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

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1 Review of Literature

1.1 Natriuretic Peptides

The history of the NP class of biomarkers dates back to 1950s when early electron microscopy studies reported dense granules in the atrial myocardium similar to glandular tissue from endocrine organs. Soon, the close interplay between atria and intravascular volume was revealed; stretching of canine left atrium increased urine output and injection of atrial tissue into rats caused diuresis and natriuresis. Atrial natriuretic peptide (ANP) was subsequently purified, sequenced, and reproduced. [Gaggin and Januzzi, 2014]

Subsequent studies were aimed at discovering family members which resulted in the isolation of two other factors which were named brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). Studies also showed that although BNP was first isolated from the brain, that it is predominantly expressed in the ventricle. ANP and BNP were therefore renamed A-type and B-type natriuretic peptide, respectively, to better reflect their position in the family and to also lessen the misleading nature of the nomenclature of BNP as a cardiovascular and not a neural factor. ANP and BNP are the natriuretic peptides which are expressed predominantly in the atria and ventricle, respectively, and are referred to as the cardiac natriuretic peptides. CNP is differentially expressed mainly in the nervous system and vasculature (e.g. endothelial cells, monocyte / macrophages) and is involved mainly in neural regulation as well as vascular control although its role is still unclear. [Suzuki et al., 2001]

Other NPs that share a common biochemical structural feature, a 17-amino-acid ring and a disulfide bridge between cysteine molecules, have been discovered since: urodilantin (an isoform of ANP), C-type natriuretic peptide, and Dendroaspis natriuretic peptide. [Gaggin and

Januzzi, 2014]

Each natriuretic peptide is coded by a separate gene but shows similar exon–intron properties. In humans, the ANP and BNP genes are located 8 kilobases apart on chromosome 1 and the CNP gene is located on chromosome 2. Each natriuretic peptide gene produces a prohormone or precursor protein. ANP is synthesized as a 126 amino acid precursor protein which is cleaved to produce a 96 amino acid amino-terminal fragment and a 28 amino acid carboxyl-terminal fragment. The carboxyl-terminal 28 amino acid fragment is the biologically active peptide and has a shorter half-life than the amino-terminal fragment. Similarly, BNP is produced as a 108 amino acid precursor protein which is cleaved into a biologically active 32 amino acid carboxyl-terminal fragment and a 76 amino acid amino-terminal fragment. CNP produces 22 and 53 amino acid fragments. The 22 amino acid fragment is the mature and more active form, and is expressed in the nervous system and endothelial cells. The common property of the natriuretic peptides is the formation of a disulfide bond which results in a ringed structure (Fig. 1).[Suzuki et al., 2001]

ANP is encoded by the NPAA gene on chromosome 1. It is translated into a 151-amino-acid pre-prohormone (preproANP) that is cleaved in the sarcoplasmic reticulum to a 126-amino-acid prohormone (proANP), which is stored in intracellular granules. When stimulated and released, proANP is further cleaved into a 28-amino-acid bioactive form (ANP) and a 98-amino-acid N-terminal fragment (NT-proANP). The half-life of ANP is approximately 2 minutes, whereas NT-proANP half-life is variable depending on the fragment measured. [Maisel and Wettersten, 2018]

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.

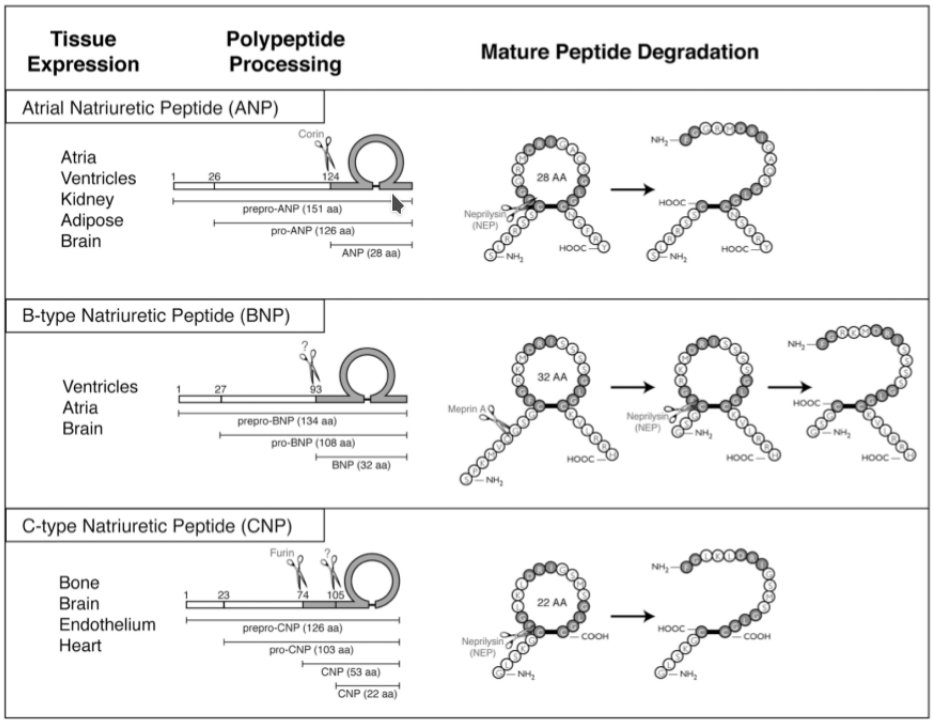

Figure 1: Structure of the human natriuretic peptides. The structure of the preprohormones for ANP, BNP and CNP are outlined on the left of each panel. The final amino acid sequence and structure of the mature peptides along with the major degradation product are shown on the right. The sites of cleavage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

Elevated natriuretic peptides levels can be 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.

1.1.1 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., 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].

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, 2003c]. 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., 1986b].

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 secretion. [Widmaier et al., 2008]
- Endothelin, a potent vasoconstrictor

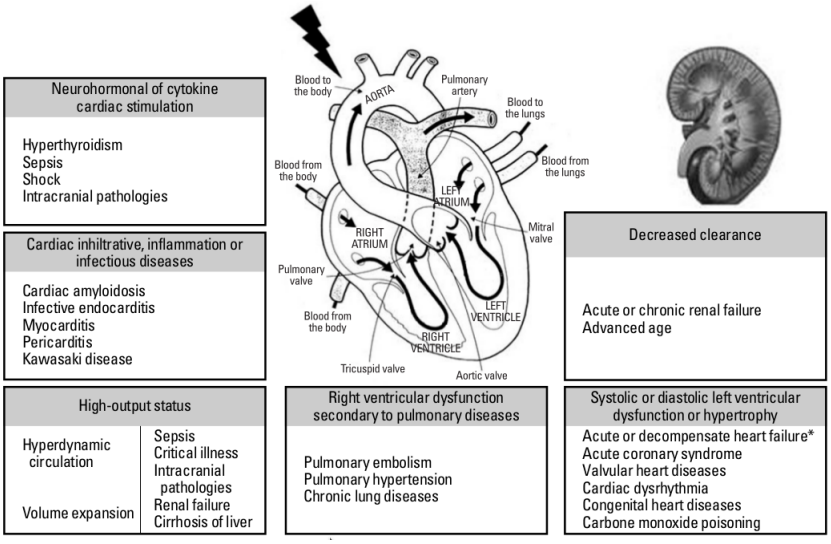

Figure 2: The causes and mechanisms of elevated natriuretic peptides levels.

- exercise [Kokkonen et al., 2002]

1.1.2 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., 1991c] [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., 1991c] [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., 1991c] [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 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. [**?**]

1.1.3 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., 1994c].

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., 2003c]. 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., 1993b] [Suga et al., 1992b] and sheer stress [Chun et al., 1997] in cultured endothelial cells. CNP expression in neo-intimal 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., 2003b].

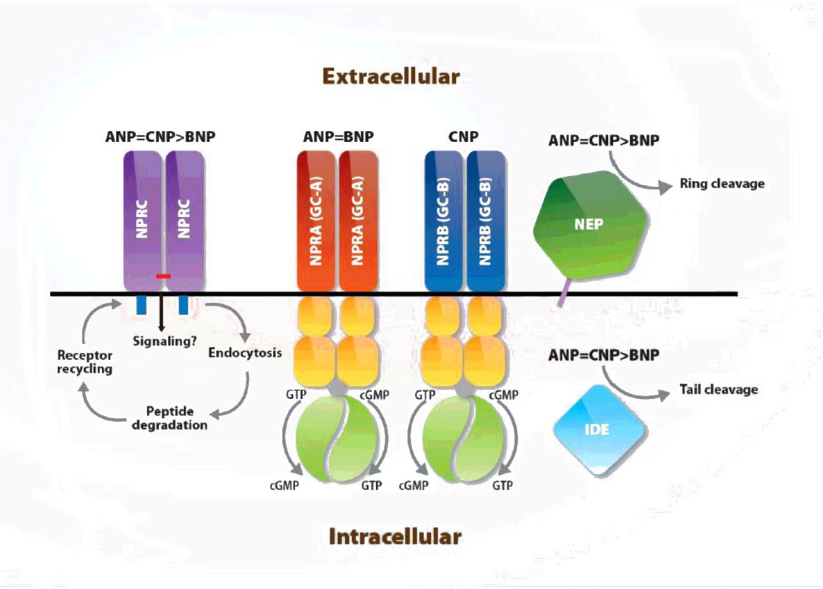

Figure 3: Schematic representation of natriuretic receptors

1.2 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 [Rose and Giles, 2008].

1.2.1 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] [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., 2005b] [Vieira et al., 2001b].

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., 2000b].

1.2.2 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.[Schulz et al., 1989].

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 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. In addition to mild growth retardation of the long bones, the rats displayed progressive, blood pressure-independent cardiac hypertrophy and an elevated heart rate [Langenickel et al., 2006].

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].

1.2.3 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., 1992b] [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].

1.3 Physiologic Effects of Natriuretic Peptides

1.3.1 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., 1995b] [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., 1994b] [Steinhelper et al., 1990b]. 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].

1.3.2 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 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].

1.3.3 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 [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].

1.4 Biological Action of CNH

Cardiac natriuretic hormones have powerful physiological effects on the cardiovascular system, body fluid, and electrolyte homeostasis [McGrath and de Bold AJ, 2005] [Clerico and Emdin, 2004a] [Clerico, 2002a] [deLemos et al., 2003] [Ruskoaho, 2003a]. CNH share a direct diuretic, natriuretic and vasodilator effect and an inhibitory action on ventricular myocyte contraction [Zhang et al., 2005] as well as remodeling and inflammatory processes of myocardium and smooth muscle cells [Nagaya et al., 1998] [Hayashi et al., 2001] [Magga et al., 2004] [Hardt and Sadoshima, 2004] (Fig. 3.14). Thus, CNH exert a protective effect on endothelial function by decreasing shear stress, modulating coagulation and fibrinolysis pathways, and inhibiting platelet activation (Fig. 3.15). They can also inhibit vascular remodeling process as well as coronary restenosis post-angioplasty [Ma et al., 2004] [Chen and Burnett] [Morishige et al., 2000] [Takeuchi et al., 2003] [Yasuda et al., 2002] [Qian et al., 2002] [Nakanishi et al., 2005].

The first evidence for a role of CNH in the immune system was given by the fact that peptide hormones and their receptors are expressed in various immune organs. Furthermore, several studies indicated that the CNH system in immune cells underlies specific regulatory mechanisms by affecting the innate as well as the adaptive immune response. In particular, ANP increases phagocytotic activity and production of reactive oxygen species of phagocytes. ANP affects the induced innate immune response by regulating the activation of macrophages at various stages. It also reduces production of pro-inflammatory medi ators by inhibition of iNOS and COX-2 as well as TNF-$\alpha synthesis. ANP also affects TNF-$

$\alpha action, i.e. it interfere s with the inflammatory effects of TNF-\alpha on the endotheliu$
$\alpha-induced endothelial permeability and adhesion and attraction of inflammatory cells$

The cited effects on the cardiovascular system and body fluid and electrolyte homeostasis can be explained at least in part by the inhibition of control systems, including the sym pathetic nervous system, the renin-angiotensin-aldosterone system (RAAS), the vaso pressin/antidiuretic hormone system, the endothelin system, cytokines and growth factors [Ventura et al., 2002] [Vatta et al., 1996] [Vatta et al., 1997] [Fermepin et al., 2000] [Rocca et al., 2001] [Tsukagoshi et al., 2001] [Kiemer et al., 2002b] [Weber et al., 2003] [Kapoun et al., 2004] [Vollmar, 2005].The endocrine action,shared by plasma ANP and BNP,can be enhanced by natriuretic peptides produced locally in target tissues (paracrine action). Indeed, endothelial cells syn thesize CNP, which in turn exerts a paracrine action on vessels [Woodard et al., 2002] [Chen and Burnett] [Morishige et al., 2000] [Takeuchi et al., 2003] [Yasuda et al., 2002] [Qian et al., 2002]. Moreover, renal tubular cells produce urodilatin, another member of the peptide natriuretic family, which has powerful diuretic and natriuretic properties [Vesely, 2003].Genes for natriuretic peptides (includ ing ANP, BNP and CNP) are also expressed in the central nervous system, where they likely act as neurotransmitters and/or neuromodulators [Vatta et al., 1996] [Vatta et al., 1997] [Fermepin et al., 2000] [Vesely, 2003] [Imura et al., 1992] [Langub et al., 1995]. In particular, it was demonstrated that intranasal ANP acts as central nervous inhibitor of the hypothalamus pituitary-adrenal stress system in humans [Perras et al., 2004]. Finally, co-expression of CNH and of their receptors was observed in rat thymus cells and macrophages [Vollmar et al., 1995] [Vollmar and Schultz, 1995],suggesting that CNH may have immunomodulatory and anti-inflammatory functions in mammals [Vollmar and AK, 2001].

