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# APPLICABILITY OF COPEPTIN AS A BIOMARKER FOR HEART FAILURE

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## ABSTRACT

Heart failure is an epidemic of vast proportions accounting for the increasing hospitalizations, mortality and increasing health care costs especially in population above 65 years of age. Diagnosis of heart failure also poses a problem in women, elderly and obese, therefore the need of biomarkers. Brain Natriuretic peptides and its more stable amino-terminal co-metabolite, N Terminal – pro BNP are markers which are Food and Drug Administration (FDA) approved. Considering the limitations of these, the quest for a perfect marker continues. Some like copeptin, which have shown promise, have been a hub of much debate and interest. In the past 5 years, since the development of copeptin assay, it has been studied as a diagnostic and prognostic marker in different diseases. In the present paper we will focus our discussion on the utility of testing copeptin in heart failure to assess their clinical role and validation of its use.

## KEY WORDS

Biomarker, Brain Natriuretic peptides, N Terminal – pro BNP, Copeptin and Heart failure.

## INTRODUCTION

### *Heart failure: the magnitude of problem and diagnostic difficulties*

Heart failure can be labelled as an epidemic of modern age. Heart failure is a syndrome which develops as a consequence of cardiac disease, and is recognised clinically by a constellation of symptoms and signs produced by complex circulatory and neurohormonal responses to cardiac dysfunction[1].The prevalence rate is about 1% at the age of 50, whilst at the age of 80 and above, almost one out of 10 persons suffers from heart failure[2]. Heart failure is the leading cause of hospitalization for patients older than 65 years[3]. There has been a steady increase in the number of hospitalizations and deaths attributed to heart failure[4] in the past decade. It also attributes to escalating costs associated with health care. Heart failure is not only widely prevalent but it is also notoriously difficult to diagnose and prognosticate. The outlook is gloomy with overall 50% of patients dying within few years of diagnosis. It remains a condition associated with high hospital readmission rates and high mortality [5].

The accuracy of diagnosis of HF by clinical means alone is often difficult, particularly in women, the elderly, and the obese[6,7]. Symptoms of heart failure in elderly may be non-specific and signs may be obscured by the presence of multiple co-morbidities[8,9,10].Presence of co-morbidities and polypharmacy also complicates the treatment strategies[11]. Several diagnostic tests are employed routinely to confirm or rule out the diagnosis of HF including electrocardiogram, chest X-ray and echocardiography to name a few. But most diagnostic tests are sensitive for the detection of patients with reduced ejection fraction. Having a preserved ejection fraction poses a diagnostic problem and moreover preserved ejection fraction (EF 45–50%) is present in almost half of the HF patients [12].

### *Biomarkers: a perfect solution*

Biochemical marker testing has revolutionized the approach to diagnosis and management of heart failure. It is not only useful to identify possible underlying (and potentially reversible) causes of heart failure but also to estimate the severity of disease and risk of its progression. Hence there is an unsurpassed excitement regarding advances in field of cardiac biomarkers.

Quest for a perfect biomarker has led to discovery of several novel cardiac, metabolic and inflammatory biomarkers[13]. Heart failure literature has a bounty of these examples including hormones like Atrial natriuretic peptide and the Brain natriuretic peptides[14], protein like high-sensitivity C-reactive protein and cardiac troponin, enzymes like myeloperoxidase and many others. Out of the above mentioned markers natriuretic peptides particularly Brain natriuretic peptides (BNP) and its more stable amino-terminal co-metabolite, N Terminal –proBNP (NT- pro BNP) [15], have emerged as an Food and Drug Administration (FDA) approved test for diagnosis of heart failure. Both of these are not only useful in confirming or refuting the diagnosis of heart failure but in stratifying long-term risk profiles [16].

### *Limitations of existing biomarkers*

But BNP is not without its own limitations. Firstly, a variety of clinical factors have been proven to influence natriuretic peptide levels in blood, including age and sex of the individual[17,18], renal function[19,20,21,22,23,24], body habitus[25,26,27], thyroid function [28,29] and anaemia [30]. Obesity, in particular, has been associated with lower blood levels across the spectrum of heart failure[31]. Pre-existing cardiac conditions such as prior history of heart failure[32], rhythm abnormalities[33,34,35], cardiac structural or functional abnormalities and recent heart surgery can significantly influence blood

natriuretic peptide levels[36, 37,38, 39]. The relative influence of the above mentioned factors in relation to the degree of cardiac dysfunction may also influence the diagnostic accuracies in various clinical settings.

Secondly, several commercial assays have become available for BNP testing and the reference ranges provided vary depending on the assay method employed and the nature of the control population. The commonly used research assay (Shionogi) often reports values that are 15% to 20% below that of the commercial assays (Biosite and Abbott)[40]. Difference in epitopes identified by antibodies has been labelled as the culprit[41]. These inter assay variations have made direct comparison among study results difficult.

***Copeptin: an alternate to existing markers***

These drawbacks has made it essential that the answers should be sought elsewhere, hence the need of research and path breaking trials using other markers to meet with multiple challenges when applied in the clinical setting. One biomarker which is generating a lot of interest and speculation is Copeptin, the C-terminal end of pro vasopressin (a precursor of arginine vasopressin).

