BNP

Daniel J. Fink, MD, MPH
Director, Core Laboratory
New York Presbyterian Hospital
Columbia University Medical Center
New York, New York

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Outline

The Biochemistry of Natriuretic Peptides

Congestive Heart Failure
  Diagnosis and Management of CHF

Clinical Chemistry of BNP Assays

Questions
Background

Heart as an Endocrine organ

1950

GFR and Aldosterone plus “Third Factor”

1981

1988

1990

2004

Natriuretic Peptides

Half-life of 3-5 minutes

Half-life of 18 minutes

Half-life of 2.6 minutes

ANP 28 aa peptide

BNP 32 aa peptide

CNP 22 aa peptide
BNP Production

- pre-proBNP (physiologically inactive)
- proBNP (physiologically inactive)
- Intracellular BNP (physiologically active)
- Intravascular BNP

Functions of the Natriuretic Hormones

- Natriuresis
- Diuresis
- Vasodilation

Cleveland Clin J of Med, Vol 71, Suppl 5, 6-2004
Characteristics of Natriuretic Peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Structure</th>
<th>Major sites of synthesis</th>
<th>Major regulators of secretion</th>
<th>Major effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP</td>
<td>28 amino acids</td>
<td>Cardiac myocytes, production shifts from atria to ventricles in cardiac overload; induction of gene expression slow</td>
<td>Myocyte stretch; vasoactive factors; stored in granules, plasma levels regulated at the level of hormone secretion</td>
<td>Natriuresis, diuresis, vasodilatation, inhibition of renin secretion and angiotensin II actions</td>
</tr>
<tr>
<td>BNP</td>
<td>32 amino acids</td>
<td>Cardiac myocytes, central nervous system; induction of gene expression rapid</td>
<td>Myocyte stretch; regulation of secretion occurs mainly at the level of synthesis, especially in ventricular myocytes</td>
<td>Natriuresis, diuresis, vasodilatation, inhibition of renin secretion and angiotensin II actions</td>
</tr>
<tr>
<td>CNP</td>
<td>22 amino acids</td>
<td>Vasculature, central nervous system</td>
<td>Cytokines, growth factors</td>
<td>Vasodilatation; inhibition of growth</td>
</tr>
</tbody>
</table>


Mechanism of Action
Outline

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Causes of Congestive Heart Failure

Hypertension

COPD

Cardiotoxic drugs

Atherosclerotic Ischemic Heart Disease (IHD)

Valvular dysfunction

Infection

Cardiomyopathy
Epidemiology of CHF

Acute CHF affects over 1,000,000 annually in the US

- Direct mortality - 42,000 deaths/year
- Indirect mortality - 220,000 deaths/year

Incidence - 500,000 new cases/year

Prevalence - 5 million (1.8%); 10% after age 75

CHF is the #1 cause of hospitalization for people over 65

Associated with a readmission rate of 30 - 40% in 90 days

CHF causes significant morbidity and mortality; 60% of men and 49% of women die within 5 years of diagnosis

Sudden death occurs at 6 - 9x the rate for the general population

Costs: $21 Billion/year

Prevalence in U.S. 1988 - 1994

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Male</th>
<th>Female</th>
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</thead>
<tbody>
<tr>
<td>20-24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25-34</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>35-44</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>45-54</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>55-64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>65-74</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>75+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
**Neurohumoral Changes**

- Sympathetic nervous system activity (Epinephrine, NE)
- Endothelin
- Arginine vasopressin
- Renin and Angiotensin II
- Aldosterone
- Neuropeptide Y
- ANP and BNP
- Insulin, Cortisol, Growth hormone, Tumor necrosis factor-α, Interleukin 6, Vasoactive intestinal peptide, Adrenomedullin, Urodilatin
- Dopamine
- Prostaglandins (PGI2, PGE2)
- Vasodilator peptides, (e.g., Bradykinin)

**NOTE:** Measurements in individual patients vary significantly; changes may not always be present.

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**BNP Levels Correlate to NYHA Class**

[Graph showing BNP levels (pg/ml) correlated with NYHA classification]

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CHF May Be Difficult to Diagnose

- Non-specific clinical signs and symptoms
- No simple near-patient diagnostic test
- Usual hospital diagnostic procedures:
  - Echocardiography
  - Cardiac catheterization
  - Radiography (radionuclide ventriculography)
- Problems: Not always available
  - Time-consuming
  - Expensive
Left Ventricular Ejection Fraction (LVEF)

\[
LVEF = \frac{\text{Stroke Volume}}{\text{End Diastolic Volume}}
\]

Stroke Volume = (End Diastolic Volume – End Systolic Volume)

Two-dimensional Doppler Echocardiogram

Blue Line – Left Ventricle
Orange Line – Blood Endocardial Border
Left Ventricular Ejection Fraction

- **Average**: 69%
- **Abnormal**: 63%
- **Dysfunctional**: 50% & 40%

Asymptomatic

Symptomatic

Why Test for Natriuretic Peptides?