A detailed review [Waschek, 2004] has highlighted a possible major role for CNH in the development of certain systems, in particular skeleton, brain, and vessels. This review cites studies showing severe skeletal defects and impaired recovery after vascu lar and renal injury in CNH

transgenic and knockout (KO) mice [108, 205]. In addition, CNH may have a role in the regulation of proliferation, survival, and neurite outgrowth of cultured neuronal and/or glial cells [108, 205].

Changes in plasma ANP are also correlated with alcohol-associated psychological vari ables [108, 205]. Acute administration of alcohol stimulates the release of ANP independent ly of volume-loading effects. Patients whose ANP levels fell markedly during abstinence also reported more intense and frequent craving as well as more anxiety [108, 205]. Several reports have shown that CNH stimulate the synthesis and release of testos terone in a dose-dependent manner in isolated and purified normal Leydig cells [Bex and Corbin, 1985] [Pandey, 2005]. It has been suggested that this effect on normal Leydig cell steroidogenesis does not involve classical mechanisms of cAMP-mediated regulation of steroidogenic activ ity by gonadotropins [Pandey, 2005]. The stimulated levels of testosterone production by ANP, BNP, and gonadotropins were comparable, whereas CNP has been found to be a weak stim ulator of testosterone production in Leydig cells [Pandey, 2005]. Moreover, testicular cells contain immunoreactive ANP-like materials and a high density of natriuretic peptide recep tor-A (NRP-A) [Pandey, 2005]. These findings suggest that CNH play paracrine and/or autocrine roles in testis and testicular cells. Furthermore, the presence of ANP and its receptors has been reported in ovarian cells, too. Increasing evidence strongly support that CNH are present and probably locally synthesized in ovarian cells of different mammalian species and also play an important physiological role in stimulating estradiol synthe sis and secretion in the female gonad [Pandey, 2005] [Gutkowska et al., 1993] [Vollmer et al., 1988] [Ivanova et al., 2003]. However, further studies are necessary in order to clarify completely the role played by CNH in the regulation of gonadal func tion and also to assess the inter-relationship between heart endocrine function and gonadal function in humans.

The huge amount of data reported above strongly supports the hypothesis that CNH are active components of the body integrative network

that includes nervous, endocrine and immune systems. According to this hypothesis, the heart can no longer be seen as a passive automaton driven by nervous, endocrine or hemodynamic inputs, but as a leading actor on the stage. Thus, CNH, together with other neuro-hormonal factors, regulate cardiovascular hemodynamics and body fluid and electrolyte homeostasis, and probably modulate inflammatory response in some districts, including the car diovascular one. This hypothesis implies that there are two counteracting systems in the body: one has sodium-retaining, vasoconstrictive, thrombophylic, pro-inflamma tory and hypertrophic actions, while the second one promotes natriuresis and vasodi latation, and inhibits thrombosis, inflammation and hypertrophy. CNH are the main effectors of the latter system, and work in concert with NO, some prostaglandins, and other vasodilator peptides (such as bradykinin) [Chatterjee et al., 1999] [Ruskoaho et al., 1997] [Gyurko et al., 2000] [Tanaka et al., 2005] [Booz, 2005]. Under physiological condi tions, the effects of these two systems are well balanced via feedback mechanisms, and result in a beat-to-beat regulation of cardiac output and blood pressure in response to endogenous and exogenous stimuli. In patients with HF, the action of the first system is predominant, as a compensatory mechanism, initially, that progressively leads to detrimental effects.

The knowledge so far accumulated regarding CNH suggests that a continuous and intense information exchange flows from the endocrine heart system to nervous and immunological systems and to other organs (including kidney, endocrine glands, liver, adipose tissue, immunocompetent cells) and vice versa (Fig. 3.16). From a pathophys iological point of view, the close link between the CNH system and counterregulatory systems could explain the increase in circulating levels of CNH in some non-cardiac-relat ed clinical conditions. Increased or decreased BNP levels were frequently reported in acute and chronic respiratory diseases [Kruger et al., 2004a] [Pruszczyk et al., 2003] [Kucher et al., 2003b] [ten Wolde et al., 2003b] [Ando et al., 1996] [Maeder et al., 2003] [Nagaya et al., 2000a] [Leuchte et al., 2004b] [Leuchte et al., 2004a], some endocrine and metabolic diseases [Kohno et al.,

1993] [Bernstein et al., 1997] [Parlapiano et al., 1998] [Schultz et al., 2004] [Jensen et al., 1997] [Sugawara et al., 1988] [Fujio et al., 1989] [Opocher et al., 1990] [Lapinski et al., 1991] [Cappuccio et al., 1989] [Straub et al., 1996] [Bhalla et al., 2004b], liver cirrhosis [Villa et al., 1995] [Salo et al., 1996] [Henriksen et al., 2003], renal failure [Vesely, 2003] [Henriksen et al., 2003], septic shock, chronic inflam matory diseases [McCullough et al., 2004b] [Castillo et al., 2004b] [Witthaut et al., 2003] [Takemura et al., 1998] [Palladini et al., 2003b], subarachnoid hemorrhage [McGirt et al., 2004] [Fukui et al., 2004] [Kurokawa et al., 1996], and some paraneo plastic syndromes [Mimura et al., 1996] [Marchioli and SL, 1997] [Johnson et al., 1997]. In addition, any myocardial damage leading to the release of sarcoplasma constituents (including CNH) in extracellular fluid, for instance that due to cardiotoxic agents [Hayakawa et al., 2001a] [Suzuki et al., 1998] [Nousiainen et al., 1998b] [Nousiainen et al., 1999b] [Okumura et al., 2000], cardiac trauma or ischemic necrosis [Mukoyama et al., 1991b] [Morita et al., 1993b], also causes an increase in plasma concentration of CNH.

Furthermore, the inter-relationships between the CNH system and proinflammatory cytokines suggest that cardiac hormones play an important role in mechanisms respon sible for cardiac and vascular adaptation, maladaptation and remodeling in response to various physiological and pathological stimuli [Tenhunen et al., 2005] [Walther et al., 2003] [Glembotski et al., 1993] [Mukoyama et al., 1991b]. Elevated BNP levels in extra-cardiac diseases reveal an endocrine heart response to a "cardiovascular stress" (Fig. 3.17). Indeed, studies reported that plasma BNP concentration is an independent risk factor for mortality (cardiac and/or total) in pul monary embolism [Kruger et al., 2004a] [Kucher et al., 2003b] [ten Wolde et al., 2003b] and hypertension [Nagaya et al., 2000a], renal failure [Clerico and Emdin, 2004a] [Vesely, 2003] [Henriksen et al., 2003], sep tic shock [McCullough et al., 2004b], amyloidosis [Palladini et al., 2003b], and diabetes mellitus [Bhalla et al., 2004b] (see Chapter 6 for more details). According to this hypothesis, a BNP assay should be considered as a marker of cardiac stress (Fig. 3.17).

In conclusion, CNH share a powerful action on the cardiovascular system, including diuretic, natriuretic and vasodilator effects and an inhibitory action on ventricular myocyte contraction, as well as on remodeling and inflammatory processes of myocardi um and smooth muscle cells. Furthermore, CNH exert a protective effect on endothelial function by decreasing shear stress, modulating coagulation and fibrinolysis pathways, and inhibiting platelet activation. They can also inhibit the vascular remodeling process as well as coronary restenosis post-angioplasty. These effects can be explained, at least in part, by the inhibition of control systems, including the sympathetic nervous system, the RAAS, the vasopressin/antidiuretic hormone system, the endothelin system, cytokines and growth factors. Finally, the endocrine action of ANP and BNP is potentiated at the periphery (target tissues) by the paracrine action of other members of the peptide natri uretic family, such as CNP (in the vascular tissue) and urodilatin (in renal tissue). Finally, some experimental studies performed in KO mice suggest a distinct patho physiological role for BNP in respect to ANP [LaPointe, 2005]. While BNP KO mice are no different from control mice with regard to blood pressure, urine volume, and urinary electrolyte excretion, they have more extensive ventricular fibrosis, accompanied by increased transforming growth factor-b3 (TGF-b3) and collagen mRNA [LaPointe, 2005]. These data suggest that BNP may function more as an autocrine/paracrine inhibitor of cell growth in the heart; while ANP may be considered as a traditional circulating hormone with pro nounced diuretic, natriuretic, and antihypertensive effects.

1.5 Natriuretic Peptide Receptors and Intracellular Second Messenger Signaling

Cardiac natriuretic hormones share their biological action by means of specific recep tors (NPR), which are present within the cell membranes

of target tissues. Three different subtypes of NPRs have so far been identified in mammalian tissues [Pandey, 2005] [Anand-Srivastava and GJ, 1993] [Kuhn, 2004].

1.5.1 Genes Encoding for NPRs

NPR1 is the gene coding the NPR-A receptor (natriuretic peptide receptor A/guany late cyclase A) and it is located on 1q21-q22 spanning 15,534 bp with 22 exons. The rel ative mRNA of 3,805 bp leads to a protein of 1,061 amino acids. The NPR-B receptor (natriuretic peptide receptor B/guanylate cyclase B) is codified by the gene NPR2. This gene of 17,303 bp is on chromosome 9 (9p21-p12) and it is organized in 22 exons, which can give two types of mRNA. NPR2 Ia is an mRNA of 3,482 bp that has a 71 nucleotide insertion relative to isoform b, which results in a dif ferent, and shorter (995 aa), carboxy-terminus that may disrupt the guanylyl cyclase activity. NPR2 Ib (3,411 bp, 1,047 aa) does not include the alternate exon found in iso form a, and thus isoform b contains a longer carboxy-terminus. The natriuretic peptide receptor C gene, also named NPR3, is on 5p14-p13 and spans 74,698 bp (8 exons), giv ing an mRNA of 1,753 bp that is translated into a protein of 540 amino acids.

1.5.2 Biological Function of NPRs

NPR-A and NPR-B are generally considered to mediate all known biological actions throughout the guanylate cyclase (GC) intracellular domain, while the third member of the natriuretic peptide receptor family, the NPR-C receptor, does not have a GC domain (Figs. 3.18, 3.19 and 3.20). The GC receptors for ANP/BNP (NPR-GC-A) and CNP (NPR-GC-B) belong to a fam ily of seven isoforms of transmembrane enzymes (from GC-A to GC-G), which all con vert guanosine triphos-

phate into the second messenger cyclic 3',5'-guanosine monophos phate (cGMP) [Anand-Srivastava and GJ, 1993].

Although partly homologous to soluble GC, the receptor for NO, the membrane GCs share a different and unique topology. The single transmembrane span domain divides the protein structure into an extracellular ligand binding domain and an intracellular region consisting of a protein kinase-homology domain, an amphipathic helical or hing region, and a cyclase-homology domain [Kuhn, 2004] (Figs. 3.18, 3.19 and 3.20). The cyclase homology domain represents the catalytic cGMP synthesizing domain. The function of the intracellular region consisting of a protein kinase-homology domain is incom pletely understood. Although it probably binds ATP and contains many residues con served in the catalytic domain of protein kinases, kinase activity has not been detect ed [Kuhn, 2004]. It represses the enzyme activity of the catalytic cGMP-synthesizing domain and at the same time is necessary for its ligand-dependent activation [Mimura et al., 1996]. The coiled-coil hing region is involved in receptor dimerization, which is also essential for the activa tion of the catalytic domain [Kuhn, 2004].

The cGMP produced modulates the activity of specific downstream regulatory pro teins, such as cGMP-regulated phosphodiesterases, ion channels and cGMP-dependent protein kinases type I (PKG I) and type II (PKG II) (Fig. 3.20). These proteins should be considered to be third messengers, which are differentially expressed in different cell types, ultimately modifying cellular functions [Lohmann et al., 1997] [Pfeifer et al., 1999]. This specific action of CNH on target tissues depends essentially on two different mechanisms.

The physiological expression of NPR-A and NPR-B differs quite significantly in human tissues (Fig. 3.21). NPR-A is found in abundance in larger, conduit blood vessels, whereas the NPR-B is found predominantly in the central nervous system [Ahluwalia et al., 2004a]. Both receptors have been localized in adrenal glands and kidney [Ahluwalia et al., 2004a]. On the other hand, several studies indicate that phos-

phorylation of the kinase homology domain is a crit ical event in the regulation of NPRs [Potter and Hunter, 1998a] [Potter and Hunter, 1998b] [Potter, 1998].

