

- of London. Treatment of adults and children with renal failure: standards and audit measures (third edition). London: UK Renal Association, 2002.
- Walker KS, Seth J. Stability of parathyroid hormone in blood from renal patients on haemodialysis. *Ann Clin Biochem* 2000;37:800–1.
  - Omar H, Chamberlin A, Walker V, Wood PJ. Immulite 2000 parathyroid hormone assay: stability of parathyroid hormone in EDTA blood kept at room temperature for 48 hours. *Ann Clin Biochem* 2001;38:561–3.
  - Teal TK, Reed M, Stevens PE, Lamb EJ. Stability of parathyroid hormone ex vivo in haemodialysis patients. *Ann Clin Biochem* 2003;40:191–3.
  - Levin GE, Nisbet JA. Stability of parathyroid hormone related protein and parathyroid hormone at room temperature. *Ann Clin Biochem* 1994;31:497–500.
  - Inaba M, Nakatsuka K, Imanishi Y, Watanabe M, Mamiya Y, Ishimura E, et al. Technical and clinical characterization of the Bio-PTH (1–84) immunochemiluminometric assay and comparison with a second-generation assay for parathyroid hormone. *Clin Chem* 2004;50:385–90.
  - Nussbaum SR, Zahradnik RL, Lavigne JR, Brennan GL, Nozawa-Ung K, Kim LY, et al. Highly sensitive two-site immunoradiometric assay of parathyrin, and its clinical utility in evaluating patients with hypercalcemia. *Clin Chem* 1987;33:1364–7.
  - D'Amour P, Brossard J-H, Rousseau L, Roy L, Gao P, Cantor T. Amino-terminal form of parathyroid hormone (PTH) with immunologic similarities to hPTH(1–84) is overproduced in primary and secondary hyperparathyroidism. *Clin Chem* 2003;49:2037–44.

Teresa K. Teal<sup>1</sup>  
 Joanna L. Wood<sup>1</sup>  
 Paul E. Stevens<sup>2</sup>  
 Edmund J. Lamb<sup>1\*</sup>

Departments of <sup>1</sup>Clinical Biochemistry  
 and <sup>2</sup>Renal Medicine  
 East Kent Hospitals NHS Trust  
 Kent, UK

\*Address correspondence to this author at: Department of Clinical Biochemistry, Kent and Canterbury Hospital, Canterbury, Kent CT1 3NG, UK. E-mail edmund.lamb@ekht.nhs.uk.

DOI: 10.1373/clinchem.2004.037994

lyze the evolving use of BNP measurements in a variety of settings at the San Diego Veteran's Affairs (VA) Hospital, the first US institution to implement the commercialized Trigate BNP Assay (2).

The study was approved by the Institutional Review Board. An analysis of the total number of BNP assays and the departments from which the assays were ordered was performed on all BNP measurements performed at the San Diego VA Hospital during the months of January through March of 2001, 2002, and 2003. Because we compared the use of BNP testing in the same quarter of the year over the 3 consecutive years, we believed it would effectively show the trends in BNP assay use.

During the period January through March of 2001, there were 537 BNP assays run (Table 1). Records were obtained from patient sample logs kept in the point-of-care laboratory at the San Diego VA Hospital. Of the 537 requests, 72% (n = 387) were ordered from the Emergency Department (ED), whereas only 11% (n = 59) were ordered from the Intensive Care Unit (ICU), 9% (n = 48) from inpatient departments, and 8% (n = 43) from outpatient departments. One year later, BNP orders rose to 1466, with only 27% from the ED. One-third (n = 542) came from outpatient departments, 20% (n = 293) from inpatient wards, and 16% (n = 235) from the ICU. By 2003, BNP assays performed grew to 2072, with the total assays ordered in the ED similar to previous years, although the percentage relative to all BNP assays decreased to 20% (n = 414). Although there was no decrease in the use of BNP in the ED, the relative percentage decreased as the

use diversified in other clinical arenas. The overall number of BNP tests ordered from the ED remained the same over the years, perhaps because of saturation, i.e., almost everyone reporting to the ED with shortness of breath might have undergone BNP testing.