- Simply, rapidly, inexpensively measured
- Potential uses

**Diagnosis**

**Prognosis**

**Guiding Therapy**
### Diagnosis

<table>
<thead>
<tr>
<th>Predictor</th>
<th>P-value</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTN</td>
<td>0.03</td>
<td>2.3</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.002</td>
<td>4.0</td>
</tr>
<tr>
<td>IHD</td>
<td>0.0003</td>
<td>6.0</td>
</tr>
<tr>
<td>BNP (&gt; 12.9 pg/mL)</td>
<td>0.005</td>
<td>13.0</td>
</tr>
<tr>
<td>NT-proBNP (&gt; 86 pg/mL)</td>
<td>&lt;0.0001</td>
<td>14.5</td>
</tr>
</tbody>
</table>

HTN, Hypertension; IHD, Ischemic Heart Disease; BNP, B-type Natriuretic Peptide, NT-proBNP, N-terminal proBNP

### Diagnosis: BNP for CHF Screening

<table>
<thead>
<tr>
<th>Type of patient population</th>
<th>General</th>
<th>Symptomatic PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>76</td>
<td>97</td>
</tr>
<tr>
<td>Specificity</td>
<td>87</td>
<td>84</td>
</tr>
<tr>
<td>PPV</td>
<td>16</td>
<td>70</td>
</tr>
<tr>
<td>NPV</td>
<td>98</td>
<td>98</td>
</tr>
</tbody>
</table>

PC, Primary Care  
PPV, Positive Predictive Value  
NPV, Negative Predictive Value

**BNP versus Physical Exam**

BNP AUC = 0.979

Physical examination AUC = 0.884


**Diagnosis: BNP Predicts Abnormal LV Function**

Source: Maisel AS et al. Am Heart J 2001; 141:367-74
1. **Controls**
2. **LVEF > 55%**
3. **LVEF 40-55%**
4. **LVEF < 40%**

**Clinical Algorithm for Interpreting BNP**

- **Patient presenting with dyspnea**
  - **Physical examination, chest x-ray, ECG, BNP level**
  - **BNP < 100 pg/mL**
    - **CHF very unlikely (2%)**
  - **BNP 100-400 pg/mL**
    - **Baseline LV dysfunction, underlying cor pulmonale or acute pulmonary embolism?**
      - **Yes**
        - **Possible CHF (25%)**
      - **No**
        - **CHF likely (75%)**
  - **BNP > 400 pg/mL**
    - **CHF very likely (95%)**

**References**

**Diagnosis: Algorithm for CHF**

- History and Physical Office EKG
  - Heart Failure?
    - Send to Hospital
      - Diagnostic Procedures
        - Electrocardiography
        - Chest X-ray
        - Radionuclide Ventriculography
        - Echocardiography
      - Abnormal
        - Other Diagnostic or Laboratory Tests
          - Select Therapy
    - Normal
      - CHF Unlikely
      - BNP Test
        - Normal
          - CHF Unlikely
        - Abnormal
          - Other Diagnostic or Laboratory Tests
            - Select Therapy

**Diagnosis: New Paradigm using BNP for CHF**

- History and Physical Office EKG
  - Heart Failure?
    - Send to Hospital
      - Diagnostic Procedures
        - Electrocardiography
        - Chest X-ray
        - Radionuclide Ventriculography
        - Echocardiography
      - Abnormal
        - Other Diagnostic or Laboratory Tests
          - Select Therapy
Heart Failure Survival Score Stratification Using BNP

- Low Risk
- Medium Risk
- High Risk

**BNP plasma concentration (pg/ml)**

<table>
<thead>
<tr>
<th>Risk Level</th>
<th>BNP Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>&lt;200 pg/ml</td>
</tr>
<tr>
<td>Medium</td>
<td>200 - 400 pg/ml</td>
</tr>
<tr>
<td>High</td>
<td>&gt;400 pg/ml</td>
</tr>
</tbody>
</table>

**p-values**

- p<0.0001
- p<0.0005


Prognosis: Survival with CHF or Post-MI

**CHF Survival**

- BNP < 73 pg/ml
- BNP > 73 pg/ml

**p-values**

- P < 0.0001


**MI Survival**

- BNP ≤ 33.3 pmol/L
- BNP > 33.3 pmol/L

**p-values**

- p < 0.001

BNP Guided Therapy for CHF

Trial

69 patients (LVEF <40% ) and symptomatic HF (NYHA class IV)

Plasma BNP (n = 33) or
standardized clinical assessment alone (n = 36).

