The predictive value of NTproBNP on postoperative outcome in patients undergoing offpump CABG

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1 Introduction

Coronary heart disease is the main cause of morbidity and mortality in developed countries, and the prevalence is increasing in developing countries. Several studies have reported biomarker clusters which are associated with CHD. The assessment of these biomarkers, alone or in combination, may improve the long-term prediction of mortality of first major cardiovascular event compared to conventional risk markers. [Zethelius et al., 2008]

Brain type natriuretic peptide (BNP) is primarily produced by cardiac myocytes. Physiological effects of BNP are a peripheral vasodilatation and inhibition of renin-angiotensin production. [Daniels and Maisel, 2007] The precursor peptide proBNP is split into the active hormone BNP and the N-terminal fragment (NT-proBNP). Both BNP and NT-proBNP are established markers for cardiac failure. NT-proBNP is also more stable, which makes its measurement more reliable. [Thay-Hsiung et al., 2013] NT-proBNP was identified as a novel and important CHD biomarker, and has prognostic value in patients with stable CHD. [Kragelund et al., 2005] A report from the BELSTRESS study suggested that NT-proBNP levels were a strong predictor of coronary events, even after adjustment for conventional risk factors. [DeSutter et al., 2005] In patients with coronary artery disease increased BNP levels are associated with an increased rate of myocardial infarction and cardiovascular death during mid-term follow-up. [Schnabel et al., 2006]

However, Other pathologies such as exacerbated chronic obstructive pulmonary disease, atrial fibrillation, and myocarditis can cause elevated BNP levels. Additionally, higher NT-proBNP levels are associated with : female gender, impaired renal fuction, and older age. Increased BNP levels are a prognostic marker associated with higher mortality in patients with myocardial infarction, cardiogenic shock, and pulmonary embolism. [Rodseth, 2009]

2 Review of Literature

2.1 Natriuretic Peptides

In 1956, Kisch found that guinea pig atrial, but not ventricular, cells contained highly developed Golgi networks, similar to those observed in secretory cells [Kisch, 1956]. Jamieson and Palade reported that atrial, but not ventricular, myocytes contain spherical, electron opaque granules [Jamieson and Palade, 1964]. At the same time, physiological experiments conducted by Henry and colleagues revealed that balloon distension of the atria correlated with increased urination in dogs [Henry et al., 1956].

In a study published in 1981, de Bold and colleagues elegantly linked the seemingly disparate studies of Kisch and Henry by showing that atrial, but not ventricular, extracts contain a potent blood pressure decreasing component that works by stimulating renal sodium and water secretion [de Bold et al., 1981]. Shortly after the publication of this landmark paper, a number of groups reported the purification and sequencing of atrial peptides of varying sizes that possessed natriuretic, diuretic, and/or smooth muscle relaxing activity [Currie et al., 1984] [Flynn et al., 1983] [Kangawa et al., 1984] [Misono et al., 1984]. Thus, the first natriuretic peptide was discovered.

Several different names were given to these peptides such as atrial natriuretic factor, atriopeptin, cardionatrin, and cardiodilatin. However, atrial natriuretic peptide (ANP) is most often used to describe this peptide in the current literature. The second member of the family to be discovered, B-type natriuretic peptide (BNP), was originally called brain natriuretic peptide because it was purified and sequenced from porcine brain [Sudoh et al., 1988]. However, subsequent studies found that it is more highly concentrated in cardiac ventricles of patients with heart failure [Mukoyama et al., 1991] [Mukoyama et al., 1990].

Therefore, it is often described as B-type natriuretic peptide today. Finally, the third member of the family, C-type natriuretic peptide (CNP) was purified in 1991 from porcine brain extracts based on its ability to relax smooth muscle. All three members are similar in primary amino acid structure, contain a 17-residue disulfide ring, and are the products of separate genes.[Sudoh et al., 1990]

The biological actions of natriuretic peptides are mediated through membrane-bound natriuretic peptide receptors (NPR) that are linked to a cyclic guanosine monophosphate-dependent signaling cascade, including NPR-A, which preferentially binds ANP and BNP, and NPR-B, which preferentially binds CNP.

Elevated natriuretic peptides levels can be also found in many circumstances involving LV dysfunction or hypertrophy; right ventricular (RV) dysfunction secondary to pulmonary diseases; cardiac inflammatory or infectious diseases; and endocrinologic diseases and high output status without decreased left ventricular ejection fraction (EF), e.g., sepsis, renal failure, cirrhosis of liver, or intracranial pathologies. The causes and mechanisms of elevated natiuretic peptides levels are summarized in Fig. 2.

2.1.1 Atrial Natriuretic Peptide

All natriuretic peptides are synthesized as preprohormones Fig 1. The resulting mRNA gives rise to a 151 amino acid polypeptide, known as preproANP. The first 25 amino acids constitute a signal sequence that is cleaved to yield a 126 amino acid peptide called proANP, which is the major form of ANP stored in the atrial granules [Oikawa et al., 1984]. Upon release from these granules, proANP is rapidly cleaved by corin, a transmembrane cardiac serine protease. Corin is highly expressed on the extracellular surface of atrial cardiomyocytes and cleaves proANP into the biologically active 28-amino acid form of ANP [Yan et al.,

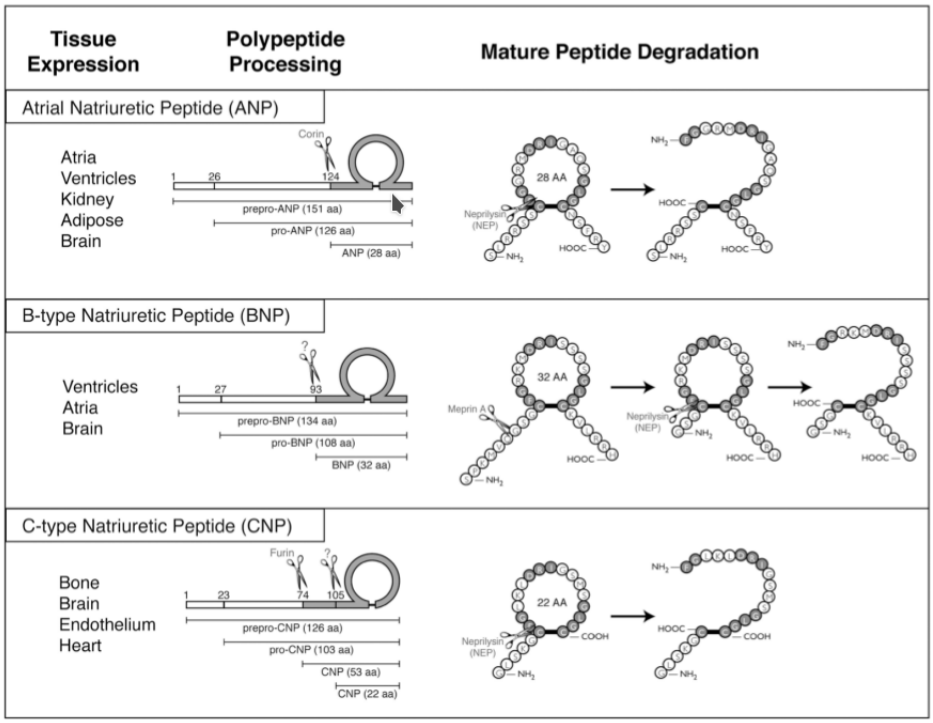

Figure 1: Structure of the human natriuretic peptides. The structure of the preprohormones for ANP, BNP and CNP are outlined on the left of each panel. The final amino acid sequence and structure of the mature peptides along with the major degradation product are shown on the right. The sites of cleavage are indicated with scissors.

2000]. Alternative processing of proANP in the kidney by an unknown protease results in a 32-amino acid peptide called urodilatin that contains four additional amino-terminal residues [Forssmann et al., 1998]. Disruption of the murine ANP gene, results in marked hypertension, which was initially described as salt-sensitive [John et al., 1995], but later found not to be correlated with dietary salt intake [John et al., 1996].

Release of proANP from the atrial granules is primarily stimulated by stretch of the atrial wall caused by increased intravascular volume [Bilder et al., 1986] [Edwards et al., 1988] [Lang et al., 1985], but pressor hormones also stimulate ANP release [Ruskoaho, 2003]. Plasma levels of ANP are relatively low (10 fmol/ml), but in patients with congestive heart failure, circulating ANP levels are elevated from 10- to 30-fold [Burnett et al., 1986] [Cody et al., 1986].

The plasma half-life of ANP in humans is approximately 2 min [Nakao et al., 1986] [Yandle et al., 1986]. Degradation of the active ANP peptide occurs through the actions of neutral endopeptidase (NEP) [Stephenson and Kenny, 1987] [Vanneste et al., 1988] as well as through binding to the natriuretic peptide clearance receptor (NPR-C), a cell surface receptor that lacks guanylyl cyclase activity and controls the local concentrations of natriuretic peptides via constitutive receptor mediated internalization and degradation. Inhibiting NEP, increases the half-life of ANP [Yandle et al., 1989], suggesting that NEP activity contributes to the rapid clearance of ANP. However, it is important to note that mice lacking functional NEP do not exhibit increased natriuretic peptide function [Lu et al., 1995]. In contrast, mice lacking NPR-C are hypotensive, exhibit skeletal overgrowth and have reduced ability to clear ANP compared to wild type mice, suggesting that NPR-C is also a physiologic regulator of circulating natriuretic peptide concentrations [Matsukawa et al., 1999].

ANP is secreted in response to:

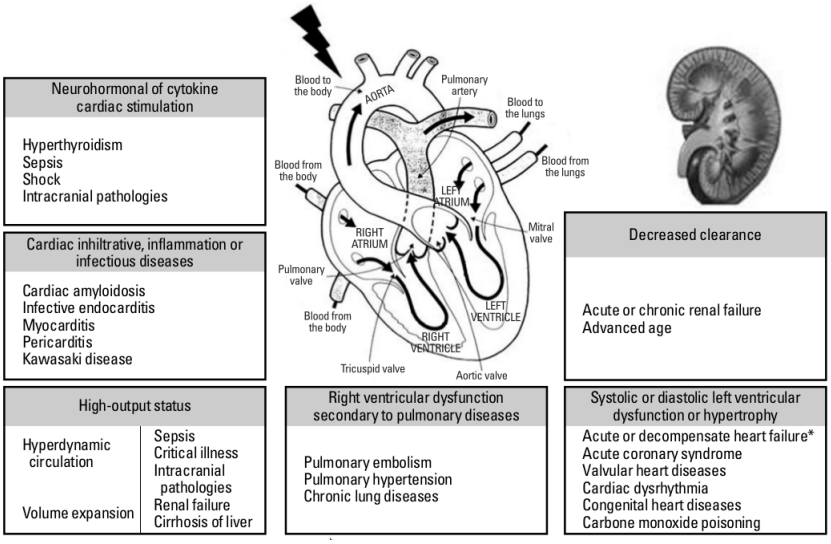

Figure 2: The causes and mechanisms of elevated natriuretic peptides levels.

- Stretching of the atrial wall [Widmaier et al., 2008]
- Reduced Sympathetic stimulation of β-adrenoceptors
- Raised sodium concentration (hypernatremia), though sodium concentration is not the direct stimulus for increased ANP secretion. [Widmaier et al., 2008]
- Endothelin, a potent vasoconstrictor
- exercise [Kokkonen et al., 2002]

2.1.2 B-Type Natriuretic Peptide

BNP was initially purified and sequenced from extracts of porcine brain tissue and hence it was named brain natriuretic peptide [Sudoh et al., 1988]. Subsequently, BNP was found at much higher concentrations in cardiac tissues [Mukoyama et al., 1991] [Mukoyama et al., 1990].

Although low levels of BNP are stored with ANP in atrial granules, BNP is found at greater concentrations in cardiac ventricles. In this tissue, BNP is not stored in granules, but rather transcribed as needed in response to cardiac stress states. [Grepin et al., 1994] [Thuerauf et al., 1994] In normal human subjects, plasma concentrations of BNP are very low (1 fmol/ml), but in response to congestive heart failure, circulating concentrations of BNP are dramatically elevated [Mukoyama et al., 1991] [Mukoyama et al., 1990].

BNP can be produced in both atria and ventricles, and is upregulated in failing ventricular myocardium. In response to increased myocardial stretch and wall stress, ventricular myocytes secret the pro-hormone pre-proBNP, which is then cleaved into biologically active BNP and the inactive byproduct N-terminal-proBNP (NT-proBNP). Elevated BNP levels have been demonstrated to be a response to increased angiotensin II and sympathetic tones. [Iwanaga et al., 2006]

BNP is eliminated by binding to the NPR-C or degradation by NEP on endothelial cells, smooth muscle cells, cardiac myocytes, renal epithelium, and fibroblasts. NT-proBNP is cleared mainly by the kidney.[Schrier and Abraham, 1999] Compared to ANP, circulating BNP has a significantly longer half-life of around 20 min in humans [Mukoyama et al., 1991] [Mukoyama et al., 1990]; the half-life of NT-proBNP is about 60-90 minutes and would be expected to be longer in the setting of renal dysfunction. Unlike ANP, BNP is not initially cleaved by NEP. Instead, the first six amino-terminal amino acids of BNP are first cleaved by the metalloprotease, meprin A in the kidney brush border, which then allows further degradation by NEP [Pankow et al., 2007].

Plastic tubes containing ethylenedinitrolotetraacetic acid (EDTA) are desirable for BNP determination and refrigeration is required if the interval between blood collection and analysis is over 4 hours; whereas NT-proBNP can be measured in both serum or plasma, collected in glass or plastic tubes, and has no significant loss of immunoreactivity after 48 hours at room temperature. Although these existing BNP

assays correlate closely, BNP assays are not currently analytical equivalent due to lack of assay standardization. A multicenter colloborative proficiency testing study conducted in 90 Italian laboratories had demonstrates that there are significant differences in analytical characteristics and measured values among the most popular commercial methods for BNP and NT-proBNP. Thus, clinicians should be very careful when comparing results obtained by laboratories that use different methods.

2.1.3 C-Type Natriuretic Peptide

C-type natriuretic peptide (CNP) was initially purified and sequenced from porcine brain extracts [Sudoh et al., 1990]. It is the most highly expressed natriuretic peptide in the brain but is also highly expressed in chondrocytes and endothelial cells. Unlike ANP and BNP, the human gene encoding CNP, NPPC, is not located on chromosome 1 but on chromosome 2 [Ogawa et al., 1994b].

Processing of proCNP to its mature form may occur through the action of the intracellular serine endoprotease, furin. In vitro, furin cleaves the 103 amino acid proCNP into a 53 amino acid carboxyl-terminal biologically active peptide [Wu et al., 2003b]. This 53 amino acid form of CNP (CNP-53) is the major active form of CNP, at the tissue level [Brown et al., 1997]. However, in the systemic circulation, a shorter 22 amino acid form dominates (CNP-22). The protease responsible for this cleavage is not known. Importantly, CNP-53 and CNP-22 appear to bind and activate their cognate receptor, NPR-B, equally well [Yeung et al., 1996].

CNP is not stored in granules and its secretion is increased by growth factors [Suga et al., 1993] [Suga et al., 1992b] and sheer stress [Chun et al., 1997] in cultured endothelial cells. CNP expression in neointimal vascular smooth muscle cells is increased in response to vascular

injury [Brown et al., 1997]. In normal human subjects, mean CNP concentration is very low (1 fmol/ml). It is elevated in patients with congestive heart failure, although to a much lower extent than ANP and BNP [Charles et al., 2006] [Del-Ry et al., 2005] [Kalra et al., 2003].

2.2 Natriuretic Peptide Receptors

There are three known natriuretic peptide binding proteins. All members contain a relatively large ($\tilde{4}$50 amino acid) extracellular ligand binding domain and a single membrane-spanning region of about 20 residues. Natriuretic peptide receptors A and B contain an equally large intracellular domain consisting of a so-called kinase homology domain, dimerization domain, and carboxyl-terminal guanylyl cyclase domain. Thus, NPR-A and NPR-B signal by catalyzing the synthesis of the intracellular signaling molecule cGMP. In contrast, NPR-C only contains a 37 residue intracellular domain and lacks guanylyl cyclase activity. It primarily controls local natriuretic peptide concentrations via receptor-mediated internalization and degradation, although many groups have reported signaling functions for NPR-C as well [Rose and Giles, 2008].

2.2.1 Natriuretic Peptide Receptor-A

Natriuretic peptide receptor-A (NPR-A) is the principal receptor of ANP and BNP. NPR-A binds natriuretic peptides at a stoichiometry of 2:1 with a rank natriuretic peptide preference of: ANP $\geq$ BNP $>$ CNP [Bennett et al., 1991] [Koller et al., 1991] [Suga et al., 1992a].

Phosphorylation is essential for activation of NPR-A and dephosphorylation is a mechanism of desensitization in response to prolonged ANP exposure or protein kinase C activation [Potter and Garbers, 1992]

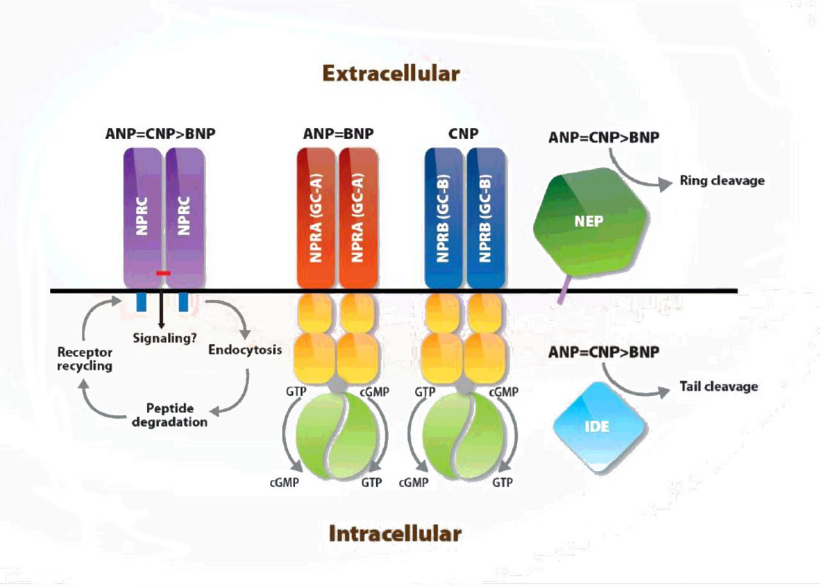

Figure 3: Schematic representation of natriuretic receptors

[Potter and Garbers, 1994]. Although ATP increases ANP-dependent guanylyl cyclase activity, the mechanism for this effect is debatable [Antos et al., 2005] [Antos and Potter, 2007] [Burczynska et al., 2007] [Joubert et al., 2005]. Data indicate that ATP reduces the Km for NPR-A [Antos and Potter, 2007].

NPR-A internalization and degradation is also controversial. One group consistently reports that the majority of internalized ANP-NPR-A complexes are degraded via a lysosomal pathway with a small portion returning intact to the plasma membrane [Pandey, 2002]. Meanwhile, studies in primary kidney and Chinese Hamster ovary indicate that NPR-A is a membrane resident protein that does not undergo acute internalization and degradation [Fan et al., 2005] [Koh et al., 1992] [Vieira et al., 2001].

NPR-A and/or its mRNA is expressed in kidney, lung, adipose, adrenal, brain, heart, testis, and vascular smooth muscle tissue [Goy et al., 2001]. NPR-A null mice exhibit chronic salt-resistant hypertension and cardiac hypertrophy and fibrosis [Kuhn et al., 2002]. A deletion

in the human NPR-A gene was identified in nine Japanese individuals, of which eight had essential hypertension; the normotensive individual with the altered allele had left ventricular hypertrophy [Nakayama et al., 2000].

2.2.2 Natriuretic Peptide Receptor-B

Natriuretic peptide receptor-B (NPR-B) is the principal receptor of C-type natriuretic peptide (CNP) and exhibits similar topology, glycosylation, and intramolecular disulfide bonding patterns as NPR-A. NPR-B binds natriuretic peptides with a selectivity preference of CNP $>$ ANP $\geq$ BNP [Bennett et al., 1991] [Koller et al., 1991] [Suga et al., 1992a].

NPR-B dephosphorylation has been shown to mediate desensitization in response to prolonged CNP exposure, protein kinase C activation, and intracellular calcium elevations [Potter, 1998] [Potter and Hunter, 2000] [Potthast et al., 2004]. ATP increases the guanylyl cyclase activity of NPR-B, by decreasing its Michaelis constant [Antos and Potter, 2007]. NPR-B and/or its mRNA is expressed in bone, brain, fibroblasts, heart, kidney, liver, lung, uterine, and vascular smooth muscle tissue [Bryan et al., 2006] [Dickey et al., 2007]. Mice with a targeted disruption of the NPR-B gene, display dwarfism and female sterility [Tamura et al., 2004].

NPR-B dominant negative mutant transgenic rats have also been generated [Langenickel et al., 2006]. In addition to mild growth retardation of the long bones, the rats displayed progressive, blood pressure-independent cardiac hypertrophy and an elevated heart rate.

Consistent with a prominent role for CNP in the heart, NPR-B, not NPR-A, is the most active natriuretic peptide receptor in the failed heart [Dickey et al., 2007]. Homologous loss-of-function mutations in

human NPR-B result in a rare form of dwarfism called acromesomelic dysplasia, type Maroteaux (AMDM) [Bartels et al., 2004].

2.2.3 Natriuretic Peptide Receptor-C

Natriuretic peptide receptor-C (NPR-C) consists of a large extracellular ligand-binding domain that is approximately 30-35% identical to NPR-A and NPR-B, a single membrane-spanning region, but only 37 intracellular amino acids [Chang et al., 1989] [Fuller et al., 1988] [Porter et al., 1990]. It has no known enzymatic activity but has been suggested to signal in a G protein-dependent manner [Rose and Giles, 2008]. It binds natriuretic peptides with a stoichiometry of two molecules of receptor to one molecule of ligand [Ammarguellat et al., 2001]. Its ligand selectivity preference is: ANP $>$ CNP $\geq$ BNP [Bennett et al., 1991] [Suga et al., 1992a].