The elevation of arginine vasopressin (AVP), in heart failure is a known phenomenon. It is released from the hypothalamus in response to changes in plasma osmolality and arterial hypovolaemia. In general, AVP plasma values are increased in patients with chronic heart failure and related to the severity of the disease[42].

Arginine vasopressin or anti diuretic hormone is a nonapeptide, synthesized within the magnocellular neurons of the hypothalamic supraoptic nuclei and paraventricular nuclei. It is transported along their axons to the posterior pituitary for storage before ultimate release into the blood stream. AVP contributes to osmoregulation and cardiovascular homeostasis and it may have a role in cardiopulmonary resuscitation[43]. Vasopressin is synthesized as part of a 166-amino acid long precursor protein called preprovasopressin. It consists of a signal peptide, AVP, neurophysin II, and copeptin[44]. This preprohormone is cleaved as it is transported along the axon [45]. The C-terminal end of provasopressin is a 39-amino acid glycopeptides present in serum[46] with a leucine-rich core segment[47,48]. It has been suggested that the function of copeptin is to help in the folding of the vasopressin precursor which, in the absence of this glycopeptide, is less stable [49].

The diagnostic use of AVP has been described in HF and septic shock[52]. There are concerns about the validity of measurement of AVP in plasma, because it is known to be unstable in isolated plasma, even when stored at -20 C[53]. AVP binds to platelet and is rapidly cleared from plasma[54]. Investigation of the role of the vasopressin was thus hampered by the instability of this peptide. Copeptin has emerged as an alternate in the same clinical settings since it is secreted in equimolar amounts to vasopressin[55]**.** This glycopeptide is stable for days after blood withdrawal and can be quickly and easily measured.

***Gene & structure***

Vasopressin gene is located in chromosome 20 (p13)[50]. The gene is composed of three exons: the first exon encodes the nonapeptide, the second exon encodes the central portion of the neurophysin II, and the third exon encodes the C-terminal part of the preprohormone, copeptin. The structure of copeptin has recently been characterized with size-exclusion chromatography and found to have a molecular mass around 5 kDa[51].

***Method of estimation of copeptin***

Currently Copeptin is measured with a sandwich immunoassay[56]. It uses two purified sheep polyclonal antibodies to the C-terminal region. Antibody raised against a peptide representing amino acids 132 to 147 of preproAVP is bound to polystyrene tubes, while other antibody raised against a peptide representing amino acids 149 to 164 of preproAVP is labelled with methyl acridinium N-hydroxysuccinimide ester and used as tracer, for chemiluminescence detection. The assay

requires 50 µl serum or plasma and yields results within 3 hours. The analyte shows ex vivo stability for at least 7 days at room temperature and for 14 days at 4°C.

***Normal copeptin levels***

The median copeptin level in 359 healthy individuals was found to be 4.2pmol/l[56]. Copeptin showed a relatively broad distribution in healthy individuals (1.0 –13.8) pmol/L. This distribution is similar to that reported by Robertson et al[57] for AVP. Stratification according to sex and age revealed lower values in females (men, 5.2 pmol/L, women, 3.7 pmol/L) but there was no major difference in median copeptin concentrations after stratification according to age groups.

***Applicability of copeptin as a marker***

In the past 4 years copeptin has been studied as a diagnostic and prognostic marker in different diseases. As a diagnostic marker copeptin has been evaluated in patients with diabetes insipidus, it offers an alternative to the laborious and ambiguous water deprivation test [58]. Huge interest has been evoked by the possibility of Copeptin improving the scenario of early diagnosis of acute MI [59, 60, 61, 62].

As a prognostic marker, Copeptin levels were found to be independent predictors of survival in critically ill patients suffering from hemorrhagic and septic shock[63], community-acquired pneumonia[64], and acute exacerbations of chronic obstructive pulmonary disease [65].

***Copeptin: prognostic implications in heart failure and post Acute Myocardial Infarction***

Copeptin levels also have prognostic implications in diseases of non infectious aetiology, foremost example being heart failure. Stoiser et al (2006) conducted the first study assessing use of copeptin as a marker in heart failure. They inferred that copeptin is an excellent predictor of outcome in advanced heart failure patients, its value being superior to that of BNP [66].

In the subsequent studies too, this novel marker was compared with well established markers like BNP. These studies have published conflicting results with some concluding copeptin to be equally efficient markers while yet others naming it to be superior than BNP. Study by Gegenhuber et al (2007) provided evidence that Copeptin measurements might have similar predictive properties as BNP determinations for one-year all-cause mortality in acute destabilized heart failure[67]. Neuhold et al (2008) reported that copeptin levels were found to escalate with New York Heart Association (NYHA) Functional class. In patients with NYHA functional classes II and III, copeptin was not only found to be most potent single predictor of mortality but was superior to BNP or NT-proBNP. They also suggested that the predictive power increased if both BNP and copeptin were added in one model[68].

