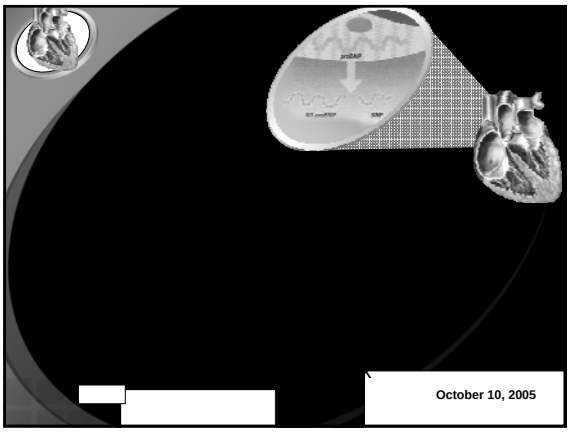
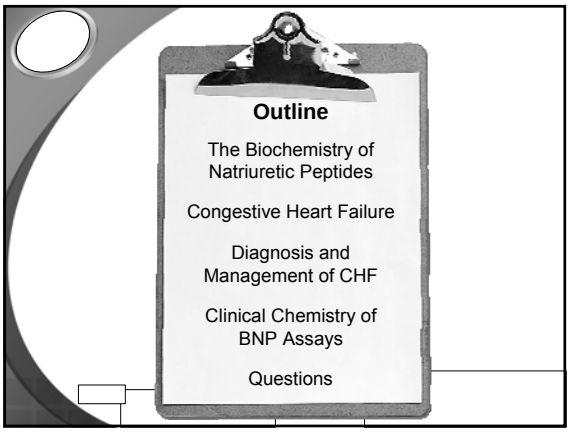
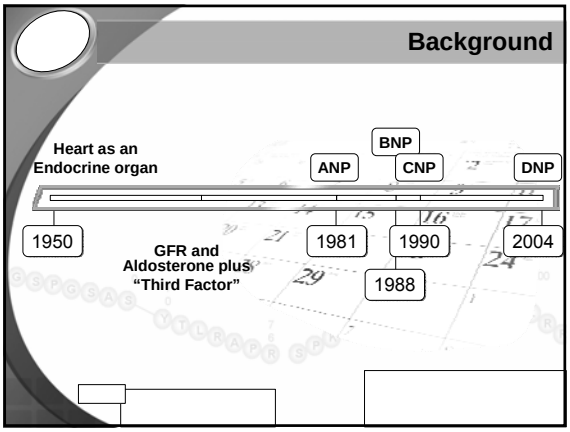
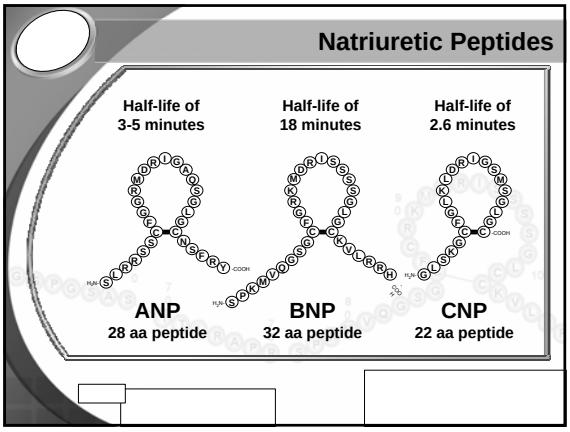

October 10, 2005


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
BNP
ANP
CNP
DNP
1950
GFR and Aldosterone plus "Third Factor"
1981
1990
2004
1988

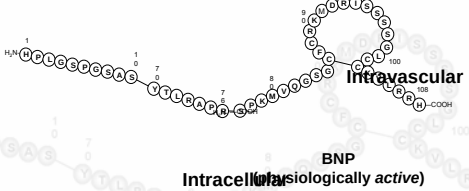

Natriuretic Peptides
Half-life of 3-5 minutes
Half-life of 18 minutes
Half-life of 2.6 minutes
ANP
28 aa peptide
BNP
32 aa peptide
CNP
22 aa peptide