The affinity for ANP, BNP and CNP also varies greatly among the different NPRs. ANP shows a greater affinity for NPR-A and NPR-C, and CNP for NPR-B, while BNP shows a lower affinity for all NPRs compared to the other two peptides (Fig. 3.21). Activation of the GC-linked NPRs is incompletely understood [Fan et al., 2005a]. NPR-A and NPR B are homo-oligomers in the absence and presence of their respective ligands, indicat ing that receptor activation does not simply result from ligand-dependent dimerization [Chinkers and EM, 1992]. However, ANP binding does cause a conformational change of each monomer closer together [Fan et al., 2005a] [Chinkers and EM, 1992] [Huo et al., 1999] [Labrecque et al., 1999] [Labrecque et al., 2001]. The stoichiometry of the ligand-receptor complex is 1:2 [Ogawa et al., 2004]. Initial in vitro data suggested that direct phosphorylation of NPR-A by protein kinase C mediated its "desensitization" (i.e., the process by which an activated receptor is turned off) [Duda and RK, 1990]. However, subsequent studies conducted in live cells indicated that desensiti zation in response to prolonged natriuretic peptide exposure or activators of protein kinase C results in a net loss of phosphate from NPR-A and NPR-B [Potter, 1998] [Potter and DL, 1992] [Potter and DL, 1994] [Foster and DL, 1998] [Joubert et al., 2001].

Although ligand-dependent internalization and degradation of NPR-A has been intense ly studied by several groups for many years, a consensus understanding of the importance of this process in the regulation of NPRs has not emerged [Joubert et al., 2001]. Early studies conducted on PC-12 pheochromocytoma cells suggested that both NPR-A and NPR-C internalize ANP and that both receptors are recycled back to the cell surface [Pandey et al., 1986]. Other studies, using Leydig, Cos, and 293 cell lines, have reported that ANP binding to NPR-A stimulates its internalization, which results in the majority of the receptors being degraded with a smaller portion being recycled to the

plasma membrane [Pandey et al., 1986] [Pandey, 1993] [Pandey et al., 2000] [Pandey, 2001]. In contrast, other stud ies performed in cultured glomerular mesangial and renomedullary interstitial cells from the rat or Chinese hamster ovary cells reported that NPR-A is a constitutively membrane resident protein that neither undergoes endocytosis nor mediates lysosomal hydrolysis of ANP [Koh et al., 1992a] [Vieira et al., 2001a]. A more study using 293T cells suggested that NPR-A and NPR B are neither internalized nor degraded in response to receptor occupation [Chinkers and EM, 1992]. Fur thermore, this study did not support the hypothesis that down-regulation is responsible for NPR desensitization observed in response to various physiological or pathological stim uli [Joubert et al., 2001].Further studies are necessary to clarify whether or not ANP binding to NPR-A stim ulates its internalization, and whether this process is tissue- and/or species-specific.

It is generally thought that the NPR-C is not linked to GC and so serves as a clearance receptor [Clerico and Emdin, 2004a] [deLemos et al., 2003] [Ruskoaho, 2003a].NPR-C is present in higher concentration than NPR-A or NPR-B in sev eral tissues (especially vascular tissue),and it is known constitutively to internalize CNH [Fan et al., 2005a] (Fig.3.22).However, studies have found that CNH interact with NPR-C to suppress the cAMP concentration by inhibition of adenylyl cyclase [MB, 2005] [Drewett et al., 1992]. Specific binding to NPR-C increases inositol triphosphate and diacylglycerol concentrations by activating phospholi pase C activity or inhibits DNA synthesis stimulated by endothelin, platelet-derived growth factor and phorbol ester by inhibiting MAPK activity,as reviewed [MB, 2005].The NPR-C mediated inhibition of adenylyl cyclase is mediated through Gi (inhibitory guanine nucleotide regulatory) proteins.According to this hypothesis,NPR-C,which is present in large amounts, especially on the endothelial cell wall,may mediate some paracrine effects of CNP on vascu lar tissue [Ahluwalia et al., 2004a] [MB, 2005].However,further studies are necessary to elucidate the possible role of NPR C receptors as modulators of CNH action and/or degradation in peripheral tissues.

1.6 Metabolic Pathways and Circulating Levels of CNH

Atrial natriuretic peptide and BNP are secreted directly from the heart. In the circula tion, CNHs are metabolized via two principal mechanisms: degradation by a membrane-bound endopeptidase (NEP 24.11) and receptor-mediated cellular uptake via NPR-C [Goetze, 2004a] (Fig. 3.22). Some biological characteristics of ANP, BNP and CNP (as well as of their precursors) are summarized in Table 3.3.

1.6.1 ANP Metabolism

Atrial natriuretic peptides are a family of peptides derived from a common precursor, called preproANP, which in humans contains 151 amino acids and has a signal peptide sequence at its amino-terminal end (Fig. 3.11). The pro-hormone is stored in secretion granules of cardiomyocytes as a 126-amino-acid peptide, proANP 1-126 , which is pro duced by cleavage of the signal peptide. When appropriate signals for hormone release are given, proANP 1-126 is further split by some proteases (especially the serine protease corin) [D'Souza et al., 2004] into N-terminal fragment NT-proANP and the COOH-terminal peptide ANP, which is generally considered to be the biologically active hormone, because it contains the cysteine ring (Figs. 3.1 and 3.11).

Studies from the group of Vesely et al. suggested that the NT-proANP can be metab olized in vivo in three peptide hormones with blood pressure-lowering, natriuretic, diuretic and/or kaliuretic properties [Vesely, 2003]. These peptide hormones, numbered by their amino acid sequences, beginning at the N-terminal end of the proANP pro-hormone, include: 1) the peptide proANP 1-30 , also called long-acting natriuretic peptide (LANP); 2) the peptide proANP 31-67 with vessel dilator properties; 3) the peptide proANP 79-98 with kaliuretic properties

[Kapoun et al., 2004]. However, these three peptides do not bind to the same NPRs of CNHs, because they do not have the cysteine ring. Further studies are neces sary to confirm and elucidate the biological action of these putative peptide hormones, as well as their in vivo metabolism.

There is some evidence that ANP is secreted according to a pulsatile pattern in humans [Haak et al., 1990] [Haak et al., 1992] [Nugent et al., 1994] [Pedersen et al., 1999] [Bentzen et al., 2003]. Upon secretion, ANP is rapidly distributed and degraded (the metabolic clearance rate of ANP is on average about 2,000 ml/min in healthy subjects) with a plasma half-life of about 4-6 minutes in healthy adult subjects. In humans, about 50% of the ANP secreted into the right atrium is extracted by the peripheral tissues during the first pass throughout the body [Pilo et al., 1998] [Iervasi et al., 1998] [Clerico and Iervasi, 1995a] [Iervasi et al., 1997]. Furthermore, circulating ANP represents only a small fraction of the total body pool (no more than 1/15) in normal subjects and plasma ANP concentration shows rapid and wide fluctuations in healthy subjects, even at rest in the recumbent position [Pilo et al., 1998] [Iervasi et al., 1998] [Clerico and Iervasi, 1995a] [Iervasi et al., 1997]. The turnover data suggest that circulating levels of ANP may not represent a close estimate of their disposal, and therefore of the activity of the CNH system, as implicitly accepted in physiological or clinical studies in which only the plasma concentration of the hormone is measured, without an estima tion of turnover rate. However, it was demonstrated that ANP clearance mechanisms are constant in the presence of rapid and large changes in endogenous ANP plasma levels induced by atrial and/or ventricular pacing, thus indicating that, at least for studies lasting only a few hours, changes in ANP circulating levels may provide a reliable esti mate of production rate variations [Iervasi et al., 1997].

1.6.2 BNP Metabolism

The biological action, metabolic pathways, and turnover parameters of BNP are not as well known as those of ANP [Goetze, 2004a]. However, it is commonly believed that the BNP turnover is less rapid than that of ANP with a plasma half-life of about 13-20 min utes; indeed, circulating levels of BNP are more stable than those of ANP in adult healthy subjects (Fig. 3.23). Bentzen et al. [Bentzen et al., 2003] analyzed the secretion pattern of ANP and BNP in 12 patients with chronic HF and in 12 healthy adult subjects. ANP and BNP in plasma were determined by radioimmunoassay (RIA) at 2 min intervals during a 2-h period and were subsequently analyzed for pulsatile behavior using the method of Fourier transformation. All patients and healthy subjects had significant rhythmic oscillations in plasma ANP levels, and 11 patients with HF and 10 healthy subjects had significant rhythmic oscillations in plasma BNP levels [Bentzen et al., 2003]. The ampli tude of the main frequency was considerably higher in patients than in healthy sub jects, but the main frequency did not differ significantly between patients and healthy subjects for either ANP or BNP. Patients with HF demonstrated pulsatile secretion of ANP and BNP with a much higher absolute amplitude, but with the same main fre quency as healthy subjects [Bentzen et al., 2003]. Finally, rhythmic oscillations in plasma ANP lev els of healthy subjects showed significantly higher mean amplitude, but not fre quency, than those of BNP [Bentzen et al., 2003].

A very small amount of immunoreactive BNP has been found in urine [Totsune et al., 1996] [Ng et al., 2004], but the precise mechanism of renal excretion has not yet been fully clarified. In contrast to BNP, the biologically active peptide, other proBNP-derived inactive fragments also cir culate in plasma. These fragments are commonly referred to as "N-terminal proBNP" (NT-proBNP), but the molecular heterogeneity also includes the intact precursor, par ticularly in patients with HF [Goetze, 2004a] [Shimizu et al., 2003b]. Cardiac secretion of proBNP

and its N-termi nal fragments has been demonstrated by blood sampling from the coronary sinus [Hunt et al., 1997]. Some data suggest that the major part of proBNP produced in myocardiocytes is appar ently processed prior to release [Goetze, 2004a]; however, intact proBNP peptide was also found in plasma of patients with HF as well as healthy adult subjects [Goetze, 2004a] [Hunt et al., 1997] [Goetze et al., 2005].

A study, employing a new method for the total and equimolar assay of all proB NP-related peptides (i.e., intact proBNP precursor plus NT-proBNP concentrations), found comparable peripheral concentrations of BNP (measured by immunoradiomet ric assay) and proBNP-related peptides in patients with HF [Goetze et al., 2005]. Moreover, the BNP concentration (median 125 pmol/l) was higher than that of total proBNP (103 pmol/l) in the coronary sinus, suggesting that the cardiac secretion of these two peptides could be different [Goetze et al., 2005].Alternatively, this finding could also reflect some difference in peripheral elimination of peptides because total proBNP concentration is significantly higher in the pulmonary artery than the aortic root in patients with right ventricular failure [Goetze et al., 2004b].

While NEP enzymes are mainly involved in natriuretic peptide inactivation in vivo, the degradation of BNP seen in vitro is most likely due to other enzymes, such as peptyl arginine aldehyde proteases, kallikrein, and serine proteases [Belenky et al., 2004b]. However, the role of these enzymes in the degradation of BNP in vivo is unclear.

A study reported that both the BNP and total proBNP concentrations were increased more than 2-fold in the coronary sinus compared to the inferior caval vein (BNP-32: median 125 pmol/l, range 21-993 vs median 52 pmol/l, range 7-705; proBNP: median 103 pmol/l, range 16-691 vs 47 pmol/l, 8-500) [Goetze et al., 2005]. These findings are in accordance with previous studies suggesting that the cardiac gradient for BNP secre tion (as estimated by the difference between BNP concentration in coronary sinus and inferior caval vein) ranges from 1.6-fold

to 2.9-fold [Shimizu et al., 2003b] [Mukoyama et al., 1991a] [Mizuno et al., 2001] [Kalra et al., 2003a]. Taking these studies as a whole, ANP and BNP share a similar peripheral extraction value (of about 30-50%). Further studies are necessary to elucidate the metabolism of BNP and in particular the predominant form of the circulating BNP-related peptides.

1.7 CNH Genes and Cardiovascular Diseases

Since CNH have a potent diuretic antihypertensive action, and the impaired action of the peptides may cause hypertension, their genes may be candidates for cardiovascu lar disease, especially arterial hypertension. Furthermore, transgenic animals (espe cially mice), overexpressing CNH or knockout for ANP/BNP genes or their specific receptors, have been used to evaluate the pathophysiological role of the CNH system in cardiovascular diseases [Nakayama, 2005].

In transgenic mice with overexpression of ANP and BNP in liver, plasma ANP and BNP levels are from 10- to 100-fold higher than in control mice, with a blood pressure of 20-25 mmHg lower. These mice also have lighter hearts, but with the same cardiac out put and rate, than controls [Nakayama, 2005] [Steinhelper et al., 1990a] [Ogawa et al., 1994a] [John et al., 1995a]. The BNP-overexpressing mice show the same hemodynamic changes; on the other hand, ANP KO mice develop NaCl-sensitive hyper tension [Nakayama, 2005]. Transgenic mice overexpressing the NPRA gene have also been created; these animals have a lower blood pressure than wild-type mice [Nakayama, 2005]. The correspon ding KO mice show an increase in blood pressure compared with controls (on average 10 mmHg in heterozygous and 30 mmHg in homozygous animals), which is not affect ed by NaCl intake [Ogawa et al., 1994a] [John et al., 1995a]. These data suggest a different pathophysiological mech anism for hypertension between KO mice for the ANP gene and its specific receptor; this difference does

not yet have an explanation [Nakayama, 2005]. NPRC heterozygous KO mice do not show blood pressure variation, whereas homozygous mice show on average a decrease in blood pressure of about 8 mmHg [Nakayama, 2005].

The function of natriuretic peptides was also studied after induction of myocardial infarction in KO mice lacking the natriuretic peptide receptor guanylyl cyclase-A, the receptor for ANP and BNP [Nakanishi et al., 2005]. KO and wild-type mice were subjected to left coronary artery ligation and then followed-up for 4 weeks. KO mice showed significantly higher mortality because of a higher incidence of acute HF, which was associated with dimin ished water and sodium excretion and with higher cardiac levels of mRNAs encoding ANP, BNP, TGF-b1, and type I collagen. By 4 weeks after infarction, left ventricular remodeling, including myocardial hypertrophy and fibrosis, and impairment of left ventricular systolic function were significantly more severe in KO than wild-type mice [Nakanishi et al., 2005]. These data confirm that the CNH system has powerful anti-remodeling properties on ventricular cardiomyocytes.