While yet another study concluded that both markers provided similar diagnostic information. Masson et al (2010) pointed out that prognostic information provided by copeptin provided prognostic information independent of natriuretic peptides[69]. Neuhold et al(2010) on performing serial measurements of neurohormones have proved that it improves prognostication in the setting of acute heart failure (HF) or chronic HF without therapeutic intervention [70].

Not only is copeptin a good diagnostic marker but also an emerging prognostic marker in patients suffering from acute myocardial infarction (AMI). AMI is associated with left ventricular (LV) dysfunction and clinical heart failure. Copeptin has been labelled as a significant independent predictor of death or heart failure in post MI settings and may provide prognostic information as pointed out by various studies. Khan et al (2007) determined plasma copeptin levels in patients with AMI and found that levels were raised in patients with HF or death after AMI as compared with those having event-free follow up [71].

Kelly et al (2008) performed a similar study in which subjects having AMI were assessed during the follow up. Remodeling of myocardium was metered by the change in LV

volumes between echo examinations. Copeptin was found to be associated with ventricular remodeling and it correlated directly with wall motion index score (WMIS) and inversely with LV ejection fraction[72]. Similarly Voors et al (2009) on analysis found that higher levels of copeptin, BNP, and NT-proBNP were all significantly related to both mortality and the composite cardiovascular endpoint [73].

## RESULTS

All the studies included in this particular review have been detailed in two tables (table1, 2) showing particulars of studies investigating role of copeptin in heart failure and statistical data for studies, comparing copeptin with BNP, and or NT pro BNP respectively. The results of all these studies point clearly towards copeptin being a good biomarker for heart failure not only in risk stratification but also having an additional prognostic role. Stoiser et al inferred that Copeptin is an excellent predictor of outcome in advanced heart failure patients. Its value is superior to that of BNP in predicting death and a combined endpoint while Gegenhuber et al rates that copeptin measurement might have similar predictive properties compared with BNP determinations. On the other hand Khan et al suggests a multimarker approach with copeptin and NTproBNP is more informative than either marker alone and may be useful for risk stratification in AMI patients. Neuhold et al maintained that Copeptin was superior to BNP or NT-proBNP in their study. Increased levels of copeptin were found to be linked to excess mortality, with this link being maintained irrespective of the clinical signs of severity of the disease. Kelly et al confirmed that copeptin is associated with LV dysfunction, volumes, and remodeling and clinical heart failure post-AMI. Voors et al had very similar results that post AMI the predictive value of copeptin were even stronger than BNP and NT-proBNP. Masson et al again pointed out that baseline concentrations were independent predictors of clinical outcome. In their study they could prove that copeptin provided prognostic information independent of natriuretic peptides which are currently the best biomarkers for risk stratification. Neuhold et al in their more recent study rates copeptin and BNP equally predictive of all-cause mortality in chronic heart failure patients. Thus though there seems a contradiction about which is better when we compare copeptin with an already FDA approved marker but none of the studies have marginalized the importance of copeptin. Statistical evaluation of these studies also gives a clear indication of copeptin being a winner biomarker for the prediction of mortality in heart failure (table 2).

### *Limitations of Copeptin: ruling out false positive?*

Though copeptin has been much studied and is being considered as a possible prognostic indicator for heart failure, this test has certain limitations also. The interpretation of copeptin levels must take into account potential confounding factors such as male gender, renal impairment and the fluid status of the subject. Exercise is known to influence the levels. Median copeptin concentration in healthy adult volunteers (12 male and 12 female) increased significantly from 3.6 pmol/L to 6.3 pmol/L after exercise[56]. After a water load, the copeptin concentration decreased and returned to original values[74] while water deprivation increased the levels .Copeptin behaves similar to AVP and seems to mirror AVP release during changes of blood volume and plasma osmolality since similar pattern after a water load has also been described for AVP[75,76]. Hence a single reference range for normal copeptin will not be valid, considering the need to adjust for the independent effects of gender and renal function. It has been suggested that the reference range in male subjects has to take renal function into account while in females the reference range may be independent of age and renal function [77].