The main function of NPR-C, also known as the clearance receptor, is to clear circulating natriuretic peptides through the process of receptor-mediated internalization and degradation [Koh et al., 1992] [Nussenzveig et al., 1990]. Internalization of NPR-C occurs in the absence of ligand; thus, this is a constitutive process [Nussenzveig et al., 1990]. Osteocrin, an endogenous protein with limited homology to members of the natriuretic peptide family, binds NPR-C, but not NPR-A or NPR-B [Moffatt et al., 2007]. Osteocrin is thought to compete with CNP for binding to NPR-C in bone, and therefore, increase local CNP levels during critical periods for bone development [Moffatt et al., 2007].

NPR-C is the most widely and abundantly expressed natriuretic peptide receptor; for example, it constitutes $\tilde{9}4\%$ of the total ANP binding sites in endothelial cells [Leitman et al., 1986]. NPR-C and/or its mRNA is expressed in adrenal, brain, heart, kidney, mesentery, and vascular smooth muscle tissue [Nagase et al., 1997] [Porter et al., 1990] [Suga et al., 1992c] [Wilcox et al., 1991]. NPR-C knockout mice exhibit

increased ANP half-lives, long bone overgrowth, hypotension, mild diuresis, dilute urine, and blood volume depletion [Matsukawa et al., 1999]. Mouse strains containing chemically induced loss-of-function mutations in the extracellular domain of NPR-C display skeletal overgrowth from endochondral ossification defects as well [Jaubert et al., 1999].

2.3 Physiologic Effects of Natriuretic Peptides

2.3.1 Natriuretic Peptide Effects on Blood Pressure

ANP binding to NPR-A is a key-signaling pathway, which regulates normal homeostatic blood pressure. This is clearly demonstrated in mice lacking ANP or its receptor NPR-A, which have blood pressures that are elevated 20-40mmHg, compared to control mice [John et al., 1995] [John et al., 1996] [Lopez et al., 1995] [Oliver et al., 1997]. The link between NPR-A and blood pressure in mice is particularly strong because Smithies and colleagues demonstrated that NPR-A copy number is inversely related to blood pressure in a remarkably linear manner [Oliver et al., 1998]. Conversely, blood pressures in transgenic mice overexpressing ANP or BNP are substantially decreased [Ogawa et al., 1994a] [Steinhelper et al., 1990]. Although infusion of supraphysiological levels of CNP into animals acutely decreases blood pressure [Clavell et al., 1993] [Sudoh et al., 1990], mice lacking functional CNP or NPR-B are normotensive [Chusho et al., 2001] [Tamura et al., 2004], suggesting that the CNP/NPR-B pathway is not a fundamental regulator of basal blood pressure in mice.

NPR-A dependent decreases in blood pressure are achieved through natriuresis and diuresis, vasorelaxation, increased endothelium permeability, and antagonism of the renin-angiotensin system. Classic experiments showed that atrial extract infusions resulted in rapid renal

excretion of water and sodium [de Bold et al., 1981]. Studies by Garbers and colleagues indicated that the renal response requires NPR-A because mice lacking this receptor do not respond to ANP, BNP, or to acute volume expansion [Kishimoto et al., 1996]. Similar studies found that NPR-A was also required for ANP- or BNP-dependent vasorelaxation in mice [Lopez et al., 1997]. Physiological experiments involving mice with severe reductions of NPR-A in vascular smooth muscle cells demonstrated that while smooth muscle NPR-A is required for acute ANP- or BNP-dependent vasorelaxation, this response does not play a significant role in controlling chronic blood pressure [Holtwick et al., 2002].

The ability of the ANP/NPR-A pathway to increase endothelial permeability is supported by the observation that hematocrit levels are elevated prior to urination and are preserved in nephrectomized animals [Almeida et al., 1986] [Fluckiger et al., 1986] [Richards et al., 1988]. Furthermore, mice with genetically engineered reductions of NPR-A in vascular endothelium exhibit volume expansion, hypertension, and reduced albumin clearance from the vascular system [Sabrane et al., 2005].

2.3.2 Effects of Natriuretic Peptides on Cardiac Hypertrophy and Fibrosis

Although prolonged hypertension can cause hypertrophy, the level of hypertrophy in NPR-A deficient mice is significantly greater than that observed in other genetic models that cause similar levels of hypertension, suggesting that NPR-A elicits a local growth inhibitory signal in the heart. Data for this idea was initially shown in NPR-A knockout mice, which have enlarged hearts even when effectively treated with antihypertensive drugs from birth [Knowles et al., 2001]. Additional studies determined that transgenic re-expression of NPR-A in the hearts of NPR-A -/- mice reduced cardiomyocyte size without affecting heart

rate or blood pressure [Kishimoto et al., 2001]. Finally, mice with reduced cardiomyocyte expression of NPR-A exhibited moderate hypertrophy even though they were slightly hypotensive [Holtwick et al., 2003] [Patel et al., 2005]. In terms of natriuretic peptides, mice lacking ANP have larger hearts, whereas mice transgenically overexpressing ANP have smaller hearts [Barbee et al., 1994] [Steinhelper et al., 1990]. In contrast, targeted deletion of BNP resulted in normotensive mice with normal heart size but with increased ventricular fibrosis - especially when subjected to pressure overload [Tamura et al., 2000]. Thus, genetic studies in mice strongly support a role for ANP activation of NPR-A in the local inhibition of cardiac hypertrophy and BNP activation of NPR-A in the inhibition of cardiac fibrosis.

Data supporting a role for the CNP/NPR-B pathway in cardiac remodeling has been reported. Although NPR-B inactivation mutations in mice have not been shown to cause hypertrophy [Tamura et al., 2004] [Tsuji and Kunieda, 2005], transgenic rats expressing a dominant negative form of NPR-B exhibit mild blood pressure-independent cardiac hypertrophy and increased heart rate [Langenickel et al., 2006]. In addition, CNP infusion was shown to reduce cardiac remodeling in response to experimentally induced myocardial infarction in rats, and transgenic expression of CNP improved outcomes in mice subjected to ischemia/reperfusion injury or myocardial infarction [Wang et al., 2007].

2.3.3 Effects of CNP and NPR-B on Bone Growth

The most obvious function of the CNP/NPR-B pathway is to stimulate long bone growth. Though undetectable at birth, mice lacking functional CNP or NPR-B develop dwarfism due to impaired endochondrial ossification [Chusho et al., 2001] [Tamura et al., 2001] [Tsuji and Kunieda, 2005]. Conversely, transgenic CNP overexpression or reduced degradation of CNP due to loss-of-function mutations in NPR-C

result in skeletal overgrowth [Jaubert et al., 1999] [Matsukawa et al., 1999] [Yasoda et al., 2004]. Growth plate histology reveals that the endochondral proliferative and hypertrophic zones are reduced in mice with impaired CNP or NPR-B signaling, whereas overexpressing mice have enlarged growth plates [Chusho et al., 2001] [Tamura et al., 2004] [Yasoda et al., 2004].

One cGMP effector involved in the long bone growth pathway is cGMP-dependent protein kinase II, also known as PKGII or cGKII. Loss-of-function mutations in the mouse or rat gene that encodes this kinase also cause dwarfism [Chikuda et al., 2004] [Pfeifer et al., 1996]. Interestingly, the growth plates of rodents with defective cGKII are enlarged, which differs from the diminished growth plates seen in the CNP or NPR-B deleted mice, suggesting that a cGKII-independent pathway is also involved in CNP-dependent long bone growth.

Humans with two loss-of-function alleles for NPR-B suffer from a rare type of autosomal recessive dwarfism, called acromesomelic dysplasia, type Maroteaux [Bartels et al., 2004]. These individuals are characterized by disproportionate limb to torso ratios that are only obvious a year or more after birth. Interestingly, although single copy carriers of a nonfunctional NPR-B allele do not suffer from disease, they are statistically shorter than comparable individuals with two wild type NPR-B alleles [Olney et al., 2006]. Thus, it is possible that NPR-B mutations could have a significant effect on the stature of the general population.

2.4 Therapeutics of Natriuretic Peptides

Measurement of serum BNP levels is used in the clinic as a diagnostic indicator for heart failure, and synthetic forms of both ANP and BNP have been approved in some countries for the treatment of heart failure [Gardner, 2003]. The extent of their usefulness, however, has

come under question due to their limited renal actions, and trials are underway to determine the most effective use of these peptides. In this section, we will explore the history of both synthetic ANP and BNP as therapeutic agents.

2.4.1 Synthetic ANP (Anaritide and Carperitide)

Studies revealed that the mature form of ANP is a 28-amino-acid peptide and that smaller versions are degradation products that maintain various levels of activity. The most widely studied of these is the 25-amino acid peptide lacking the first three amino-terminal residues. This peptide is referred to as ANF IV and its synthetic form is called anaritide. Since the activities of the 25-amino acid and mature 28-amino acid peptide were similar, many studies were conducted with the smaller peptide. Studies by Cody and colleagues indicated that infusion of anaritide in healthy male volunteers resulted in natriuresis, diuresis, and reduction in systolic blood pressure; however, in seven patients with congestive heart failure, the changes in urine volume and sodium excretion were minimal [Cody et al., 1986]. Saito and colleagues observed a similar lack of diuresis and natriuresis, when congestive heart failure patients were infused with the mature form of ANP [Saito et al., 1987].

Meanwhile, others acknowledged the renal hyporesponsiveness to anaritide in congestive heart failure patients, but indicated that the renal parameters did show a statistically significant increase in larger patient samples [Fifer et al., 1990]. In Japan, clinical studies on the effectiveness of mature ANP continued; and in 1995, synthetic full length ANP (carperitide) was approved for the treatment of acute decompensated heart failure. In the United States, clinical use of BNP, not ANP, was explored for the treatment of heart failure due to its larger renal responsiveness, and possibly due to unique patient opportunities.

Investigations were also initiated to study the effectiveness of ANP in the treatment of human renal disease. Specifically, trials were conducted to evaluate the ability of anaritide infusion to reduce the need for dialysis in patients with acute tubular necrosis. The initial study with 53 patients suggested a positive outcome for patients receiving anaritide because they had increased creatinine clearance and a decreased need for dialysis [Rahman et al., 1994]. This led to the formation of a multicenter placebo-controlled clinical trial in 504 patients with acute tubular necrosis. While 24-h infusion of anaritide did not improve the overall survival of the patients without dialysis, it appeared that a subset of patients might have benefited [Allgren et al., 1997]. Thus, a second trial was conducted in patients with oliguric acute renal failure. However, this 222 patient trial indicated no statistically significant benefit of anaritide in dialysis-free survival [Lewis et al., 2000]. Both trials remarked on the severe hypotension that often occurred as a result of the anaritide infusion. In fact, it is this severe hypotension that appears to be limiting the utility of anaritide or nesiritide as a therapy for either heart failure or renal disease. The authors stated in their discussion, it is possible that if this hypotension could have been avoided, anaritide would have been efficacious [Lewis et al., 2000]. Anaritide was also investigated for its ability to prevent radiocontrast-induced nephropathy. However, in a 247 person clinical trial anaritide along with hydration was no more effective at preventing radiocontrast-induced nephropathy than hydration alone [Kurnik et al., 1998].

Finally, in 2004, studies conducted in Sweden compared the ability of the loop diuretic, furosemide, or mature ANP (1-28) to increase GFR, renal blood flow, and reduce renal oxygen consumption in patients with acute renal failure. They concluded that furosemide was a more effective agent [Sward et al., 2005]. Therefore, despite its potent natriuretic and diuretic effects in normal, healthy subjects, clinical studies conducted to date indicate little or no therapeutic benefit of ANP analogs in the successful treatment of renal disease.

2.4.2 Synthetic BNP (Nesiritide)

Given the natriuretic effects of ANP, the related peptide BNP, was assumed to elicit a similar response. McGregor and colleagues demonstrated that administration of porcine BNP resulted in a natriuretic response and an increase in urinary excretion of cGMP [McGregor et al., 1990]. Yoshimura and colleagues reported the same response in healthy volunteers to infusion of human BNP [Yoshimura et al., 1991].

Patients with congestive heart failure also responded to infusion of BNP. The effectiveness of 24-h infusion of nesiritide to patients with congestive heart failure was examined in a multicenter, placebo-controlled trial. The peptide resulted in a reduction of both preload and afterload resulting in an increase in stroke volume and cardiac output [Mills et al., 1999]. The results of a second multicenter trial, called the Vasodilation in the Management of Acute Congestive Heart Failure (VMAC) study, compared the effects of the addition of nitroglycerin or nesiritide versus placebo to standard therapy. The group treated with nesiritide had improved dyspnea after 3 h treatment, while there was no difference in the other groups. The nitroglycerin group reported more adverse effects than the nesiritide group. Additionally, patients receiving nesiritide had less adverse cardiovascular effects at either the 0.015 or 0.03mcg/kg/min infusion rate compared to patients receiving dobutamine as determined by the 246-patient PRECEDENT Trial [deLissovoy et al., 2003].

With the approval of the first new intravenous compound for the treatment of heart failure in many years, use of nesiritide was immediate. After approval, the number of patients treated with nesiritide was larger than any clinical trial and with the larger sample population came some unpleasant findings. Initially, Wang and colleagues reported in 2004 that nesiritide does not improve renal function in patients with chronic heart failure [Wang et al., 2004a], but more damaging were two meta-analysis studies by Sackner-Bernstein and colleagues

indicating that nesiritide worsened renal function and increased the likelihood of death [Sackner-Bernstein et al., 2005a] [Sackner-Bernstein et al., 2005b].

The results of a 75-person study (BNP-CARDS study), however, suggest nesiritide has no detrimental effect on renal function, when cohorts of similar baseline renal function were compared [Witteles et al., 2007]. The number of persons in this study was small, however, so a more definitive conclusion on whether nesiritide impairs renal function will have to wait until the result of more detailed, larger studies are released. Several such studies are currently in progress. One is a clinical trial enlisting at least 1,900 patients throughout Europe and Latin America - the ETNA (Evaluating Treatment with Nesiritide in Acute Decompensated Heart Failure) trial. This trial was scheduled to begin in 2006 to study the efficacy of nesiritide on treatment of acutely decompensated heart failure. Results from the trial are not yet available. The second study involving about 900 patients, called FUSION II, was conducted to determine the safety and efficacy of outpatient administration of nesiritide to patients with heart failure. Preliminary analysis indicates that nesiritide did not induce renal complications or increase patient mortality [Cleland et al., 2007].

Finally, there is the ASCEND HF trial (Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure). This trial is scheduled to compare the effects of nesiritide treatment versus placebo for a minimum of 24 h up to a maximum of 7 days in 7,000 heart failure patients. Meanwhile, other therapeutic applications of nesiritide have also been investigated. Given that nesiritide was often reported to decrease pulmonary capillary wedge pressure, Michaels and colleagues tested its effectiveness in pulmonary hypertension, however, they found no effect for a 30 min infusion [Michaels et al., 2005]. Chen and colleagues have investigated the effectiveness of subcutaneous injections of nesiritide. Their most recent paper on effects in a dog heart failure pacing model suggest that subcutaneous injection of nesiritide reduces both preload and afterload but has no effect on cardiac output [Chen

et al., 2006].

2.5 BNP and NT-proBNP

2.5.1 Differences in Physiology

BNP is a hormonally active natriuretic peptide that is mainly released from the cardio-myocytes in the left ventricular wall. In reaction to stretch and tension of the myocardial wall the pro-hormone proBNP splits into BNP and the hormonally inactive remnant N-terminal proBNP (NT-proBNP) by proteolytic cleavage Fig. 4. [Pfister and Schneider, 2004] This process occurs under influence of integrins, structures at the Z-disc of sarcomeres, that measure stretch of these sarcomeres [Liang et al., 2000, Pyle and Solaro, 2004] after which both peptides will be secreted in equimolar amounts into the circulation.

Circulating BNP acts as an antagonist of the renin angiotensine aldosterone system, and protects the body from plasma overload by inducing diuresis, natriuresis, vascular dilatation and inhibition of the sympathetic nervous system. [Sudoh et al., 1988] The half life of BNP is around 20 minutes and the half life of NT-proBNP is around 120 minutes. BNP is known to be cleared from the blood by natriuretic peptide clearance receptors, by neuro endopeptidases and by the kidneys. Little is known on the exact clearance mechanism of NT-proBNP, although it has been suggested that the kidneys play a major role in this clearance. [Hall, 2005] Absolute values of BNP are significantly lower than values of NT-proBNP, despite equimolar secretion. The reference ranges for BNP and NT-proBNP vary depending on the assay that is used and the nature of the control population. In general, the suggested normal range for circulating BNP is 0.5-30 pg/ml and for circulating NT-proBNP the suggested normal range is 68-112 pg/ml. [Cowie et al., 2003] These natriuretic peptides may be beneficial in

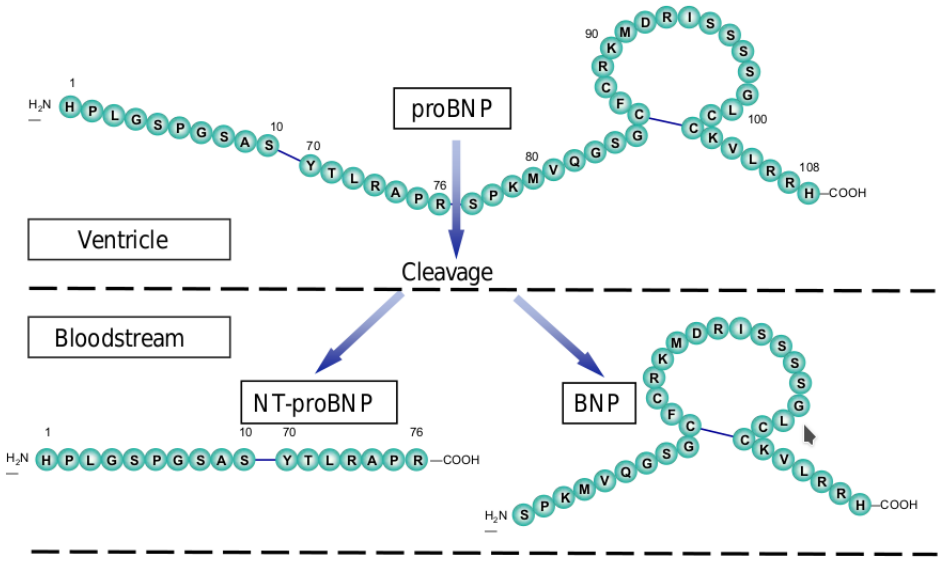

Figure 4: Secretion of BNP and NtproBNP

clinical practice since plasma levels of BNP and NT-proBNP are elevated in patients with HF and are related to the severity of the disease. [Mukoyama et al., 1990]

2.5.2 BNP and NT-proBNP in clinical practice

BNP and NT-proBNP plasma levels are promising tools in the daily management of suspected or established HF. Most studies on the use of BNP and NT-proBNP in clinical practice addressed their diagnostic properties. However, an increasing amount of evidence is available on the prognostic value of BNP and NT-proBNP and some studies provided hopeful results for the benefits of NT-proBNP guided medical treatment.

Diagnosis

Recent trials provided strong evidence that BNP and NT-proBNP are powerful diagnostic tools in exclusion and diagnosis of HF. The Breathing Not Properly study showed, by means of receiver operating characteristics analyses, that a BNP value of 100 pg/ml was the optimal value to differentiate patients with dyspnoea caused by HF from patients with dyspnoea due to pulmonary pathology at the emergency department Fig. 5. [Maisel et al., 2002]

This value of 100 pg/ml also discriminated non-systolic HF (LVEF ¡45%) from non-HF patients at the emergency department. It has also been suggested that BNP could be used to discriminate systolic from diastolic HF. Although non-systolic HF patients had significantly lower BNP plasma levels than systolic HF patients (LVEF ¿45%), BNP only had modest added value in differentiating non-systolic from systolic HF. In another study, a BNP value of 100 pg/ml added significant value to the diagnosis of HF on top of clinical judgement. [McCullough et al., 2002]

An international pooled analysis of 1256 patients provided cut off values for NT-proBNP in an emergency department setting. An age independent cut point of 300 pg/ml had a negative predictive value of 98%. Additionally, an optimal strategy to identify acute HF was to use age stratified cut off points of 450, 900 and 1800 pg/ml for ages ¡50, 50-75, and ¿75 respectively which yielded 90% sensitivity and 84% specificity for acute HF Fig. 6. [Januzzi et al., 2006, JL et al., 2005] Furthermore, BNP and NT-proBNP seem useful as diagnostic tools in primary care (where most patients with suspected HF are encountered and where only limited diagnostic tools are available) and as such are recommended in recent guidelines. [Swedberg et al., 2005]

The added value of these natriuretic peptides on top of established diagnostic tools, including symptoms and signs, has not been properly studied, in particular in relevant subgroups, and currently large studies are underway addressing this issue. Discharge diagnoses have been instrumental in providing estimates and time trends in prevalence and

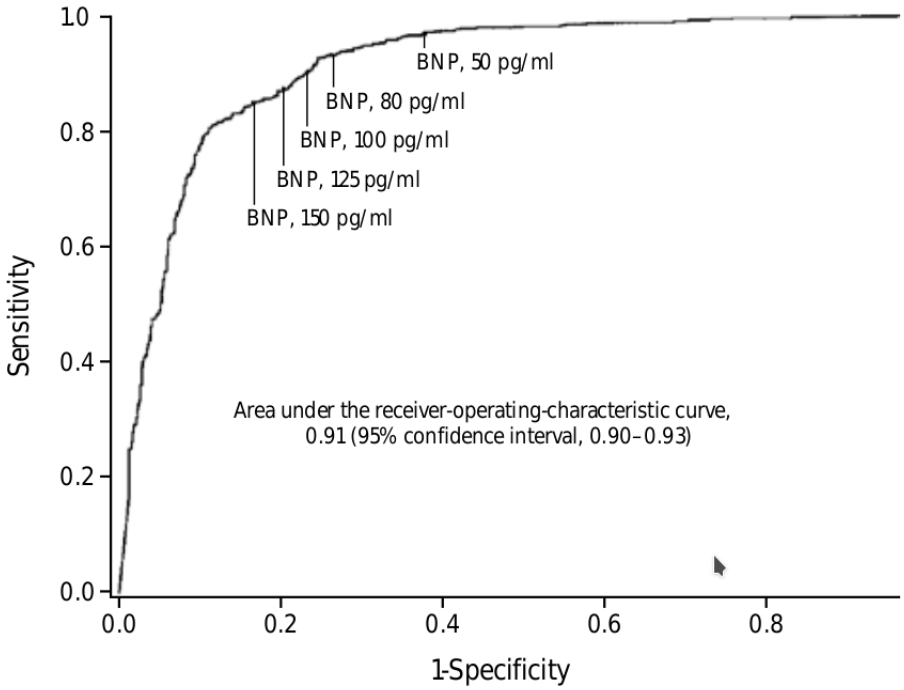

Figure 5: ROC curves for BNP in the diagnosis of heart failure at the emergency department.

incidence of HF. However, previous studies in the Netherlands, Sweden and in the United States showed that respectively 20%, 18% and 33% of the patients that were given the discharge diagnosis 'HF' at close examination did not have HF at all. [**?**Ingelsson et al., 2005, Goff et al., 2000] It is unknown whether the established BNP cut off value of 100 pg/ml can also be used at discharge after admission for HF.