In years, molecular genetic techniques have been introduced in etiological stud ies of polygenetic diseases, in linkage studies, in sib-pair linkage studies of various candi date genes, and in related studies [Nakayama, 2005] [Clerico, 2003]. The association between some abnormalities in genes, coding for the CNH and their receptors, and some cardiovascular (in particular hypertension) and metabolic (such as diabetes mellitus) diseases has been tested in a large number of clinical studies (see also Chapter 6 for more details). To date, the results obtained are conflicting and seem to depend strictly on the ethnic population of the study. The restriction fragment length polymorphism for the enzyme HpaII, located in intron 2 of NPPA (polymorphism also called Sma I), was reported to be more common in hypertensive African-Americans than in normotensive black controls [Rutledge et al., 1995]; these data were then confirmed in two [Ramasawmy et al., 1993] [Schorr et al., 1997], but not a third [Widecka et al., 1998], Caucasian popula tions.

Furthermore, another study found that the HpaII polymorphism was not asso ciated with hypertension in the Chinese population of Hong Kong [Cheung et al., 1999].

Regarding other NPPA polymorphisms, Japanese studies reported that both G1837A and T2238C polymorphisms are associated with essential hypertension [Rahmutula et al., 2001], while only a marginally significant association was found with an ANP polymorphism locat ed in the 5'-untranslated region (C664G) [Kato et al., 2000].

Several allelic variants have also been described for genes coding for CNH recep tors (see the revew by Nakayama [Nakayama, 2005] for a more detailed discussion of this topic). The clearance receptor for natriuretic peptides (NPR-C) is highly expressed in adipose tissue, and its bi-allelic (A/C) polymorphism was detected at position -55 in the conserved promoter element named P1. This variant of the NPR-C P1 promoter is associated with lower ANP levels and higher systolic blood pressure and mean blood pres sure in obese hypertensives: the C(-55) variant, in the presence of increased adiposity, might reduce plasma ANP through increased NPR-C-mediated ANP clearance, con tributing to higher blood pressure [Sarzani et al., 1999].

In the Japanese population an insertion/deletion (GCTGAGCC) polymorphism has been identified in the 5'-flanking region of the NPRA gene that is associated with essen tial hypertension and left ventricular hypertrophy [Nakayama et al., 2000a]. Another insertion/deletion polymorphism is on the 3'-untranslated region of the NPRA gene, on exon 22, and it seems to be associated with familial hypertension [Lucarelli et al., 2001]. However, these data should be confirmed in larger studies, including other ethnic populations.

3.9 An Integrated Neuro-Hormonal System Regulates Vascular Function

Endothelial cells release an array of vasoactive mediators that alter

the tone and growth of the underlying smooth muscle and regulate the reactivity of circulating white blood cells, erythrocytes and platelets. These endogenous factors are usually called endothe lium-derived vasorelaxant mediators [Ahluwalia and AJ, 2005]. Moreover, it appears that alterations in the capacity of the endothelium to release some mediators in response to pathophysiolog ical stimuli (the so-called endothelium dysfunction) are a major precipitating factor in many cardiovascular diseases. Perhaps the most important of these paracrine medi ators are prostacyclin (PGI 2) and nitric oxide (NO). More , a third endotheli um-derived vasorelaxant mediator has been described [Ahluwalia and AJ, 2005]. This is termed endothe lium-derived hyperpolarizing factor (EDHF) because it elicits a characteristic smooth muscle hyperpolarization and relaxation. Much attention has focused on identifying EDHF(s), with diverse candidates, including cytochrome P450 metabolites, KC ions, anandamide and hydrogen peroxide [Ahluwalia and AJ, 2005]. However, the role of each of these as EDHF remains unsubstantiated.

There is now compelling evidence that CNH (and especially CNP) act as EDHFs in some vascular beds [Ahluwalia and AJ, 2005] [Han and Hasin, 2003] [Houben et al., 2005] [Scotland et al., 2005]. Indeed, numerous studies have demonstrated that ANP, BNP and CNP bind to NPR-A and NPR-B receptors on vascular smooth muscle cells (either freshly isolated or in culture), stimulate cGMP accumulation, and cause a dose dependent vasodilation [Han and Hasin, 2003] [Houben et al., 2005] [Scotland et al., 2005]. This increase in cGMP causes vasodilatation by reducing intracellular calcium levels, as occur when cGMP accumulation is stimulated by NO and its analogs .

It is theoretically conceivable that ANP and BNP act like hormones in vascular tis sue by reaching the smooth muscle cells from the circulation after secretion by the heart, while CNP shows a paracrine action, being secreted by endothelial cells [Woodard et al., 2002] [Chen and Burnett] [Yasuda et al., 2002] [Qian et al., 2002] (Fig. 3.15). However, Casco et al. [Casco et al., 2002] demonstrated the existence of a com-

plete CNH system (including the production and secretion of ANP, BNP and CNP) in ather osclerotic human coronary vessels by means of in situ hybridization and immunocy tochemistry methods. In particular, the expression of mRNAs of ANP, BNP and CNP, measured by RT PCR, tended to be increased in macroscopically diseased arteries com pared to normal vessels, although only the values for BNP expression were significant ly different [Casco et al., 2002]. This study suggests that the CNH system is involved in the pathobi ology of intimal plaque formation as well as in vascular remodeling in humans. Some studies indicated that there are complex interactions even among CNH them selves. Nazario et al. [Nazario et al., 1995] reported that ANP and BNP can stimulate CNP production through a guanylate cyclase receptor on endothelial cells. As a result, vasodilatory, and anti-mitogenic effects of ANP and BNP in the vasculature could occur in part through CNP production and subsequent action if these interactions occur in vivo. In other words, ANP/BNP and CNP paracrine system should share a synergic action on vascu lar tissues.

Several studies have demonstrated complex interactions betwen CNH and the other endothelium-derived vasorelaxant mediators [Ahluwalia and AJ, 2005]. Indeed, evidence from cellular, ani mal, and human studies suggests that all CNH are able to stimulate NO production by endothelial NO synthase (eNOS); this effect is probably mediated by clearance recep tor NPR-C [Houben et al., 2005]. Stimulation of this NPR-C receptor results in decreased cAMP levels by adenyl cyclase inhibition through an inhibitory guanine nucleotide-regulating pro tein [Houben et al., 2005]. Furthermore, ANP treatment increases renal and cardiac NO synthesis in rats [los Angeles Costa et al., 2004]. On the other hand, NO, released from endothelial cells, negatively modulates ANP secretion from atrial myocytes, induced by mechanical stretch in perfused rat heart preparation [**?**]. Furthermore, ANP expression is markedly upregulated in eNOS -/- mice, and exogenous ANP restores ventricular relaxation in wild-type mice treated with NOS inhibitors [**?**]. These data suggest that the CNH and NO systems are linked by a negative feedback mechanism. Finally, CNH (and espe-

cially CNP) mimic many of the anti-atherogenic actions of PGI 2 and NO [Ahluwalia and AJ, 2005]. This gives rise to the pos sibility that CNP might compensate for the loss of these mediators in cardiovascular pathologies to restore the vasodilator capacity of the endothelium, in addition to its anti-adhesive and anti-aggregatory influences.

CNH also strongly interact with the effectors of counter-regulatory systems at the vas cular tissue level [McGrath and de Bold AJ, 2005] [Clerico and Emdin, 2004a] [Clerico, 2002a] [deLemos et al., 2003] [Ruskoaho, 2003a] [Ventura et al., 2002] [Vatta et al., 1996] [Vatta et al., 1997] [Fermepin et al., 2000] [Rocca et al., 2001] [Tsukagoshi et al., 2001] [Kiemer et al., 2002b] [Weber et al., 2003] [Kapoun et al., 2004] [Vollmar, 2005]. In particular, interactions between CNH and ET-1 also appear to be important physiologically; indeed, the vascular effects of CNH are directly opposite to those of ET-1 [Ahluwalia and AJ, 2005] [Han and Hasin, 2003]; in particular, ET-1-induced vasocon striction and myocyte hypertrophy is inhibited by CNH. While CNP has little natri uretic and diuretic action compared to ANP or BNP, it is capable of modulating the vascular effects of the local RAAS by opposing potent vasoconstriction to angiotensin II [Han and Hasin, 2003]. CNP not only functionally antagonizes ET-1 and angiotensin II, but it also directly modulates ET-1 [Kohno et al., 1992] and angiotensin II [Davidson et al., 1996a] synthesis. On the other hand, ET 1 induces an increase in the number of endothelial cells that secrete CNP [Evans et al., 2002]. There fore, the parallel production and activity of vasodilator CNP and vasoconstrictors such as ET-1 and angiotensin II allows for tight local regulation of these vasoactive peptides and thus blood flow [Ahluwalia and AJ, 2005] [Han and Hasin, 2003] [Evans et al., 2002].

Furthermore, the inter-relationships between the CNH system and proinflammatory cytokines suggest that cardiac hormones play an important role in mechanisms respon sible for cardiac and vascular adaptation, maladaptation and remodeling in response to various physiological and pathological stimuli [Tenhunen et al., 2005] [Walther et al.,

2003] [Glembotski et al., 1993] [Mukoyama et al., 1991b]. The identification of CNP as an EDHF, combined with its expression in endothelial cells, indicates that CNP is suited to modulate the activity of circulating cells, particularly leukocytes and platelets. Moreover, inflammatory stimuli such as IL-1b, TNF and lipopolysaccharide [Suga et al., 1993a] stim ulate the release of CNP from isolated endothelial cells. As a result, modulation of the biological activity of CNP is likely to have a profound influence on the development of an inflammatory response. Certainly, an anti-atherogenic activity of CNP fits with the cytoprotective, anti-inflammatory actions of NO and PGI 2 , the other major endotheli um-derived vasorelaxants [Ahluwalia and AJ, 2005] [Han and Hasin, 2003] [Houben et al., 2005] [Scotland et al., 2005] [Suga et al., 1993a].

From a clinical point of view, it is important to note that exogenous application of CNP in situations where endothelial NO production is compromised might be therapeutic in disorders that are associated with endothelial dysfunction. For example, overex pression of CNP by adenoviral-gene delivery in veins dramatically reduces the lumi nal narrowing (neointimal hyperplasia) that develops when it is grafted to the carotid artery, thereby retaining patency of the graft [Ohno et al., 2002]. CNH, including CNP, also suppress the production of pro-inflammatory cyclooxygenase 2 metabolites in isolated cells [Vollmar and AK, 2001] [Kiemer et al., 2002a]. Other studies demonstrated a direct effect of CNP on immune-cell recruitment in vivo [Ahluwalia and AJ, 2005] [Scotland et al., 2005]. Therefore, like NO, endothelial CNP (like ANP and BNP) exerts a pro tective anti-inflammatory effect [Vollmar et al., 1995] [Vollmar and Schultz, 1995] [Vollmar and AK, 2001] [Ahluwalia and AJ, 2005] [Scotland et al., 2005] [Kiemer et al., 2005]. This inhibitory effect of CNH on leukocytes indicates that these peptides modulate the expression of adhesion mol ecules on either the endothelium or leukocytes.

Several data support the thesis that CNH (and especially CNP) are important, endoge nous, anti-atherogenic mediators. CNP is a potent

inhibitor of vascular smooth muscle migration and proliferation that is stimulated by oxidized LDL [Kohno et al., 1992]. CNP also inhibits the proliferation of vascular smooth muscle [Furuya et al., 1991], and enhances endothelial cell regen eration in vitro and in vivo [Ohno et al., 2002]. The observation that CNP alters leukocyte-endothe lial interactions indicates that it might also affect platelet function. In accordance with this, thrombus formation is suppressed significantly in the presence of CNP [Suga et al., 1993a], which indicates that inhibition of coagulation might contribute to the vasoprotective proper ties of this peptide. Observations that CNP blocks platelet aggregation, induced by throm bin, confirm that endothelium-derived CNP also exerts an anti-thrombotic effect [Ahluwalia and AJ, 2005].

All the above-mentioned studies demonstrate that CNH (and especially CNP) exert a protective effect on endothelial function by decreasing shear stress, modulating coagulation and fibrinolysis pathways, and inhibiting platelet activation (Fig. 3.15). They can also inhibit the vascular remodeling process as well as coronary restenosis post-angioplasty [Ma et al., 2004] [Chen and Burnett] [Morishige et al., 2000] [Takeuchi et al., 2003] [Yasuda et al., 2002] [Qian et al., 2002] [Nakanishi et al., 2005] [Ahluwalia and AJ, 2005] [Ohno et al., 2002] [Kiemer et al., 2005]. These vasoprotective actions should be considered as a result of complex inter-relationships between the CNH system and both the synergic (including NO, PGI 2 , and other endothelium-derived vasoactive mediators) and the counter-regulatory systems (including endothelins, RAAS, cytokines, and growth factors).