## Table 1

*Details of studies investigating role of copeptin in heart failure.*

| Study | Population selected | No of participants | follow-up | Model | Conclusion |
|---|---|---|---|---|---|
| Stoiser et al (2006) | patients with advanced heart failure after they had been discharged from the hospital. | 268 | 15.8 months | Univariate and multivariate analysis | Copeptin is an excellent predictor of outcome in advanced heart failure patients. Its value is superior to that of BNP in predicting death and a combined endpoint, |
| Gegenhuber et al (2007) | patients with acute destabilized heart failure attending a tertiary care hospital | 137 | 365 days | multivariable Cox proportional-hazards regression analyses | Copeptin measurements might have similar predictive properties compared with BNP determinations for one-year all-cause mortality in acute destabilized heart failure. . |
| Khan et al (2007) | single-hospital study recruiting post–acute myocardial infarction patients66 (24 to 95)years | 980 | 342 (range 0 to 764) days | Cox Regression Analysis for Death or HF After AMI | A multimarker approach with copeptin and NTproBNP is more informative than either marker alone and may be useful for risk stratification in AMI patients. |
| Neuhold et al (2008) | Patients included from several clinical trials in this long-term observational study (57 +-11 years) | 786 | 2 yrs | stepwise Cox regression model. | Increased levels of copeptin are linked to excess mortality, and this link is maintained irrespective of the clinical signs of severity of the disease. Copeptin was superior to BNP or NT-proBNP in this study, but the markers seem to be closely related. |
| Kelly et al (2008) | subjects with AMI | 274 | 381 days | Cox proportional hazards model | Copeptin is associated with LV dysfunction, volumes, and remodeling and clinical heart failure post-AMI. |
| Voors et al (2009) | A subset of patients from the OPTIMAAL (Optimal Trial in Myocardial Infarction with Angiotensin II Antagonist Losartan) trial | 224 | 33+7 months. | univariable Cox proportional hazards survival analysis | Post AMI the predictive value of copeptin was even stronger than BNP and NT-proBNP. |
| Masson et al (2010) | patients with chronic and stable HF enrolled in the GISSI-heart failure trial68 yrs | 1237 | 3.9 yrs | multivariable Cox proportional-hazards regression analyses | Baseline concentrations were independent predictors of clinical outcome. Copeptin provided prognostic information independent of natriuretic peptides which are currently the best biomarkers for risk stratification. |
| Neuhold et al (2010) | cohort of chronic HF patients undergoing increases in HF-specific therapy | 181 | 2 yrs | stepwise Cox regression analysis | Copeptin and BNP equally predictive of all-cause mortality |

**Table 2**
**Statistical data for studies comparing copeptin with BNP, and or NT pro BNP**

| Author(year of publication) | Statistical analysis | Copeptin | BNP | NTproBNP (if included) | Additional information |
|---|---|---|---|---|---|
| **Stoiser et al (2006)** | 1.Univariate predictors of death<br>2.Multivariate predictors of death 3.Univariate predictors of re-hospitalization 4.multivariate analysis of re-hospitalization | yes<br>Yes<br>Yes<br>(chi(2) = 4.2, P < 0.05) | No<br>No<br>Yes<br>(chi(2) = 18, P < 0.0001) | Not included | |
| **Gegenhuber et al (2007)** | 1.ROC curve analysis for the prediction of 1-year mortality<br>2. Kaplan-Meier curve analyses | (0.688, 95% CI 0.603-0.764).log-rank test for trend, P < .001 | BNP (0.716, 95% CI 0.633-0.790) log-rank test for trend, P < .001 | Not included | |
| **Neuhold et al (2008)** | 1. ROC curves. with respect to 2-year all-cause mortality<br>2.Single-Predictor Regression Analysis for 24-Month Mortality for Various Clinical Variables | 0.711<br>Independently related to mortality, In NYHA functional class IV, copeptin provided independent additional information | 0.711<br>No independent relation to mortality | Not included | 0.744 if both variables were added in one model |
| **Neuhold et al (2010)** | 1.Stepwise Cox regression analysis (adjusted for age, sex, glomerular filtration rate, diabetes mellitus, and ischemic HF, baseline and follow-up) | 1.92, 95% CI 1.233-3.007, P = 0.004 | 1.46, 95% CI 1.039-2.050, P = 0.029. | Not included | |
| **Khan et al** | 1.ROC curve | *0.75, 95% CI, 0.69 to 0.81) | Not included | 0.76, 95% CI, 0.71 to 0.82 | If both variables 0.84 (95% CI, 0.79 to 0.89, *P*_0.001), |
| **Voors et al** | 1. Univariable Cox regression survival analysis for prediction of death | 2.27 (1.76–2.93)0.0001 | 4.07(1.80–.18)0.0007 | 6.46 (2.02–20.6) 0.0016 | |
| | 2. Univariable Cox regression survival analysis for prediction of composite endpoint ( death/acute myocardial infarction/stroke/resuscitated cardiac arrest) | 1.55 (1.28–1.87)0.0001 | 2.31(1.33–4.00) 0.0028 | 3.18 (1.52–6.66) 0.0021 | |
| | 3. Multivariable Cox regression survival analysis(After adjustment for age and gender) | 1.83 (1.36–2.46) 0.0001 | 1.91 (0.85–4.36) | 2.0 (0.61–6.53)0.25 | |
| | 4. Multivariable Cox regression survival analysis(After adjustment for age and gender,renal fuction, previous MI,diabetes and treatment group) | 1.83 (1.26–2.64) 0.0014 | 1.85 (0.79–4.31)0.15 | 1.30 (0.37–4.58)0.69 | |
| | 5. Receiver operating characterristic analysis | 0.81 (0.75–0.86) | 0.66 (0.60–0.73) | 0.67 (0.61–0.74) | |

Data are presented as hazard ratios, (95% confidence intervals) and p values

# CONCLUSION

What makes vasopressin and its precursor copeptin exciting for heart failure research today is not only the prognostic information provided by these markers, but also a possibility of their use for optimization of Heart failure therapy[70] and the role of vasopressin blockade as a potential new therapeutic target [66,74]. Newly developed agents targeting vasopressin receptors are under investigation and might result in a novel adjunct treatment of both acute and chronic heart failure [78, 79, 80].
But thorough understanding of the physiology as well as the patho-physiology of this marker in a large population based study is required in order to derive normal reference ranges, to know exact cut off values for heart failure and to know the release kinetics of copeptin in disease.