Prognosis

The prognostic value of BNP and NT-proBNP is well established in several groups of patients. An early study on 85 patients with chronic HF revealed that BNP is a strong independent predictor of mortality. [Tsutamoto et al., 1997] Another study confirmed these results in a larger research population of 452 systolic HF patients (LVEF ¡35%).

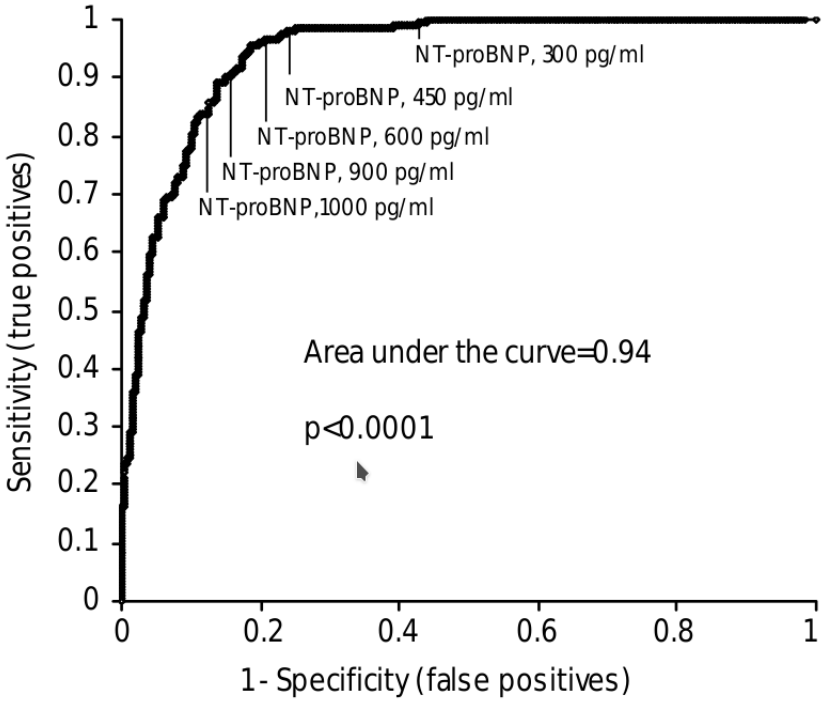

Figure 6: ROC curves for NTproBNP in the diagnosis of heart failure at the emergency department.

In this study BNP was found to be a strong independent predictor of sudden death during a follow up period of 3 years. [Berger et al., 2002] Furthermore, NT-proBNP was a predictor of sudden death in this study population. A substudy of the COPERNICUS trial (n=1011) revealed that NT-proBNP was consistently associated with an increased risk for all-cause mortality and hospitalisation for HF in patients with severe HF (LVEF ¡25%). [Hartmann et al., 2004] Another study on 142 patients with advanced HF also reported that NT-proBNP was an independent predictor of all cause mortality. [Gardner et al., 2003]

Guidance of treatment

BNP and NT-proBNP are influenced by drugs that are prescribed to HF patients like diuretics, [Tsutamoto et al., 2004] beta blockers, [Richards et al., 1999] ACE inhibitors or angiotensine II receptor blockers [Latini et al., 2002] and therefore these natriuretic peptides could possibly be used to guide medical treatment. A small study by the Australia-New Zealand Heart Failure Group including 69 patients with symptomatic HF provided evidence of the possible benefit of a NT-proBNP guided approach to therapy. Half of the patients received therapy guided by plasma NT-proBNP, therapy in the remaining patients was guided by clinical monitoring at the same frequency, but with the physician blinded to the NT-proBNP result. Clinical monitoring was based on scores assigned to 10 symptoms or signs of HF used in the Framingham criteria for HF. The study found significantly lower mortality, fewer hospitalisations and episodes of decompensated HF in the NT-proBNP-guided therapy group (target 1680 pg/ml). [Troughton et al., 2000] Larger studies are underway that are about to provide firmer evidence as to whether or not BNP and NT-proBNP can be used as a marker in the monitoring of treatment of HF patients. [Buckley et al., 1999]

2.5.3 Variables influencing BNP and NT-proBNP levels: potential limitations?

Although natriuretic peptide levels are of value in the diagnosis and prognosis of HF patients, several clinical conditions other than HF influence BNP and NT-proBNP plasma levels as well. These influences may be a disadvantage for the use of BNP and NT-proBNP in clinical practice of HF since it may lead to biased interpretations of the test results.

Cardiac variables: BNP and NT-proBNP are also elevated in patients with acute coronary syndrome. After acute myocardial infarction, levels of BNP rise rapidly during the first 24 hours and then tend to stabilize, [deLemos et al., 2001] and in patients with a Q-wave infarction, a peak in NT-proBNP levels was found after 12-48 hours. [Talwar et al., 2000] In patients with unstable angina pectoris, BNP levels were found to be four times higher compared to patients with stable angina pectoris. [Kikuta et al., 1996] Moreover, atrial fibrillation resulted in increased BNP levels in patients without, but not in patients with HF. [Knudsen et al., 2005] Right ventricular failure due to acute pulmonary embolism can also be determined by BNP. [Tulevski et al., 2002] Furthermore, hypertensive patients have higher BNP and NT-proBNP levels compared to non-hypertensive subjects. [Boomsma and van den Meiracker, 2001]

Non-cardiac variables: A few studies in relatively small study populations without HF showed that anaemia causes elevated BNP levels [Tsuji et al., 2004, Willis et al., 2005, Wold et al., 2005] and in a study on a small group of HF patients anaemia was also related to increased NT-proBNP levels. [Wu et al., 2005] However, besides that these studies were limited in sample size, they only investigated one of the two peptides and the effect of anaemia on NT-proBNP was investigated in HF patients only. Furthermore, anaemia is often caused by renal dysfunction, but this co-morbidity has not been investigated in detail

in these studies. Since both BNP and NT-proBNP are known to be elevated in case of renal dysfunction, [Luchner et al., 2005] and because renal function and HF are interrelated, a study investigating the effect of anaemia and renal function on both BNP and NT-proBNP in HF patients is needed. An additional variable that is related to both BNP and NT-proBNP is obesity. In several large studies lower natriuretic peptide levels were associated with higher body mass indexes. [Das et al., 2005, Krauser et al., 2005, Mehra et al., 2004, Wang et al., 2004b] As far as diabetes is concerned, results are conflicting between BNP and NT-proBNP; BNP levels did not differ between patients with or without diabetes, [Wu et al., 2004] but NT-proBNP levels seem to be higher in diabetic patients compared to non diabetics. [Magnusson et al., 2004] Furthermore, ascitic cirrhosis, hyperaldosteronism, hypercortisolism, carcinoma, subarachnoid hemorrhage, [Pfister and Schneider, 2004] lung cancer, tuberculosis and pulmonary embolism [**?**] are clinical conditions with reported elevated natriuretic peptide levels.

Patient related variables: Studies showed that both BNP and NT-proBNP levels are influenced by biological variation, with the biological variation of BNP being higher compared to NT-proBNP (up to 44% and up to 35% respectively). [Bruins et al., 2004, Wu et al., 2003a] Both BNP and NT-proBNP increase with advancing age and are higher in females compared to males in healthy subjects. [Raymond et al., 2003]

Previous research shows that BNP is related to maximal exercise performance. [Kruger et al., 2002] Moreover, the influence of moderate physical activity (75% of the maximum) on BNP levels was investigated in 10 healthy subjects, 10 HF patients with NYHA class I-II and in 10 HF patients with NYHA class III-IV. A significant increase in BNP levels was observed directly after exercise. [McNairy et al., 2002] However, it is not known whether B-type natriuretic peptide levels also reflect sub-maximal functional capacity during daily activities and whether they are related to quality of life.

3 Material and Methods

60 cases registered for elective off-pump coronary artery bypass grafting OPCAB were recruited in this study. Patients were interviewed preoperatively for history taking and clinical examination. EuroScore II was calculated. Demographic, past medical and surgical history, medications and baseline laboratory results (labs. on admission to hopsital) and preoperative angiography results were recorded. No specific attempts were made to standardize the anesthetic and surgical management. Venous samples for measuring NT-proBNP were collected on the day of surgery before induction. Samples were sent for analysis in Cairo University Clinical Pathology department. Intra-operative and postoperative data were recorded, including: duration of surgery, number of grafts, intraoperative blood transfusion and, in case CPB was need arotic cross clamp time, CPB time : lowest naso-pharyngeal temp on CPB. Patients were followed during their hospital stay [1] and events recorded including: death from cardiovascular causes, ischemic stroke , low output heart failure, myocardial infarction, prolonged intubaton and arrhythmias.

3.0.1 Exclusion Criteria

Patients with signicant valvular heart disease, dilated or hypertophic cardiomyopathy, NYHA III or IV, EF $< 40\%$, preoperative atrial fibrillation, inotropic support or intra-aortic balloon pump, creatinine clearance < 60 ml/min/1.73 m2, hyperthyoidism and hypothyroidism, and moderate to severe COPD were excluded to eliminate potential confounding factors which may influence heart function and plasma biomarkers.

[1] whenever we mention ICU or hospital stay, we mean postoperative duration in ICU and postoperative duration till discharge respectively

Hyperthyroidism and hypothyroidism are defined as serum TSH levels above or below reference ranges respectively. It was measured only upon clinical suspicion.

3.0.2 Definitions

The following definitions were used in this Study

Moderate to severe COPD Shortness of breath at own pace on the level, FEV1 $< 80\%$ of predicted, or continuous use of bronchodilators for > 2 weeks.

ischemic cerebral stroke New neurologic deficit lasting for 24hours with definite image evidence of cerebrovascular accident by computed tomography

low output heart failure Need of CPB during surgery, intra-aortic balloon pump and/or inotropes at 48 hours post-operatively

Myocardial infarction Elevated tropoponin 10x99th percentile URL at 12 hours after surgery associated with characteristic ECG changes or echocardiographically documented new regional wall motion abnormality

Prolonged intubation Intubation more than 24 hours postoperatively and/or reintubation following planned extubation

3.0.3 Primary and Secondary Outcomes

Primary outcomes:

- low output heart failure and myocardial infarction

Secondary outcome parameters:

- mortality
- arrhythmias
- length of ICU and hospital stay
- prolonged intubation

3.0.4 Data Analysis and Statistical Methods

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 24. Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Mann-Whitney test [Chan, 2003a]. Correlations between quantitative variables were done using Spearman correlation coefficient [Chan, 2003b]. ROC curve was constructed with area under curve analysis performed to detect best cutoff value of NTproBNP for detection of outcomes. P-values less than 0.05 were considered as statistically significant.

Table 1: pre-operative characteristics of study patients

		count	%
Gender	f	9	13.6
	m	57	86.4
Diabetic	yes		
	no		
Hypertensive	yes		
	no		

4 Results

Patients mean age was 57.4 ± 7.3 years, and 86.4% were male. One patient didn't undergo surgery; due to failure to obtain consent. Only two patients died; one of sepsis and the other of respiratory failure. Three required prolonged mechanical ventilation, one of whom was due to delayed recovery from anaesthesia (the only patient suffering from such complication). Three suffered recent onset arrhythmia (3 Atrial fibrillation, One Ventricular Tachycardia) during their ICU stay. One patient was re-admitted to the ICU for atrial fibrillation. Five patients had low output heart failure, and four had perioperative myocardial infarction. The mean ICU stay was 3.37 ± 0.84 days and mean hospital stay was 6.38 ± 1.3 (range 3-12) days. The preoperative NTproBNP levels ranged 100-14400 pg/mL, with a mean of 3096.83. It didn't correlate with any of the measured outcome parameters. See tables 3 and 4

Table 2: frequency of monitored postoperative outcomes

		count	%
low CO	yes	5	7.7
	no	60	92.3
Arrhythmia	yes	4	6.2
	no	61	93.8
perioperative MI	yes	4	6.2
	no	61	93.8
Prolonged Ventilation	yes	3	4.6
	no	62	95.4
Delayed Recovery	yes	1	1.5
	no	64	98.5
Moratality	yes	2	3.1
	no	63	96.9

Table 3: Relation between NTBNP and other parameters

		NTproBNP					
		Mean	Standard Deviation	Median	Minimum	Maximum	P value
low Co	yes	490.00	307.97	650.00	60.00	750.00	0.168
	no	296.84	329.75	160.00	10.00	1440.00	
Arrhythmia	yes	400.00	292.91	410.00	60.00	720.00	0.462
	no	306.37	333.77	160.00	10.00	1440.00	
perioperative MI	yes	437.50	326.22	485.00	60.00	720.00	0.397
	no	303.79	331.23	160.00	10.00	1440.00	
proloonged vent	yes	550.00	244.33	660.00	270.00	720.00	0.121
	no	300.33	330.69	160.00	10.00	1440.00	
Delayed Recovery	yes	1030.00		1030.00	1030.00	1030.00	0.129
	no	300.65	319.29	160.00	10.00	1440.00	
mortality	yes	495.00	318.19	495.00	270.00	720.00	0.306
	no	306.33	331.15	160.00	10.00	1440.00	

Table 4: Correlation between NTproBNP and other parameters

		NTproBNP
AGE	Correlation Coefficient	0.081
	P value	0.518
	N	66
EUROSCORE II	Correlation Coefficient	0.217
	P value	0.080
	N	66
ICU stay	Correlation Coefficient	-0.022-
	P value	0.861
	N	65
hospital stay	Correlation Coefficient	-0.017-
	P value	0.896
	N	65

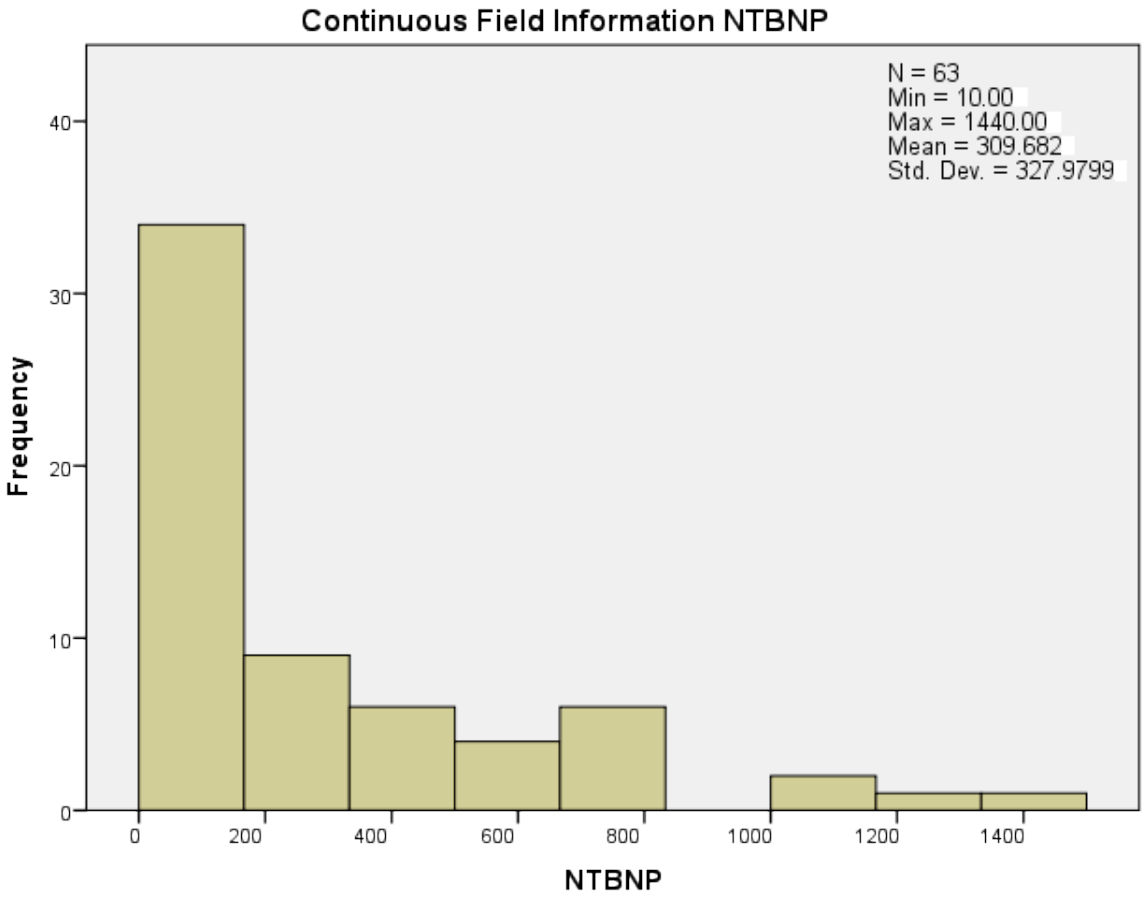
Continuous Field Information NTBNP
N = 63
Min = 10.00
Max = 1440.00
Mean = 309.682
Std. Dev. = 327.9799
Frequency
0
10
20
30
40
0
200
400
600
800
1000
1200
1400
NTBNP

Figure 7:

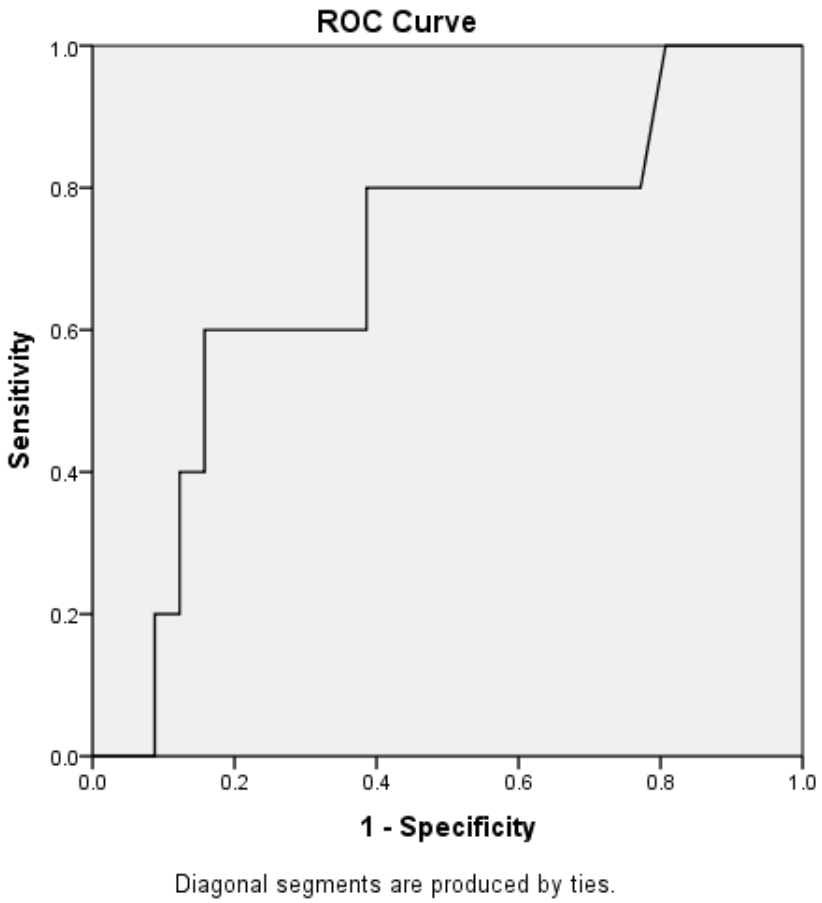

Figure 8: Receiver operating characteristic curve for the ability of NT-proBNP to predict postoperative low output heart failure. area under curve = 0.69; 95%CI = 0.44-0.93