BNP Production

pre-proBNP
(physiologically *inactive*)

proBNP
(physiologically *inactive*)

NT-proBNP
(physiologically *inactive*)

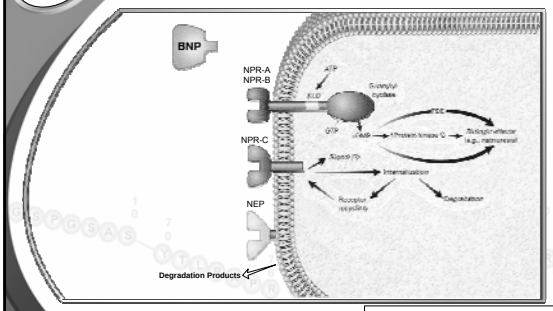


Intracellular

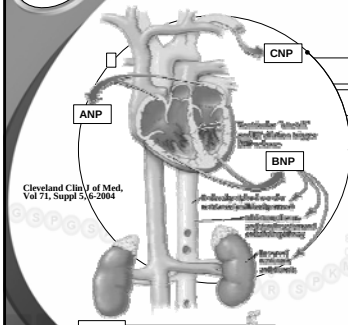
Intravascular

BNP
(physiologically *active*)

Mechanism of Action



Functions of the Natriuretic Hormones



Natriuresis

Diuresis

Vasodilation

Outline

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Characteristics of Natriuretic Peptides

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

Ruskoaho H et al. J Mol Med 1997;75:876-886

Causes of Congestive Heart Failure

•Hypertension

COPD

Cardiotoxic drugs

•***Atherosclerotic Ischemic Heart Disease (IHD)***

Valvular dysfunction

Infection

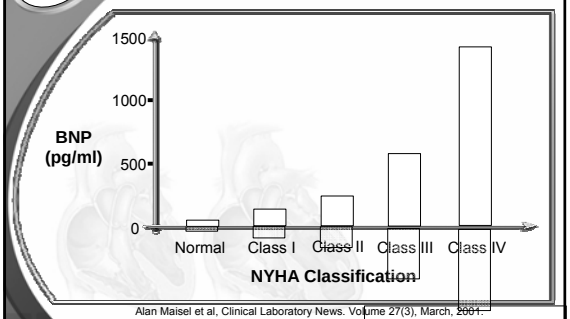
Cardiomyopathy

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

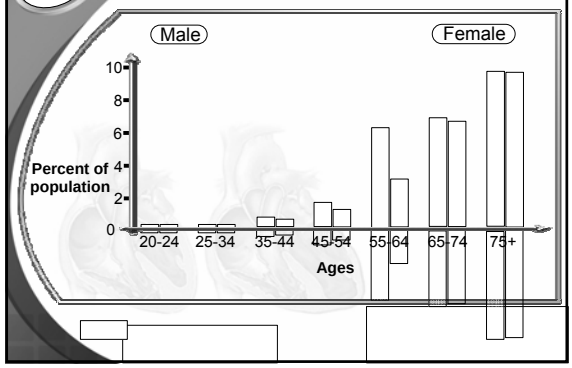
Costs: $21 Billion/year

BNP Levels Correlate to NYHA Class



Alan Maisel et al, Clinical Laboratory News. Volume 27(3), March, 2001.

Prevalence in U.S. 1988 - 1994



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Neurohumoral Changes

Heart Failure

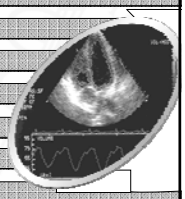
Increases…

- 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.