***Perspective***
It is not currently standard clinical practice to measure these peptides to determine prognosis in patients with heart failure. Our aim in this study was to review the literature to determine how well copeptin predicts mortality and morbidity in patients with heart failure, and to determine if this varied with the clinical setting or severity of heart failure. Copeptin use will simplify, and therefore complement the judgment of clinicians and/or validated clinical severity scores.

Copeptin can be possibly used in the panel of investigations in those at risk of heart failure especially elderly, those diagnosed with MI or those presenting to emergency stetting with dyspnoea.

## REFERENCES


1. PA Poole-Wilson. Chronic heart failure: cause, pathophysiology, prognosis, clinical manifestations, investigations. In: DG Julian, AJ Camm, KF Fox, RJC Hall, PA Poole-Wilson (eds.), Diseases of the Heart, London: Balliere-Tindall, 1989, pp.24-36.
2. Eriksson H. Heart failure: a growing public health problem. Journal of Internal Medicine, 2: 135 – 141, (1995).
3. LW Stevenson and E Braunwald. Recognition and management of patients with heart failure. In: L. Goldman, E. Braunwald (eds.), Primary Cardiology, Philadelphia: WB Saunders, 1983, pp.310-329.
4. Braunwald E. Shattuck Lecture — cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. N Engl J Med, 337:1360-1369, (1997).
5. Barretto AC, Del Carlo CH, Cardoso JN, Morgado PC, Munhoz RT, Eid MO, et al.Hospital readmissions and death from Heart Failure--rates still alarming. Arq Bras Cardiol., 91(5):335-41, (2008).
6. Remes J, Miettinen H, Reunanen A, Pyorala K. Validity of clinical diagnosis of heart failure in primary health care. Eur Heart J, 12:315–321, (1991).
7. Wheeldon NM, MacDonald TM, Flucker CJ, McKendrick AD, McDevitt DG, Struthers AD. Chronic heart failure in the community: an echocardiographic study of its prevalence and an assessment of the workload it generates for primary heath care and hospital physicians. Quart J Med, 86:17-23, (1993).
8. Wolinsky FD, Smith DM, Stump TE, Overhage JM, Lubitz RM. The sequelae of hospitalization for congestive heart failure among older adults. J Am Ger Soc,45:558-563, (1997).
9. Morgan S, Smith H, Simpson I, Liddiard GS, Raphael H, Pickering RM, et al. Prevalence and clinical characteristics of left ventricular dysfunction among elderly patients in general practice setting: cross sectional survey. Br Med J, 318:368-72, (1999).
10. Williamson J, Chopin JM. Adverse reactions to prescribed drugs in the elderly: a multicentre investigation. Age Ageing, 9:73-80, (1980).
11. Lien C T C, Gillespie N D, Struthers A D, McMurdo M ET. Heart failure in frail elderly patients: diagnostic difficulties, co-morbidities, polypharmacy and treatment dilemmas. European Journal of Heart Failure, 4: 91-98, (2002).
12. Owan T E, Hodge D O, Herges R M, Jacobsen S J, Roger V L, Redfield M M. Trends in Prevalence and Outcome of Heart Failure with Preserved Ejection Fraction. N Engl J Med, 355:251-259, (2006).
13. Tang W W H, Francis GS, Morrow DA, Newby LK, Cannon CP, Jesse RL, et al. National Academy of Clinical Biochemistry laboratory Medicine Practice Guidelines: Clinical Utilization of Cardiac Biomarker Testing in Heart Failure .Circulation, 116:e99-e109, (2007).
14. Doust JA, Glasziou PP, Pietrzak E, Dobson AJ. A systematic review of the diagnostic accuracy of natriuretic peptides for heart failure. Arch Intern Med, 164:1978-1984, (2004).
15. Richards AM, Nicholls MG, Yandle TG, Frampton C, Espiner EA, Turner JG, et al. Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: new neurohormonal predictors of left

