combined

Go et al, Circulation 2013
Scope of the Problem: Morbidity

- In 2009, total cost of CVD and stroke ~ $312.6 billion
- Accounts for 15% of total health costs

What is the role for biomarkers?

- 1980 to 2002: age-adjusted CVD mortality decreased in men and women
- Morbidity and mortality remains high
  - Existing therapies are static: pharmacologic, device-based and interventional
- Use of cardiac biomarkers may improve outcomes by allowing for personalized or biologically-based care
What is a biomarker?

- Indicator of disease:
  - Trait: risk factor/marker
    - Antecedent
  - State: preclinical or clinical
    - Screening
    - Diagnostic
  - Rate: progression
    - Staging
    - Prognostic

What Makes a Cardiac Biomarker Useful?

- Goal: improve ability of clinician to optimize care of patient
- Characteristics:
  - Accurate, reproducible, available, interpretable
  - Known normal distribution and abnormal values
  - Acceptable to patient
  - Predicts outcome of interest
  - Adds to existing clinical and laboratory assessment
  - Knowledge of levels changes management
Biomarkers in CVD states

- Coronary heart disease: Troponin
- Heart failure: NT-proBNP
- Emerging biomarkers: Galectin-3, sST2, proneurotensin
Two-compartment troponin biology

- MYOCARDIAL INJURY
  - Cytosolic troponin
  - Responsible for "early" troponin release

- Contractile apparatus
  - Responsible for "delayed/persistent" troponin release

Cytosolic troponin

Contractile apparatus

Cardiomyocyte


- cTnT Levels are Associated with Outcomes in ACS

Ohman et al, NEJM 1996

Graph showing probability of death within 30 days against troponin T levels (ng/ml).
Diagnosis of MI

- Detection of rise/fall of cTnI or cTnT
  - At least one value > 99th percentile of the URL

- Evidence of myocardial ischemia with at least one of the following:
  - Symptoms
  - ECG changes (new ischemia, Q waves)
  - Imaging evidence of new loss of myocardium or new RWMA

99th Percentile for Troponin T

- Blood donors (n=1251)
- Apparently healthy individuals (n=500)

- 99th percentile = 13 pg/mL (imprecision 10%)
- Old URL = 0.03 ng/mL (imprecision 10%)
Sex Differences in 99th Percentile Values for cTnI and cTnT

• cTnI in healthy males > females
  – Differences between assays

• cTnT in healthy males similar to females
  – Single assay

• Role of assay imprecision?

Apple et al, Clin Chem 2012

Newer ‘high sensitivity’ troponin assays

• New assays for troponin
• Extreme precision (i.e. <10% co-efficient of variation) at the 99th percentile for a normal patient population
Sex Differences in 99th Percentile Values for hs-cTnl and hs-cTnT

- hs-cTnl in healthy males > females
- hs-cTnT in healthy males > females

Apple et al, Clin Chem 2012

Risk Factors Associated with Detectable hs-cTn

- Age
- Sex (male > female)
- Race (black > others)
- Lower eGFR
- History of HF
- Increased LV mass and LV thickness
- Hypertension
- Diabetes mellitus

de Lemos et al, JAMA 2010
hsTn Elevation in Apparently Healthy

- cTnI and cTnT are 100% specific for heart
  - High-sensitivity assay detects cTn in nearly all presumably healthy subjects
  - May reflect detection of subclinical disease
  - Indirect assessment of CV health
    - Structural/functional changes, CAD severity
    - Relevance of gender differences

Association with CV Mortality

Category 5 (>99th percentile)
Category 4
Category 3
Category 2
Category 1 (undetectable)

de Lemos et al, JAMA 2010
Highly Sensitive Troponins Improve the Early Diagnosis of AMI

hs-Tn + ECG + History → Rule out ↑ + Rule in ↑

Reichlin et al, NEJM 2009

hsTnT is Superior to cTnT for Diagnostic Evaluation of Chest Pain

<table>
<thead>
<tr>
<th>Analyte, cut-point</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsTnT, 13 pg/mL</td>
<td>62%</td>
<td>89%</td>
<td>38%</td>
<td>96%</td>
</tr>
<tr>
<td>cTnT, 0.01 ng/mL</td>
<td>49%</td>
<td>97%</td>
<td>67%</td>
<td>95%</td>
</tr>
<tr>
<td>cTnT, 0.03 ng/mL</td>
<td>35%</td>
<td>99%</td>
<td>72%</td>
<td>93%</td>
</tr>
</tbody>
</table>

hsTnT ~30% of ACS patients were reclassified from UA to NSTEMI by hsTnT

NRI and IDI analyses demonstrate significant improvement in diagnostic accuracy with hsTnT

