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

Key cell biological observations that predicted the existence of natriuretic peptides were reported over fifty years ago. 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]; the heart is recognized not only as the pump of the circulatory system but also an endocrine organ[de Bold, 1979]. In a seminal 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]. Thus, the first natriuretic peptide was discovered. 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]. 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) [Sudoh et al., 1990] 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.

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). The biological actions of NPs 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 BNP levels have been demonstrated to responsed to increased angiotensin II and sympathetic tones. [Iwanaga et al., 2006]

Elevated NPs 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 endocrinology diseases and high output status without decreased left ventricular ejection fraction (EF), e.g., sepsis, renal failure, cirrhosis of liver, or intracranial pathologies. Even in the absence of significant clinical evidence of volume overload or LV dysfunction, markedly elevated NP levels can be found in patients with multiple comorbidities with certain degree of prognostic value. The causes and mechanisms of elevated NPs levels are summarized in Fig. 1. The potential clinical applications in the non-HF settings are summarized in Table 1.

2.1.1 Atrial Natriuretic Peptide

All natriuretic peptides are synthesized as preprohormones **??**. The human gene encoding ANP is called NPPA (GeneID 4878) and is located on chromosome 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 has been shown to cleave proANP into the biologically active 28-amino acid form of ANP in vitro [Yan et al., 2000]. Mice lacking functional corin have dramatically reduced levels of fully processed ANP in their hearts and are mildly hypertensive [Chan et al., 2005]. 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, NPPA, 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].

ANP is well conserved between species **??**. The 28-amino acid mature form of ANP in humans and rats differs by only one amino acid at position 12, where the human peptide contains a methionine and the rat peptide contains an isoleucine. The mature, circulating form of ANP is identical in humans, chimps, dogs, pigs, horses, and sheep. The sequence of mature rat ANP is identical in mice and rabbits. Additionally, the entire length of the preproANP polypeptide, not just the carboxyl terminal biologically active end, is well conserved.

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]. Upon secretion and cleavage into the mature peptide, ANP enters the coronary sinus and is distributed to its target organs via the circulation. 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, with reported values falling between 1.7 and 3.1 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).

NPR-C is 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 both in vitro [Stephenson and Kenny, 1987] [Yandle et al., 1989] and in vivo [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 skel 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:

- 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 secre-

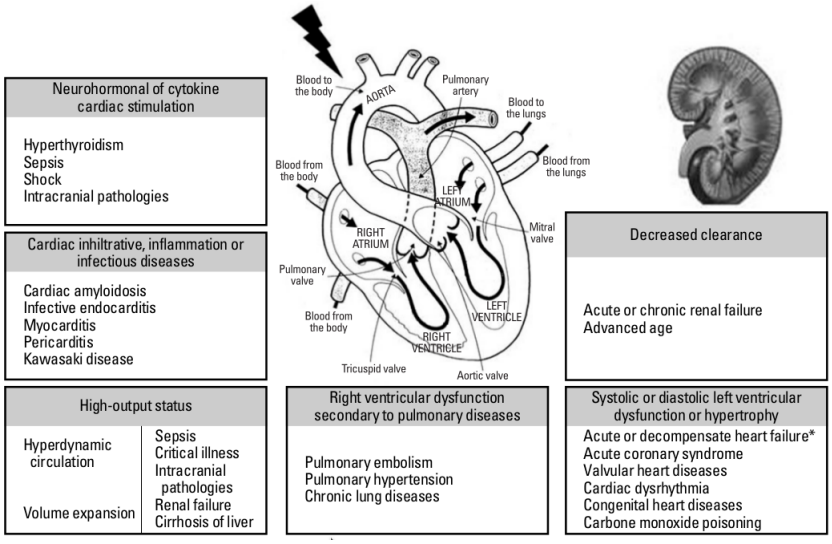

Figure 1: The causes and mechanisms of elevated natriuretic peptides levels.

tion. [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]. The gene encoding human BNP, NPPB (GeneID 4879), is located on chromosome 1.