1.8 Summary and Conclusion

Natriuretic peptides (including ANP, BNP, CNP, DNP and urodilatin) constitute a fam ily of peptide hormones and neurotransmitters, sharing a similar peptide chain, char acterized by a cysteine bridge (Fig. 3.1). The physiological relevance of these peptides is well demonstrated

by their presence since the first dawning of life, from unicellular to pluricellular organisms, including plants and all animals. Furthermore, their genes have been repeatedly doubled during evolution, starting from an ancestral gene, thus sug gesting that these peptides are indispensable for life (Fig. 3.6).

CNH have powerful physiological effects on the cardiovascular system, body fluid, and electrolyte homeostasis. These effects can be explained at least in part by the inhibition of counter-regulatory systems, including the sympathetic nervous system, RAAS, the vaso pressin/antidiuretic hormone system, the endothelin system, cytokines and growth factors. The endocrine action shared by plasma ANP and BNP can be enhanced by natri uretic peptides produced locally in target tissues (paracrine action). Indeed, endothe lial cells synthesize CNP, which exerts a paracrine action on vessels. Moreover, renal tubular cells produce urodilatin, another member of the peptide natriuretic family, which shows powerful diuretic and natriuretic properties. Genes for natriuretic peptides (including ANP, BNP and CNP) are also expressed in the central nervous system, where they likely act as neurotransmitters and/or neuromodulators. Finally, co-expres sion of CNH and their receptors was observed in immunocompetent cells, suggesting that CNH may have immunomodulatory and anti-inflammatory functions in mam mals. Furthermore, CNH are expressed in almost all the body tissues as well as their specific receptors, including organs and tissues not discussed in this chapter, such as gut [Bosc et al., 2000], skeletal [Vollmar and AK, 2001] and ocular [Rollin et al., 2004] tissues. In all tissues, CNH could also act as a local mediator or paracrine effector of tissue-specific functions.

These data, taken as a whole, strongly suggest that natriuretic peptides constitute a family sharing endocrine, paracrine and autocrine actions and neurotransmitter and immunomodulator functions. Therefore, it can be hypothesized that the CNH system is closely related to the other regulatory systems (nervous, endocrine and immuno logical) in a biological hierarchical network (Fig. 3.16) [Barabasi and ZN, 2004]

[Clerico et al., 2006b].

1.9 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.

1.9.1 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., 1986b]. 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., 1987b].

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.

1.9.2 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., 2004b], 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].

1.10 BNP and NT-proBNP

1.10.1 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

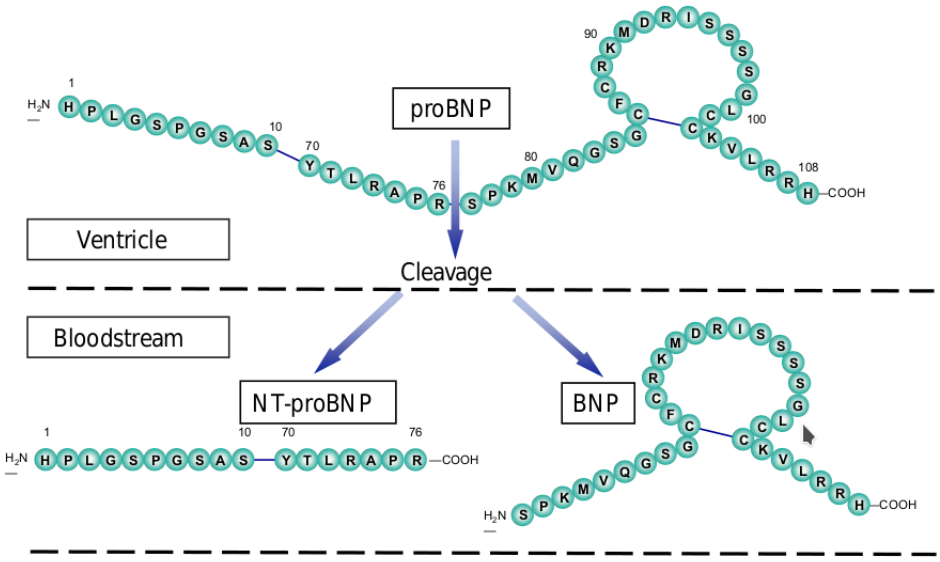

Figure 4: Secretion of BNP and NtproBNP

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 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]

1.10.2 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., 2002b]. 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 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%). 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., 2003b]

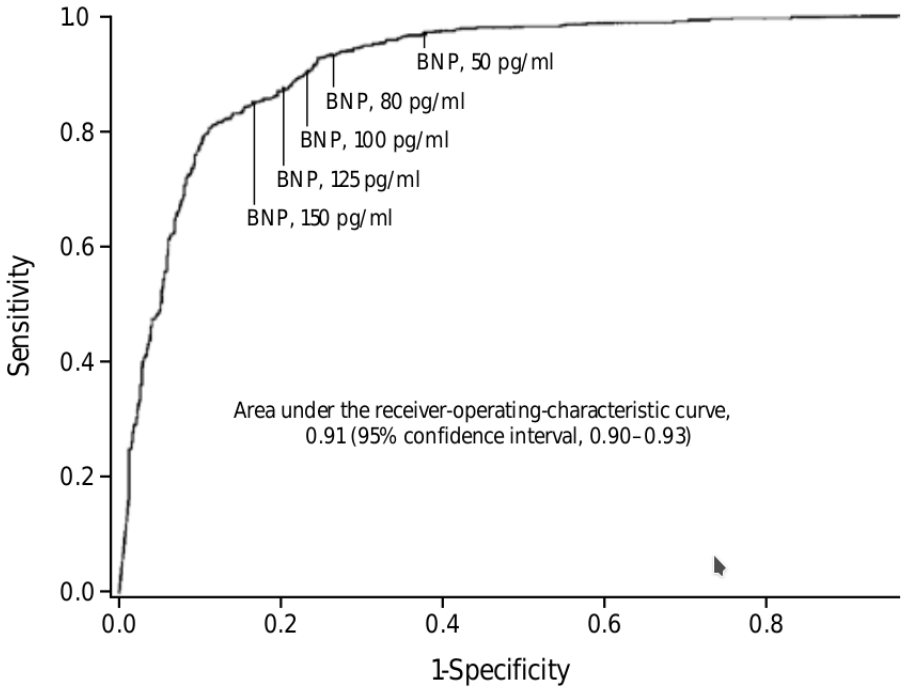

Figure 5: ROC curves for BNP in the diagnosis of heart failure at the emergency department.

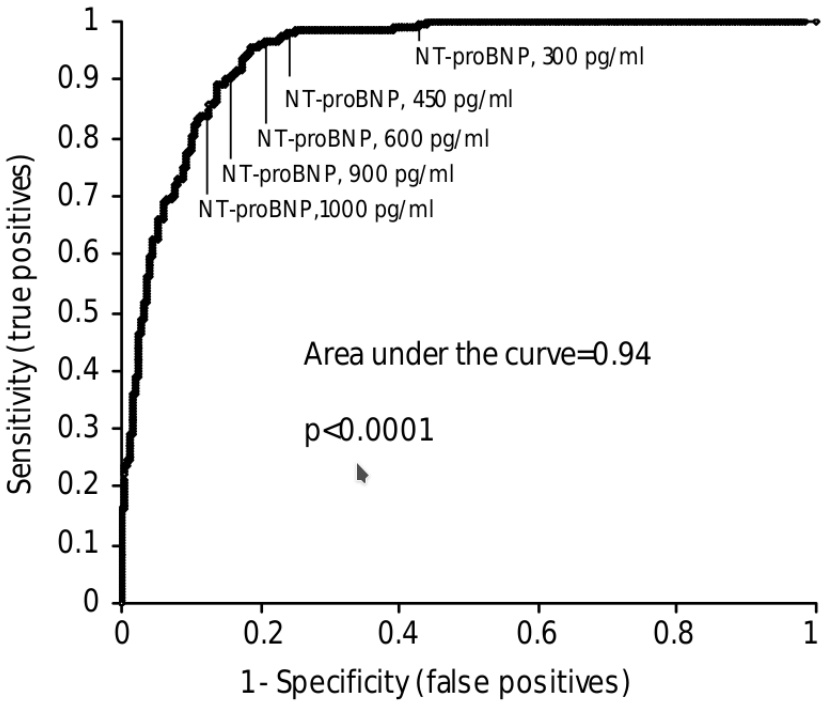

Figure 6: ROC curves for NTproBNP in the diagnosis of heart failure at the emergency department.

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., 2002b] 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]

1.10.3 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., 2004b, Wang et al., 2004c] 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., 2004b] 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 [Arden et al., 1995] 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., 2004b, Wu et al., 2003b] 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.

2 Clinical Considerations and Applications in Cardiac Diseases

2.1 Circulating Levels of Cardiac Natriuretic Hormones

2.1.1 Physiological Considerations and Clinical Interpretation

2.1.2 Influence of Age and Gender

The circulating levels of CNH are regulated or modified by several physiological factors (such as circadian variations, age, gender, exercise, body posture, and water immersion), eating habits (especially sodium intake), clinical conditions (Table 5.1), and drugs (including corticosteroids, sex steroid hormones, thyroid hormones, diuretics, angiotensin-converting enzyme [ACE] inhibitors, and adrenergic agonists and antagonists) [Bold et al., 2001] [Clerico, 2002b] [Ruskoaho, 2003b] [de Lemos et al., 2003] [Clerico and Emdin, 2004b] [Goetze, 2004b].

The wide variations of circulating levels of CNH in adult healthy subjects in relation to aging and gender could have a particular clinical relevance [Clerico et al., 2002] [Redfield et al., 2002] [Vasan et al., 2002] [Wang et al., 2002] (Figs. 5.1 and 5.2, Table 5.2). Indeed, Vasan et al. [Vasan et al., 2002] demonstrated that the diagnostic accuracy of CNH assay for community screening is gender-dependent.

In order to explain these variations, the possible influence of sex steroid hormones on the CNH system, as well as the modification of the cardiovascular system with aging, should be taken into account [Friesinger,

1999] [Bold et al., 1996] [de Bold, 1999] [Maffei et al., 2001]. According to these mechanisms, the higher CNH values of women during the fertile adult period could be explained by the physiological stimulation of female sex steroid hormones. In particular, the BNP concentration is on average 36% higher in women than in men aged less than 50 years [Clerico et al., 2002] (Figs. 5.1 and 5.2, Table 5.2). The increase in CNH with aging may be due to the decline in myocardial function and other organs (including kidney), typical of senescence [Sayama et al., 1999]. In this case, the CNH assay may be considered as a biochemical marker of increased risk of cardiac morbidity in old age [Ueda et al., 2003]. Moreover, the increase in CNH with aging may be due to a decrease in their clearance rate. Indeed, an age modulation of maximum binding capacity of clearance (C-type) receptors for CNH was reported in platelets of elderly persons [Giannessi et al., 2001].

All CNH derive from pre-pro-hormones (i.e., preproANP and preproBNP), containing a signal peptide sequence at the amino-terminal end. The pro-hormones (i.e., proANP and proBNP) are produced by cleavage of signal peptide, and then are further split into inactive longer NH-2 -terminal fragments (i.e., NT-proANP or NT proBNP), and a biologically active shorter COOH-terminal peptide (i.e., ANP or BNP), which are secreted in the blood in equimolar amounts (Figs. 3.11 and 3.12). However, ANP and BNP have a shorter plasma half-life and consequently lower plasma concentration, compared to NT-proANP and NT-proBNP (Table 4.1) [Bold et al., 2001] [Clerico, 2002b] [Ruskoaho, 2003b] [de Lemos et al., 2003] [Clerico and Emdin, 2004b] [Goetze, 2004b] [Clerico et al., 2002] [Clerico et al., 2000b].

Studies on structure-activity relationships have shown the importance for the binding to the specific receptors of the central ring structure of CNH, formed by a disulfide bridge between the two cysteine residues. For this reason, only ANP and BNP, which present the disulfide bridge in the peptide chain, share the typical hormonal activity of CNH, while the NT-proANP and NT-proBNP do not [Bold et al., 2001] [Clerico, 2002b] [Ruskoaho, 2003b] [de Lemos et al., 2003] [Clerico and Emdin,

2004b] [Goetze, 2004b] [Clerico et al., 2002].

Theoretically, setting up an immunoassay for NT-proANP and NT-proBNP should be easier because their plasma concentrations are higher than ANP and BNP [Clerico et al., 2000b]. On the other hand, NT-proANP and NT-proBNP immunoassays may be affected by several analytical problems, mainly concerning the different assay specificities; consequently, very different results are produced by different methods with a large bias [Clerico, 2002b] [Clerico and Emdin, 2004b] [Goetze, 2004b] [Clerico et al., 2000b] (Table 4.1). The different analytical performance might affect the diagnostic accuracy of the assays, in discriminating between subjects with or without cardiac disease [Clerico, 2002b] [Clerico and Emdin, 2004b] [Goetze, 2004b].

The respective advantages of measuring biologically active peptide hormones (ANP and BNP), or inactive peptides (NT-proANP and NT-proBNP) are summarized in Table. The assay of the inactive propeptides better fits the definition of disease marker than the assay of circulating levels of ANP or BNP, which, on the other hand, may be considered a more reliable index of the activation status of the CNH system. Considering the biochemical and physiological characteristics of the different peptides, it is conceivable that ANP is a better marker of acute overload and/or rapid cardiovascular hemodynamic changes than BNP and, especially, than NT-proANP or NT-proBNP [Clerico, 2002b] [Clerico and Emdin, 2004b]. For example, circulating levels of ANP are known to be more affected by body position and decreased to a greater extent by a hemodialysis session in patients with chronic renal failure than those of BNP, while plasma NT-proANP is unchanged [Clerico et al., 2001].