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)

$$LVEF = \frac{Stroke\ Volume}{End\ Diastolic\ Volume}$$

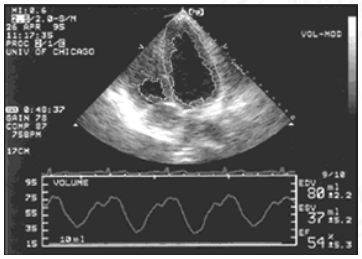
Stroke Volume = (End Diastolic Volume – End Systolic Volume)

Why Test for Natriuretic Peptides?

Simply, rapidly, inexpensively measured potential uses

- Diagnosis
- Prognosis
- Guiding Therapy

Two-dimensional Doppler Echocardiogram



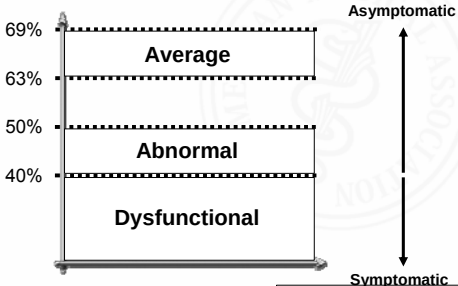
Blue Line – Left Ventricle
Orange Line – Blood Endocardial Border

Diagnosis

Predictor	P-value	Odds Ratio
HTN	0.03	2.3
Male sex	0.002	4.0
IHD	0.0003	6.0
BNP (> 12.9 pg/mL)	0.005	13.0
NT-proBNP (> 86 pg/mL)	<0.0001	14.5

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

Left Ventricular Ejection Fraction



Diagnosis: BNP for CHF Screening

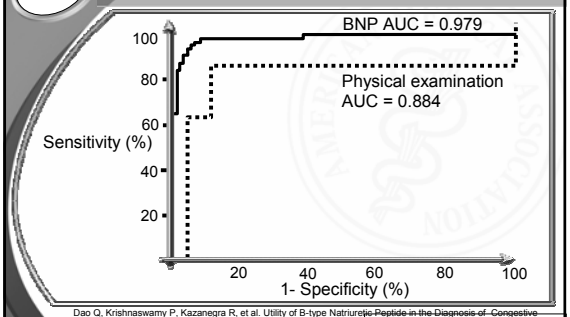
Type of patient population

	General	Symptomatic PC
Sensitivity	76	97
Specificity	87	84
PPV	16	70
NPV	98	98

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

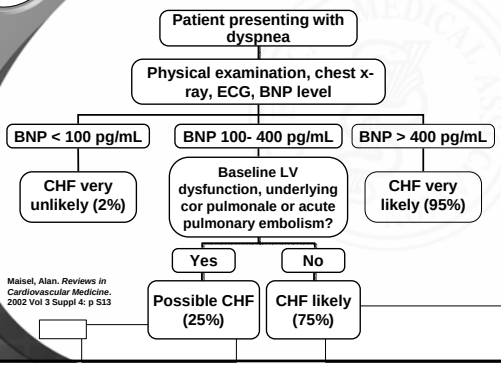
Source: Struthers A. Heart 2000;84:334-38.

BNP versus Physical Exam



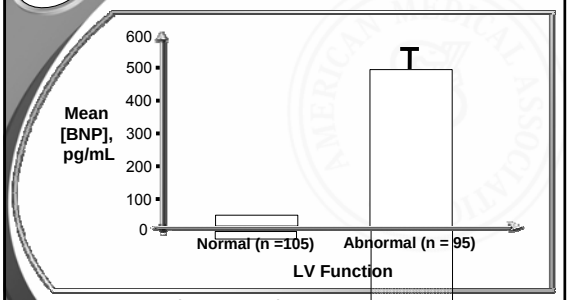
Dao Q, Krishnaswamy P, Kazanegra R, et al. Utility of B-type Natriuretic Peptide in the Diagnosis of Congestive Heart Failure in an Urgent-care Setting. J Am Coll Cardiol 2001; 37:379–385.