Like NPPA , the NPPB gene consists of 3 exons and 2 introns [Ogawa et al., 1994a]. PreproBNP is 134 amino acids in length, consisting of

Table 1: Potential Clinical Applications of Natriuretic Peptides in Selected Diseases

Diseases	Screening [1]		Prognosis
Heart failure [2]	+	+	+
Acute coronary syndrome	+	+	+
Cardiac procedures	+	+	+
Pulmonary embolism	+	+	+
Pulmonary hypertension	+	+	-
Chronic lung diseases	+	+	+
Valvular heart diseases	+	+	N/A
Cardiac dysrhythmia	+	+	+/-
Cardiac inflammatory or infectious diseases	+	+	N/A
Cardiogenic syncope	+	N/A	N/A
Sleep apnea	+	+	N/A
Hypertension	+	+	N/A
Sepsis	+	+	+
Renal failure	+	+	+
Cirrhosis of liver	+	+	+
Hyperthyroidism	+	+	N/A
Intracranial pathologies	+	+	+
Epilepsy / Seizures	+	-	-
Carbone monoxide poisoning	+	N/A	N/A

a 26 amino acid signal sequence followed by 108 amino acids that constitute proBNP. Unlike preproANP, which has high species homology throughout the entire polypeptide sequence, preproBNP sequences in mammals only have high homology at the amino and carboxyl terminal ends of the polypeptide. For example, the homology of canine preproBNP to human preproBNP is 53%, whereas the homology for preproANP between these species is 85%. This lower level of homology gives rise to differing lengths of the active, circulating BNP between mammalian species. For example, in humans and pigs circulating BNP is 32 amino acids in length, while in rats and mice the circulating form is 45 amino acids. The peptidase that cleaves proBNP to its active form has not been identified, but corin is a reasonable suspect.

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 such as volume overload. The transcription of BNP is under the regulatory control of GATA4, a transcription factor [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 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]. Obese patients (especially those who have body mass index greater than 30) tend to have lower BNP levels than others. Neural endopeptidases that can be secreted by adipose tissue may be related to increased BNP clearance in obese patients.[**?**]

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.[**?**]

Both knockout and overexpression models of NPPB have been generated in mice. The knockout model of Nppb was created by targeted deletion of exons 1 and 2 [Tamura et al., 2000]. In contrast to ANP knockout mice, Nppb -/- mice showed no signs of systemic hypertension or ventricular hypertrophy on standard or high salt diets. However, Nppb -/- mice had ventricular fibrotic lesions that increased in size and number in response to pressure overload, compared to wild type animals. Thus, these studies suggest that BNP is not a regulator of blood pressure, at least in mice. Rather, it is a paracrine regulator of cardiac remodeling. In murine overexpression models of BNP, blood pressure reduction of 20 mmHg was seen with 10- to 100-fold increases in plasma BNP levels [Ogawa et al., 1994a]. Interestingly, these mice had marked increases in long bone length compared to their wild-type littermates, which most likely resulted from overactivation of NPR-B, the receptor of CNP

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 (GeneID 4880), is not located on chromosome 1 but on chromosome 2 [Ogawa et al., 1994b]. Another difference of NPPC is that it consists of only 2 exons and 1 intron. In the murine genome, Nppc is also separated spatially from both ANP and BNP being located on chromosome 2 [Ogawa et al., 1994b].

NPPC encodes a polypeptide of 126 amino acids, with a 23 amino acid signal sequence followed by a 103 amino acid proCNP [Tawaragi et al., 1991]. PreproCNP shows remarkable homology between species, even more so than preproANP. The preproCNP polypeptides of mammalian species show 99, 96, 91, and 94% homology to the human form in chimpanzees, dogs, mice, and rats, respectively. Perhaps even more telling is that the circulating 22 amino acid carboxyl terminal form of CNP is absolutely identical in all of the above species. 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., 2003]. 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].