ventricular function and prognosis after myocardial infarction. Circulation, 97: 1921–1929, (1998).
16. Omland T, Persson A, Ng L, O'Brien R, Karlsson T, Herlitz J, et al. N-terminal pro-B-type natriuretic peptide and long-term mortality in acute coronary syndromes. Circulation,106: 2913–2918, (2002).
17. Emdin M, Passino C, Del Ry S, Prontera C, Galetta F, Clerico A. Influence of gender on circulating cardiac natriuretic hormones in patients with heart failure. Clin Chem Lab Med, 41:686–692, (2003).
18. Loke I, Squire IB, Davies JE, Ng LL. Reference ranges for natriuretic peptides for diagnostic use are dependent on age, gender and heart rate. Eur J Heart Fail, 5:599–606, (2003).
19. McLean AS, Huang SJ, Nalos M, Tang B, Stewart DE. The confounding effects of age, gender, serum creatinine, and electrolyte concentrationson plasma B-type natriuretic peptide concentrations in critically ill patients. Crit Care Med., 31:2611–2618, (2003).
20. McCullough PA, Duc P, Omland T, McCord J, Nowak RM, Hollander JE, et al. B-type natriuretic peptide and renal function in the diagnosis of heart failure: an analysis from the Breathing Not Properly Multinational Study. Am J Kidney Dis,41:571–579, (2003).
21. Vesely DL. Natriuretic peptides and acute renal failure. Am J Physiol Renal Physiol, 285:F167–F177, (2003).
22. McCollough PA, Kuncheria J, Mathur VS. Diagnostic and therapeutic utility of B-type natriuretic peptide in patients with renal insufficiency and decompensated heart failure. Rev Cardiovasc Med , 4(suppl 7):S3–S12, (2003).
23. Herrmann Z, Uhl W, Steinberg HW, Dworschack R. The influence of renal function on NT-proBNP levels in various disease groups. Clin Lab, 49:649–656, (2003).
24. Anwaruddin S, Lloyd-Jones DM, Baggish A, Chen A, Krauser D, Tung R, et al . Renal function, congestive heart failure, and amino-terminal pro-brain natriuretic peptide measurement: results from the ProBNP Investigation of Dyspnea in the Emergency Department (PRIDE) Study. J Am Coll Cardiol., 47:91–97, (2006).
25. Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Wilson PW, et al. Impact of obesity on plasma natriuretic peptide levels. Circulation, 109:594–600, (2004).
26. Taylor JA, Christenson RH, Rao K, Jorge M, Gottlieb SS. B-type natriuretic peptide and N-terminal pro B-type natriuretic peptide are depressed in obesity despite higher left ventricular end diastolic pressures. Am Heart J, 152:1071–1076, (2006).
27. Krauser DG, Lloyd-Jones DM, Chae CU, Cameron R, Anwaruddin S, Baggish AL, et al. Effect of body mass index on natriuretic peptide levels in patients with acute congestive heart failure: a ProBNP Investigation of Dyspnea in the Emergency Department (PRIDE) substudy. Am Heart J,149:744 –750, (2005).
28. Schultz M, Faber J, Kistorp C, Jarlov A, Pedersen F, Wiinberg N, et al. N-terminal-pro-B-type natriuretic peptide (NT-pro-BNP) in different thyroid function states. Clin Endocrinol (Oxf),60:54–59, (2004).
29. Missouris CG, Grouzmann E, Buckley MG, Barron J, MacGregor GA, Singer DR. How does treatment influence endocrine mechanisms in acute severe heart failure? Effects on cardiac natriuretic peptides, the renin system, neuropeptide Y and catecholamines. Clin Sci (Lond)., 94:591–599, (1998).
30. Ralli S, Horwich TB, Fonarow GC. Relationship between anemia,cardiac troponin I, and B-type natriuretic peptide levels and mortality in patients with advanced heart failure. Am Heart J, 150:1220 –1227, (2005).
31. Mehra R M, Uber A P, Park H M, Scott L R, Ventura O H, Harris C B, et al. Obesity and suppressed B-type natriuretic peptide levels in heart failure J Am Coll Cardiol, 43:1590-1595, (2004).