Clinical Algorithm for Interpreting BNP



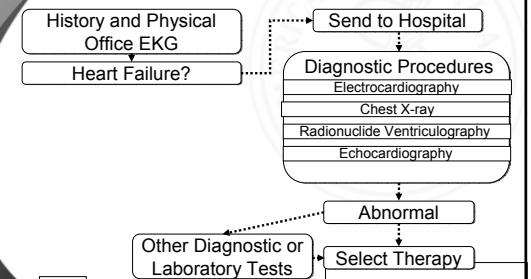
Maisel, Alan. *Reviews in Cardiovascular Medicine.* 2002 Vol 3 Suppl 4: p S13

Diagnosis: BNP Predicts Abnormal LV Function

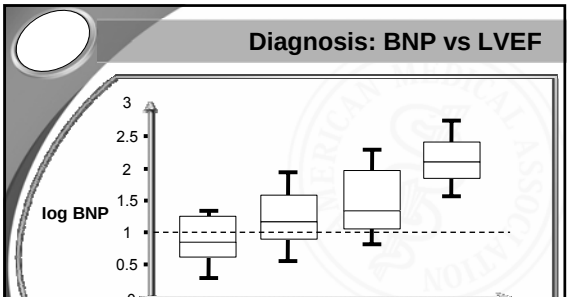


Source: Maisel AS et al. Am Heart J 2001;141:367-74

Diagnosis: Algorithm for CHF

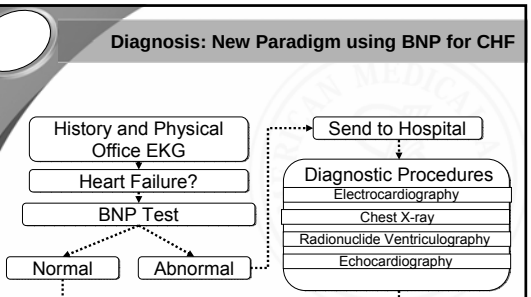


Diagnosis: BNP vs LVEF

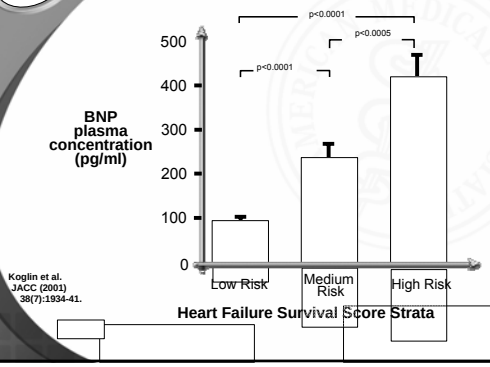


Valli et al. Clin Chim Acta 2001;306:19-26

Diagnosis: New Paradigm using BNP for CHF



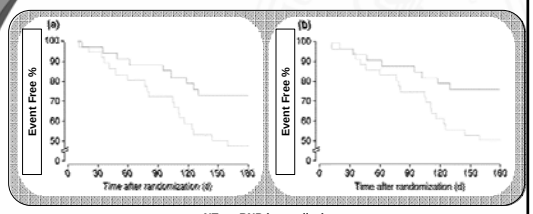
Prognosis: Risk Stratification Using BNP



Koglin et al. JACC (2001) 38(7):1934-41.

Guided Therapy for CHF

Total Cardiac Events **Episodes of CHF**

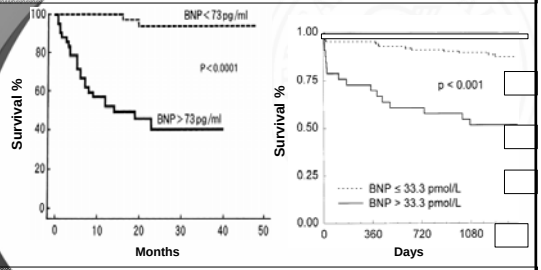


NT-proBNP (upper line) vs. Usual Clinical Care

Richards et al. Trends in Endo & Metab 2002;13:151-5

Prognosis: Survival with CHF or Post-MI

CHF Survival MI Survival



Tsutamoto et al. Circulation 1997;96:509-16. Omland et al. Circulation 1996;93:1963-69.