The majority of BNP assays (47%) ordered in this last period were from the outpatient setting (n = 974). Although we could not segregate the outpatient use based on the origin of the BNP test request, i.e., whether the test was ordered by an internist and a cardiologist, the overall increase in outpatient use of BNP testing was likely to be evenly distributed between the two because the ordering of BNP tests by medical residents is a common practice at the VA Medical Center. The BNP assays ordered from ICU and inpatient wards amounted to 18% (n = 373) and 15% (n = 311), respectively. The distribution of assays by location changed significantly across years ( $\chi^2 = 614.32$ ;  $df = 6$ ;  $P < 0.001$ ).

Over the past 3 years the large increase in BNP assays ordered has transcended use of the assays in the ED. Some of this increase has occurred in conjunction with a wealth of emerging clinical data describing biomarker-guided diagnostic, management, and risk stratification strategies (3). Much of this literature has discussed the many uses and information provided by obtaining and monitoring BNP concentrations in patients admitted to the hospital. BNP is the first biomarker that has found value in monitoring of patients, in tailoring management and titrating therapy (4), in providing objectivity in assessing discharge and admission criteria, and in pre-

## Evolving Use of B-Type Natriuretic Peptide in Clinical Practice

To the Editor:

Point-of-care B-natriuretic peptide (BNP) assays have become an accepted part of the diagnostic armamentarium for the physician attending to acutely dyspneic patients (1). The present study was done to ana-

Table 1. Distribution of BNP tests ordered from different locations by year.

Departments	Distribution, % (n)		
	2001	2002	2003
ED	72 (387)	27 (396)	20 (414)
ICU	11 (59)	16 (235)	18 (373)
Inpatient	9 (48)	20 (293)	15 (311)
Outpatient	8 (43)	37 (542)	47 (974)
Total	100 (537)	100 (1466)	100 (2072)
Increase from 2001		+173%	+286%

dicting both adverse cardiac events and readmissions in congestive heart failure inpatients (5).

Interestingly, as clinicians learned to use BNP results in various clinical settings, it was in the outpatient setting that BNP test usage experienced the most growth in (8% in 2001 to 47% in 2003), accounting for the majority of assays ordered in the time period assessed in 2003. Although monitoring of BNP in the outpatient setting has not yet been shown effective in tailoring treatment of congestive heart failure, several large-scale studies are underway to test this hypothesis. The fact that effective treatments for heart failure have been correlated to decreasing BNP concentrations bodes well for future applications in tailoring treatment and in determining the overall stability of the patient outside the hospital (6). These results may also help delineate the appropriateness of the use of BNP testing in the outpatient setting and avoid any overuse that might occur. BNP concentrations have also been shown to be of value in several outpatient situations, including assessing volume overload and prognosis in renal failure (7), as well as in assessing the severity of valvular heart disease (8).

Dr. Maisel is a consultant for Biosite Inc., and has received research support from Biosite Inc., Roche, and Bayer Diagnostics

#### References

1. Maisel AS, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, Duc P, et al. Breathing Not Properly Multinational Study Investigators. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. *N Engl J Med* 2002;347:161-7.
2. Maisel A. B-Type natriuretic peptide measurements in diagnosing congestive heart failure in the dyspneic emergency department patient [Review]. *Rev Cardiovasc Med* 2002;3(Suppl 4):S10-7.
3. Bhalla V, Willis S, Maisel AS. B-Type natriuretic peptide: the level and the drug-partners in the diagnosis of congestive heart failure. *Congest Heart Fail* 2004;10:3-27.
4. Kazanegra R, Cheng V, Garcia A, Krishnaswamy P, Gardetto N, Clopton P, et al. A rapid assay for B-type natriuretic peptide correlates with falling wedge pressures in patients treated for decompensated heart failure: a pilot study. *J Card Fail* 2001;7:21-9.
5. Harrison A, Morrison LK, Krishnaswamy P, Kazanegra R, Clopton P, Dao Q, et al. B-type natri-

uretic peptide predicts future cardiac events in patients presenting to the emergency department with dyspnea. *Ann Emerg Med* 2002;39:131-8.