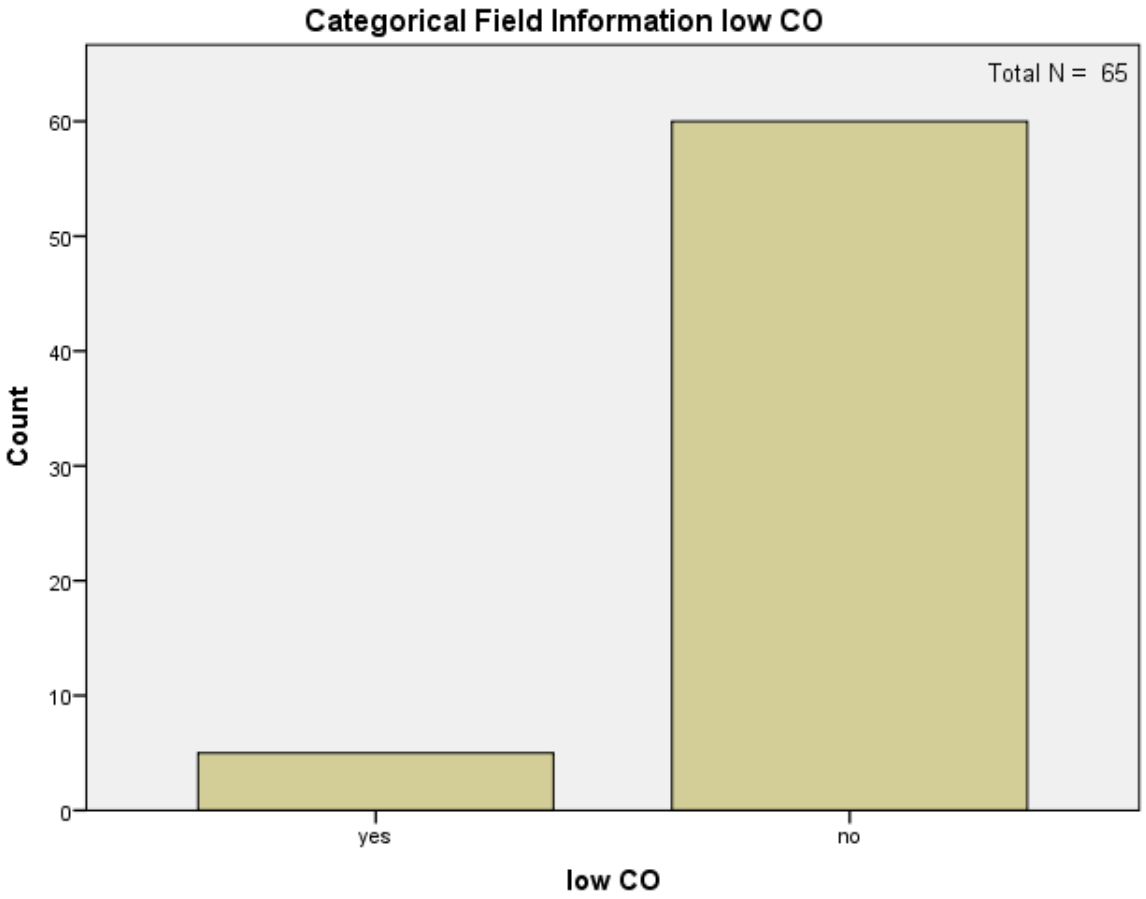
Categorical Field Information low CO
Total N = 65
Count
60
50
40
30
20
10
0
yes
no
low CO

Figure 9:

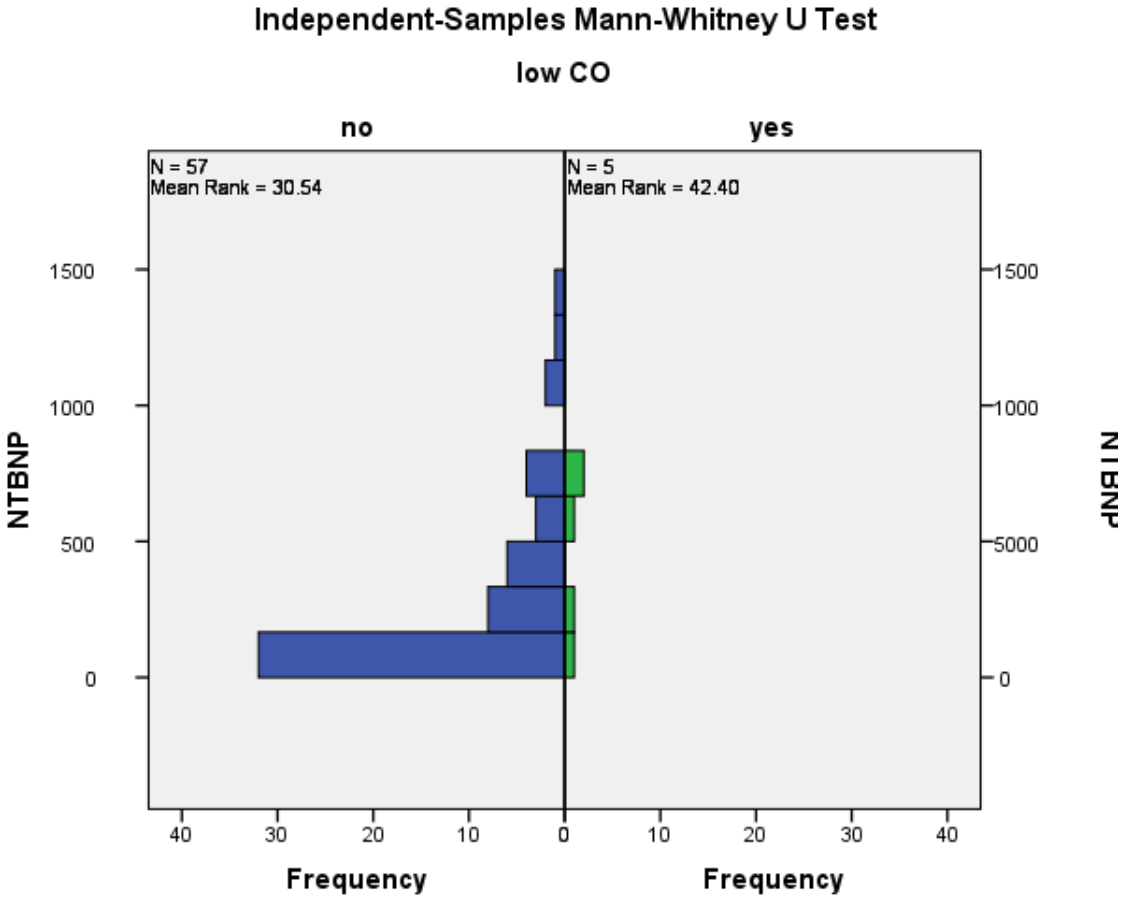
Independent-Samples Mann-Whitney U Test
low CO
no
yes
N = 57
Mean Rank = 30.54
N = 5
Mean Rank = 42.40
NTBNP
1500
1000
500
0
5000
40
30
20
10
0
Frequency

Figure 10:

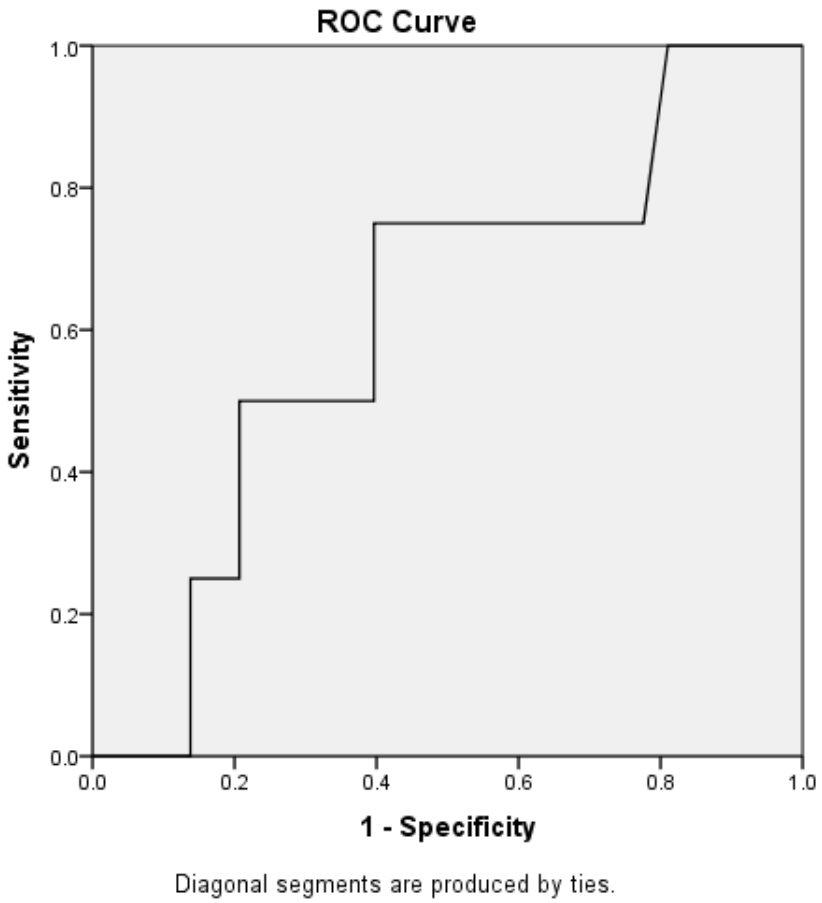

Figure 11: Receiver operating characteristic curve for the ability of NT-proBNP to predict postoperative arrhythmia. area under curve = 0.61; 95%CI = 0.35-0.88

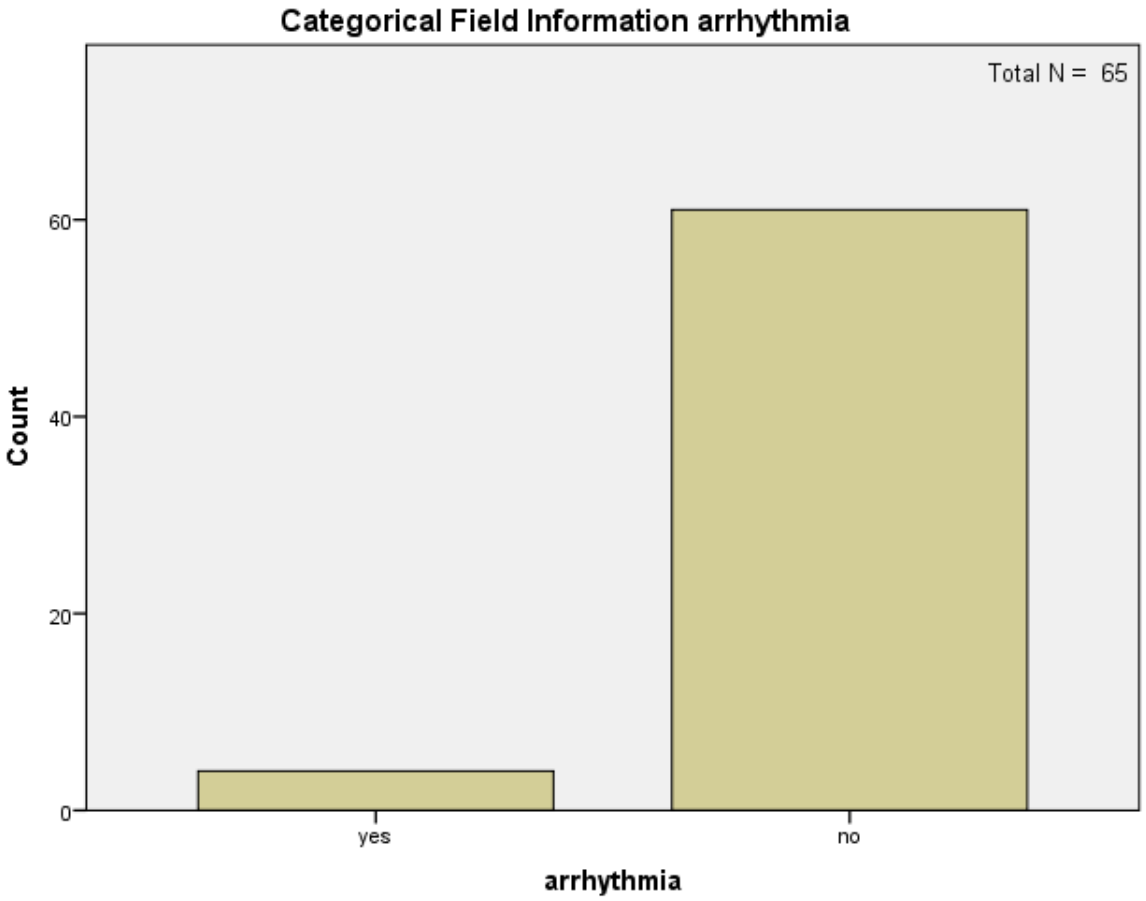
Categorical Field Information arrhythmia
Total N = 65
Count
60
40
20
0
yes
no
arrhythmia

Figure 12:

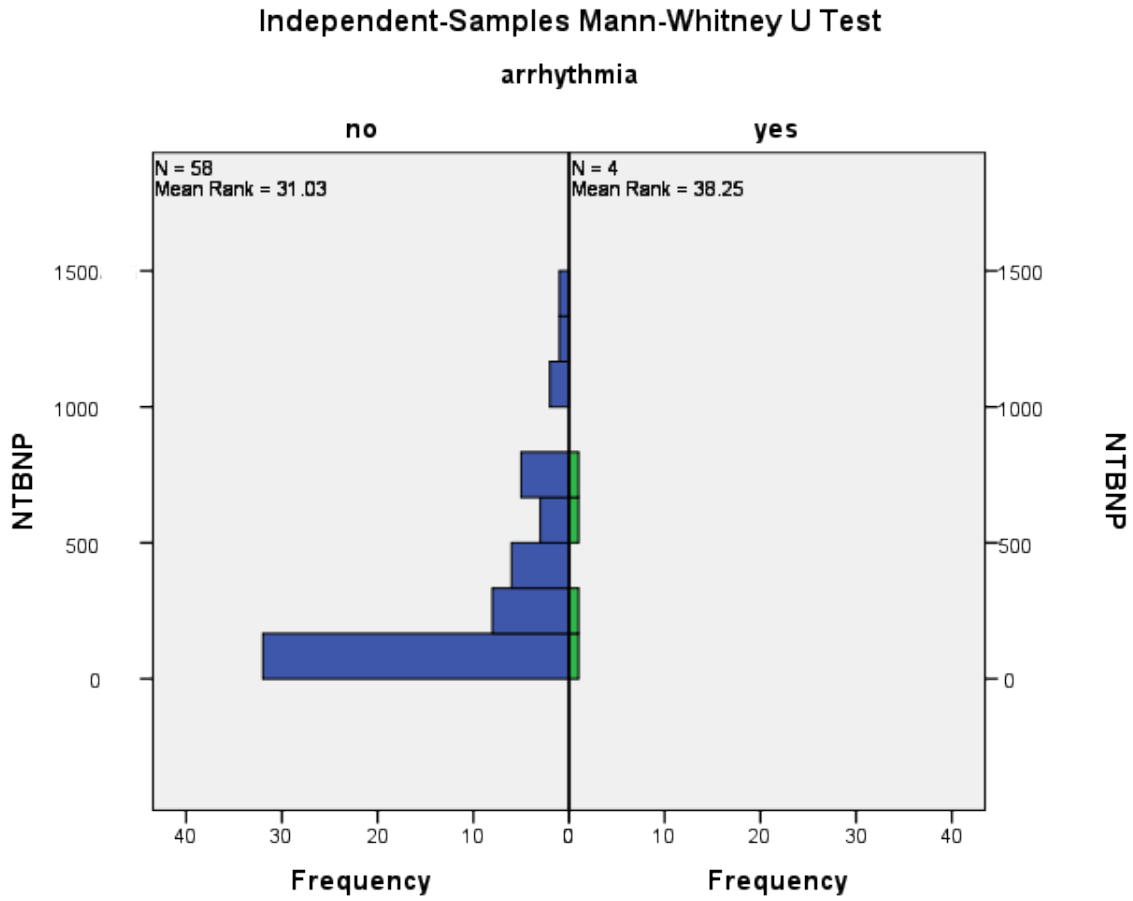
Independent-Samples Mann-Whitney U Test
arrhythmia
no
yes
N = 58
Mean Rank = 31.03
N = 4
Mean Rank = 38.25
NTBNP
1500
1000
500
0
40
30
20
10
0
Frequency

Figure 13:

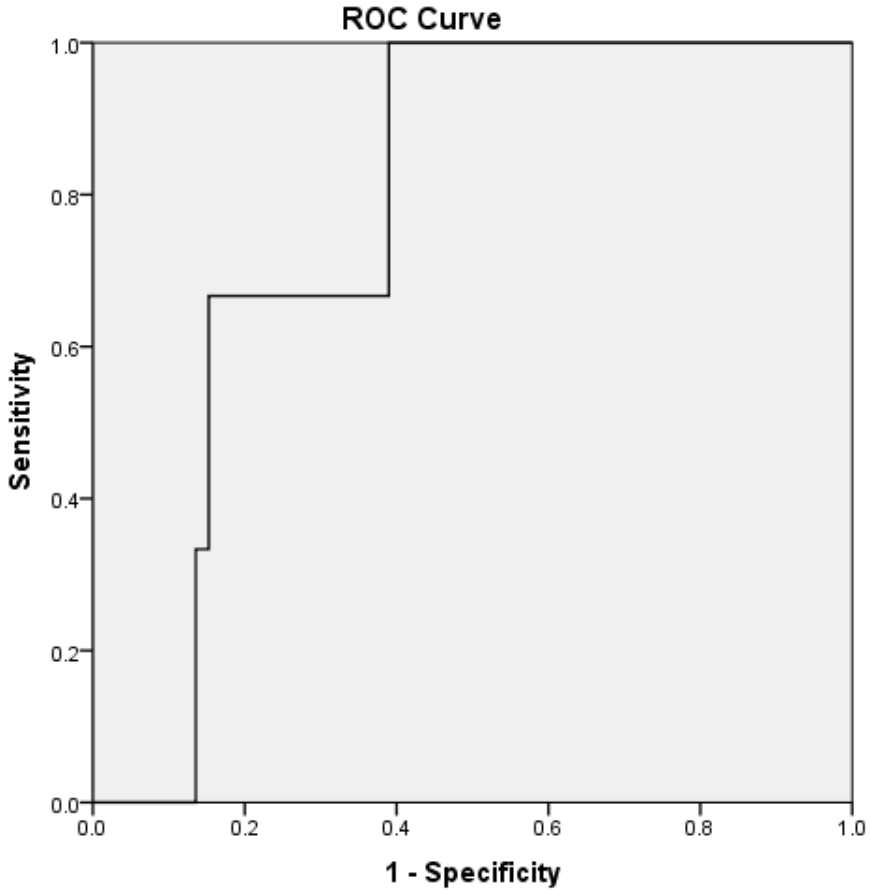

Figure 14: Receiver operating characteristic curve for the ability of NT-proBNP to predict prolonged postoperative mechanical ventilation. area under curve = 0.77; 95%CI = 0.61-0.93

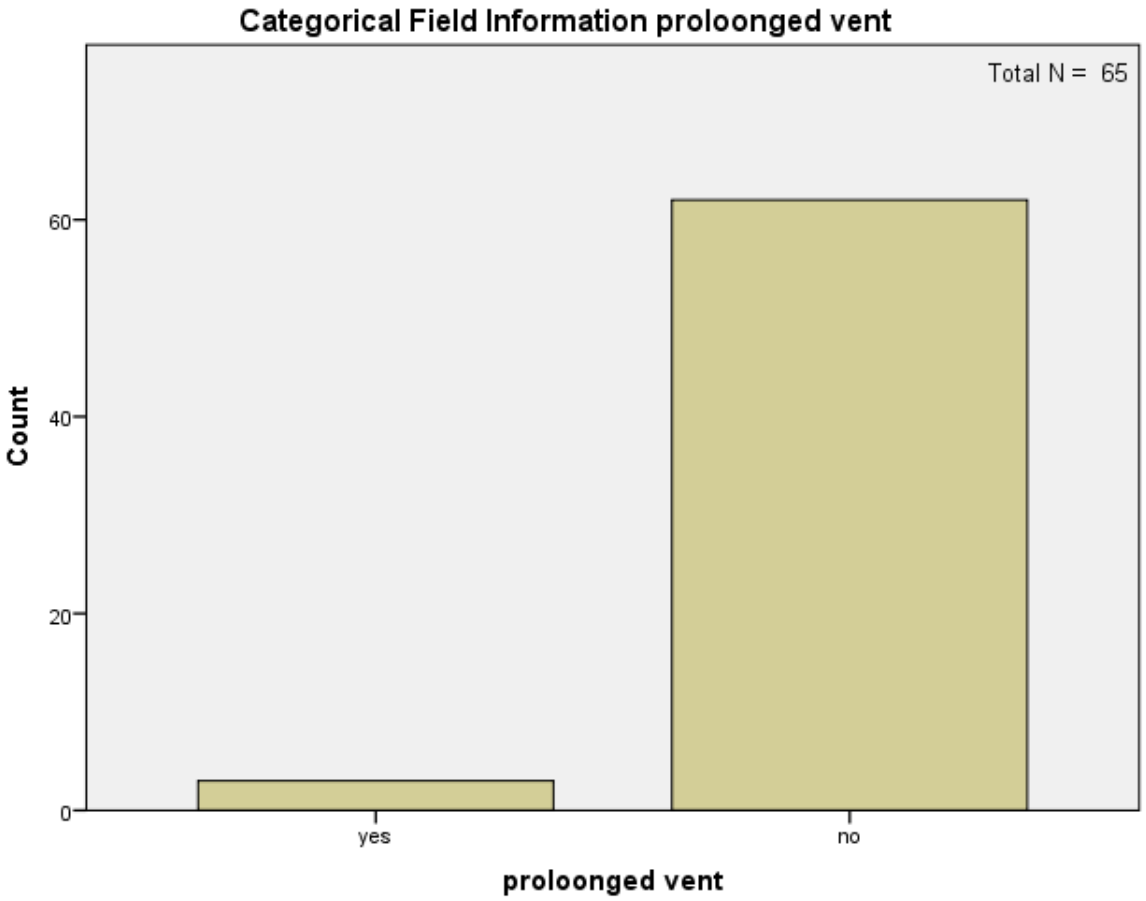
Categorical Field Information proloonged vent
Total N = 65
Count
60
40
20
0
yes
no
proloonged vent

Figure 15:

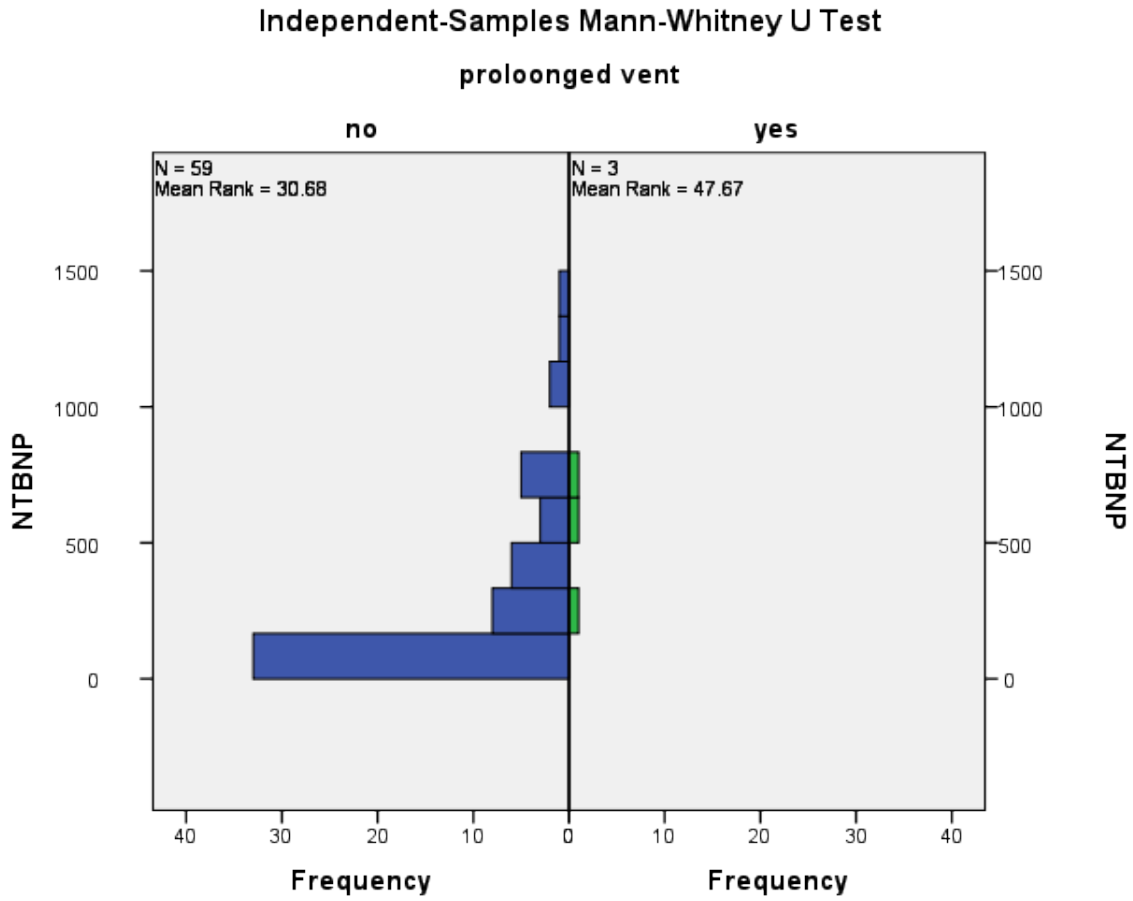
Independent-Samples Mann-Whitney U Test
proloonged vent
no
yes
N = 59
Mean Rank = 30.68
N = 3
Mean Rank = 47.67
NTBNP
1500
1000
500
0
40
30
20
10
0
Frequency

Figure 16:

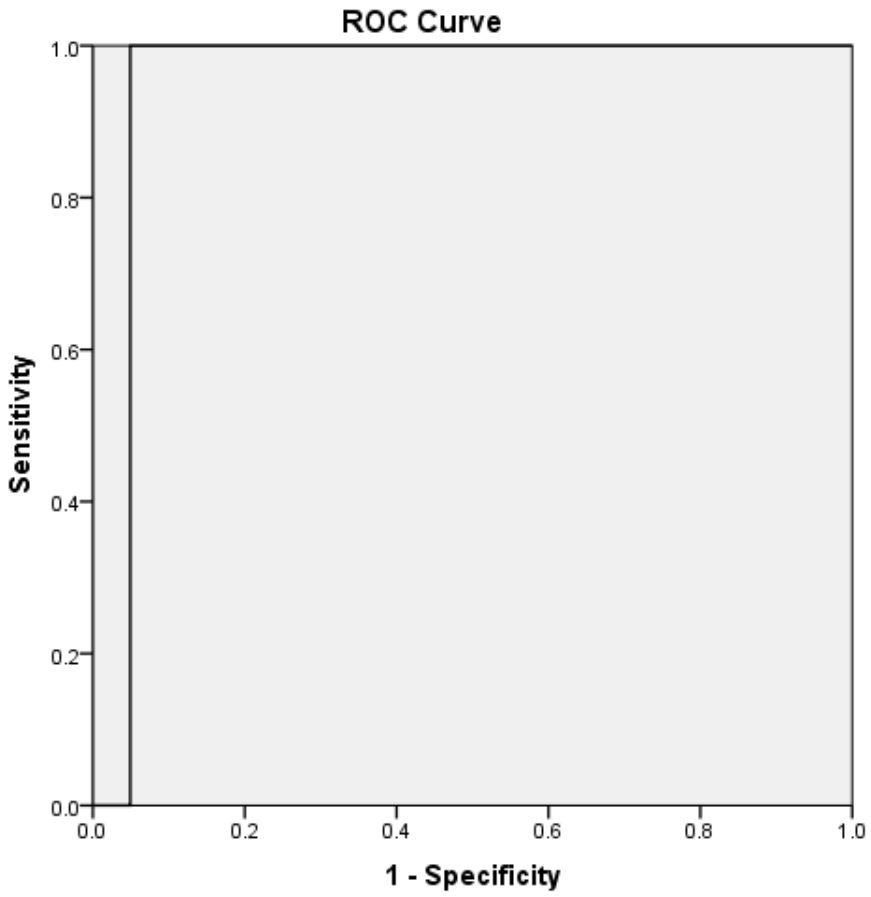

Figure 17: Receiver operating characteristic curve for the ability of NT-proBNP to predict delayed postoperative neurological recovery. area under curve = 0.95; 95%CI = 0.89-1.0

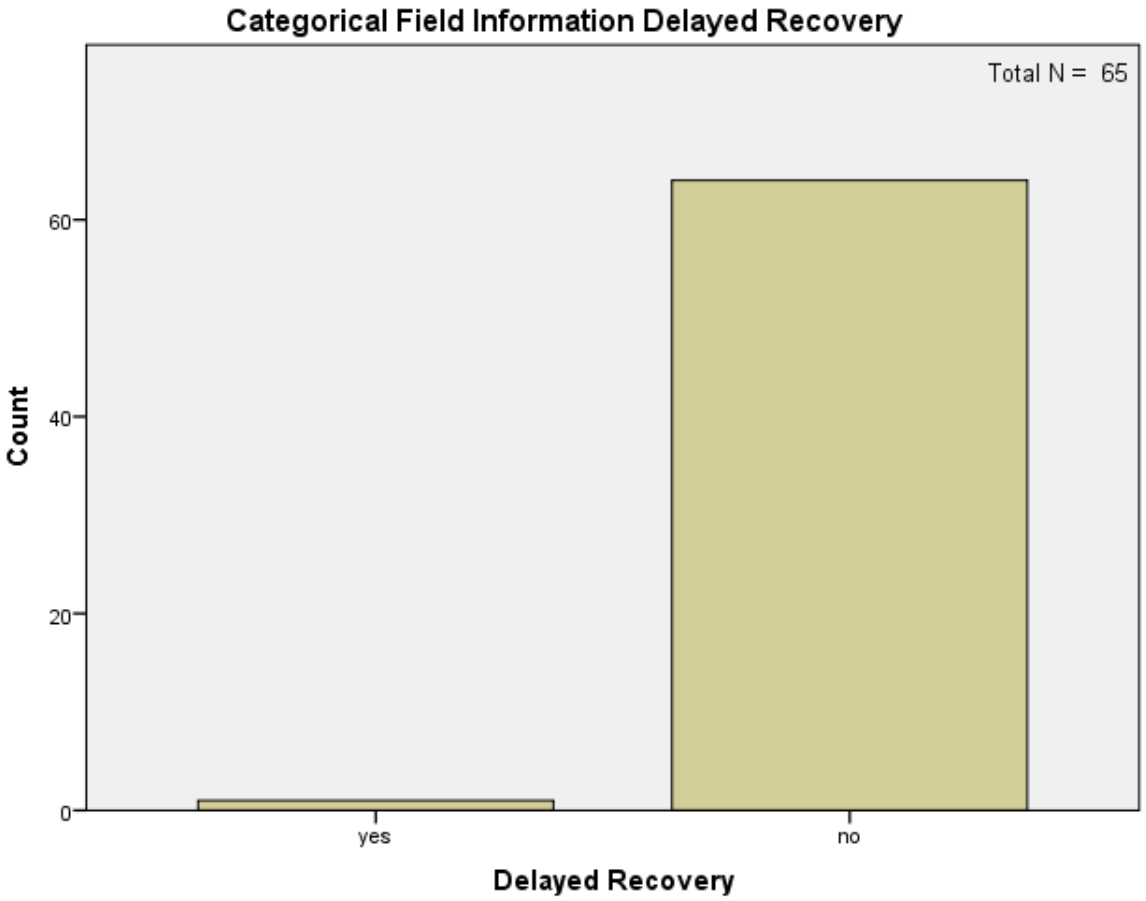
Categorical Field Information Delayed Recovery
Total N = 65
Count
60
40
20
0
yes
no
Delayed Recovery

Figure 18:

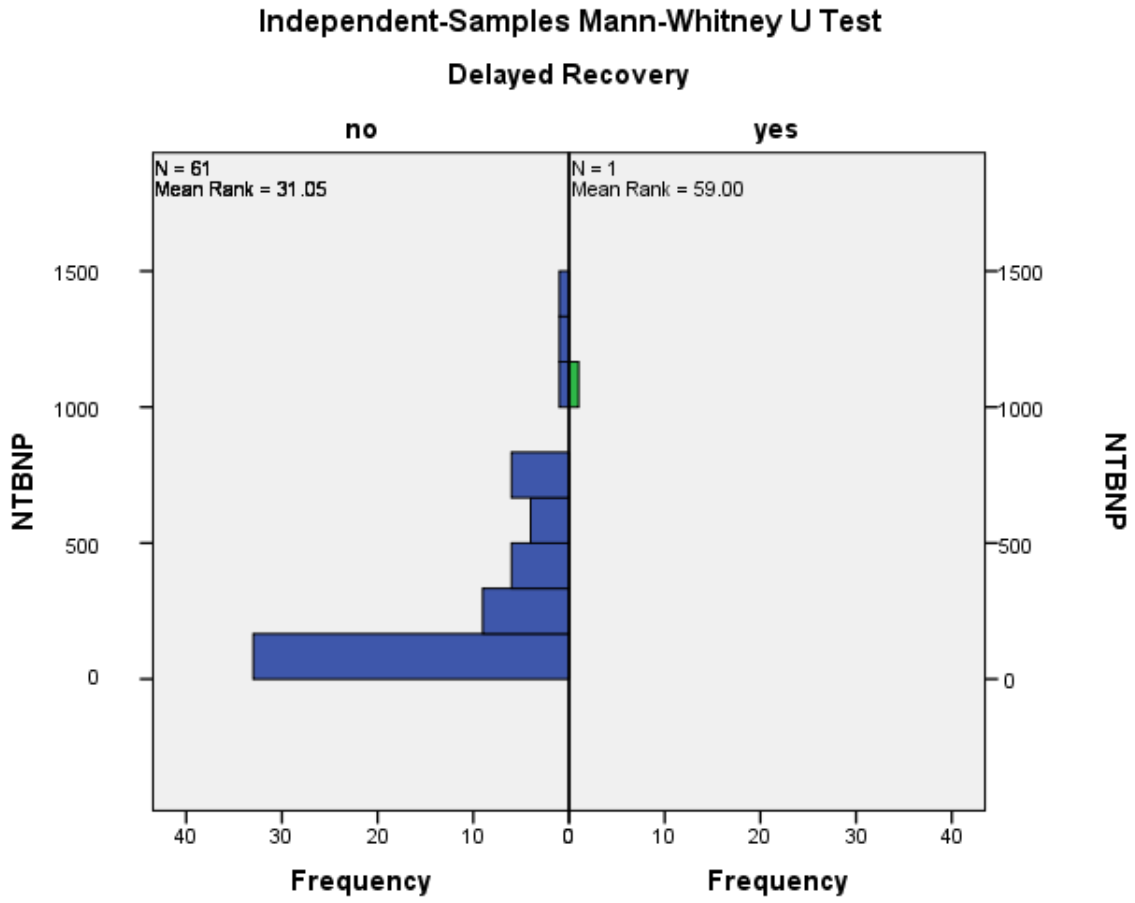
Independent-Samples Mann-Whitney U Test
Delayed Recovery
no
yes
N = 61
Mean Rank = 31.05
N = 1
Mean Rank = 59.00
NTBNP
1500
1000
500
0
40
30
20
10
0
Frequency

Figure 19:

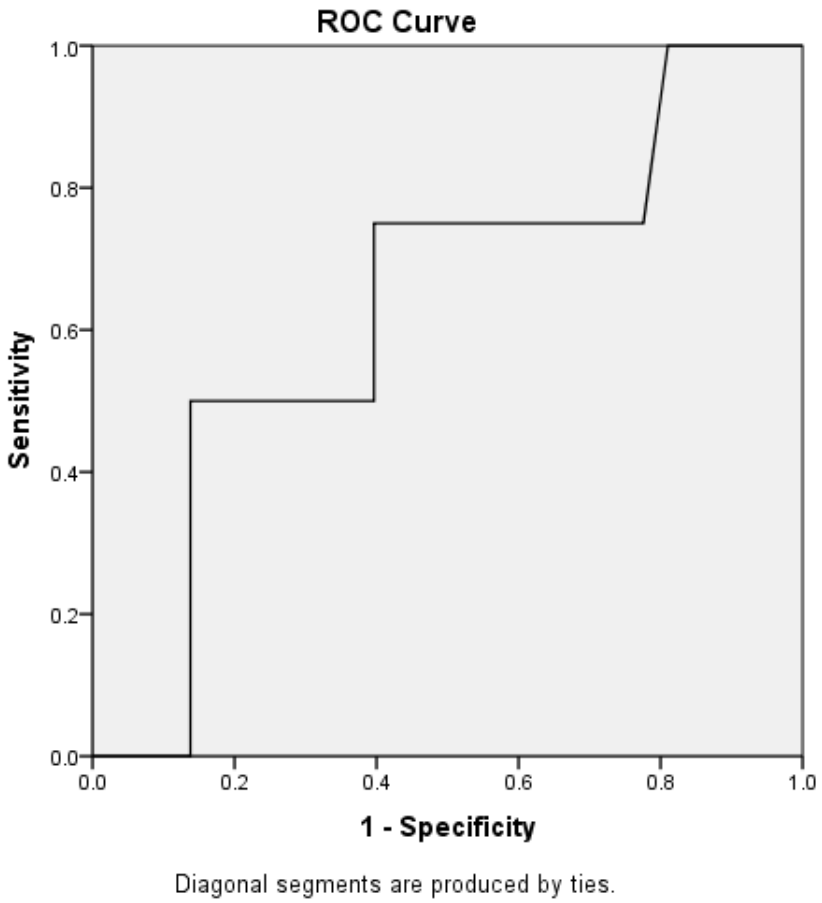

Figure 20: Receiver operating characteristic curve for the ability of NT-proBNP to predict perioperative myocardial infarction. area under curve = 0.63; 95%CI = 0.35-0.91

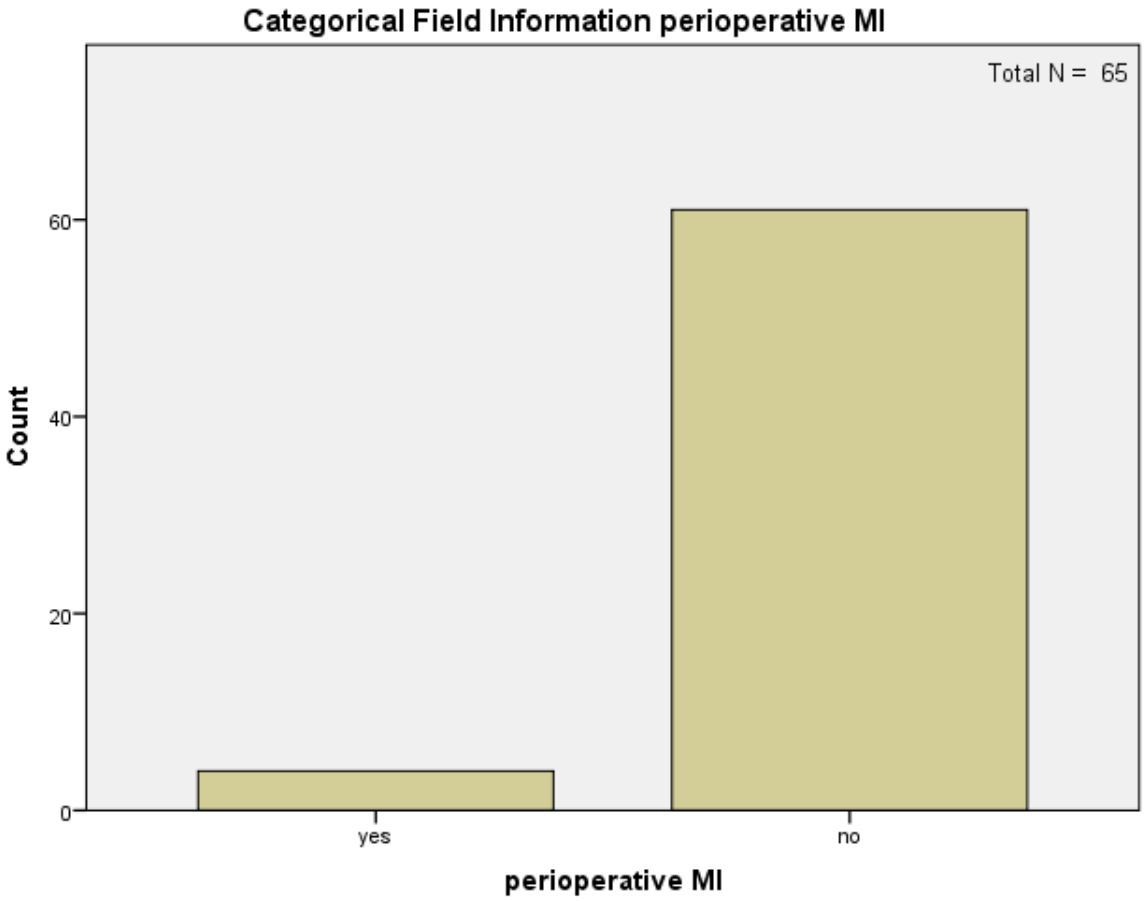
Categorical Field Information perioperative MI
Total N = 65
Count
60
40
20
0
yes
no
perioperative MI

Figure 21:

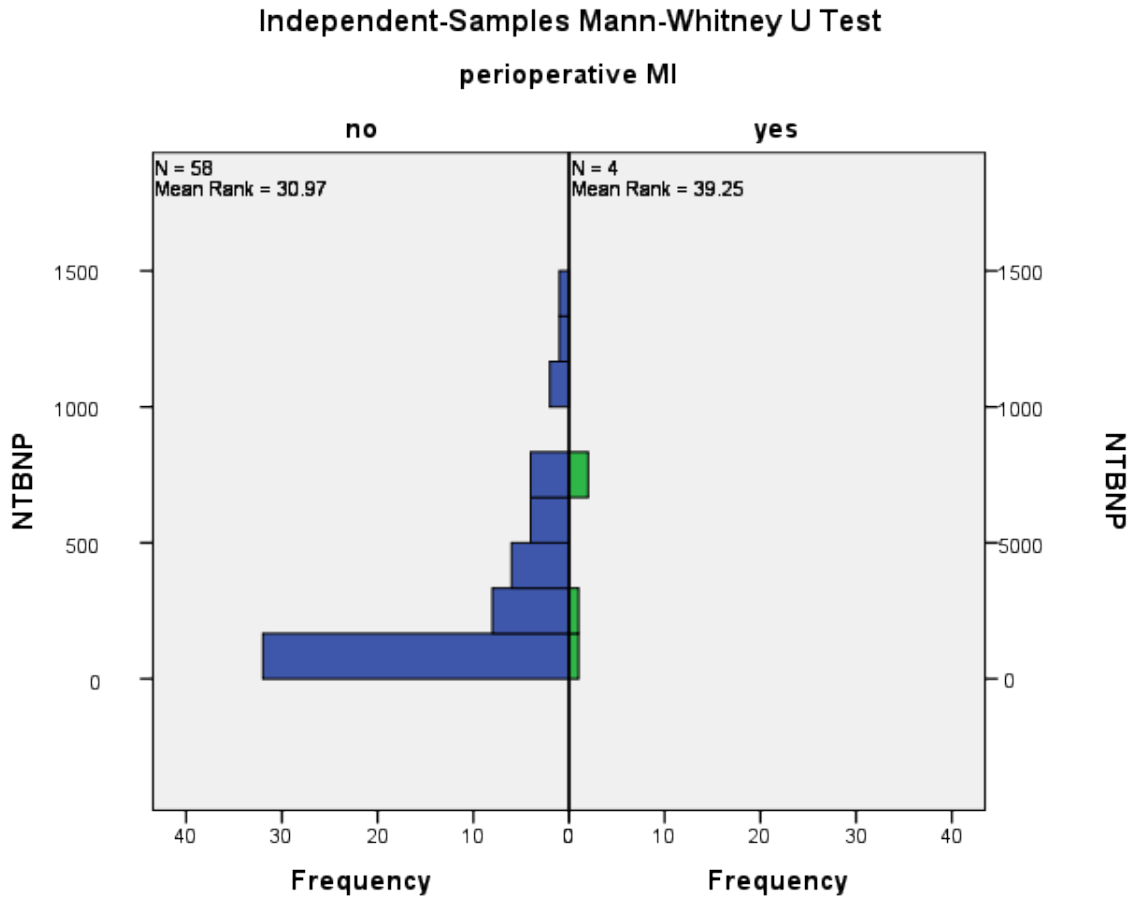
Independent-Samples Mann-Whitney U Test
perioperative MI
no
yes
N = 58
Mean Rank = 30.97
N = 4
Mean Rank = 39.25
NTBNP
1500
1000
500
0
5000
40
30
20
10
0
Frequency