The clearance of CNP-22 in human plasma is very rapid, with a calculated half-life of 2.6 min [Hunt et al., 1994]. Like ANP, CNP has been shown in vitro to be inactivated by neutral endopeptidase [Kenny et al., 1993] and is internalized and degraded by NPR-C. The NPR-C route of CNP degradation is especially important at the tissue level, as can be seen in NPR-C knockout models. Mice lacking functional NPR-C receptors have disproportionately long bones, most likely due to the failed clearance of CNP from specific regions in the growth plate [Jaubert et al., 1999] [Matsukawa et al., 1999].

Both over and under expression models of CNP have been created in mice. Knockout animals were generated by disruption of exons 1 and 2 of Nppc [Chusho et al., 2001]. The Nppc -/- mice are dwarfs due to impaired endochondral ossification and have severely reduced life spans. Targeted expression of CNP in the growth plate chondrocytes rescued the skeletal defect of Nppc -/- mice and prolonged their survival. Importantly, the Nppc transgene partially rescued skeletal growth in a murine model of achondroplasia caused by a gain of function mutation in the fibroblast growth factor receptor 3 (Fgf r3 ach), which accounts for most types of human dwarfism and may indicate that CNP analogs could be used to treat various forms of human dwarfism [Yasoda et al., 2004]. A recent spontaneous point mutation in Nppc was characterized by a dwarfism phenotype similar to that seen in the Nppc -/- mice [Jiao et al., 2007]. The mutation resulted in a missense mutation in proCNP, which is one of the highly conserved residues in the cysteine ring found in all natriuretic peptides. Our recent data indicate that the mutant CNP has reduced ability to bind and activate NPR-B [Yoder et al., 2008]. Finally, balanced translocations of chromosome 2, which separate putative negative transcriptional regulators from the CNP promoter, cause abnormal elevations in CNP and asso-

ciated Marfanoid-like skeletal overgrowth in humans [Bocciardi et al., 2007] [Moncla et al., 2007]. Hence, moderate elevations of CNP are correlated with demonstrative skeletal overgrowth.

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 atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP). Its extracellular domain contains three intramolecular disulfide bonds and five N-linked glycosylation sites [Miyagi and Misono, 2000]. NPR-A exists as a homodimer or homotetramer in its native state, and oligomerization is ligand-independent [Chinkers and Wilson, 1992] [Iwata et al., 1991], although ligand binding does bring the juxtamembrane regions of each monomer closer together [Labrecque et al., 2001]. NPR-A binds natriuretic peptides at a stoichiometry of 2:1 with a rank natri-

uretic 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] [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]. Recent 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] [Lowe et al., 1989] [Nagase et al., 1997] [Wilcox et al., 1991]. NPR-A null mice exhibit chronic salt-resistant hypertension and cardiac hypertrophy and fibrosis [Kuhn et al., 2002] [Lopez et al., 1995] [Oliver et al., 1997]. 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, glycosy-

lation, and intramolecular disulfide bonding patterns as NPR-A. The extracellular and intracellular regions of rat NPR-B are 43 and 78% identical to rat NPR-A at the amino acid level, respectively [Schulz et al., 1989]. NPR-B binds natriuretic peptides with a selectivity preference of CNP > ANP ≥ BNP [Bennett et al., 1991] [Koller et al., 1991] [Suga et al., 1992a].

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] [Chrisman et al., 1993] [Dickey et al., 2007] [Herman et al., 1996] [Langub et al., 1995]. 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]. Individuals with single defect NPR-B alleles are statistically shorter than the average person [**?**].

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]. Unlike NPR-A and NPR-B, it contains one or two juxtamembrane intermolecular disulfide bonds. Hence, it is a disulfide-linked dimer. It has no known enzymatic activity but has

been suggested to signal in a G protein-dependent manner [Rose and Giles, 2008]. It contains three known N-linked extracellular glycosylation sites [Stults et al., 1994] and 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 20–40mm mercury, 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)

The natriuretic peptide field emerged with the key discovery that specific peptides present in atrial extracts cause natriuresis and diuresis. 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]. Furthermore, 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].