Januzzi et al, Circulation 2010
Ischemia & Necrosis: old cTn assay

- Stable CAD
- Non-coronary cardiac necrosis
- Unstable Angina
- AMI

Ischemia & Necrosis: hs-cTn assay

- Stable CAD
- Increase 25-75%
- Non-coronary cardiac necrosis
- Unstable Angina
- AMI
- Increase >200%

Christ et al, Am J Med 2010
Application of hsTn for differential dx

What’s the problem?
$cTn_{AMI} = cTn_{Myocarditis} = cTn_{Tachycardia} = cTn_{AHF}$ etc.

- hsTnT strongly associated with the presence and severity of CAD, as well as cardiac structure and function…

...independent of a diagnosis of ACS

Januzzi et al, Circulation 2010

Non-ACS Troponin Elevation

- A real false positive
- Normal variants?
- Pericarditis
- Severe illness
  - Sepsis
- Blunt chest trauma
- Radiofrequency ablation
- DC Cardioversion
- Transplant rejection
- Aortic dissection
- Myocarditis
- Pulmonary embolism
- Myocardial abscess
- CHF and LVH
- Arrhythmias and LVH
- Idiopathic CMP
- Chemotherapy or other toxic/metabolic insults
- Cirrhosis
- Renal failure
Biomarkers in CVD states

- Coronary heart disease: Troponin
- Heart failure: NT-proBNP
- Emerging biomarkers: Galectin-3, sST2, proneurotensin

Why should we care about heart failure?

*Lifetime Risk for CHF by Sex and Age*

Lloyd-Jones et al, Circulation 2002
Assessment of Heart Failure

No gold standard for the evaluation of HF exists!

History and Physical

Laboratory Testing

What is NT-proBNP?

Motiwala and Januzzi, Cardiol Rev 2012
Natriuretic Peptides: Major Clinical Utilities

- To supplement clinical judgment
  - Acute evaluation and diagnosis
  - Grading HF severity
- To provide prognostication using processes not obvious at the bedside
- To offer unique information regarding therapeutic intervention
  - To judge therapeutic success
  - To guide therapy?

Diagnostic Uncertainty in Dyspnea is Associated with Poor Prognosis

31% of subjects in PRIDE were judged uncertainly by the managing physician re: likelihood for HF as cause of dyspnea.

Their prognosis was significantly worse, with higher rates of death and re-hospitalization and longer lengths of stay.

Green et al, Arch Intern Med 2008
NT-proBNP Levels are Higher in Acute Heart Failure

Januzzi et al, Am J Cardiol 2005

NT-proBNP Levels are Associated with Symptoms

Januzzi et al, Am J Cardiol 2005
### Know the Differential Diagnosis of an Elevated NP

<table>
<thead>
<tr>
<th>Unrecognized HF</th>
<th>Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior HF</td>
<td>Pulmonary embolism</td>
</tr>
<tr>
<td>LVH</td>
<td>Cardiac surgery</td>
</tr>
<tr>
<td>Valvular heart disease</td>
<td>Sleep apnea</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>Critical illness</td>
</tr>
<tr>
<td>Advancing age</td>
<td>Sepsis</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>Burns</td>
</tr>
<tr>
<td>ACS</td>
<td>Renal failure</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>Toxic-metabolic insults</td>
</tr>
<tr>
<td>Congenital heart disease</td>
<td></td>
</tr>
</tbody>
</table>

### Know the Differential Diagnosis of an Unexpectedly Low NP

- Obesity
- Non-systolic heart failure
- Mild acute heart failure
- Isolated right heart failure
- Partially treated heart failure
Logical use of natriuretic peptide values

Patient presents with acute dyspnea

History, physical exam, CXR, ECG
Measure NT-proBNP

NT-proBNP <300 ng/L
HF very unlikely
Evaluation for a non-cardiac cause of dyspnea is recommended

NT-proBNP >300 ng/L
HF possible
Clinical correlation necessary
Triage and treat as appropriate, possible early die

NT-proBNP >age-adjusted positive
HF likely
Triage and treat as appropriate
If prior HF, evaluate for a >5% hemodynamic “dry” NT-proBNP

NP values are Lower in HFpEF

O'Donoghue et al, J Card Fail 2005
Sex Differences in NT-proBNP and BNP Levels

- Healthy: female > men
  - Lower BMI
  - Lower androgen (testosterone) levels

- Disease: female < male
  - Increased incidence of HFpEF

Natriuretic Peptides: Major Clinical Utilities

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  - Acute evaluation and diagnosis
  - Grading severity of HF

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  - To judge therapeutic success
  - To guide therapy?
NPs are Associated with Long-term Risk of Death in Dyspneic Patients

A single natriuretic peptide measurement at presentation offers prognostic value out to over 4 years!