Figure 22:

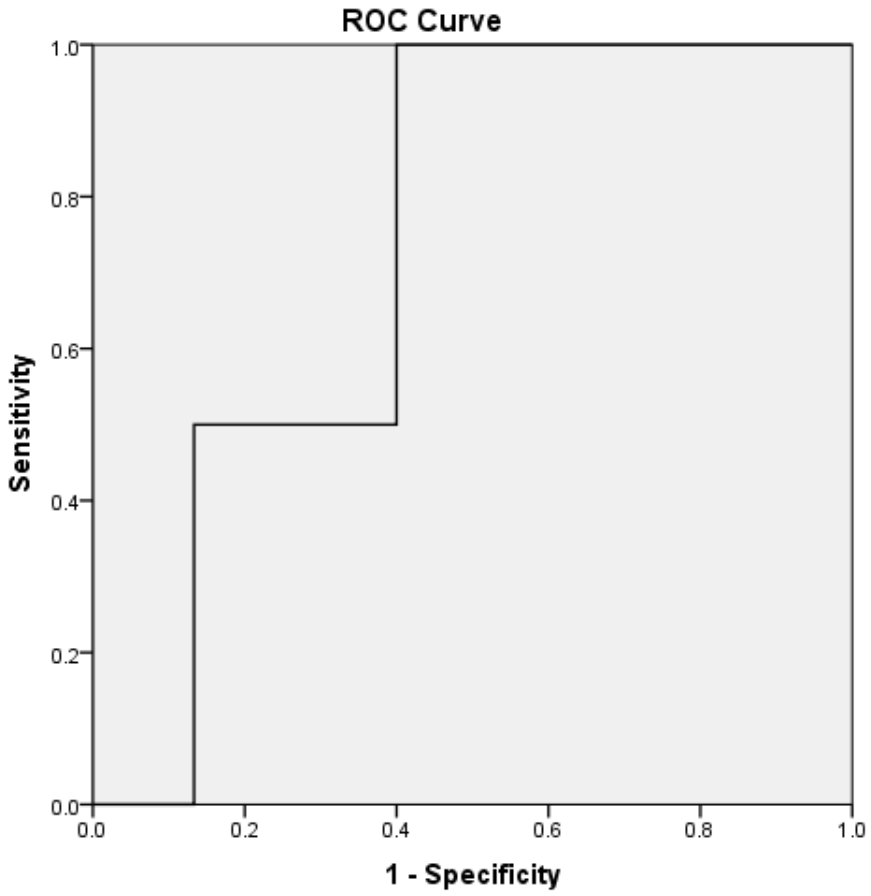

Figure 23: Receiver operating characteristic curve for the ability of NT-proBNP to predict in-hospital mortality. area under curve = 0.73; 95%CI = 0.52-0.94

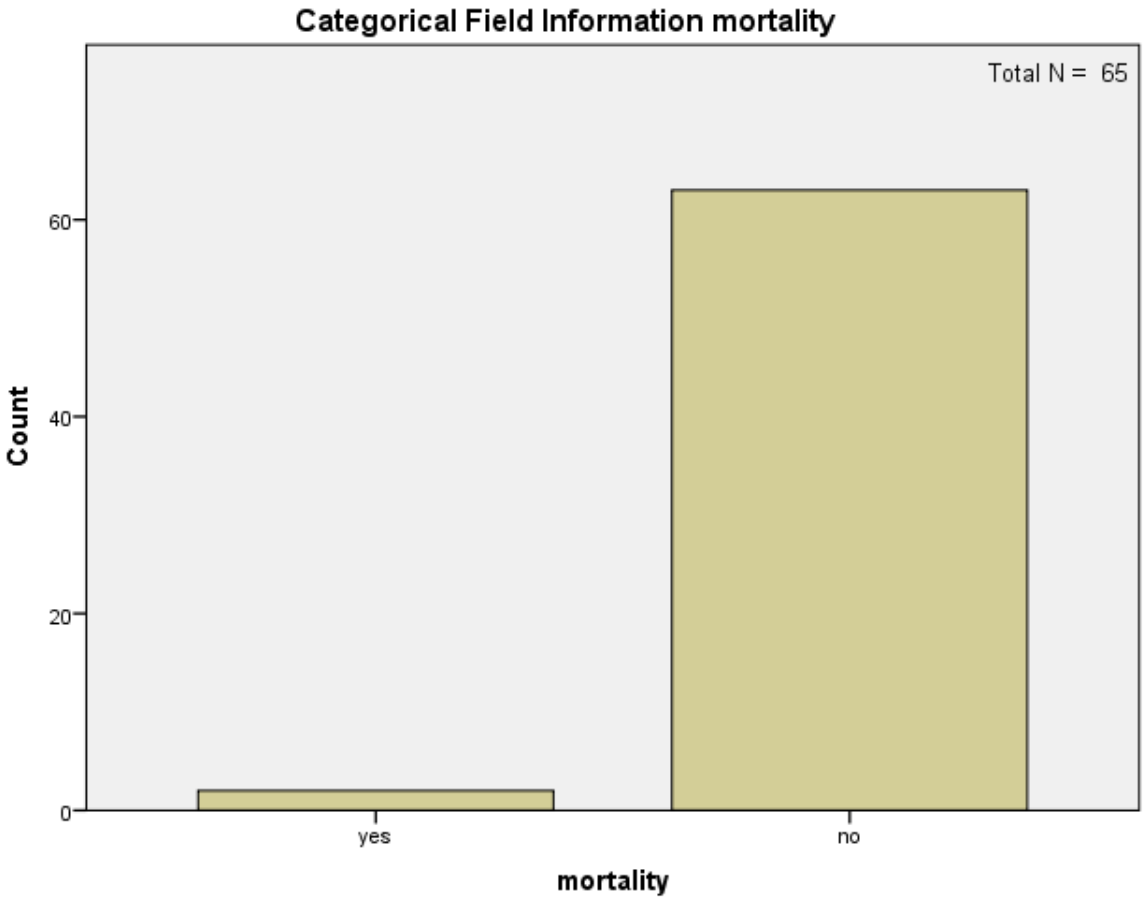
Categorical Field Information mortality
Total N = 65
Count
60
40
20
0
yes
no
mortality

Figure 24:

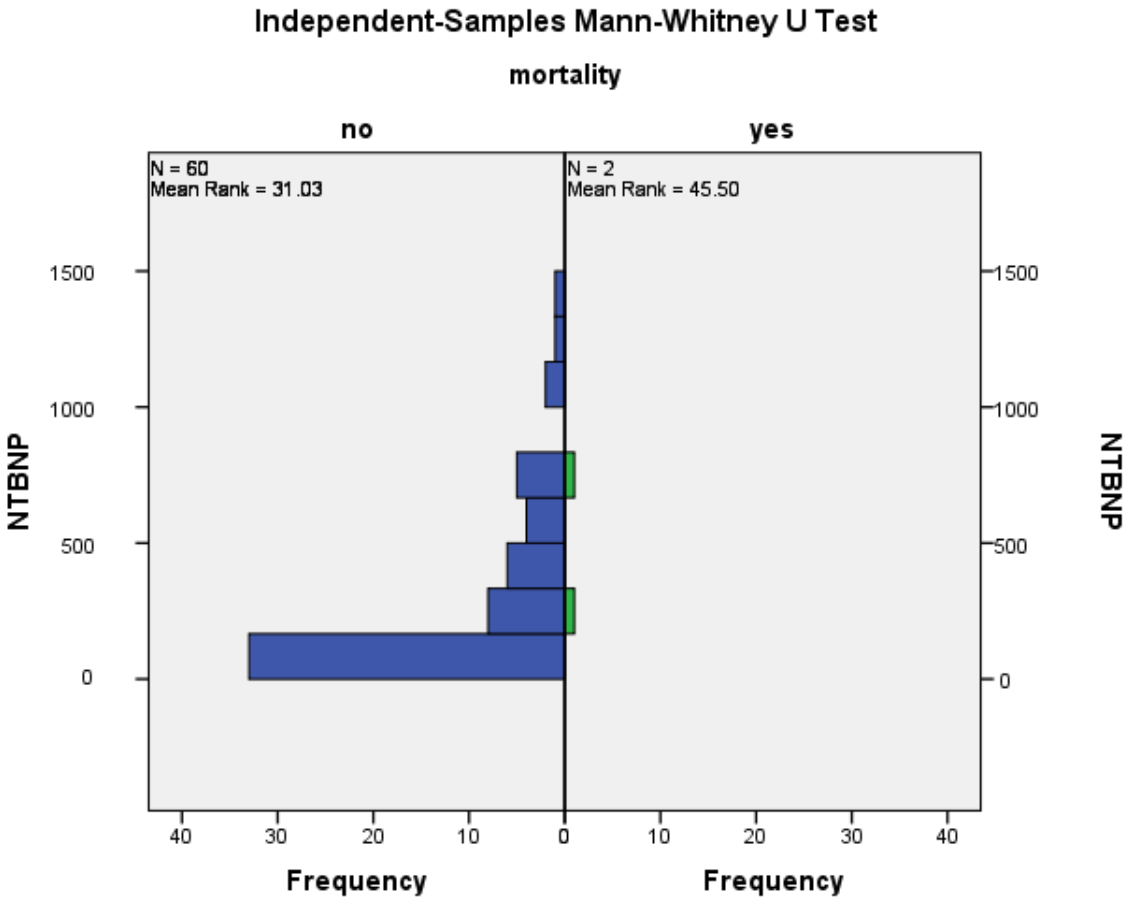
Independent-Samples Mann-Whitney U Test
mortality
no
yes
N = 60
Mean Rank = 31.03
N = 2
Mean Rank = 45.50
NTBNP
1500
1000
500
0
40
30
20
10
0
Frequency

Figure 25:

5 Discussion

Steady-state levels of NT-proBNP are as much as four-to six-fold higher than BNP. We used NT-proBNP instead of BNP because of its longer plasma half-life (60-120 min). The longer half-life of NT-proBNP suggests it is more independent of inter- and intra-individual variations [Clerico et al., 2006]. Furthermore, molecules of BNP are unstable at room temperature and start degrading immediately after blood draw if not processed. And in their relation to clinical characteristics and prognostic performance in a large population of patients with heart failure, BNP and NT-proBNP showed subtle differences [Masson et al., 2006].

BNP and NTproBNP are simple laboratory tests with the results easily reproduced and corroborated. Several investigators demonstrated that plasma BNP and NT-proBNP concentrations are good predictors for outcome of cardiac surgery [Hutfless et al., 2004]. Eliasdottir et al. showed that preoperative NT- proBNP concentration was significantly higher in patients with prolonged ICU stay, death within 28 postoperative days, required inotropic agents or IABP, and new development of postoperative renal failure and also found a good correlation between NT-proBNP and euroSCORE [Eliasdottir et al., 2008]. However, these two studies were not adjusted for the risk factors that could influence the BNP level, NT-proBNP level, morbidity, and mortality.

We are aware of four previous studies that tried to answer the same question of the current thesis; is preoperative natriuretic peptides of prognostic value when it comes to cardiac surgery patients. The tables shown in this section shows the differences and similarities between those studies in design; cohort characteristics, peptide used and frequency and timing of samples, variables observed and duration, and their results.

Table 5: design of compared studies and preoperative Variables

	Eliasdottir et al. [2008]	Schachner et al. [2010]	Krzych et al. [2011]	Chen et al. [2013]	current study
year	2008	2010	2011	2013	2017
population/cohort	elective cardiac surgery	isolated CABG	elective on-pump CABG	elective CABG	OPCAB
Number	135	819	100	76	65
peptide measured	NTproBNP	NTproBNP	NTproBNP	BNP and NTproBNP	NTproBNP
sampling time	preoperative	preoperative	preoperative	preoperative and postoperative days 1 and 7	preoperative
Follow-up	28 days	3 yrs	30 days		till discharge
male	76%	77%	76%	85.5%	86.3%
age	67 [56 − 88]	67 [27 − 89]	65.9 ± 9.1	64 ± 10.2	57.4 ± 7.3
NYHA I			2%		
NYHA II			81%		
NYHA III			17%	5%	-
NYHA IV			-		-
EF				61 ± 11.2	51.1 ± 8.3
		51 [10 − 84]	52.5(45-60)		49.5(44-57)
HTN		85%	67%		65.1%
DM		24%	33%		
(on insulin)		6%			15.15%
GFR			90(75.5-90)		
Creat.		1 [0.5 − 6.2]			
CRF (on dialysis)		1%			-
PVD		13%	12%		1.5%
carotid disease		7%	4%		
COPD			28%		
EUROSCORE (logarithmic)	8.15	2.51 [1 − 63]			0.75 ± 0.34^2
operation					
on-pump CABG	56%			71%	
OPCAB	12%			14.47%	
mininally invasive CABG				14.47%	
valve repair	12%				
CABG and valve repair	20%				
urgent operation	-	17%			-
peptide level pg/mL					
BNP				103.8 ± 184	
NTproBNP	1223 ± 470	[6 − 65998]		621.3 ± 1050.7	309.68 ± 327.9
	237 (75-899)	291(123-808)	526(156-1150)		160(80-395)

Table 6: post-operative variables in compared studies

	Eliasdottir et al. [2008]	Schachner et al. [2010]	Krzych et al. [2011]	Chen et al. [2013]	current study
prolonged ventilation		8 [0 − 1900] h	15%		4.62%
respiratory failure			2%		
atrial fibrillation		23%	34%		6.15%
perioperative MI	14.1%	1%	2%		6.15%
low CO			9%		7.69%
inotropes	33%		61%		
IABP	10%	4%	7%		
stroke/delerium		0.5%	1%		1.54%
ICU stay	22%	22 [7 − 1919] h			3.37 ± 0.84 days 23%
Hospital stay					6.38 ± 1.33
mortality	5.9%	4%	-		3%

Table 7: correlation of peptide levels with post-operative variables in compared studies

	Schachner et al. [2010]	Krzych et al. [2011]	Chen et al. [2013]	current study

Numerical data are shown in the tables with the following conventions:

- $[range]$
- median(2nd quartile - 3rd quartile)
- $average \pm SD$
- percentage%
- a dash - where values are not applicable
- blank cells where values are not available

We extracted the data from the published papers and didn't try to contact any of the authors for further clarification.

Only Chen et al. [2013] measured postoperative levels of natriuretic peptides but, those results will be disregarded in this discussion since they are of no comparative values.

Schachner et al. concluded in their study (N=819) that high preoperative levels , with cutoff value of 504 pg/mL; of NTproBNP predict mid-term mortality after CABG and are associated with significantly higher hospital mortality and perioperative complications. Age, preoperative serum creatinine, peripheral vascular disease, and high NT-proBNP levels were significantly associated with hospital mortality. Their study was more permissive than ours, including all patients undergoing isolated CABG. [Schachner et al., 2010]

Krzych et al.; in a study published 2011 (N=100) found the preoperative NT-proBNP level was a predictor of postoperative prolonged mechanical ventilation, respiratory failure, AF, IABP use, inotropic support and postoperative platelet transfusions. However, good or

very good diagnostic accuracy was found only in relation to mechanical ventilation, respiratory insufficiency, IABP use, and milrinone use, with cutoff value of 1032-1443 pg/mL. No deaths were observed in their study. [Krzych et al., 2011] Like our study, they included only patients scheduled for elective surgery with EF $>$ 30% and without renal failure.

In a study, Chen et al. (N=76) demonstrated that postoperative Day 1 BNP and NT-proBNP concentrations were significantly higher in patients with prolonged ICU stay and hospitalization. However, the correlation became insignificant after adjusting for age, sex, renal function, and LVEF [Chen et al., 2013]. Moreover, they stated that high postoperative Day 1 BNP and NT-proBNP concentrations did not show significant correlation with prognosis after other risk factor adjustments. In their study preoperative BNP and NTproBNP were not significantly associated with outcomes.

6 Conclusion

This study suggests that preoperative NTproBNP levels don't correlate with postoperative outcomes for patients who have good ejection fraction, are not in heart failure, and are undergoing elective isolated CABG surgery. This is supported by similar, albeit small-sample studies. However, the small sample size may result in statistical bias. Therefore, a large-scale study and long-term follow-up should be performed in the future.

7 Summary

References

RL Allgren, TC Marbury, SN Rahman, LS Weisberg, AZ Fenves, RA Lafayette, RM Sweet, FC Genter, BR Kurnik, JD Conger, and MH Sayegh. Anaritide in acute tubular necrosis; auriculin anaritide acute renal failure study group. *N Engl J Med*, 336:828–834, 1997.

FA Almeida, M Suzuki, and T Maack. Atrial natriuretic factor increases hematocrit and decreases plasma volume in nephrectomized rats. *Life Sci*, 39:1193–1199, 1986.

F Ammarguellat, I Larouche, and EL Schiffrin. Myocardial fibrosis in doca-salt hypertensive rats: effect of endothelin et(a) receptor antagonism. *Circulation*, 103:319–324, 2001.

LK Antos and LR Potter. Adenine nucleotides decrease the apparent km of endogenous natriuretic peptide receptors for gtp. *Am J Physiol Endocrinol Metab*, 293:E1756–1763, 2007.

LK Antos, SE Abbey-Hosch, DR Flora, and LR Potter. Atp-independent activation of natriuretic peptide receptors. *J Biol Chem*, 280:26928–26932, 2005.

RW Barbee, BD Perry, RN Re, JP Murgo, and LJ Field. Hemodynamics in transgenic mice with overexpression of atrial natriuretic factor. *Circ Res*, 74:747–751, 1994.

CF Bartels, H Bukulmez, P Padayatti, DK Rhee, C van Ravenswaaij-Arts, RM Pauli, S Mundlos, D Chitayat, LY Shih, LI Al-Gazali, S Kant, T Cole, J Morton, V Cormier-Daire, L Faivre, M Lees, J Kirk, GR Mortier, J Leroy, B Zabel, CA Kim, Y Crow, NE Braverman, F van-den Akker, and MLA Warman. Mutations in the transmembrane natriuretic peptide receptor npr-b impair skeletal growth and cause acromesomelic dysplasia, type maroteaux. *Am J Hum Genet*, 75:27–34, 2004.

BD Bennett, GL Bennett, RV Vitangcol, JR Jewett, J Burnier, W Henzel, and DG Lowe. Extracellular domain-igg fusion proteins for three human natriuretic peptide receptors; hormone pharmacology and application to solid phase screening of synthetic peptide antisera. *J Biol Chem*, 266:23060–23067, 1991.

R Berger, M Huelsman, K Strecker, A Bojic, P Moser, B Stanek, and R Pacher. B-type natriuretic peptide predicts sudden death in patients with chronic heart failure. *Circulation*, 105:2392–7, 2002.

GE Bilder, TL Schofield, and EH Blaine. Release of atrial natriuretic factor; effects of repetitive stretch and temperature. *Am J Physiol*, 251:F817–F821, 1986.

F Boomsma and AH van den Meiracker. Plasma a- and b-type natriuretic peptides: physiology, methodology and clinical use. *Cardiovasc Res*, 51:442–9, 2001.

J Brown, Q Chen, and G Hong. An autocrine system for c-type natriuretic peptide within rat carotid neointima during arterial repair. *Am J Physiol*, 272:H2919–H2931, 1997.

S Bruins, MR Fokkema, JW Romer, MJ Dejongste, FP van der Dijs, JM van den Ouweland, and FA Muskiet. High intraindividual variation of b-type natriuretic peptide (bnp) and amino-terminal probnp in patients with stable chronic heart failure. *Clin Chem*, 50:2052–8, 2004.

PM Bryan, D Smirnov, A Smolenski, S Feil, R Feil, F Hofmann, S Lohmann, and LR Potter. A sensitive method for determining the phosphorylation status of natriuretic peptide receptors: cgk-ialpha does not regulate npr-a. *Biochemistry*, 45:1295–1303, 2006.

MG Buckley, NJ Marcus, and MH Yacoub. Cardiac peptide stability, aprotinin and room temperature: importance for assessing cardiac function in clinical practice. *Clin Sci (Lond)*, 97:689–95, 1999.

B Burczynska, T Duda, and RK Sharma. Atp signaling site in the arm domain of atrial natriuretic factor receptor guanylate cyclase. *Mol Cell Biochem*, 301:93–107, 2007.

JC Burnett, PC Kao, DC Hu, DW Heser, D Heublein, JP Granger, TJ Opgenorth, and GS Reeder. Atrial natriuretic peptide elevation in congestive heart failure in the human. *Science*, 231:1145– 1147, 1986.

YH Chan. Biostatictcs102: Quantitative data - parametric and non-parametric tests. *Singapre Med J*, 44:391–396, 2003a.

YH Chan. Biostatistics 104: Correlational analysis. *Singapore Med J*, 44:614–619, 2003b.

MS Chang, DG Lowe, M Lewis, R Hellmiss, E Chen, and DV Goeddel. Differential activation by atrial and brain natriuretic peptides of two different receptor guanylate cyclases. *Nature*, 341:68–72, 1989.

CJ Charles, TC Prickett, EA Espiner, MT Rademaker, AM Richards, and TG Yandle. Regional sampling and the effects of experimental heart failure in sheep: differential responses in a, b and c-type natriuretic peptides. *Peptides*, 27:62–68, 2006.

HH Chen, JA Schirger, A Cataliotti, and JC Burnett. Intact acute cardiorenal and humoral responsiveness following chronic subcutaneous administration of the cardiac peptide bnp in experimental heart failure. *Eur J Heart Fail*, 8:681–686, 2006.

Thay-Hsiung Chen, Ching-Ling Lin, Joseph Jaey-Ming Shih, James Yao-Ming Shih, Chung-Huo Chen, Mei-Ling Chang, and Chih-Hui Chin. Plasma b-type natriuretic peptide in predicting outcomes of elective coronary artery bypass surgery. *Kaohsiung Journal of Medical Sciences*, 29:254–258, 2013.