2.1.3 Resistance to the Biological Action of CNH

Patients with chronic HF show increased CNH plasma levels compared to normal subjects (Table 3.1, Fig. 3.13). These findings have been defined the "endocrine paradox" in HF [Goetze, 2004b], i.e., extremely high circulating levels of hormones with powerful natriuretic activity in patients with congestive HF, who show physical signs of fluid retention and vasoconstriction due to a relatively poor biological activity of the CNH system. A blunted natriuretic response after pharmacological doses of ANP and BNP has been observed in experimental models and in patients with chronic HF, suggesting a resistance to the biological effects of CNH, principally natriuresis [Clerico and Iervasi, 1995b] [Charloux et al., 2003] [Clerico et al., 2000a] [Clerico and Iervasi, 1995b] [Cody et al., 1986a] [Saito et al., 1987a] [Komeichi et al., 1995] [Zeidel, 2000]. This resistance syndrome was also demonstrated by in vivo turnover studies using radioactive tracers in patients with HF [Iervasi et al., 1995] [Clerico et al., 2000a].

Resistance to the biological action of CNH could, theoretically, have three different causes (Table 5.3). First, circulating CNH could be, at least in part, inactive. Furthermore, a great fraction of CNH could be inactivated by plasma and tissue proteases before they bind to specific receptors. These two conditions account for all possible mechanisms acting at the pre-receptor level. Second, down-regulation of specific receptors could explain a reduced CNH activity. Finally, some mechanisms can act at postreceptor level, counteracting the biological effects of CNH.

A resistance to the biological action of CNH may be theoretically due to an increase in degradation (turnover) of circulating biologically active peptides. CNH are degraded in vivo and in vitro by several types of proteolytic enzymes, including serin-proteases, peptidyl arginine aldehyde proteases, kallikrein like proteases, and neutral endopeptidases (NEP) [Clerico and Emdin, 2004b] [Goetze, 2004b] [Clerico et al.,

2000b] [Apple et al., 2005] [Panteghini and A, 2004] [Belenky et al., 2004a] [Shimizu et al., 2002] [Shimizu et al., 2003a].

Individual differences in the ability of heart tissue to mature the precursor of CNH peptides, or of peripheral tissues to degrade them, may help to explain why there are some differences in the clinical presentation among patients with HF with similar clinical severity and ventricular function [Goetze, 2004b].However,further studies are necessary to confirm this hypothesis.From a clinical point of view,it is important to note that some drugs sharing an inhibitory action on both NEP and ACE (so called vasopeptidase inhibitors) may have some beneficial effects in patients with arterial hypertension and/or HF because the administration of these durgs can potentiate the biological activity of CNH system by increasing the concentration of biologically active peptides [Trindade and JL, 2001] [Sagnella, 2002] [Dawson and AD, 2002] [Floras, 2002].

It is important to note that renal function can affect the biological action of CNH in different ways .CNH are small peptides freely filtrated by renal glomerulus; the kidneys are probably responsible for about 50% of metabolic clerance rate of plasma ANP and BNP and in this way renal diseases can affect the circulating levels of CNH. Indeed,a decreased renal function greatly increases the plasma CNH concentration and consequently more peptide hormones are available for other target tissues (such as brain,vascular tissue,adrenal gland and so on) [Clerico and Emdin, 2004b].However,luminal perfusion with ANP has been shown to reduce sodium efflux from the inner medullar collecting duct, suggesting that this hormone has also luminal sites of action. As a consequence, a reduction in the filtration can potentially induce renal hypo-responsiveness to CNH. To date, however,ANP has been detected only on tubular basolateral membranes [Charloux et al., 2003].

Some studies suggest that the resistance to biological effects of CNH in HF may be due,in part,to variations in the relative amount of the three different types of natriuretic peptide-specific receptors.In particu-

lar,there could be an upregulation of type C receptors (NPR-C) with a parallel down regulation of type A and B receptors (NPR-A and NPR-B) [Andreassi et al., 2001] [Tsunoda et al., 1988] [Tsutamoto et al., 1993] [Mukkaddam-Daher et al., 1996] [Kuhn et al., 2004]. NPR-A and NPR-B mediate all known hormonal actions of CNH, therefore their down-regulation should induce a deactivation of the CNH system. The upregulation of NPR-C receptors that strongly contribute to the clearance of biologically active peptides could further increase the resistance to CNH in patients with HF [Andreassi et al., 2001].

These findings are well in accordance with the results of in vivo kinetic studies obtained using radioactive tracers in patients with HF [Iervasi et al., 1995] [Clerico et al., 2000a]. Moreover, a study confirmed that mRNA expression levels of ANP, BNP and the NPR-C receptor were markedly increased in human failing hearts [Mukkaddam-Daher et al., 1996]. Reversal of cardiomyocyte hypertrophy during left ventricular assist device support was accompanied by normalization of ANP, BNP and NPR-C mRNA levels and a significant recovery of responsiveness to ANP [Kuhn et al., 2004]. However, [Fan et al., 2004] found that neither NPR-A nor NPR-B were internalized or degraded in response tonatriuretic peptide binding in cultured cells.

Another well-characterized deactivation mechanism is the process by which an activated receptor is turned off, commonly referred to as "desensitization". Phosphorylation of the intracellular kinase homology domain of NRP-A and NPR-B is required for hormone-dependent activation of the receptor, while dephosphorylation at this site causes desensitization. Deactivation of the CNH system via desensitization of NRP-A and NPR-B can occur in response to various pathophysiological stimuli [Fan et al., 2004] [Bryan and LR, 2002] [Potter and T, 1998] .

A peripheral resistance to the biological effects of CNH may play an important role in other clinical conditions, besides HF. For example, NPR-C is also present on cellular membranes of adipose tissue. It

was suggested that the increase in NPR-C receptors observed in obese subjects can in turn increase the peripheral degradation of CNH and consequently blunt the action of the CNH system [Dessi-Fulgheri et al., 1998] [Sarzani et al., 2004]. Indeed, studies have documented decreased circulating levels of CNH (especially BNP) in obese subjects, compared to nonobese subjects matched for age and gender [Dessi-Fulgheri et al., 1998] [Wang et al., 2004a]. This reduced activity of the CNH system may increase the risk of developing arterial hypertension and other cardiovascular diseases due to the non-contrasted and therefore prevailing effects of the counter regulatory system with sodium-retentive and vasoconstrictive properties [Sarzani et al., 2004] [Mehra et al., 2004a] [Wang et al., 2004a]

However, studies found that the NPR-C receptor could be coupled to a G-protein that inhibits cyclic AMP synthesis. These receptors, which are present in great amount especially on the endothelial cell wall, may mediate some paracrine effects of CNP on vascular tissue [Ahluwalia et al., 2004b] [Drewett et al., 1990] [Anand-Srivastava et al., 1988]. Therefore, further studies will be necessary to elucidate the possible role of NPR-C receptors as modulators of CNH action and/or degradation in peripheral tissues.

A large number of studies demonstrated that the activation of the neuro-hormonal system accelerates the left ventricular functional impairment in patients with HF [Bold et al., 2001] [Clerico, 2002b] [Ruskoaho, 2003b] [de Lemos et al., 2003] [Clerico and Emdin, 2004b] [Goetze, 2004b] [Packer, 1992] [Benedict, 1994] [Emdin et al., 2004]. Drugs that contrast the detrimental effects of the neuro-hormonal system activation play a key role for the current pharmacological treatment of HF. Some of these, such as ACE inhibitors, angiotensin II receptor blockers, β-blockers, and spironolactone decrease the circulating levels of CNH [Clerico and Emdin, 2004b] [Richards et al., 2002] [Latini et al., 2002a] [Richards and RW, 2004] [Bettencourt, 2004] [Cowie and GF, 2002],"normalize" their kinetics, and increase their biological activity. Furthermore, they enhance the natriuretic effect of ANP or BNP

analogs administered to patients. In other words, the treatment with this type of pharmacological agents decreases the systemic resistance to the biological effects of CNH [Clerico and Iervasi, 1995b] [Clerico et al., 2000a].

Patients with HF show a progressive and parallel increase in CNH levels and in some neuro-hormones and cytokines. This increase can be closely related to disease severity, as assessed by functional NYHA class (Table 3.1, Fig. 3.13). Plasma BNP values, normalized by mean values found in healthy subjects, are significantly higher than other normalized neuro-hormone and cytokine values in HF (Fig. 5.3, Table 5.4) [Emdin et al., 2004].

On average, the response of the CNH system to the increasing challenge of disease severity may not be linear (Fig. 5.4). The curve reported in Figure 5.4 suggests that the CNH system responds with a sharp increase in BNP plasma concentration in the early phase of HF (NYHA class I-II patients), followed, with the clinical progression of the disease, by a blunted increase (NYHA class III), and finally by a plateau (NYHA class IV) (Table 3.1, Fig. 5.4). These findings are consistent with the results from in vivo kinetic studies, indicating that ANP turnover (i.e., metabolic clearance rate and production rate) and natriuresis are both increased in patients in the early phase of HF (NYHA class I), as compared to patients with congestive HF [Clerico and Iervasi, 1995b] [Clerico et al., 2000a]. This suggests that resistance to the biological action of CNH is characteristic only of the congestive stage of HF. During the asymptomatic, early phase of the disease, the CNH system is able to compensate for the overactivity of the counter regulatory system (Fig. 5.4).

2.1.4 Diagnostic Accuracy of CNH Assay in Plasma from Patients with Cardiac Diseases

The CNH system activation is modulated not only by hemodynamic factors, but also by the activity of the counter-regulatory neuro-hormonal system.Consequently, it is likely that very small changes in hemodynamics, not assessable by echocardiographic examination, may produce significant (and measurable) variations in plasma concentrations of CNH. Moreover, studies have indicated that very small changes in some neuro-hormones and cytokines can produce wider variations in BNP circulating levels [Emdin et al., 2004] (Fig. 5.3).

It is well known that changes in hemodynamic parameters (such as left ventricular ejection fraction, EF) and plasma CNH levels (expressed in a log scale) are closely related in patients with cardiovascular diseases (Fig. 5.5) [Clerico, 2002b] [Ruskoaho, 2003b] [de Lemos et al., 2003] [Clerico and Emdin, 2004b] [Hammerer-Lercher et al., 2001] [Packer, 1992] [Benedict, 1994] [Emdin et al., 2004] [Richards et al., 2002] [Latini et al., 2002a] [Richards and RW, 2004] [Bettencourt, 2004] [Cowie and GF, 2002]. However, correlations between plasma CNH levels and echocardiographically measured parameters, such as left ventricular EF, myocardial mass and chamber volumes, are usually less close in the general population (large community-based sample, including healthy subjects with or without individuals with asymptomatic myocardial dysfunction) [Redfield et al., 2002] [Vasan et al., 2002] [Nakamura et al., 2002] [Kawai et al., 2004], as well shown dy data reported in Figure 5.6.

It should be emphasized that the recognition of HF syndrome is not equivalent to the clinical diagnosis of cardiomyopathy or to the assessment of left ventricular dysfunction, these latter terms describing possible structural reasons for the development of HF. Instead, HF is a clinical syndrome that is characterized by specific symptoms (namely dyspnea and fatigue) and signs (namely fluid retention). There is no

diagnostic test for HF, because it is largely a clinical diagnosis based on a careful history and physical examination [bib, 2001]. Therefore, there are no objective criteria for the identification and/or clinical stratification of patients with suspected HF; several "reference (gold) standards" [Bossuyt, 2003], based on clinical, laboratory and instrumental examinations, can be used to evaluate the diagnostic accuracy of the CNH assay. For this evaluation, some studies took into consideration only echocardiographic assessment of ventricular dysfunction, while in others either results of echocardiography or all clinical data have been used for the diagnosis of HF [Clerico and Emdin, 2004b].

Unfortunately, using echocardiography as the unique reference standard may lead to misinterpretations when evaluating the diagnostic accuracy of the CNH assay. A systematic review of clinical studies in patient populations with a prevalence of HF ranging from 3.8% to 51% to determine the diagnostic accuracy of BNP assays found that the sensitivity (ranging from 90 to 97%) was much less variable (by 4.4-fold) than the specificity (ranging from 53 to 84%) [Clerico and Emdin, 2004b].

Furthermore, in a meta-analysis [Doust et al., 2004], diagnostic accuracy of BNP assays greatly varied according not only to the group of patients studied, but also to the reference standard used (left ventricular EF ¡40% or ¡55%, diagnosis of diastolic dysfunction, diagnosis of systolic + diastolic dysfunction, or integrative clinical criteria). These data indicate that the choice of a suitable and accurate reference standard for evaluation of the diagnostic accuracy of the BNP assay in patients with HF may be a problem that is actually underestimated in the literature. For a proper definition of diagnostic accuracy, tested individuals should be grouped into those with and without disease, by means of an independent clinical judgment, considering both the CNH assay and echocardiographic data.

Careful echocardiographic examinations usually show slightly better or even similar diagnostic accuracy than the BNP assay in patients

with cardiac diseases [Logeart et al., 2002] [Dokainish et al., 2004]. On the other hand, the CNH assay may have some advantages compared to echocardiographic examinations alone in specific clinical settings. For example, Williams et al. suggested that in patients with chronic HF, the NT-proBNP assay reflects functional cardiac impairment and decreased exercise capacity (measured by peak exercise oxygen consumption) better than the left ventricular EF [Williams et al., 2004].

