**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

GFR and Aldosterone plus “Third Factor”

ANP

BNP

CNP

DNP

1950

1981

1990

2004

**Natriuretic Peptides**

- ANP: 28 aa peptide
- BNP: 32 aa peptide
- CNP: 22 aa peptide

Half-life of 3-5 minutes

Half-life of 18 minutes

Half-life of 2.6 minutes
**Functions of the Natriuretic Hormones**

- **Natriuresis**
- **Diuresis**
- **Vasodilation**

**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, stimulation of gene expression slow</td>
<td>Myocyte stretch, cytokines, growth factors, stored in granules, regulated at the level of hormone secretion</td>
<td>Natriuresis, diuresis, vasodilation, 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, production 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, inhibition of renin secretion and angiotensin II actions</td>
</tr>
<tr>
<td>CNP</td>
<td>22 amino acids</td>
<td>Vasodilation, central nervous system</td>
<td>Cytokines, growth factors</td>
<td>Vasodilation, inhibition of growth</td>
</tr>
</tbody>
</table>

**Causes of Congestive Heart Failure**

- **Hypertension**
- **COPD**
- **Atherosclerotic Ischemic Heart Disease (IHD)**
- **Valvular dysfunction**
- **Infection**
- **Cardiotoxic drugs**
- **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

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**Prevalence in U.S. 1988 - 1994**

- Graph showing prevalence by age and gender.

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**Neurohumoral Changes**

- Sympathetic nervous system activity (Epinephrine, NE)
- Eosphelin
- 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)

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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)**

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

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

**Why Test for Natriuretic Peptides?**

Simply, rapidly, inexpensively measured potential uses

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Prognosis</th>
<th>Guiding Therapy</th>
</tr>
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</table>

**Two-dimensional Doppler Echocardiogram**

Blue Line – Left Ventricle
Orange Line – Blood Endocardial Border

**Left Ventricular Ejection Fraction**

- Average
- Abnormal
- Dysfunctional

**Dysfunctional**

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- Abnormal
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- Diagnosis
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**Left Ventricular Ejection Fraction**

- Average
- Abnormal
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**Diagnostic**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>P-value</th>
<th>Odds Ratio</th>
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</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>
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</table>

PPV, Positive Predictive Value; NPV, Negative Predictive Value

Source: Struthers A. Heart 2000;84:334-38
**BNP versus Physical Exam**

- BNP AUC = 0.979
- Physical examination AUC = 0.884

**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**
        - CHF unlikely (25%)
      - **No**
        - CHF likely (75%)

**Diagnosis: BNP Predicts Abnormal LV Function**

- **Normal (n = 105)**
- **Abnormal (n = 95)**

**Diagnosis: Algorithm for CHF**

- **History and Physical Office EKG**
  - **Heart Failure?**
    - **Send to Hospital**
  - **Diagnostic Procedures**
    - Electrocardiography
    - Chest x-ray
    - Radionuclide ventriculography
    - Echocardiography

**Diagnosis: BNP vs LVEF**

- **Log BNP**
  - Controls > 55%
  - 40 - 55%
  - < 40%

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

- **History and Physical Office EKG**
  - **Heart Failure?**
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**Source:**
- Maisel AS et al. Am Heart J 2001;141:367-74

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**Source:**
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Heart Failure Survival Score Strata

Prognosis: Risk Stratification Using BNP

- Low Risk
- Medium Risk
- High Risk

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

- Low Risk: BNP < 100 pg/ml
- Medium Risk: 100 pg/ml < BNP < 300 pg/ml
- High Risk: BNP > 300 pg/ml


CHF Survival

- Survival %
- Days
- Months

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

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

**BNP Guided Therapy for CHF**

- No CV events after 9.5 months

53% BNP Guided Therapy

27% Usual Care

**Outline**

- The Biochemistry of Natriuretic Peptides
- Congestive Heart Failure Management of CHF
- Diagnosis and Clinical Chemistry of BNP Assays
- Questions
Availability of Assays for BNP and NT-proBNP

**Beckman - BioSite BNP Assay**
Oct 2003

**SHIONORIA BNP Assay**

**ADVIA Centaur BNP Assay**
Two Site Immunoassay Format

**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

Centaur Compared to ShionoRIA BNP

0.9892 Shionogi - 0.7628 r = 0.98
(Linear regression) N=148

Centaur Compared To Triage BNP

Centaur = 1.0365 * Biosite - 0.2566
r = 0.92
N=135

**SHIONORIA BNP Assay**

G-Terminal specific
US-205 Antibody
Coated Bead

**Centaur Compared to ShionoRIA BNP**

0 200 400 600 800 1000 1200 1400
Shionogi BNP pg/mL

0 200 400 600 800 1000 1200 1400 1600 1800
Centaur Compared to ShionoRIA BNP Assay

**Centaur Compared To Triage BNP**

0 200 400 600 800 1000 1200 1400
Biosite BNP pg/mL

**ADVIA Centaur BNP Assay**

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BNP and pro-BNP Degradation

**In vivo:**

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

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

From: Allen Wu

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.

**Clinical Chemistry / Clinical Analysis**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Access 2</th>
<th>ACCESS Control</th>
<th>ACCESS yynm</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>59</td>
<td>140</td>
<td>110</td>
</tr>
<tr>
<td>4-32</td>
<td>159</td>
<td>170</td>
<td>164</td>
</tr>
<tr>
<td>13-32</td>
<td>97</td>
<td>160</td>
<td>162</td>
</tr>
<tr>
<td>1-101</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

*Data are given as percentages. For proprietary information, see the text.

**Stability of Natriuretic Peptides in EDTA Whole Blood**

<table>
<thead>
<tr>
<th>Stability of Natriuretic Peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Storage Temperature</strong></td>
</tr>
<tr>
<td>Room temperature</td>
</tr>
<tr>
<td>4 °C (2 - 8 °C)</td>
</tr>
<tr>
<td>-20 °C</td>
</tr>
</tbody>
</table>

**Caveats**

- Age and Sex
- Renal failure
- Cirrhosis
- Obesity
- Other Conditions
- Assays may not compare
Influence of Age and Sex on BNP

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)

Other Diseases with Increases in BNP

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

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
<table>
<thead>
<tr>
<th>Summary</th>
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<tbody>
<tr>
<td><strong>Diagnosis</strong></td>
</tr>
<tr>
<td>Strong NPV (~98%) for R/O of CHF</td>
</tr>
<tr>
<td>Potential use as a screening test (~70% PPV) in “at risk” population</td>
</tr>
<tr>
<td><strong>Prognosis</strong></td>
</tr>
<tr>
<td>BNP and NT-proBNP levels increase proportionately with CHF disease severity. Correlates to NYHA classification system</td>
</tr>
<tr>
<td>Correlates with Left Ventricular Ejection Fraction (LVEF)</td>
</tr>
<tr>
<td>Assess risk of future episodes of CHF and Cardiac Events</td>
</tr>
<tr>
<td><strong>Guidance and monitoring of drug therapy</strong></td>
</tr>
<tr>
<td>Guide the selection therapy and monitor its efficacy</td>
</tr>
<tr>
<td>Aids the physician in the choice and dosage of medication</td>
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