32. Chung T, Sindone A, Foo F, Dwyer A, Paoloni R, Janu MR, et al. Influence of history of heart failure on diagnostic performance and utility of B-type natriuretic peptide testing for acute dyspnea in the emergency department. Am Heart J, 152:949 –955, (2006).
33. Albage A, Kenneback G, van der Linden J, Berglund H. Improved neurohormonal markers of ventricular function after restoring sinus rhythm by the Maze procedure. Ann Thorac Surg,75:790 –795, (2003).
34. Inoue S, Murakami Y, Sano K, Katoh H, Shimada T. Atrium as a source of brain natriuretic polypeptide in patients with atrial fibrillation. J Card Fail, 6:92–96, (2000).
35. Rossi A, Enriquez-Sarano M, Burnett JC Jr, Lerman A, Abel MD, Seward JB. Natriuretic peptide levels in atrial fibrillation: a prospective hormonal and Doppler-echocardiographic study. J Am Coll Cardiol, 35:1256 –1262, (2000).
36. Troughton RW, Prior DL, Pereira JJ, Martin M, Fogarty A, Morehead A, et al. Plasma B-type natriuretic peptide levels in systolic heart failure: importance of left ventricular diastolic function and right ventricular systolic function. J Am Coll Cardiol, 43:416–422, (2004).
37. Lubien E, DeMaria A, Krishnaswamy P, Clopton P, Koon J, Kazanegra R, et al. Utility of B-natriuretic peptide in detecting diastolic dysfunction: comparison with Doppler velocity recordings. Circulation, 105:595– 601, (2002).
38. Maisel AS, McCord J, Nowak RM, Hollander JE, Wu AH, Duc P, et al. Bedside B-Type natriuretic peptide in the emergency diagnosis of heart failure with reduced or preserved ejection fraction: results from the Breathing Not Properly multinational study. J Am Coll Cardiol, 41:2010 –2017, (2003).
39. Cheung BM. Plasma concentration of brain natriuretic peptide is related to diastolic function in hypertension. Clin Exp Pharmacol Physiol, 24:966 –968, (1997).
40. Redfield MM, Rodeheffer RJ, Jacobsen SJ, Mahoney DW, Bailey KR, Burnett JC. Plasma brain natriuretic peptide concentration: impact of age and gender. J Am Coll Cardiol, 40:976 –982, (2002).
41. Tetin SY, Ruan Q, Saldana SC, Pope MR, Chen Y, Wu H, et al. Interactions of two monoclonal antibodies with BNP: high resolution epitope mapping using fluorescence correlation spectroscopy. Biochemistry, 45:14155–14165, (2006).
42. Chatterjee K. Neurohormonal activation in congestive heart failure and the role of vasopressin. Am J Cardiol, 95:8B–13B, (2005).
43. Wenzel V, Krismer AC, Arntz HR, Sitter H, Stadlbauer KH, Lindner KH. European Resuscitation Council Vasopressor during Cardiopulmonary Resuscitation Study Group. A Comparison of vasopressin and epinephrine for out-of-hospital cardiopulmonary resuscitation. N Engl J Med, 350:105–113, (2004).
44. de Bree FM, Burbach JP. Structure-function relationships of the vasopressin prohormone domains. Cell Mol Neurobiol,18:173–191, (1998).
45. Brownstein MJ, Russel JT, Gainer H. Synthesis, transport, and release of posterior pituitary hormones. Science, 207: 373-378, (1980).
46. Altstein M, Whitnalla M H, Housea S, Keya S, Gainer H. An immunochemical analysis of oxytocin and vasopressin prohormone processing in vivo. Peptides, 9 (1) : 87-105, (1988).
47. Holwerda DA. A glycopeptide from the posterior lobe of pig pituitaries.I. Isolation and characterization. Eur J Biochem, 28(3):334–339, (1972).
48. Holwerda DA. A glycopeptide from the posterior lobe of pig pituitaries. 2. Primary structure. Eur J Biochem, 28 (3):340–346, (1972).
49. Barat C, Simpson L, Breslow E. Properties of human vasopressin precursor constructs: inefficient monomer

folding in the absence of copeptin as a potential contributor to diabetes insipidus. Biochemistry, 43: 8191-8203, (2004).
50. Dutil J, Moujahidine M, Lemieux C, Jankowski M, Gutkowska J, Deng AY. Chromosomal and comparative mapping of rat oxytocin, oxytocin receptor and vasopressin genes. Cytogenet Cell Genet, 93: 57-59, (2001).
51. Struck J, Morgenthaler NG, Bergmann A. Copeptin, a stable peptide derived from the vasopressin precursor, is elevated in serum of sepsis patients. Peptides, 26(12):2500-2504, (2005).
52. Dunser MW, Mayr AJ, Ulmer H, Knotzer H, Sumann G, Pajk W, et al. Arginine vasopressin in advanced vasodilatory shock: a prospective, randomized, controlled study. Circulation, 107:2313–2319, (2003).
53. Robertson GL, Klein LA, Roth J, Gorden P. Immunoassay of plasma vasopressin in man. Proc Natl Acad Sci U S A, 66(4):1298–1305, (1970).
54. Preibisz JJ, Sealey JE, Laragh JH, Cody RJ, Weksler BB. Plasma and platelet vasopressin in essential hypertension and congestive heart failure. Hypertension ,5: 1129–1138, (1983).
55. Smyth DG, Massey DE. A new glycopeptide in pig, ox, and sheep pituitary. Biochem Biophys Res Commun, 87:1006-1010, (1979).
56. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clin Chem, 52:112–119, (2006).
57. Robertson GL, Mahr EA, Athar S, Sinha T. Development and clinical application of a new method for the radioimmunoassay of arginine vasopressin in human plasma. J Clin Invest, 52: 2340–2352, (1973).
58. Katan M, Morgenthaler N, Dixit K C, Rutishauser J, Brabant G, Muller B, et al. Anterior and posterior pituitary function testing with simultaneous insulin tolerance test and a novel copeptin assay. J Clin Endocrinol Metab, 92:2640-2643, (2007).
59. Keller T, Tzikas S, Zeller T, Czyz E, Lillpopp L, Ojeda FM, et al. Copeptin improves early diagnosis of acute myocardial infarction. J Am Coll Cardiol, 55(19):2096-2106, (2010).
60. Reichlin T, Hochholzer W, Stelzig C, Laule K, Freidank H, Morgenthaler NG, et al. Incremental value of copeptin for rapid rule out of acute myocardial infarction. J Am Coll Cardiol , 54(1):60-68, (2009).
61. Shand JA, Menown IB, McEneaney DJ. A timely diagnosis of myocardial infarction. Biomarkers in Medicine, 4(3): 385-393, (2010).
62. Chan D, Ng LL. Biomarkers in acute myocardial infarction. BMC Med., 8(1): 34, (2010).
63. Morgenthaler NG, Muller B, Struck J, Bergmann A, Redl H, Christ- Crain M. Copeptin, a stable peptide of the arginine vasopressin precursor, is elevated in hemorrhagic and septic shock. Shock, 28:219-226, (2007).
64. Muller B, Morgenthaler N, Stolz D, Schuetz P, Muller C, Bingisser R, et al. Circulating levels of copeptin, a novel biomarker, in lower respiratory tract infections. Eur J Clin Invest, 37:145-152, (2007).
65. Stolz D, Christ-Crain M, Morgenthaler NG, Leuppi J, Miedinger D, Bingisser R, et al. Copeptin, C reactive protein, and procalcitonin as prognostic biomarkers in acute exacerbation of COPD. Chest, 131:1058-1067, (2007).
66. Stoiser B, Mörtl D, Hülsmann M, Berger R, Struck J, Morgenthaler NG, et al. Copeptin, a fragment of the vasopressin precursor, as a novel predictor of outcome in heart failure. Eur J Clin Invest, 36(11):771-778, (2006).
67. Gegenhuber A, Struck J, Dieplinger B, Poelz W, Pacher R, Morgenthaler NG, et al. Comparative evaluation of B-type natriuretic peptide, mid-regional pro-A-type natriuretic peptide, mid-regional pro-adrenomedullin, and Copeptin to predict 1-year mortality in patients with