Several studies indicate that BNP and NT-proBNP are powerful and independent risk markers of cardiovascular events (especially mortality) not only in patients with HF, but also in those with acute coronary syndrome, as reported in and systematic reviews [Clerico and Emdin, 2004b] [Galvani et al., 2004] [Jernberg et al., 2004b] [Wiviott et al., 2004]. Some studies also suggested that the cardiovascular risk increases progressively to CNH concentration [Jernberg et al., 2004b] [Wiviott et al., 2004] [Kellett, 2004]; that is, there is no threshold that actually identifies patients with null risk.

Diagnostic sensitivity of BNP/NT-proBNP assays in detecting left ventricular systolic dysfunction could be suboptimal in asymptomatic or low-risk individuals, especially in women [Vasan et al., 2002]. Specificity of BNPassays in patients with HF ranged from 53 to84% and positive predictive values from 3 to 85% inseveral studies. These data indicate that CNH assays can produce a relatively large number of false-positive results. Consequently, many individuals, actually without HF (about 15-60% of those with positive CNH tests),mightundergo expensive and/or harmful investigations to rule out the disease, or even be inappropriately labeled as cardiac patients [Clerico and Emdin, 2004b].

Several false-positive results can be observed in patients with various clinical diagnoses, as reported in Table 5.1. In these patients, increased plasma BNP may predict, even in the presence of a normal echocardiographic examination, an increased risk of mortality or major cardiac events, including pulmonary embolism [Kruger et al., 2004b]

[Kucher et al., 2003a] [ten Wolde et al., 2003a] and hypertension [Nagaya et al., 2000b], renal failure [**?**] [McCullough et al., 2004a], septic shock [Castillo et al., 2004a], some chronic inflammatory diseases (such as amyloidosis and sarcoidosis) [Palladini et al., 2003a] [Yasutake et al., 2005], and diabetes mellitus [Bhalla et al., 2004a].

On the other hand, false-negative results could be found in patients on treatment with anti-adrenergic agents, diuretics and/or ACE inhibitors, all drugs that can reduce CNH levels [Clerico and Emdin, 2004b]. As shown in Figure 5.7, a large number of patients with only mild HF (NYHA classes I and II) may have values slightly above or even under the 99th percentile of distribution values of BNP concentration in healthy subjects. In these patients, successful treatment and consequent improvement in cardiac function and exercise capacity, and reduction in filling pressure and cardiac volumes, is usually associated with a marked fall in CNH levels: thus, a larger number of patients could have BNP values within the reference range values [Clerico and Emdin, 2004b] [Takeda et al., 2004].

However, at matched echocardiographic alterations, patients in whom BNP levels drop in response to therapy have a reduced rate of major cardiac events or mortality, compared to untreated hypertensive patients, who could have similar echocardiographic abnormalities. This represents the rationale for using the CNH assay for therapy decision making and for monitoring HF patients [Clerico and Emdin, 2004b] [Richards et al., 2002] [Latini et al., 2002a] [Richards and RW, 2004] [Bettencourt, 2004] [Cowie and GF, 2002].

In populations with a higher prevalence of cardiac diseases, including only individuals with a clinical suspicion of HF, the diagnostic sensitivity of BNP can improve up to 95%, or even more, as long as appropriate cut-off values are selected [Clerico and Emdin, 2004b] [Doust et al., 2004]. In this case, a strategy called "SnNout", which maximizes test sensitivity, could be used to rule out the presence of HF [Sackett and RB, 2002]. Furthermore, CNH assay also shows high (¿95%) negative

predictive values [Clerico and Emdin, 2004b] [Prontera et al., 2003], which can help to confirm the absence of HF. This is the rationale for choosing the CNH assay as the first step for an algorithm for the diagnosis of HF [Remme and K, 2001] [bib, 2003]. Such a clinical strategy has proved successful in some studies evaluating the cost-effectiveness of using plasma BNP measurements for screening of cardiac dysfunction in the general population [Nielsen et al., 2003] [Sim et al., 2003] [Ng et al., 2003].

2.1.5 Biological Variation of Plasma BNP: a Problem or a Clinical Resource?

The variability of measured plasma concentrations of many substances is due to three different sources: pre-analytical, analytical and inherent biological variation. The latter is usually described as a random variation around a homeostatic setting point, and defined as the intra-individual or within-subject biological variation [Fraser, 2004].

In order to achieve a correct interpretation of serial test results that are collected for follow-up or tailored treatment of HF patients, several studies [Pagani et al., 2003] [d'Eril et al., 2003] [Wu et al., 2003a] [Wu and A, 2004] [Bruins et al., 2004a] evaluated the biological variation of BNP and its related peptides, in both healthy subjects and cardiac patients. Due to secretory bursts and its rapid turnover (half-life about 15-20 min) [Bold et al., 2001] [Vanderheyden et al., 2004] , it is not surprising that intra-individual biological variations of plasma BNP levels were found to be very large, in both healthy subjects and patients with heart failure (ranging from 30 to 50%) [Pagani et al., 2003] [Wu et al., 2003a] [Wu and A, 2004] [Bruins et al., 2004a]. According to this, only a decrease of more than 50% or a more than 2-fold increase in plasma BNP should be assumed to be statistically significant in an individual patient.

In contrast with this assumption, a clinical trial [Takeda et al., 2004] has suggested that a BNP decrease inferior to this calculated reference change could be clinically relevant in patients with heart failure. In this study, only the group of patients treated with the $\beta -$ $blockeragentcarvedilol, whorespondonaveragewithadecreaseofonly38\%inplasmaB$

Furthermore, several studies have demonstrated that cardiovascular risk (mortality or major cardiovascular events) increases continuously and progressively throughout the whole range of BNP concentrations in patients with cardiovascular diseases [Clerico and Emdin, 2004b] [Jernberg et al., 2004b] [Wiviott et al., 2004] [Kellett, 2004].

In order to explain these conflicting findings, it should be taken into account that BNP secretion is closely regulated by specific pathophysiological mechanisms. Thus, changes in plasma hormone levels cannot be interpreted as random variations around a setting point, but as strictly determined by the activation level of the counter-regulatory system and by changes in hemodynamics. The clinician should look at the intra-individual variation in BNP as a mirror of variation in neuro-endocrine network activity. According to this hypothesis, it was suggested to consider all changes in BNP concentration as potentially clinically relevant, even when narrower than the calculated intra-individual biological variation [Clerico et al., 2005]. In other words, BNP variations should be interpreted and considered by physicians, as the variability of heart rhythm and blood pressure, by taking into account clinical history and examination, comprehensive of the response to specific treatments, as well as of laboratory and instrumental test findings.

2.2 CNH Assay as Diagnostic and Prognostic Tool in Cardiac Diseases

In this second part of the Chapter 5, we will review the clinical relevance of CNH assay (especially of BNP and NT-proBNP). There has been an explosion of clinical researches and trials concerning the routinary use of CNH (especially BNP) assay in the diagnosis and risk stratification of patients with cardiovascular diseases in the first years of the new century . It is clearly impossible to cover all these scientific contributes. In order to reduce the number of references without reducing the efficacy and objectiveness, we have used systematic reviews and meta-analyses, when available. First, we will review the clinical relevance of CNH assay in the screening and classification of patients with cardiac dysfunction, divided according to the severity of disesae and age, or associated to some particular clinical conditions (such as myocardial infarction or treatment with cardiotoxic drugs) . Moreover, we will discuss the diagnostic accuracy and clinical relevance of assay in coronary artery disease , where more BNP and NT-proBNP are increasingly frequently assayed with (almost in part) unexpected results. Finally, the diagnostic accuracy of CNH assay will be compared to that of other clinical tests, such as ECG, echocardiogram and chest radiogram . The last part of the Chapter is dedicated to prognostic relevance of CNH assay in cardiac diseases and general population , to relevance of measurement of CNH in tailoring the therapy , and to its cost-effectiveness in patients with HF.

2.2.1 Use of CNH Assay in the Screening and Classification of Patients with Cardiac Dysfunction

The diagnosis of HF can often be difficult, mainly in primary care settings, where patients may present with non-specific symptoms and signs, such as dyspnea, fatigue, and ankle swelling [bib, 2001] [Remme

and K, 2001] [bib, 2003]. In several population-based studies, fewer than 40% of patients with a suspected diagnosis of HF in primary care had this diagnosis confirmed by more specific and accurate clinical investigations, which are often expensive, timeconsuming and demanding for the patient [bib, 2001] [Remme and K, 2001] [bib, 2003] [Remes et al., 1991] [Fox and andWood DA et al, 2001]. As a result, a relatively simple and inexpensive biochemical test (such as the CNH assay) may be very useful to confirm the clinical suspicion of HF in this clinical setting [Clerico and Emdin, 2004b] [Apple et al., 2005] [Panteghini and A, 2004].

2.2.2 Diagnostic Accuracy of CNH Assay in Asymptomatic,

Mild Ventricular Systolic Dysfunction

Patients with asymptomatic left ventricular systolic dysfunction are likely to have lower plasma BNP than those with overt HF [Clerico, 2002b] [Ruskoaho, 2003b] [de Lemos et al., 2003] [Clerico and Emdin, 2004b] [Apple et al., 2005] [Panteghini and A, 2004] [Latini et al., 2002a] [Richards and RW, 2004] [Bettencourt, 2004] [Cowie and GF, 2002] [Nakamura et al., 2002] [Doust et al., 2004], as shown in (Fig. 3.13.)

Two large studies [Vasan et al., 2002] [Nakamura et al., 2002] evaluated the diagnostic accuracy of the CNH assay as a screening method in a general population. The first study analyzed the Framingham Heart Study cohort (3,177 individuals) using BNP and NT-proANP in the evaluation of left ventricular hypertrophy and systolic dysfunction in a community population [Vasan et al., 2002].

The presence of the disease was evaluated by using echocardiographic findings (the prevalence of left ventricular systolic dysfunction was 9.3% in the 1,470 men and 2.5% in the 1,707 women tested, respec-

tively). The area under the curve (AUC) of receiver operating characteristic (ROC) analysis for CNH assay for identifying both left ventricular hypertrophy and systolic dysfunction was on average about 0.75, with a good specificity (assumed 95% both for men and women) and negative predictive value (NPV, on average ranging from 92% to 97% in men, and from 91% to 98% in women), but a poor sensitivity (i.e., ranging from 27% to 28% in men, and from 13% to 40% in women) and positive predictive value (PPV, from 22% to 38% in men, and from 5% to 40% in women), using gender-related BNP cut-off values [Vasan et al., 2002].

The aim of the second study was to examine the validity of plasma BNP measurement for detection of various cardiac abnormalities in a rural Japanese population (1,098 subjects, 693 men and 405 women), with a low prevalence of coronary heart disease and left ventricular systolic dysfunction (i.e., only 37 participants, corresponding to 3.0%, showed an EF ¡30%). The diagnosis was carried out by two independent cardiologists based on a medical questionnaire, chest radiogram, electrocardiogram (ECG), and echocardiographic report. The optimal threshold for identification of disease was a BNP concentration of 50 ng/l (14.4 pmol/l), with an area under the ROC curve of 0.970, a sensitivity of 89.7%, a specificity of 95.7%, PPV of 44.3%, and NPV of 99.6%, respectively. [Nakamura et al., 2002]

The conclusions of these two studies, though similar in aim, as well as in clinical and experimental protocols, were strongly conflicting. The Japanese study suggested that the BNP assay is a very efficient and cost-effective mass screening technique for identifying patients with various cardiac abnormalities regardless of etiology and degree of left ventricular systolic dysfunction [Nakamura et al., 2002], while the Framingham study suggested only limited usefulness of the CNH assay as a mass screening tool for this clinical condition, especially in women [Vasan et al., 2002].

This discrepancy may be due to the different gold standard used for

the diagnosis of heart failure adopted in the two studies, as discussed in a previous paragraph . However, these two studies, taken as a whole, indicate that the CNH assay may have only a limited usefulness as a screening method for HF in a general population, owing to the poor sensitivity and PPV. However, both studies also found good specificity and NPV, thus suggesting that the CNH assay may be used to rule out HF in an asymptomatic individual.

2.2.3 Diagnostic Accuracy of CNH Assay in Patients with Suspected HF

Many studies [Nielsen et al., 2003] [Omland et al., 1996b] [Hobbs et al., 2002] [Cowie et al., 1997] [Maisel et al., 2002a] [Maisel et al., 2003] [Fisher et al., 2001] [Apple et al., 2003] [Bay et al., 2003] [McLean et al., 2003] [Richards et al., 1998] [andYoshimura M and et al, 1994] [Nakagawa et al., 1995] [Vasan et al., 1995] [Bonow and JE, 1992] [Bonow and JE, 1992] [Suzuki et al., 2000] [Maisel et al., 2001] [Lubien et al., 2002] [Vanderheyden and andVerstreken S et al, 2004] [Hayakawa et al., 2001b] [Ono et al., 2001] have suggested that the CNH assay could be useful as a screening method and/or for the differential diagnosis in patients suspected of HF in different clinical settings:

- randomly selected high-risk community populations
- primary-care patients with a new diagnosis of HF
- patients with acute dyspnea in the emergency department
- consecutive unselected hospital inpatients
- patients admitted to the intensive care unit.