BNP Guided Therapy for CHF

No CV events after 9.5 months

BNP Guided Therapy

Usual Care

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

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

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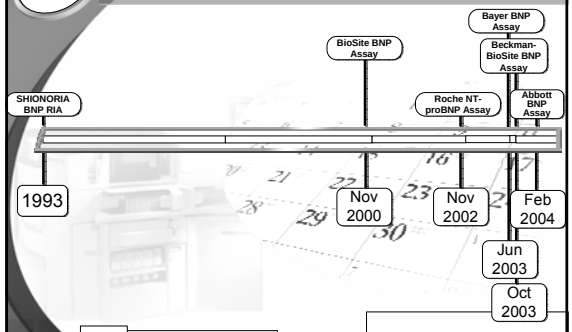
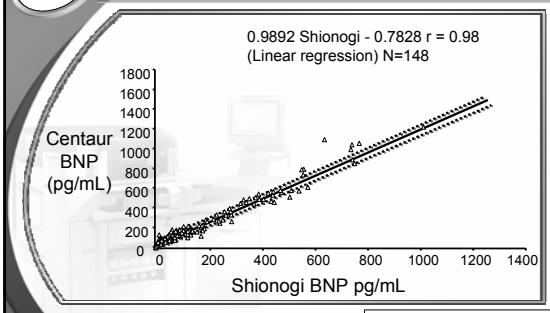
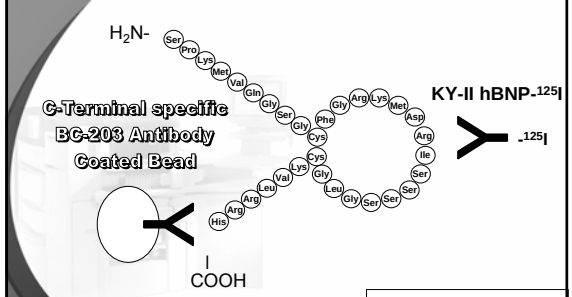
Availability of Assays for BNP and NT-proBNP)



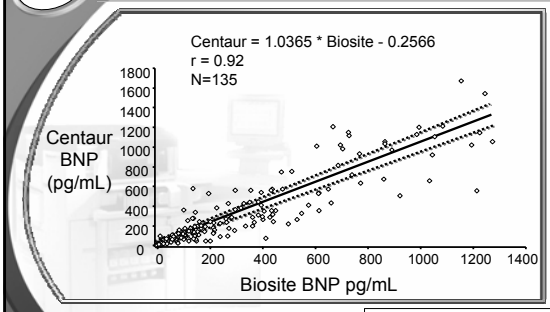
Centaur Compared to ShionoRIA BNP



SHIONORIA BNP Assay

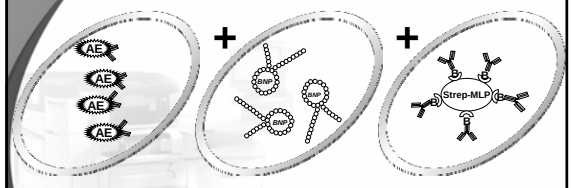


Centaur Compared To Triage BNP



ADVIA Centaur BNP Assay

Two Site Immunoassay Format



Lite Reagent: DMAE Labeled KY-hBNP-II $(Fab')_2$

Sample: EDTA plasma 100 µL

Solid Phase: Streptavidin coated Magnetic Latex Particles with Biotinylated BC-203

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

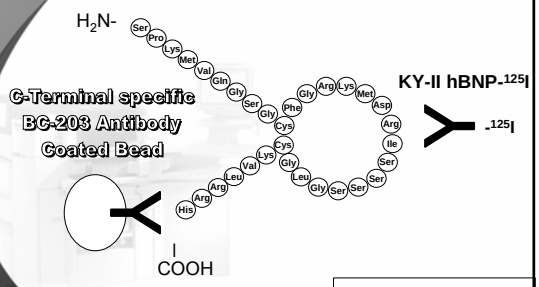
In vivo:

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

proBNP: Reticulo-endothelial system
Renal Excretion

From: Allen Wu

SHIONORIA BNP Assay



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.