acute destabilized heart failure. J Card Failure, 13(1):42-49, (2007).
68. Neuhold S, Huelsmann M, Strunk G, Stoiser B, Struck J, Morgenthaler N G, et al.Comparison of Copeptin, B-Type Natriuretic Peptide, and Amino-Terminal Pro-B-Type Natriuretic Peptide in Patients With Chronic Heart Failure Prediction of Death at Different Stages of the Disease. J Am Coll Cardiol, 52: 266-272, (2008).
69. Masson S, Latini R, Carbonieri E, Moretti L, Rossi MG, Ciricugno S, et al. The predictive value of stable precursor fragments of vasoactive peptides in patients with chronic heart failure: data from the GISSI-heart failure (GISSI-HF) trial. Eur J Heart Failure, 12(4):338-47, (2010).
70. Neuhold S, Huelsmann M, Strunk G, Struck J, Adlbrecht C, Gouya G, et al. Prognostic value of emerging neurohormones in chronic heart failure during optimization of heart failure-specific therapy. Clin Chem, 56:121-126, (2010).
71. Khan SQ, Dhillon OS, O'Brien RJ, Struck J, Quinn PA, Morgenthaler NG, et al. C-terminal provasopressin (copeptin) as a novel and prognostic marker in acute myocardial infarction: Leicester Acute Myocardial Infarction Peptide (LAMP) study. Circulation, 115:2103-2110, (2007).
72. Kelly D, Squire IB, Khan SQ, Quinn P, Struck J, Morgenthaler NG, et al. C-terminal provasopressin (copeptin) is associated with left ventricular dysfunction, remodeling, and clinical heart failure in survivors of myocardial infarction. J Card Failure, 14:739-745, (2008).
73. Voors AA, von Haehling, S, Anker SD, Hillege HL, Struck S, Hartmann O, et al. C-Terminal Provasopressin (Copeptin) is a strong prognostic marker in patients with heart failure after an acute myocardial infarction. Results from the OPTIMAAL study. Eur Heart Journal, 30: 1187-1194, (2009).
74. Szinnai G, Morgenthaler N G, Berneis K, Struck J, Müller B, Keller U, et al. Changes in Plasma Copeptin, the C-Terminal Portion of Arginine Vasopressin during Water Deprivation and Excess in Healthy Subjects. The Journal of Clinical Endocrinology & Metabolism, 92: 3973-3978, (2007).
75. Robertson GL. The use of vasopressin assays in physiology and pathophysiology. Semin Nephrol, 14: 368–383, (1994).
76. Robertson GL. Thirst and vasopressin function in normal and disordered states of water balance. J Lab Clin Med, 101: 351–371, (1983).
77. Bhandari SS, Loke I, Davies JE, Squire IB, Struck J, Ng LL. Gender and Renal function influence plasma levels of Copeptin in Healthy Individuals. Clinical Science, 116:257–263, (2009).
78. Gheorghiade M, Niazi I, Ouyang J, Czerwiec F, Kambayashi J chi, Zampino M, et al. Vasopressin V2-receptor blockade with tolvaptan in patients with chronic heart failure: results from a double-blind randomized trial. Circulation, 107:2690–2696, (2003).
79. Gheorghiade M, Gattis WA, O'Connor CM, Adams KF Jr, Elkayam U, Barbagelata A, et al. Acute and Chronic Therapeutic Impact of Vasopressin Antagonism in Congestive Heart Failure (ACTIV in CHF) Investigators. Acute and chronic therapeutic impact of a vasopressin 2 antagonist (tolvaptan) in congestive heart failure. JAMA, 291: 1963–1971, (2004).
80. Thibonnier M. Vasopressin receptor antagonists in heart failure. Curr Opin Pharmacol, 3:683–689, (2003).