Troughton et al. The Lancet 2000;355:1126-1130

Guided Therapy for CHF

<table>
<thead>
<tr>
<th>Total Cardiac Events</th>
<th>Episodes of CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT-proBNP (upper line)</td>
<td>Usual Clinical Care</td>
</tr>
</tbody>
</table>

BNP Guided Therapy for CHF

No CV events after 9.5 months

53%

Usual Care

27%

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**ADVIA Centaur BNP Assay**

**Two Site Immunoassay Format**

- **Lite Reagent:** DMAE Labeled KY-hBNP-II (Fab')
- **Sample:** EDTA plasma 100 µL
- **Solid Phase:** Streptavidin coated Magnetic Latex Particles with Biotinylated BC-203

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**Centaur Compared to ShionoRIA BNP**

- Linear regression: 0.9892 Shionogi - 0.7828 \( r = 0.98 \)  
  \( N=148 \)
**Use of NT-proBNP Versus BNP**

- **BNP** has been used in more studies & seems to correlate better with disease status.
- **NT-proBNP** circulates at higher levels
- **NT-proBNP** has a longer half-life (1-2 hours)
- **BNP** has a short half-life (<20 minutes)
- **NT-proBNP** will not cross-react with exogenous BNP
- Clearance of NT-proBNP dependent upon renal function
**BNP and pro-BNP Degradation**

*In vivo:*

- **BNP:** Natriuretic peptide receptors (A,B,C)
  - Neutral Zn\(^{2+}\)-dependent glycoprotein metalloproteinases
  - Renal Excretion?

- **proBNP:** Reticulo-endothelial system
  - Renal Excretion

From: Allen Wu

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**BNP and NT-proBNP Degradation**

*In vitro:*

- **BNP:** Shimizu et al. suggested that BNP is degraded by contact activation of the kallikrein system (extrinsic clotting).
  - Glass collection tubes can activate this extrinsic system
  - Arginine and kallikrein-specific inhibitors superior to serine proteinase inhibitors

- **NT-proBNP:** More stable in vitro because it is not degraded by proteinases.
### Stability of Natriuretic Peptides in EDTA Whole Blood

#### Stability of Natriuretic Peptides

<table>
<thead>
<tr>
<th>Storage Temperature</th>
<th>BNP</th>
<th>NT-proBNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temperature</td>
<td>24 h</td>
<td>5 - 72 h</td>
</tr>
<tr>
<td>4 °C (2 - 8 °C)</td>
<td>24 - 48 h</td>
<td>48 - 72 h</td>
</tr>
<tr>
<td>-20 °C</td>
<td>9 mo</td>
<td>12 mo</td>
</tr>
</tbody>
</table>

### SHIONORIA BNP Assay

- **C-Terminal specific BC-203 Antibody Coated Bead**
- **$H_2N-$**
- **$COOH-$**
- **KY-II hBNP-$^{125}I$**
- **$^{125}I$**
Table 3
Summary of Molar Immunoreactivity of B-Type Natriuretic Peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Access 2</th>
<th>ADVIA Contour</th>
<th>AxSYM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-32</td>
<td>82</td>
<td>126</td>
<td>106</td>
</tr>
<tr>
<td>3-32</td>
<td>69</td>
<td>126</td>
<td>118</td>
</tr>
<tr>
<td>4-32</td>
<td>159</td>
<td>175</td>
<td>164</td>
</tr>
<tr>
<td>10-32</td>
<td>&lt;1</td>
<td>163</td>
<td>&lt;1</td>
</tr>
<tr>
<td>1-31</td>
<td>87</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* Data are given as percentages. For proprietary information, see the text.
Influence of Age and Sex on BNP

Relative to males, female plasma BNP levels are significantly lower:

- **20-29 years:** p=0.0037
- **30-39 years:** p=0.0321
- **40-49 years:** p=0.0105
- **50-59 years:** p=0.0321
- **60+ years:** p=0.0105

Possible Reasons for Sex/Age Differences:

1. Women have thicker walled hearts than men
2. Estrogen effect (HRT) with postmenopausal women
3. Declining GFR with age
4. Obesity effect (BMI decrease with age)
BNP Results Interpretation

Caveats

- Age and Sex
- Renal failure
- Cirrhosis
- Obesity
- Other Conditions
- Assays may not compare

Other Diseases with Increases in BNP

- Left Ventricular Hypertrophy
- Cardiac Inflammation (e.g., Myocarditis, Cardiac Allograft Rejection)
- Arrhythmogenic Right Ventricle With Reduced Ejection Fraction
- Kawasaki Disease
- Primary Pulmonary Hypertension
- Pulmonary Embolism
- Renal Failure
- Ascitic Cirrhosis
- Endocrine (Primary Hyperaldosteronism, Cushing Syndrome)

BNP is secreted by the ventricular myocardium in response to volume overload and increase stretch.

BNP is more commonly used but NT-proBNP will probably be equally useful.

The effectiveness of nesiritide validates the basic pharmacological properties of endogenously-produced BNP.
## Summary

### Diagnosis

- Strong NPV (~98%) for R/O of CHF
- Potential use as a screening test (~70% PPV) in “at risk” population

### Prognosis

- BNP and NT-proBNP levels increase proportionately with CHF disease severity. Correlates to the NYHA classification system
- Correlates with Left Ventricular Ejection Fraction (LVEF)
- Assess risk of future episodes of CHF and Cardiac Events

### Guidance and monitoring of drug therapy

- Guide the selection therapy and monitor its efficacy.
- Aids the physician in the choice and dosage of medication

## Questions?