H Chikuda, F Kugimiya, K Hoshi, T Ikeda, T Ogasawara, T Shimoaka, H Kawano, S Kamekura, A Tsuchida, N Yokoi, K Nakamura,

K Komeda, UI Chung, and H Kawaguchi. Cyclic gmp-dependent protein kinase ii is a molecular switch from proliferation to hypertrophic differentiation of chondrocytes. *Genes Dev*, 18:2418–2429, 2004.

TH Chun, H Itoh, Y Ogawa, N Tamura, K Takaya, T Igaki, J Yamashita, K Doi, M Inoue, K Masatsugu, R Korenaga, J Ando, and K Nakao. Shear stress augments expression of c-type natriuretic peptide and adrenomedullin. *Hypertension*, 29:1296–1302, 1997.

H Chusho, N Tamura, Y Ogawa, A Yasoda, M Suda, T Miyazawa, K Nakamura, K Nakao, T Kurihara, Y Komatsu, H Itoh, K Tanaka, Y Saito, and M Katsuki. Dwarfism and early death in mice lacking c- type natriuretic peptide. *Proc Natl Acad Sci U S A*, 98:4016–4021, 2001.

AL Clavell, AJ Stingo, CM Wei, DM Heublein, and JC Burnett. C-type natriuretic peptide: a selective cardiovascular peptide. *Am J Physiol*, 264:R290–295, 1993.

JG Cleland, AP Coletta, and AL Clark. Clinical trials update from the american college of cardiology 2007: Alpha, everest, fusion ii, validd, parr-2, remodel, spice, courage, coach, remadhe, pro-bnp for the evaluation of dyspnoea and this-diet. *Eur J Heart Fail*, 9:740–745, 2007.

A Clerico, G Carlo Zucchelli, A Pilo, C Passino, and M Emdin. Clinical relevance of biological variation: the lesson of brain natriuretic peptide (bnp) and nt-probnp assay. *Clin Chem Lab Med*, 44:366–378, 2006.

RJ Cody, SA Atlas, JH Laragh, SH Kubo, AB Covit, KS Ryman, A Shaknovich, K Pondolfino, M Clark, MJ Camargo, RM Scarborough, and JA Lewicki. Atrial natriuretic factor in normal subjects and heart failure patients; plasma levels and renal, hormonal, and hemodynamic responses to peptide infusion. *J Clin Invest*, 78:1362–1374, 1986.

MR Cowie, P Jourdain, A Maisel, U Dahlstrom, F Follath, R Isnard, A Luchner, T McDonagh, J Mair, M Nieminen, and G Francis. Clinical applications of b-type natriuretic peptide (bnp) testing. *Eur Heart J*, 24:1710–8, 2003.

MG Currie, DM Geller, BR Cole, NR Siegel, KF Fok, SP Adams, SR Eubanks, GR Galluppi, and P Needleman. Purification and sequence analysis of bioactive atrial peptides (atriopeptins). *Science*, 223:67–69, 1984.

LB Daniels and AS Maisel. natriuretics peptides. *Journal of American College of Cardiology*, 50:2357–2368, 2007.

SR Das, MH Drazner, DL Dries, GL Vega, HG Stanek, SM Abdullah, RM Canham, AK Chung, D Leonard, Jr Fh Wians, and JA deLemos. Impact of body mass and body composition on circulating levels of natriuretic peptides: results from the dallas heart study. *Circulation*, 112:2163–8, 2005.

AJ de Bold, HB Borenstein, AH Veress, and H Sonnenberg. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sciences*, 28(1):89–94, 1981.

S Del-Ry, C Passino, M Maltinti, M Emdin, and D Giannessi. C-type natriuretic peptide plasma levels increase in patients with chronic heart failure as a function of clinical severity. *Eur J Heart Fail*, 7: 1145–1148, 2005.

JA deLemos, DA Morrow, JH Bentley, T Omland, MS Sabatine, CH McCabe, C Hall, CP Cannon, and E Braunwald. The prognostic value of b-type natriuretic peptide in patients with acute coronary syndromes. *N Engl J Med*, 345:1014–21, 2001.

G deLissovoy, DM Stier, G Ciesla, M Munger, and AJ Burger. Economic implications of nesiritide versus dobutamine in the treatment of patients with acutely decompensated congestive heart failure. *Am J Cardiol*, 92:631–633, 2003.

J DeSutter, D DeBacquer, S Cuypers, J Delanghe, and M DeBuyzere. Plasma n-terminal pro-brain natriuretic peptide concentration predicts coronary events in men at work: a report from the BELSTRESS study. *European Heart Journal*, 26:2664–2649, 2005.

DM Dickey, DR Flora, PM Bryan, X Xu, Y Chen, and LR Potter. Differential regulation of membrane guanylyl cyclases in congestive heart failure: natriuretic peptide receptor (npr)-b, not npr-a, is the predominant natriuretic peptide receptor in the failing heart. *Endocrinology*, 148:3518– 3522, 2007.

BS Edwards, RS Zimmerman, TR Schwab, DM Heublein, and JC Burnett. Atrial stretch, not pressure, is the principal determinant controlling the acute release of atrial natriuretic factor. *Circ Res*, 62: 191–195, 1988.

SB Eliasdottir, G Klemenzson, B Torfason, and F Valsson. Brain natriuretic peptide is a good predictor for outcome in cardiac surgery. *Acta Anaesthesiol Scand*, 52:182–7, 2008.

D Fan, PM Bryan, LK Antos, RJ Potthast, and LR Potter. Downregulation does not mediate natriuretic peptide-dependent desensitization of natriuretic peptide receptor (npr)-a or npr-b: Guanylyl cyclase-linked natriuretic peptide receptors do not internalize. *Mol Pharmacol*, 67:174– 183, 2005.

MA Fifer, CR Molina, AC Quiroz, TD Giles, HC Herrmann, IR DeScheerder, DL Clement, S Kubo, RJ Cody, and JN Cohn. Hemodynamic and renal effects of atrial natriuretic peptide in congestive heart failure. *Am J Cardiol*, 65:211–216, 1990.

JP Fluckiger, B Waeber, G Matsueda, B Delaloye, J Nussberger, and HR Brunner. Effect of atriopeptin iii on hematocrit and volemia of nephrectomized rats. *Am J Physiol*, 251:H880–H883, 1986.

TG Flynn, ML deBold, and AJ deBold. The amino acid sequence

of an atrial peptide with potent diuretic and natriuretic properties. *Biochem Biophys Res Commun*, 117:859–865, 1983.

WG Forssmann, R Richter, and M Meyer. The endocrine heart and natriuretic peptides: histochemistry, cell biology, and functional aspects of the renal urodilatin system. *Histochem Cell Biol*, 110:335–357, 1998.

F Fuller, JG Porter, AE Arfsten, J Miller, JW Schilling, RM Scarborough, JA Lewicki, and DB Schenk. Atrial natriuretic peptide clearance receptor; complete sequence and functional expression of cdna clones. *J Biol Chem*, 263:9395–9401, 1988.

DG Gardner. Natriuretic peptides: markers or modulators of cardiac hypertrophy? *Trends Endocrinol Metab*, 14:411–416, 2003.

RS Gardner, F Ozalp, AJ Murday, SD Robb, and TA McDonagh. N-terminal pro-brain natriuretic peptide a new gold standard in predicting mortality in patients with advanced heart failure. *Eur Heart J*, 24:1735–43, 2003.

DC Goff, Jr, DK Pandey, FA Chan, C Ortiz, and MZ Nichaman. Congestive heart failure in the united states: is there more than meets the i(cd code)? the corpus christi heart project. *Arch Intern Med*, 160:197–202, 2000.

MF Goy, PM Oliver, KE Purdy, JW Knowles, JE Fox, PJ Mohler, X Qian, O Smithies, and N Maeda. Evidence for a novel natriuretic peptide receptor that prefers brain natriuretic peptide over atrial natriuretic peptide. *Biochem J*, 358:379–387, 2001.

C Grepin, L Dagnino, L Robitaille, L Haberstroh, T Antakly, and M Nemer. A hormone-encoding gene identifies a pathway for cardiac but not skeletal muscle gene transcription. *Mol Cell Biol*, 14:3115–3129, 1994.

C Hall. Nt-probnp: the mechanism behind the marker. *J Card Fail*, 11:S81–3, 2005.

F Hartmann, M Packer, AJ Coats, MB Fowler, H Krum, P Mohacsi, JL Rouleau, M Tendera, A Castaigne, SD Anker, I Amann-Zalan, S Hoersch, and HA Katus. Prognostic impact of plasma n-terminal pro-brain natriuretic peptide in severe chronic congestive heart failure: a substudy of the carvedilol prospective randomized cumulative survival (copernicus) trial. *Circulation*, 110:1780–6, 2004.

JP Henry, OH Gauer, and JL Reeves. Evidence of the atrial location of receptors influencing urine flow. *Circ Res*, 4:85–90, 1956.

R Holtwick, M Gotthardt, B Skryabin, M Steinmetz, R Potthast, B Zetsche, RE Hammer, J Herz, and M Kuhn. Smooth muscle-selective deletion of guanylyl cyclase-a prevents the acute but not chronic effects of anp on blood pressure. *Proc Natl Acad Sci U S A*, 99:7142–7147, 2002.

R Holtwick, M Van-Eickels, BV Skryabin, HA Baba, A Bubikat, F Begrow, MD Schneider, DL Garbers, and M Kuhn. Pressure-independent cardiac hypertrophy in mice with cardiomyocyte-restricted inactivation of the atrial natriuretic peptide receptor guanylyl cyclase-a. *J Clin Invest*, 111:1399–1407, 2003.

R Hutfless, R Kazanegra, M Madani, MA Bhalla, A Tulua-Tata, A Chen, P Clopton, C James, A Chiu, and AS Maisel. Utility of b-type natriuretic peptide in predicting postoperative complications and outcomes in patients undergoing heart surgery. *J Am Coll Cardiol*, 43:1873–1879, 2004.

E Ingelsson, J Arnlov, J Sundstrom, and L Lind. The validity of a diagnosis of heart failure in a hospital discharge register. *Eur J Heart Fail*, 7:787–91, 2005.

Y Iwanaga, I Nishi, S Furuichi, T Noguchi, K Sase, and Y Kihara. B-type natriuretic peptide strongly reflects diastolic wall stress in patients with chronic heart failure: comparison between systolic and diastolic heart failure. *Journal of American College of Cardiology*, 47:742–8, 2006.

JD Jamieson and GE Palade. Specific granules in atrial muscle cells. *J Cell Biol*, 23:151–172, 1964.

JL Januzzi, KR van, J Lainchbury, A Bayes-Genis, J Ordonez-Llanos, M Santalo-Bel, YM Pinto, and M Richards. Nt-probnp testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: the international collaborative of nt-probnp study. *Eur Heart J*, 27:330–7, 2006.

J Jaubert, F Jaubert, N Martin, LL Washburn, BK Lee, EM Eicher, and JL Guenet. Three new allelic mouse mutations that cause skeletal overgrowth involve the natriuretic peptide receptor c gene (npr3). *Proc Natl Acad Sci U S A*, 96:10278–10283, 1999.

Jr Januzzi JL, CA Camargo, S Anwaruddin, AL Baggish, AA Chen, DG Krauser, R Tung, R Cameron, JT Nagurney, CU Chae, DM Lloyd-Jones, DF Brown, S Foran-Melanson, PM Sluss, E Lee-Lewandrowski, and KB Lewandrowski. The n-terminal pro-bnp investigation of dyspnea in the emergency department (pride) study. *Am J Cardiol*, 95:948–54, 2005.

SW John, JH Krege, PM Oliver, JR Hagaman, JB Hodgin, SC Pang, TG Flynn, and O Smithies. Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension [published erratum appears in science 1995 mar 24;267(5205):1753]. *Science*, 267:679–681, 1995.

SW John, AT Veress, U Honrath, CK Chong, L Peng, O Smithies, and H Sonnenberg. Blood pressure and fluid-electrolyte balance in mice with reduced or absent anp. *Am J Physiol*, 271:R109–114, 1996.

S Joubert, C Jossart, N McNicoll, and A De-Lean. Atrial natriuretic peptide-dependent photolabeling of a regulatory atp-binding site on the natriuretic peptide receptor-a. *FEBS J*, 272:5572–5583, 2005.

PR Kalra, JR Clague, AP Bolger, SD Anker, PA Poole-Wilson, AD Struthers, and AJ Coats. Myocardial production of c-type na-

triuretic peptide in chronic heart failure. *Circulation*, 107:571–573, 2003.

K Kangawa, Y Tawaragi, S Oikawa, A Mizuno, Y Sakuragawa, H Nakazato, A Fukuda, N Minamino, and H Matsuo. Identification of rat gamma atrial natriuretic polypeptide and characterization of the cdna encoding its precursor. *Nature*, 312:152–155, 1984.

K Kikuta, H Yasue, M Yoshimura, E Morita, H Sumida, H Kato, K Kugiyama, H Ogawa, K Okumura, Y Ogawa, and K Nakao. Increased plasma levels of b-type natriuretic peptide in patients with unstable angina. *Am Heart J*, 132:101–7, 1996.

B Kisch. Electron microscopy of the atrium of the heart in guineo pig. *Exp Med Surg*, 14:99– 112, 1956.

I Kishimoto, SK Dubois, and DL Garbers. The heart communicates with the kidney exclusively through the guanylyl cyclase-a receptor: acute handling of sodium and water in response to volume expansion. *Proc Natl Acad Sci U S A*, 93:6215–6219, 1996.

I Kishimoto, K Rossi, and DL Garbers. A genetic model provides evidence that the receptor for atrial natriuretic peptide (guanylyl cyclase-a) inhibits cardiac ventricular myocyte hypertrophy. *Proc Natl Acad Sci U S A*, 98:2703–2706, 2001.

JW Knowles, G Esposito, L Mao, JR Hagaman, JE Fox, O Smithies, HA Rockman, and N Maeda. Pressure-independent enhancement of cardiac hypertrophy in natriuretic peptide receptor a- deficient mice. *J Clin Invest*, 107:975–984, 2001.

CW Knudsen, T Omland, P Clopton, A Westheim, AH Wu, P Duc, J McCord, RM Nowak, JE Hollander, AB Storrow, WT Abraham, PA McCullough, and A Maisel. Impact of atrial fibrillation on the diagnostic performance of b-type natriuretic peptide concentration in dyspneic patients: an analysis from the breathing not properly multinational study. *J Am Coll Cardiol*, 46:838–44, 2005.

GY Koh, DR Nussenzveig, J Okolicany, DA Price, and T Maack. Dynamics of atrial natriuretic factor- guanylate cyclase receptors and receptor-ligand complexes in cultured glomerular mesangial and renomedullary interstitial cells. *J Biol Chem*, 267:11987–11994, 1992.

UM Kokkonen, AR Pösö, S Hyyppä, P Huttunen, and J Leppäluoto. Exercise-induced changes in atrial peptides in relation to neuroendocrine responses and fluid balance in the horse. *Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine*, 49(3):144–50, 4 2002.

KJ Koller, DG Lowe, GL Bennett, N Minamino, K Kangawa, H Matsuo, and DV Goeddel. Selective activation of the b natriuretic peptide receptor by c-type natriuretic peptide (cnp). *Science*, 252:120–123, 1991.

C Kragelund, B Gronning, L Kober, P Hildebrandt, and R Steffensen. N-terminal pro-B-type natriuretic peptide and long-term mortality in stable coronary heart disease. *New-England Journal of Medicine*, 352:666–675, 2005.

DG Krauser, DM Lloyd-Jones, CU Chae, R Cameron, S Anwaruddin, AL Baggish, A Chen, R Tung, and Jr Januzzi JL. 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*, 149:744–50, 2005.

S Kruger, J Graf, D Kunz, T Stickel, P Hanrath, and U Janssens. brain natriuretic peptide levels predict functional capacity in patients with chronic heart failure. *J Am Coll Cardiol*, 40:718–722, 2002.

LJ Krzych, D Szurlej, T Kolodziej, L Machej, A Weglarzy, A Blach, M Wilczynski, S Wos, and A Bochenek. Diagnostic accuracy of pre-operative nt-probnp level in predicting short-term outcomes in coronary surgery: a pilot study. *Kardiol Pol*, 69:1121–1127, 2011.

M Kuhn, R Holtwick, HA Baba, JC Perriard, W Schmitz, and E Ehler. Progressive cardiac hypertrophy and dysfunction in atrial natriuretic peptide receptor (gc-a) deficient mice. *Heart*, 87:368–374, 2002.

BR Kurnik, RL Allgren, FC Genter, RJ Solomon, ER Bates, and LS Weisberg. Prospective study of atrial natriuretic peptide for the prevention of radiocontrast-induced nephropathy. *Am J Kidney Dis*, 31:674–680, 1998.

RE Lang, H Tholken, D Ganten, FC Luft, H Ruskoaho, and T Unger. Atrial natriuretic factor-a circulating hormone stimulated by volume loading. *Nature*, 314:264–266, 1985.

TH Langenickel, J Buttgereit, I Pagel-Langenickel, M Lindner, J Monti, K Beuerlein, N Al-Saadi, R Plehm, E Popova, J Tank, R Dietz, R Willenbrock, and M Bader. Cardiac hypertrophy in transgenic rats expressing a dominant-negative mutant of the natriuretic peptide receptor b. *Proc Natl Acad Sci U S A*, 103:4735–4740, 2006.

R Latini, S Masson, I Anand, D Judd, AP Maggioni, YT Chiang, M Bevilacqua, M Salio, P Cardano, PH Dunselman, NJ Holwerda, G Tognoni, and Cohn JN; Valsartan Heart Failure Trial Investigators. Effects of valsartan on circulating brain natriuretic peptide and norepinephrine in symptomatic chronic heart failure - the valsartan heart failure trial (val-heft). *Circulation*, 106:2454–8, 2002.

DC Leitman, JW Andresen, T Kuno, Y Kamisaki, JK Chang, and F Murad. Identification of multiple binding sites for atrial natriuretic factor by affinity cross-linking in cultured endothelial cells. *J Biol Chem*, 261:11650–11655, 1986.

J Lewis, MM Salem, GM Chertow, LS Weisberg, F McGrew, TC Marbury, and RL Allgren. Atrial natriuretic factor in oliguric acute renal failure; anaritide acute renal failure study group. *Am J Kidney Dis*, 36:767–774, 2000.

F Liang, A Atakilit, and DG Gardner. Integrin dependence of brain natriuretic peptide gene promoter activation by mechanical strain. *J Biol Chem*, 275:20355–60, 2000.

MJ Lopez, SK Wong, I Kishimoto, S Dubois, V Mach, J Friesen, DL Garbers, and A Beuve. Salt-resistant hypertension in mice lacking the guanylyl cyclase-a receptor for atrial natriuretic peptide. *Nature*, 378:65–68, 1995.

MJ Lopez, DL Garbers, and M Kuhn. The guanylyl cyclase-deficient mouse defines differential pathways of natriuretic peptide signaling. *J Biol Chem*, 272:23064–23068, 1997.

B Lu, NP Gerard, LF Kolakowski, M Bozza, D Zurakowski, O Finco, MC Carroll, and C Gerard. Neutral endopeptidase modulation of septic shock. *J Exp Med*, 181:2271–2275, 1995.

A Luchner, C Hengstenberg, H Lowel, GA Riegger, H Schunkert, and S Holmer. Effect of compensated renal dysfunction on approved heart failure markers: direct comparison of brain natriuretic peptide (bnp) and n-terminal pro-bnp. *Hypertension*, 46:118–23, 2005.

M Magnusson, O Melander, B Israelsson, A Grubb, L Groop, and S Jovinge. Elevated plasma levels of nt-probnp in patients with type 2 diabetes without overt cardiovascular disease. *Diabetes Care*, 27: 1929–35, 2004.

AS Maisel, P Krishnaswamy, RM Nowak, J McCord, JE Hollander, P Duc, T Omland, AB Storrow, WT Abraham, AH Wu, P Clopton, PG Steg, A Westheim, CW Knudsen, A Perez, R Kazanegra, HC Herrmann, and Investigators McCullough PA; Breathing Not Properly Multinational Study. Rapid measurement of b-type natriuretic peptide in the emergency diagnosis of heart failure. *N Engl J Med*, 347:161–7, 2002.

S Masson, R Latini, IS Anand, T Vago, L Angelici, S Barlera, ED Missov, A Clerico, G Tognoni, and JN Cohn. (on behalf of

the val-heft investigators) direct comparison of b-type natriuretic peptide (bnp) and amino-terminal probnp in a larger population of patients with chronic and symptomatic heart failure: the valsartan heart failure data. *Clin Chem*, 52:1528–1538, 2006.

N Matsukawa, WJ Grzesik, N Takahashi, KN Pandey, S Pang, M Yamauchi, and O Smithies. The natriuretic peptide clearance receptor locally modulates the physiological effects of the natriuretic peptide system. *Proc Natl Acad Sci U S A*, 96:7403–7408, 1999.

PA McCullough, RM Nowak, J McCord, JE Hollander, HC Herrmann, PG Steg, P Duc, A Westheim, T Omland, CW Knudsen, AB Storrow, WT Abraham, S Lamba, AH Wu, A Perez, P Clopton, P Krishnaswamy, R Kazanegra, and AS Maisel. B-type natriuretic peptide and clinical judgment in emergency diagnosis of heart failure: analysis from breathing not properly (bnp) multinational study. *Circulation*, 106:416–22, 2002.

A McGregor, M Richards, E Espiner, T Yandle, and H Ikram. Brain natriuretic peptide administered to man: actions and metabolism. *J Clin Endocrinol Metab*, 70:1103–1107, 1990.

M McNairy, N Gardetto, P Clopton, A Garcia, P Krishnaswamy, R Kazanegra, M Ziegler, and AS Maisel. Stability of b-type natriuretic peptide levels during exercise in patients with congestive heart failure: implications for outpatient monitoring with b-type natriuretic peptide. *Am Heart J*, 143:406–11, 2002.