BNP ASSAY EPITOPES

Table 3.
Summary of Molar Immunoreactivity of B-Type Natriuretic Peptides*

Peptide	Method		
	Access 2	ADVIA Centaur	AxSYM
1-32	82	126	106
3-32	69	126	118
4-32	159	175	164
10-32	<1	163	<1
1-31	87	<1	<1

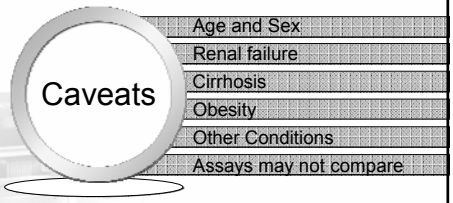
* Data are given as percentages. For proprietary information, see the text.

Stability of Natriuretic Peptides in EDTA Whole Blood

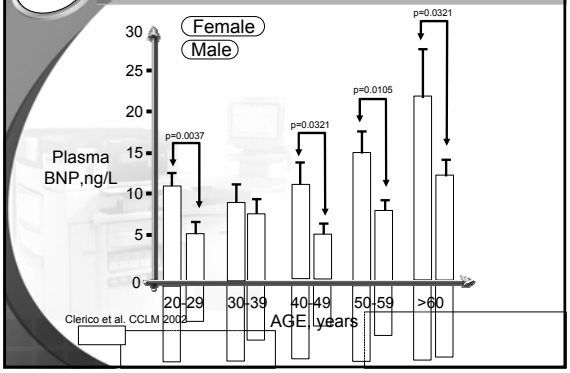
Stability of Natriuretic Peptides

Storage Temperature	BNP	NT-proBNP
Room temperature	24 h	5 - 72 h
4 ^{0}C (2 - 8 ^{0}C)	24 - 48 h	48 - 72 h
-20 ^{0}C	9 mo	12 mo

BNP Results Interpretation



Influence of Age and Sex on BNP



Clerico et al. CCLM 2003

Other Diseases with Increases in BNP

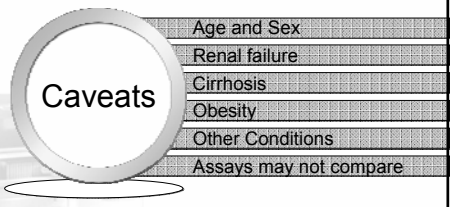
Left Ventricular Hypertrophy
Cardiac Inflammation (eg, Myocarditis, Cardiac Allograft Rejection)
Arrhythmogenic Right Ventricle With Reduced Ejection Fraction
Kawasaki Disease
Primary Pulmonary Hypertension
Pulmonary Embolism
Renal Failure
Ascitic Cirrhosis
Endocrine (Primary Hyperaldosteronism, Cushing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

Adapted, With Permission, From: Peacock WF IV. The B-type Natriuretic Peptide Assay: A Rapid Test For Heart Failure. Cleve Clin J Med 2002; 69:245-251.

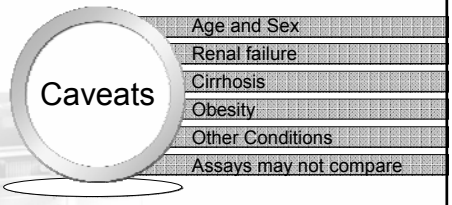
Possible Reasons for Sex/Age Differences

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 Results Interpretation



Summary

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