Rehman et al, in preparation

Remodeling in ACS and HF

- Remodeling is not felt
- Remodeling is not detectable with physical exam, until too late
- Imaging can see remodeling, but only too late
- Predicting remodeling with imaging is imperfect
Higher NT-proBNP is Associated with Risk for Remodeling

<table>
<thead>
<tr>
<th>Remodeling index</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in LVEDVi</td>
<td>1.43</td>
<td>1.10-1.86</td>
<td>0.007</td>
</tr>
<tr>
<td>Increase in LVESVi</td>
<td>1.54</td>
<td>1.10-1.91</td>
<td>0.01</td>
</tr>
<tr>
<td>Fall in LVEF</td>
<td>1.53</td>
<td>1.12-1.89</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Hazard ratio refers to risk for remodeling per log-unit of NT-proBNP at the end of the study.

NT-proBNP was entered as log-transformed due to non-normality. Model adjusted for baseline log-transformed NT-proBNP, age, diabetes, ischemic heart disease and New York Heart Association Symptom Severity.

Weiner et al, Eur J Heart Fail 2012

Natriuretic Peptides: Major Clinical Utilities

- To supplement clinical judgment
  - Acute evaluation and diagnosis
  - Grading severity of HF
- To provide prognostication using processes not obvious at the bedside
- To offer unique information regarding therapeutic intervention
  - To judge therapeutic success
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Serial NP Measurements are Useful for Prognostication in Chronic HF

“Shouldn’t we be maximizing all heart failure meds in all patients?”

- Even for the skilled HF specialist with resources for close follow up, the addition of NP measurement is valuable
- The majority of heart failure care is not in the hands of heart failure specialists.
- Opportunities exist for achieving guideline-derived medical therapy goals
Risk-Treatment Mismatch in HF: Canadian EFFECT Study

At Hospital Discharge 90-Day Follow-Up 1-Year Follow-Up

Use rates in absence of contraindications. For all drug classes, P < 0.001 for trend.

Therapies with Effects on B-Type Natriuretic Peptide Levels

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Effect on BNP/NT-proBNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuresis</td>
<td>↓</td>
</tr>
<tr>
<td>ACE-I</td>
<td>↓</td>
</tr>
<tr>
<td>ARB</td>
<td>↓</td>
</tr>
<tr>
<td>β-blockers</td>
<td>Some transiently ↑, most ↓</td>
</tr>
<tr>
<td>Aldosterone antagonists</td>
<td>↓</td>
</tr>
<tr>
<td>BiV pacing</td>
<td>↓</td>
</tr>
<tr>
<td>Exercise</td>
<td>↓</td>
</tr>
<tr>
<td>Rate control of AF</td>
<td>↓</td>
</tr>
<tr>
<td>BNP infusions</td>
<td>↓ N-BNP, ↑BNP then ↓</td>
</tr>
</tbody>
</table>
Characteristics of “guided therapy” trials

- Well tolerated
- Up-titration of therapies more often seen in biomarker guided arm
- When a low target was selected and natriuretic peptide lowering was achieved, better outcomes were observed

Januzzi, J Card Fail 2011

Guided therapy Combined Analyses Demonstrate Benefit

Meta analysis of publication data
Pooled patient data from all available trials

Felker et al, Am Heart Journal 2009
Troughton et al, ESC 2011
Biomarkers in CVD states

- Coronary heart disease: Troponin
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Galectin-3 in HF  
*Scientific Discovery*

- In animal models of HF, galectin-3 is highly expressed in failing versus functionally compensated hearts
- Intrapericardial administration of galectin-3 significantly increases LV collagen content and reduces LV EF

Sharma et al, Circulation 2004
Joint effect of NT-proBNP and galectin 3 in chronic heart failure

Galectin-3 Levels are Associated with Outcomes in HF

Response status relative to 20 ng/mL
High/Low vs. Low/High, p = 0.5

Motiwala et al, submitted
What is ST2?