The results of these studies likely led to the approval of nesiritide, marketed under the trade name Natrecor, for the treatment of acute decompensated heart failure in the United States in 2001. In 2003, nesiritide became commercially available in Israel and Switzerland, under the trade name Noratak. However, approval in the rest of Europe was delayed pending further investigations into the renal responses of nesiritide infusion – a request that in retrospect is prophetic.

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., 2004], 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 capil-

lary wedge pressure, Michaels and colleagues tested its effectiveness in pulmonary hypertension [Michaels et al., 2005]. However, they found no effect of a 30 min infusion. 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 Coronary Artery Disease

An extremely short chapter. No time to be wasted on it.

2.6 Coronary Artery Bypass Grafting

Also short

2.6.1 on-pump vs OPCAB

3 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 aneshetic 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 in-hospital stay and events recorded including: death from cardiovascular causes, ischemic stroke (defined as new neurologic deficit lasting for 24hours with definite image evidence of cerebrovascular accident by computed tomography), low output heart failure (defined as need of any of the following: CPB during off-pump surgery, intra-aortic balloon pump, inotropes at 48 hours post-operatively), myocardial infarction (defined as elevated tropoponin 10x99th percentile URL at 12 hours after surgery associated with characteristic ECG changes or echocardiographically documented new regional wall motion abnormality). prolonged intubaton (24 hours postoperatively and reintubation) and arrhythmias.

Primary outcomes:

- low output heart failure and myocardial infarction

Secondary outcome parameters:

- moratlity
- arrhythmias
- length of ICU and in-hospital stay
- prolonged intubation

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.

4 Results

Patients' mean age was 57.4 ± 7.3 years, and 86.4% were male. 2 patients died 3.1% 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). Four suffered post-operative arrhythmia (3 Atrial fibrillation, One Ventricular Tachycardia), with one patient readmitted to the ICU. The rest of the results are summarized in table 2

The preoperative NTproBNP levels ranged 100-14400 pg/mL, with a mean of 3096.83. The mean ICU stay was 3.37 ± 0.84 days and mean

Table 2:

		count	%
Gender	f	9	13.6
	m	57	86.4
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

hospitalization was 6.38 ± 1.3 (range 3-12) days. It didn't correlate with any of the measured outcome parameters. See tables 3 and 4

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].

Table 3: Relation between NTBNP and other parameters

		NTproBNP				
		Mean	Standard Deviation	Median	Minimum	Ma
low Co	yes	4900.00	3079.77	6500.00	600.00	75
	no	2968.42	3297.52	1600.00	100.00	14
Arrhythmia	yes	4000.00	2929.16	4100.00	600.00	72
	no	3063.79	3337.70	1600.00	100.00	14
perioperative MI	yes	4375.00	3262.28	4850.00	600.00	72
	no	3037.93	3312.38	1600.00	100.00	14
proloonged vent	yes	5500.00	2443.36	6600.00	2700.00	72
	no	3003.39	3306.94	1600.00	100.00	14
Delayed Recovery	yes	10300.00	.	10300.00	10300.00	10
	no	3006.56	3192.90	1600.00	100.0	14
mortality	yes	4950.00	3181.98	4950.00	2700.00	72
	no	3063.33	3311.51	1600.00	100.00	14

Table 4: Correlation between NTproBNP and other parameters

		NTproBNP
AGE	Correlation Coefficient	.081
	P value	.518
	N	66
GENSINI	Correlation Coefficient	.084
	P value	.501
	N	66
EUROSCORE II	Correlation Coefficient	.217
	P value	.080
	N	66
ICU stay	Correlation Coefficient	-.022-
	P value	.861
	N	65
in-hospital stay	Correlation Coefficient	-.017-
	P value	.896
	N	65

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.

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 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 func-

tion, 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 and preoperative BNP and NTproBNP were not significantly associated with outcomes.

5.0.1 Limitations of study

This study demonstrated that preoperative NTproBNP levels don't correlate with postoperative outcomes for patients who have undergone elective CABG surgery. 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.

6 Conclusion

7 Summary

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