6. Anand IS, Fisher LD, Chiang YT, Latini R, Masson S, Maggioni AP, et al. Changes in brain natriuretic peptide and norepinephrine over time and mortality and morbidity in the Valsartan Heart Failure Trial (Val-HeFT). *Circulation* 2003;107:1278-83.
7. Naganuma T, Sugimura K, Wada S, Yasumoto R, Sugimura T, Masuda C, et al. The prognostic role of brain natriuretic peptides in hemodialysis patients. *Am J Nephrol* 2002;22:437-44.
8. Qi W, Mathisen P, Kjekshus J, Simonsen S, Bjornerheim R, Endresen K, et al. Natriuretic peptides in patients with aortic stenosis. *Am Heart J* 2001;142:725-32.

Alex Harrison  
Vikas Bhalla  
Nancy Gardetto  
Alan S. Maisel\*

Division of Cardiology  
Department of Medicine  
Veteran's Affairs Medical Center  
and  
University of California  
San Diego, CA

Address correspondence to this author at: VAMC Cardiology 111-A, 3350 La Jolla Village Dr., San Diego, CA 92161. Fax 858-552-7490; e-mail amaisel@ucsd.edu.

DOI: 10.1373/clinchem.2004.034769

#### Measurements, Zymographic Analysis, and Characterization of Matrix Metalloproteinase-2 and -9 in Healthy Human Umbilical Cord Blood

To the Editor:

Matrix metalloproteinases (MMPs) are zinc/calcium-dependent effectors/mediators of physiologic and pathologic reproductive processes (1); MMPs may alter fetal homeostasis and be involved in pathologic syndromes of pregnancy (2). No information is available on MMPs circulating in healthy umbilical cord plasma (UCP). To characterize the effect(s) of umbilical cord blood (UCB) sampling and to identify possible cellular source(s) of MMP-2 (EC 3.4.24.24) and MMP-9 (EC 3.4.24.35) circulating in UCB, we investigated their concentrations, biochemical

characteristics, and isoform distributions by immunoassay and gelatin zymography.

We collected UCB (n = 20) immediately after delivery from women with uncomplicated healthy pregnancies; mean (SD) gestation age at delivery was 38 (2) weeks. We used plastic tubes containing lithium heparin, dipotassium EDTA, or buffered sodium citrate (9NC); sera were obtained from clot-activator-gel tubes (SST; Becton Dickinson). After centrifugation at 1000g for 15 min, supernatants were collected. Leukocyte subpopulations were tested for their subsets and gelatinase content after sedimentation on Lympholyte® gradient (Cedarlane) (3).

Umbilical cord vein sections (n = 5) were excised, separated from the Wharton's jelly, homogenized, and centrifuged at 8000g for 30 min; the supernatants were then analyzed (4).

Gelatinase calibrators were prepared from healthy capillary peripheral blood (3). MMP molecular isoforms were analyzed under non-reducing conditions on gelatin-copolymerized polyacrylamide gels (5). Western blotting was performed with anti-human MMP-2 and -9 monoclonal antibodies (clones 75-7F7 and GE-213, respectively; Calbiochem). We measured UCP gelatinases by Biotrak™ MMP-2 and MMP-9 assays (Amersham Pharmacia) (5). Mean (SE) values of three independent experiments performed in duplicate were calculated; statistical analysis was performed with the Student *t*-test and Mann-Whitney *U*-test. *P* values <0.05 were considered statistically significant.

Mean (SE) UCP gelatinase concentrations differed among the collection methods, with the highest concentrations in SST tubes [192 (18) and 46 (4) μg/L for MMP-2 and -9, respectively] and the lowest concentrations obtained from 9NC tubes [102 (8) and 5 (1) μg/L for MMP-2 and -9, respectively]. MMP-2 concentrations appeared unaffected by anticoagulants, and MMP-2 was constitutively present in all UCB samples at 124 (11) μg/L.

Gelatin zymography of whole UCB revealed a constant band at