Kakkar and Lee, Nat Rev Drug Discov 2008

sST2 is Additive to NT-proBNP for Long Term Prognosis

Januzzi et al, Clin Chem 2010
Ramification of change in ST2 in PROTECT: 3 months

Across the entire trial, the HR_{adj} for events when changing from response to non-response was 3.64 (p = .009)

ST2 Predicts HF in Community Cohort

*Adjusted for: age, gender, BMI, systolic blood pressure, hypertension therapy, total cholesterol, HDL cholesterol, regular cigarette smoking, presence of diabetes, BNP, CRP, eGFR, prevalent atrial fibrillation, prevalent cardiovascular disease, electrocardiographic left ventricular hypertrophy, and heart murmur

Wang et al, Circulation 2012
What is (Pro-) Neurotensin (P-NT)?

- Neurotensin is a satiety hormone

Sites of Synthesis and Function

Central nervous system
- Amygdala
- Hypothalamus

Gastrointestinal tract
- enteroendocrine cells (N-cells) in the mucosa of ileum, jejunum, colon and duodenum

SATIETY, HYPOTHERMIC, ANTINOCICEPTIVE

STIMULATION OF GROWTH, SECRETION
INHIBITION OF MOTILITY, SATIETY (?)
Neurotensin Has 3 Different Receptors in Humans

- **NTSR1**: G-coupled
  - Breast cancer progression (trophic and antiapoptotic)

- **NTSR2**: G-coupled

- **NTSR3**: Not G-coupled
  - (=SORT1)
  - One of the major human CAD susceptibility gene variants

P-NT Strongly Interacts With Gender on Risk of Incident CVD

<table>
<thead>
<tr>
<th>Sample size / first events</th>
<th>HR per 1 SD (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (4362 / 519)</td>
<td>1.17 (1.07-1.27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women (2559 / 224)</td>
<td>1.33 (1.17-1.51)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men (1803 / 295)</td>
<td>1.06 (0.95-1.19)</td>
<td>0.310</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, use of antihypertensive medication, systolic blood pressure, BMI, current smoking, diabetes mellitus and fasting concentrations of HDL and LDL

Melander et al, JAMA 2012
### P-NT Strongly Predicts CVD in Women

<table>
<thead>
<tr>
<th>Q1 (of P-NT)</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 (ref)</td>
<td>0.91 (0.59-1.41)</td>
<td>1.58 (1.08-2.30)</td>
<td>1.65 (1.13-2.41)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, use of antihypertensive medication, systolic blood pressure, BMI, current smoking, diabetes mellitus and fasting concentrations of HDL and LDL.

Melander et al, JAMA 2012

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### P-NT Predicts New-Onset Diabetes Mellitus in Women

<table>
<thead>
<tr>
<th>Sample size / first events</th>
<th>HR per 1 SD (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women non-DM (2200 / 74)</td>
<td>1.41 (1.12-1.77)</td>
<td>0.003</td>
</tr>
<tr>
<td>Women non-IFG (1950 / 38)</td>
<td>1.47 (1.08-2.00)</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Adjusted for age, use of antihypertensive medication, systolic blood pressure, BMI, waist circumference, prevalent cardiovascular disease, current smoking and fasting concentrations of glucose, HDL, LDL, triglycerides and insulin.

Melander et al, JAMA 2012
### P-NT Predicts Breast Cancer (113 first events)

<table>
<thead>
<tr>
<th>Q1 (of P-NT)</th>
<th>Q2 (95% CI)</th>
<th>Q3 (95% CI)</th>
<th>Q4 (95% CI)</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 (ref)</td>
<td>1.32 (0.70-2.50)</td>
<td>1.89 (1.03-3.46)</td>
<td>2.80 (1.59-4.92)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Adjusted for age, use of antihypertensive medication, use of hormone replacement therapy, ever use of oral contraceptives, educational level, age at menarche, age at first child birth, number of children, menopausal status, systolic blood pressure, BMI, diabetes mellitus, current smoking, prevalent cardiovascular disease, heredity for cancer, and fasting concentrations of HDL, LDL and insulin

Melander et al, JAMA 2012

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**Incident breast cancer**

![](image)
P-NT/Neurotensin Might Be Broad Markers of Women’s Health Risk

- Associations with DM, CVD, death and breast cancer
- Identification of women at high risk (>20%) in absence of traditional risk factors
- Can this change management and therapeutic choices?