MR Mehra, PA Uber, MH Park, RL Scott, HO Ventura, BC Harris, and ED Frohlich. Obesity and suppressed b-type natriuretic peptide levels in heart failure. *J Am Coll Cardiol*, 43:1590–5, 2004.

AD Michaels, K Chatterjee, and T De-Marco. Effects of intravenous nesiritide on pulmonary vascular hemodynamics in pulmonary hypertension. *J Card Fail*, 11:425–431, 2005.

RM Mills, TH LeJemtel, DP Horton, C Liang, R Lang, MA Silver, C Lui, and K Chatterjee. Sustained hemodynamic effects of an infusion of nesiritide (human b-type natriuretic peptide) in heart failure: a randomized, double-blind, placebo-controlled clinical trial; natrecor study group. *J Am Coll Cardiol*, 34:155–162, 1999.

KS Misono, RT Grammer, H Fukumi, and T Inagami. Rat atrial natriuretic factor: isolation, structure and biological activities of four major peptides. *Biochem Biophys Res Commun*, 123:444–451, 1984.

P Moffatt, G Thomas, K Sellin, MC Bessette, F Lafreniere, O Akhouayri, R St-Arnaud, and C Lanctot. Osteocrin is a specific ligand of the natriuretic peptide clearance receptor that modulates bone growth. *J Biol Chem*, 282:36454–36462, 2007.

M Mukoyama, K Nakao, Y Saito, Y Ogawa, K Hosoda, S Suga, G Shirakami, M Jougasaki, and H Imura. Increased human brain natriuretic peptide in congestive heart failure. *N Engl J Med*, 323: 757–758, 1990.

M Mukoyama, K Nakao, K Hosoda, S Suga, Y Saito, Y Ogawa, G Shirakami, M Jougasaki, K Obata, and H Yasue. Brain natriuretic peptide as a novel cardiac hormone in humans; evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. *J Clin Invest*, 87:1402–1412, 1991.

M Nagase, T Katafuchi, S Hirose, and T Fujita. Tissue distribution and localization of natriuretic peptide receptor subtypes in stroke-prone spontaneously hypertensive rats. *J Hypertens*, 15:1235– 1243, 1997.

K Nakao, A Sugawara, N Morii, M Sakamoto, T Yamada, H Itoh, S Shiono, Y Saito, K Nishimura, and T Ban. The pharmacokinetics of alpha-human atrial natriuretic polypeptide in healthy subjects. *Eur J Clin Pharmacol*, 31:101–103, 1986.

T Nakayama, M Soma, Y Takahashi, D Rehemudula, K Kanmatsuse, and K Furuya. Functional deletion mutation of the 5-flanking region of type a human natriuretic peptide receptor gene and its association with essential hypertension and left ventricular hypertrophy in the japanese. *Circ Res*, 86:841–845, 2000.

DR Nussenzveig, JA Lewicki, and T Maack. Cellular mechanisms of the clearance function of type c receptors of atrial natriuretic factor. *J Biol Chem*, 265:20952–20958, 1990.

Y Ogawa, H Itoh, N Tamura, S Suga, T Yoshimasa, M Uehira, S Matsuda, S Shiono, H Nishimoto, and K Nakao. Molecular cloning of the complementary dna and gene that encode mouse brain natriuretic peptide and generation of transgenic mice that overexpress the brain natriuretic peptide gene. *J Clin Invest*, 93:1911–1921, 1994a.

Y Ogawa, H Itoh, Y Yoshitake, M Inoue, T Yoshimasa, T Serikawa, and K Nakao. Molecular cloning and chromosomal assignment of the mouse c-type natriuretic peptide (cnp) gene (nppc): comparison with the human cnp gene (nppc). *Genomics*, 24:383–387, 1994b.

S Oikawa, M Imai, A Ueno, S Tanaka, T Noguchi, H Nakazato, K Kangawa, A Fukuda, and H Matsuo. Cloning and sequence analysis of cdna encoding a precursor for human atrial natriuretic polypeptide. *Nature*, 309:724–726, 1984.

PM Oliver, JE Fox, R Kim, HA Rockman, HS Kim, RL Reddick, KN Pandey, SL Milgram, O Smithies, and N Maeda. Hypertension, cardiac hypertrophy, and sudden death in mice lacking natriuretic peptide receptor a. *Proc Natl Acad Sci U S A*, 94:14730–14735, 1997.

PM Oliver, SW John, KE Purdy, R Kim, N Maeda, MF Goy, and O Smithies. Natriuretic peptide receptor 1 expression influences blood pressures of mice in a dose-dependent manner. *Proc Natl Acad Sci U S A*, 95:2547–2551, 1998.

RC Olney, H Bukulmez, CF Bartels, TC Prickett, EA Espiner, LR Potter, and ML Warman. Heterozygous mutations in natriuretic peptide receptor-b (npr2) are associated with short stature. *J Clin Endocrinol Metab*, 91:1229–1232, 2006.

KN Pandey. Intracellular trafficking and metabolic turnover of ligand-bound guanylyl cyclase/atrial natriuretic peptide receptor-a into subcellular compartments. *Mol Cell Biochem*, 230:61– 72, 2002.

K Pankow, Y Wang, F Gembardt, E Krause, X Sun, G Krause, HP Schultheiss, WE Siems, and T Walther. Successive action of meprin a and neprilysin catabolizes b-type natriuretic peptide. *Circ Res*, 101:875–882, 2007.

JB Patel, ML Valencik, AM Pritchett, JC Burnett, JA McDonald, and MM Redfield. Cardiac-specific attenuation of natriuretic peptide a receptor activity accentuates adverse cardiac remodeling and mortality in response to pressure overload. *Am J Physiol Heart Circ Physiol*, 289:H777– H784, 2005.

A Pfeifer, A Aszodi, U Seidler, P Ruth, F Hofmann, and R Fassler. Intestinal secretory defects and dwarfism in mice lacking cgmp- dependent protein kinase ii. *Science*, 274:2082–2086, 1996.

R Pfister and CA Schneider. Natriuretic peptides bnp and nt-pro-bnp: established laboratory markers in clinical practice or just perspectives? *Clin Chim Acta*, 349:25–38, 2004.

JG Porter, A Arfsten, F Fuller, JA Miller, LC Gregory, and JA Lewicki. Isolation and functional expression of the human atrial natriuretic peptide clearance receptor cdna. *Biochem Biophys Res Commun*, 171:796–803, 1990.

LR Potter. Phosphorylation-dependent regulation of the guanylyl cyclase-linked natriuretic peptide receptor b: dephosphorylation is a mechanism of desensitization. *Biochemistry*, 37:2422– 2429, 1998.

LR Potter and DL Garbers. Dephosphorylation of the guanylyl cyclase-a receptor causes desensitization. *J Biol Chem*, 267:14531–14534, 1992.

LR Potter and DL Garbers. Protein kinase c-dependent desensitization of the atrial natriuretic peptide receptor is mediated by dephosphorylation. *J Biol Chem*, 269:14636–14642, 1994.

LR Potter and T Hunter. Activation of pkc stimulates the dephosphorylation of natriuretic peptide receptor-b at a single serine residue: a possible mechanism of heterologous desensitization. *J Biol Chem*, 275:31099–31106, 2000.

R Potthast, SE Abbey-Hosch, LK Antos, JS Marchant, M Kuhn, and LR Potter. Calcium-dependent dephosphorylation mediates the hyperosmotic and lysophosphatidic acid-dependent inhibition of natriuretic peptide receptor-b/guanylyl cyclase-b. *J Biol Chem*, 279: 48513–48519, 2004.

WG Pyle and RJ Solaro. At the crossroads of myocardial signaling: the role of z-discs in intracellular signaling and cardiac function. *Circ Res*, 94:296–305, 2004.

SN Rahman, GE Kim, AS Mathew, CA Goldberg, R Allgren, RW Schrier, and JD Conger. Effects of atrial natriuretic peptide in clinical acute renal failure. *Kidney Int*, 45:1731–1738, 1994.

I Raymond, BA Groenning, PR Hildebrandt, JC Nilsson, M Baumann, J Trawinski, and F Pedersen. The influence of age, sex and other variables on the plasma level of n- terminal pro brain natriuretic peptide in a large sample of the general population. *Heart*, 89:745–751, 2003.

AM Richards, G Tonolo, P Montorsi, J Finlayson, R Fraser, G Inglis, A Towrie, and JJ Morton. Low dose infusions of 26- and 28-amino acid human atrial natriuretic peptides in normal man. *J Clin Endocrinol Metab*, 66:465–472, 1988.

AM Richards, R Doughty, MG Nicholls, S Macmahon, H Ikram, N Sharpe, EA Espiner, C Frampton, and TG Yandle. Neurohumoral prediction of benefit from carvedilol in ischemic left ventricular dysfunction, australia-new zealand heart failure group. *Circulation*, 99: 786–92, 1999.

RN Rodseth. B type natriuretic peptide - a diagnostic breathrough in peri-operative cardiac risk assessment? *Anaesthesia*, 64:165–178, 2009.

RA Rose and WR Giles. Natriuretic peptide c receptor signalling in the heart and vasculature. *J Physiol*, 586:353–366, 2008.

H Ruskoaho. Cardiac hormones as diagnostic tools in heart failure. *Endocr Rev*, 24:341–356, 2003.

K Sabrane, MN Kruse, L Fabritz, B Zetsche, D Mitko, BV Skryabin, M Zwiener, HA Baba, M Yanagisawa, and M Kuhn. Vascular endothelium is critically involved in the hypotensive and hypovolemic actions of atrial natriuretic peptide. *J Clin Invest*, 115:1666–1674, 2005.

JD Sackner-Bernstein, M Kowalski, M Fox, and K Aaronson. Short-term risk of death after treatment with nesiritide for decompensated heart failure: a pooled analysis of randomized controlled trials. *Jama*, 293:1900–1905, 2005a.

JD Sackner-Bernstein, HA Skopicki, and KD Aaronson. Risk of worsening renal function with nesiritide in patients with acutely decompensated heart failure. *Circulation*, 111:1487–1491, 2005b.

Y Saito, K Nakao, K Nishimura, A Sugawara, K Okumura, K Obata, R Sonoda, T Ban, H Yasue, and H Imura. Clinical application of atrial natriuretic polypeptide in patients with congestive heart failure: beneficial effects on left ventricular function. *Circulation*, 76:115–124, 1987.

T Schachner, D Wiedemann, H Fetz, G Laufer, and N Kocher A Bonaros. Influence of preoperative serum n-terminal pro-brain type natriuretic peptide on the postoperative outcome and survival rates of coronary artery bypass patients. *Clinics*, 65:1239–1245, 2010.

R Schnabel, E Lubos, HJ Rupprecht, C Espinola-Klein, C Bickel, and KJ Lacker. B-type natriuretic peptide and the risk of cardiovascular events and death in patients with stable angina: results from the AtheroGene study. *Journal of American College of Cardiology*, 47: 552–558, 2006.

RW Schrier and WT Abraham. Hormones and hemodynamics in heart failure. *New England Journal of Medicine*, 341:577–85, 1999.

ME Steinhelper, KL Cochrane, and LJ Field. Hypotension in transgenic mice expressing atrial natriuretic factor fusion genes. *Hypertension*, 16:301–307, 1990.

SL Stephenson and AJ Kenny. The hydrolysis of alpha-human atrial natriuretic peptide by pig kidney microvillar membranes is initiated by endopeptidase-24,11. *Biochem J*, 243:183–187, 1987.

T Sudoh, K Kangawa, N Minamino, and H Matsuo. A new natriuretic peptide in porcine brain. *Nature*, 332:78–81, 1988.

T Sudoh, N Minamino, K Kangawa, and H Matsuo. C-type natriuretic peptide (cnp): a new member of natriuretic peptide family identified in porcine brain. *Biochem Biophys Res Commun*, 168:863–870, 1990.

S Suga, K Nakao, K Hosoda, M Mukoyama, Y Ogawa, G Shirakami, H Arai, Y Saito, Y Kambayashi, K Inouye, and H Imura. Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and c-type natriuretic peptide. *Endocrinology*, 130:229–239, 1992a.

S Suga, K Nakao, H Itoh, Y Komatsu, Y Ogawa, N Hama, and H Imura. Endothelial production of c-type natriuretic peptide and

its marked augmentation by transforming growth factor-beta; possible existence of vascular natriuretic peptide system. *J Clin Invest*, 90:1145–1149, 1992b.

S Suga, K Nakao, I Kishimoto, K Hosoda, M Mukoyama, H Arai, G Shirakami, Y Ogawa, Y Komatsu, and O Nakagawa. Phenotype-related alteration in expression of natriuretic peptide receptors in aortic smooth muscle cells. *Circ Res*, 71:34–39, 1992c.

S Suga, H Itoh, Y Komatsu, Y Ogawa, N Hama, T Yoshimasa, and K Nakao. Cytokine-induced c-type natriuretic peptide (cnp) secretion from vascular endothelial cells-evidence for cnp as a novel autocrine/paracrine regulator from endothelial cells. *Endocrinology*, 133:3038–3041, 1993.

K Sward, F Valsson, J Sellgren, and SE Ricksten. Differential effects of human atrial natriuretic peptide and furosemide on glomerular filtration rate and renal oxygen consumption in humans. *Intensive Care Med*, 31:79–85, 2005.

K Swedberg, J Cleland, H Dargie, H Drexler, F Follath, M Komajda, L Tavazzi, OA Smiseth, A Gavazzi, A Haverich, A Hoes, T Jaarsma, J Korewicki, S Levy, C Linde, JL Lopez-Sendon, MS Nieminen, L Pierard, and WJ Remme. Guidelines for the diagnosis and treatment of chronic heart failure: executive summary (update 2005). *Eur Heart J*, 26:1115–40, 2005.

S Talwar, IB Squire, PF Downie, AM Mccullough, MC Campton, JE Davies, DB Barnett, and LL Ng. Profile of plasma n-terminal probnp following acute myocardial infarction; correlation with left ventricular systolic dysfunction. *Eur Heart J*, 21:1514–21, 2000.

N Tamura, Y Ogawa, H Chusho, K Nakamura, K Nakao, M Suda, M Kasahara, R Hashimoto, G Katsuura, M Mukoyama, H Itoh, Y Saito, I Tanaka, H Otani, and M Katsuki. Cardiac fibrosis in mice lacking brain natriuretic peptide. *Proc Natl Acad Sci U S A*, 97:4239–4244, 2000.

N Tamura, TD Chrisman, and DL Garbers. The regulation and physiological roles of the guanylyl cyclase receptors. *Endocr J*, 48:611–634, 2001.

N Tamura, LK Doolittle, RE Hammer, JM Shelton, JA Richardson, and DL Garbers. Critical roles of the guanylyl cyclase b receptor in endochondral ossification and development of female reproductive organs. *Proc Natl Acad Sci U S A*, 101:17300–17305, 2004.

C Thay-Hsiung, L Ching-Ling, JS Joseph, YS James, C Chung-Huo, C Mei-Ling, and C Chih-Hui. Plasma B-type natriuretic peptide in predicting outcomes of elective coronary artery bypass surgery. *Kaohsiung Journal of Medical Sciences*, 29:254–258, 2013.

DJ Thuerauf, DS Hanford, and CC Glembotski. Regulation of rat brain natriuretic peptide transcription; a potential role for gata-related transcription factors in myocardial cell gene expression. *J Biol Chem*, 269:17772–17775, 1994.

RW Troughton, CM Frampton, TG Yandle, EA Espiner, MG Nicholls, and AM Richards. Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (n-bnp) concentrations. *Lancet*, 355:1126–30, 2000.

H Tsuji, N Nishino, Y Kimura, K Yamada, M Nukui, S Yamamoto, T Iwasaka, and H Takahashi. Haemoglobin level influences plasma brain natriuretic peptide concentration. *Acta Cardiol*, 59:527–31, 2004.

T Tsuji and T Kunieda. A loss-of-function mutation in natriuretic peptide receptor 2 (npr2) gene is responsible for disproportionate dwarfism in cn/cn mouse. *J Biol Chem*, 280:14288– 14292, 2005.

T Tsutamoto, A Wada, K Maeda, T Hisanaga, Y Maeda, D Fukai, M Ohnishi, Y Sugimoto, and M Kinoshita. Attenuation of compensation of endogenous cardiac natriuretic peptide system in chronic heart failure: prognostic role of plasma brain natriuretic peptide

concentration in patients with chronic symptomatic left ventricular dysfunction. *Circulation*, 96:509–16, 1997.

T Tsutamoto, H Sakai, Wada, C Ishikawa, K Ohno, M Fujii, T Yamamoto, T Takayama, T Dohke, and M Horie. Torasemide inhibits transcardiac extraction of aldosterone in patients with congestive heart failure. *J Am Coll Cardiol*, 44:2252–3, 2004.

II Tulevski, BJ Mulder, and DJ van Veldhuisen. Utility of a bnp as a marker for rv dysfunction in acute pulmonary embolism. *J Am Coll Cardiol*, 39:2080, 2002.

Y Vanneste, A Michel, R Dimaline, T Najdovski, and M Deschodt-Lanckman. Hydrolysis of alpha-human atrial natriuretic peptide in vitro by human kidney membranes and purified endopeptidase-24,11 evidence for a novel cleavage site. *Biochem J*, 254:531–537, 1988.

MA Vieira, M Gao, LN Nikonova, and T Maack. Molecular and cellular physiology of the dissociation of atrial natriuretic peptide from guanylyl cyclase a receptors. *J Biol Chem*, 276:36438– 36445, 2001.

DJ Wang, TC Dowling, D Meadows, T Ayala, J Marshall, S Minshall, N Greenberg, E Thattassery, ML Fisher, K Rao, and SS Gottlieb. Nesiritide does not improve renal function in patients with chronic heart failure and worsening serum creatinine. *Circulation*, 110:1620–1625, 2004a.

TJ Wang, MG Larson, D Levy, EJ Benjamin, EP Leip, PW Wilson, and RS Vasan. Impact of obesity on plasma natriuretic peptide levels. *Circulation*, 109:594–600, 2004b.

Y Wang, MC deWaard, A Sterner-Kock, H Stepan, HP Schultheiss, DJ Duncker, and T Walther. Cardiomyocyte-restricted overexpression of c-type natriuretic peptide prevents cardiac hypertrophy induced by myocardial infarction in mice. *Eur J Heart Fail*, 9: 548–557, 2007.

EP Widmaier, H Raff, and KT Strang. *Vander's Human Physiology*. 11 edition, 2008.

JN Wilcox, A Augustine, DV Goeddel, and DG Lowe. Differential regional expression of three natriuretic peptide receptor genes within primate tissues. *Mol Cell Biol*, 11:3454–3462, 1991.

MS Willis, ES Lee, and DG Grenache. Effect of anemia on plasma concentrations of nt-probnp. *Clin Chim Acta*, 358:175–81, 2005.

RM Witteles, D Kao, D Christopherson, K Matsuda, RH Vagelos, D Schreiber, and MB Fowler. Impact of nesiritide on renal function in patients with acute decompensated heart failure and pre-existing renal dysfunction a randomized, double-blind, placebo-controlled clinical trial. *J Am Coll Cardiol*, 50:1835–1840, 2007.

KC Wold, H Vik-Mo, and T Omland. Blood haemoglobin is an independent predictor of b-type natriuretic peptide (bnp). *Clin Sci (Lond)*, 109:69–74, 2005.

AH Wu, T Omland, P Duc, J McCord, RM Nowak, and JE Hollander. The effect of diabetes on b-type natriuretic peptide concentrations in patients with acute dyspnea: an analysis from the breathing not properly multinational study. *Diabetes Care*, 27:2398–404, 2004.

AH Wu, T Omland, C Wold Knudsen, J McCord, RM Nowak, and JE Hollander. Relationship of b-type natriuretic peptide and anemia in patients with and without heart failure: A substudy from the breathing not properly (bnp) multinational study. *Am J Hematol*, 80:174–80, 2005.

AHB Wu, A Smith, S Wieczorek, JF Mather, B Duncan, CM White, C McGill, D Katten, and G Heller. Biological variation for n-terminal pro- and b-type natriuretic peptides and implications for therapeutic monitoring of patients with congestive heart failure. *Am J Cardiol*, 92:628–31, 2003a.

C Wu, F Wu, J Pan, J Morser, and Q Wu. Furin-mediated processing of pro-c-type natriuretic peptide. *J Biol Chem*, 278:25847–25852, 2003b.

W Yan, F Wu, J Morser, and Q Wu. Corin, a transmembrane cardiac serine protease, acts as a pro-atrial natriuretic peptide-converting enzyme. *Proc Natl Acad Sci U S A*, 97:8525–8529, 2000.

TG Yandle, AM Richards, MG Nicholls, R Cuneo, EA Espiner, and JH Livesey. Metabolic clearance rate and plasma half life of alpha-human atrial natriuretic peptide in man. *Life Sci*, 38:1827– 1833, 1986.

TG Yandle, SO Brennan, EA Espiner, MG Nicholls, and AM Richards. Endopeptidase-24,11 in human plasma degrades atrial natriuretic factor (anf) to anf(99-105/106-126). *Peptides*, 10:891–894, 1989.

A Yasoda, Y Komatsu, H Chusho, T Miyazawa, A Ozasa, M Miura, T Kurihara, T Rogi, S Tanaka, M Suda, N Tamura, Y Ogawa, and K Nakao. Overexpression of cnp in chondrocytes rescues achondroplasia through a mapk-dependent pathway. *Nat Med*, 10:80–86, 2004.

VT Yeung, SK Ho, MG Nicholls, and CS Cockram. Binding of cnp-22 and cnp-53 to cultured mouse astrocytes and effects on cyclic gmp. *Peptides*, 17:101–106, 1996.

M Yoshimura, H Yasue, E Morita, N Sakaino, M Jougasaki, M Kurose, M Mukoyama, Y Saito, K Nakao, and H Imura. Hemodynamic, renal, and hormonal responses to brain natriuretic peptide infusion in patients with congestive heart failure. *Circulation*, 84:1581–1588, 1991.

B Zethelius, L Berglund, J Sundström, E Ingelsson, and S Basu. Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. *New-England Journal of Medicine*, 358:2107–2116, 2008.