The studies concerning the diagnostic accuracy of the CNH assay in patients with HF have also been analyzed by two systematic reviews

[Clerico and Emdin, 2004b] [Doust et al., 2004]; unfortunately, these studies showed quite heterogeneous data, thus introducing some bias in the statistical analysis. In particular, even some high quality studies were not designed with the primary goal of evaluating the diagnostic accuracy of the CNH assay. Indeed, this aim was considered only at a post-hoc analysis and retrospectively assessed in blood samples collected for different original purposes, some years before the actual evaluation of diagnostic accuracy. This may introduce a significant bias, although its true clinical relevance is difficult to assess [Clerico and Emdin, 2004b].

The second most important cause of heterogeneous data is the different reference (gold) standard used to evaluate the diagnostic accuracy of the CNH assay. In some studies, the patients studied were stratified and grouped according to clinical severity, as described by functional classification (usually NYHA classification). In other studies, only echocardiographic measurements were used as "gold standard" for the accuracy of the CNH assay for the diagnosis of left ventricular dysfunction (and not for the clinical diagnosis of HF) [Clerico and Emdin, 2004b] [Doust et al., 2004].

Furthermore, comparison of studies concerning the diagnostic accuracy of CNH assay is also difficult because different populations were enrolled and different immunoassays were used. Indeed, diagnostic accuracy (especially predictive values) is strictly dependent on disease prevalence (pre-test probability), which greatly varies according to the clinical setting considered (i.e., screening for general population, outpatients seen by a general practitioner, or in primary care, emergency department, coronary care unit, and so on). In particular, the prevalence of HF in the populations studied varied from less than 5% (in studies of screening of asymptomatic population) to more than 50% (in studies including patients referred to hospital with suspicion of HF) [Clerico and Emdin, 2004b].

Finally, data reported in the literature suggested that diagnostic accu-

racy may vary significantly in relation with the specific cardiac peptide measured and/or immunoassay used. Unfortunately, some studies do not clearly indicate the type of immunoassay used to measure CNH, while the majority do not report the assay performance (and often even the reference values) evaluated in their own laboratory.[Clerico and Emdin, 2004b]

Taking all studies as a whole, a meta-analysis showed that the odds ratio for diagnostic accuracy of BNP assay in different groups of patients with suspected HF is highly significant. In particular, the pooled diagnostic odds ratio, when clinical criteria were used as gold standard for HF, was 30.9 (95% confidence interval 27.0-35.4), while it fell to 11.9 (8.4-16.1) when a value $\leq$40% of left ventricular EF, estimated by echocardiography, was used as reference standard [Doust et al., 2004].

In a metaanalysis, including data of three studies comparing the diagnostic accuracy of BNP assay in patients with diastolic dysfunction, the pooled odds ratio was 28.3 (95% confidence interval 2.66-300.5). However, a bias may affect these studies, as suggested by the significant test of heterogeneity [Doust et al., 2004].

Finally, [Wright et al., 2003] evaluated the effect of NT-proBNP assay on the clinical diagnostic accuracy of HF in primary care by means of a prospective, randomized controlled trial in 305 patients. Each patient was randomized in two groups, one in which the general practioner had at their disposal the NT-proBNP assay results (NT-proBNP assay group), while the other did not (control group). The diagnostic accuracy improved by 21% in the NT-proBNP assay group and by 8% in the control group ($p = 0.002$). This study indicates that NT-proBNP measurement significantly improves the clinical diagnostic accuracy of HF in general practice [Wright et al., 2003].

2.2.4 Diagnostic Accuracy of CNH Assay in Patients with Acute Myocardial Infarction

Circulating levels of CNH increase after acute myocardial infarction (AMI); the extent of the increase is related to the size of the infarct [Horio et al., 1993] [Morita et al., 1993a] [Arakawa et al., 1994] [Uusimaa et al., 1999]. Patients with smaller infarcts tend to have a monophasic increase in plasma BNP, peaking at 20 hours after the onset of symptoms; on the other hand, those with larger infarcts, lower EF, and clinical signs of HF may present a further peak at 5 days after admission [Morita et al., 1993a]. Other studies are less convincing regarding the ability of the CNH assay to identify patients with significant myocardial dysfunction after AMI [Omland et al., 1996a] [Panteghini et al., 2003]. These conflicting results could be due to the differences in sample collection time, type of CNH (ANP, BNP, or NT-proBNP) measured, type of assay, and inclusion criteria adopted. However, persisting elevation of CNH levels at 1 or 2 months after AMI usually suggests a high risk of adverse remodeling and subsequent HF [Clerico and Emdin, 2004b].

The diagnostic accuracy of the BNP assay in patients with myocardial infarction was evaluated in a meta analysis [Doust et al., 2004] taking into account only two studies [Bettencourt et al., 2000] [Choy et al., 1994], fitting the inclusion criteria of this analysis; the pooled odds ratio was 9.4 (95% confidence interval 4.5-19.4).

2.2.5 Diagnostic Accuracy of CNH Assay in Elderly People

Heart failure is primarily a disease of old age; chronic HF increases in prevalence with aging from ¡1% in people aged ¡65 years to ¿5% in those ¿65 years of age, and this clinical condition is the first cause of morbidity and mortality in older people [bib, 2001] [Remme and K,

2001] [bib, 2003] [Baruch et al., 2004]. A study demonstrated that elderly patients present with more advanced HF, as evidenced by their higher morbidity and mortality rate along with greater neurohormonal activation [Baruch et al., 2004]. According to these findings, elderly people should be considered to be a population with high risk for developing HF and so the BNP/NT-proBNP assay may be useful as a screening test for HF in older age. Indeed, several studies reported that the BNP/NT-proBNP assay could be clinically useful in elderly people suspected to have HF [Ueda et al., 2003] [Baruch et al., 2004] [Hutcheon et al., 2002] [**?**] [Heidenreich et al., 2004] [Ray et al., 2004] [Valle et al., 2005].

Two studies compared the diagnostic accuracy of the BNP assay and that of ECG in elderly people screened for HF. A prospective cohort study specifically evaluated the diagnostic accuracy for HF of BNP assay in 299 consecutive patients (mean age 79 years, 65% women) attending day-hospital over a period of 13 months. This study suggested that both BNP assay and ECG were sensitive in detecting left ventricular systolic dysfunction, but lacked specificity (but the combination of the two tests improved diagnostic accuracy) [Hutcheon et al., 2002].

The other study reported that both the ECG and the plasma concentration of BNP were highly efficient in excluding left ventricular systolic dysfunction in 407 75-year-old subjects. However, compared with the BNP, the ECG yields a lower number of false-positive cases. Therefore, this study suggested that in screening for left ventricular systolic dysfunction in elderly people, the BNP assay has a diagnostic value in addition to the ECG, but only in individuals with abnormal ECG [**?**].

Another study indicated that the BNP assay may be particularly useful in ederly patients, especially in differentiating cardiogenic pulmonary edema from respiratory causes of dyspnea [Ray et al., 2004]. Screening of populations with more than 1% prevalence of HF (such as people with age more than 60 years) with BNP followed by echocardiogra-

phy should provide a health benefit at a cost that is comparable to or less than other accepted health interventions [Heidenreich et al., 2004]. Finally, the NT-proBNP assay was demonstrated to be useful for detecting HF among people living in elderly nursing homes [Valle et al., 2005].

2.2.6 Detection of Drug Cardiotoxicity by means of BNP Assay

Several studies demonstrated that the BNP assay may also be a marker of chemotherapy cardiotoxicity [Suzuki and andYamazaki T et al, 1998] [Nousiainen et al., 1998a] [Nousiainen et al., 1999a] [Okumura and andYoshida T et al, 2000] [Hayakawa et al., 2001b]. In particular, two studies ([Pichon et al., 2005] and [Sandri et al., 2005]) also suggested that BNP/NT-proBNP assay is a predictive marker of cardiac dysfunction in patients affected by aggressive malignancies and treated with high-dose chemotherapy. The acute release of circulating levels of troponin should be only a mirror of the death of myocardiocytes, while the persistent increase in BNP, after several days or weeks from the administration of cardiotoxic drug, should be specifically related to ventricular remodeling and myocardial dysfunction. Further studies are necessary to confirm these data, and to evaluate the respective diagnostic accuracy and clinical relevance of troponin and BNP assays in the follow-up of patients treated with potentially cardiotoxic drugs.

2.2.7 Diagnostic Accuracy of CNH Assay in Coronary Artery Disease

Two very studies suggested that the BNP assay could be a reliable marker of ischemia in patients with coronary artery disease [Foote et al., 2004] [Sabatine et al., 2004]. It could be hypothesized that

exercise-induced ischemia results in increased wall stress and triggers release of CNH from myocardiocytes. According to this hypothesis, Foote et al. [Foote et al., 2004] measured NT proBNP and BNP in blood samples from a group of normal volunteers, and two groups of patients, one with and the other without coronary artery disease, before and after maximal exercise. Post-exercise increases in NT-proBNP and BNP were approximately 4-fold higher in the ischemic group than in the nonischemic group; while in volunteers, the increase was almost identical to that of the non-ischemic patient group. At equal specificity to the ECG (58.8%), the sensitivities of the BNP/NT-proBNP assay in detecting ischemia were 90 and 80%, respectively; in contrast, the sensitivity of the exercise ECG was only 37.5%. In the study by Sabatine et al. [Sabatine et al., 2004], transient myocardial ischemia was associated with an immediate rise in circulating BNP levels, and the magnitude of the rise was proportional to the severity of ischemia. These findings demonstrate an important link between the severity of an acute ischemic insult and the circulating levels of BNP. However, further studies are necessary to evaluate the relevance of the BNP/NT-proBNP assay.

2.3 Comparison between the Diagnostic Accuracy of CNH Assay and that of Other Tests and Clinical Investigations

Signs and symptoms correlate poorly with the presence of HF, for this reason diagnosis relies on clinical judgment based on a combination of history, physical examination and appropriate investigation [Cowie and GF, 2002] [Remme and K, 2001] [bib, 2003] [Cardarelli and TG, 2003].

Cowie et al. [Cowie et al., 1997] reported that ROC curves for BNP (AUC = 0.96), ANP (0.93), and NT-proANP (0.89) were better than

that for cardiothoracic ratio on chest radiogram (0.79) in screening for patients likely to have HF and requiring further clinical assessment. Nielsen et al. [Nielsen et al., 2003] found that BNP assay showed a diagnostic accuracy better than ECG in a random sample of 1,257 community subjects. Another study suggested that BNP assay together with the presence of major ECG abnormalities and history reduced by a factor of six (in comparison to consideration of BNP assay in isolation) the number of subjects requiring echocardiography to detect one case of myocardial dysfunction ina large population screening (1,360 patients tested) [Ng et al., 2003].

Several studies have compared the diagnostic accuracy of BNP assay and ECG in the elderly population. In 75-year-old subjects both the ECG and the plasma concentration of BNP are highly efficient in excluding left ventricular systolic dysfunction, as suggested [Talwar et al., 1999]. In another study, several types of structural heart disease, in particular valvular heart disease, could be identified exclusively by BNP testing, suggesting that BNP measurement can make a significant contribution to screening for CHF precursors when used in combination with ECG in elderly populations (856 subjects enrolled, with age $\geq$ 65 years) [Nakamura et al., 2005]. However, compared with the BNP assay, the ECG yielded a lower number of false positive cases in another study [Hedberg et al., 2004]. In screening for left ventricular systolic dysfunction, the BNP has a diagnostic value in addition to the ECG, but only in individuals with abnormal ECG [Hedberg et al., 2004].

NT-proBNP alone was a better predictor of left ventricular dysfunction than any other single or combination of factors, while the ECG had a poor predictive value for left ventricular systolic dysfunction in identifying patients with left ventricular systolic dysfunction in a high-risk population (243 patients, 129 men, median age 73 years, range 20-94) [Talwar et al., 1999].

Another study [Ogawa et al., 2002] compared the diagnostic accuracy

of BNP assay, ECG, chest radiography and echocardiography; the results of this study confirmed that sensitivity, specificity and accuracy of BNP assay and echocardiography were significantly better than those of ECG and chest radiography [Ogawa et al., 2002].

A huge number of studies have compared the diagnostic accuracy of BNP assay and echocardiography in patients with acute or chronic HF [Clerico and Emdin, 2004b] [Latini et al., 2002a] [Richards and RW, 2004] [Bettencourt, 2004] [Cowie and GF, 2002] [Doust et al., 2004] [Struthers, 1993]. According to the guidelines for the diagnosis of heart failure proposed by the Task Force on Heart Failure of the European Society of Cardiology [Remme and K, 2001], echocardiography is recommended as the most practical tool to demonstrate cardiac dysfunction. However, a epidemiological study [Thomas et al., 2004] suggested that the diagnosis of HF might be better defined in terms of symptoms, elevated neuro-hormones and impaired cardiac workload. In more than 50% of the clinical studies, the echocardiographic investigation has been considered as the gold standard (more exactly the reference standard method) for the evaluation of diagnostic accuracy of CNH assay in patients with heart failure [Clerico and Emdin, 2004b] [Doust et al., 2004].When we use the echocardiography investigation as reference method, we assume that BNP assay cannot theoretically have a better diagnostic accuracy than it. Indeed, conflicting results were found when different clinical settings (patient's selection) and/or gold standard were used [Clerico and Emdin, 2004b] [Doust et al., 2004].

Choy et al. [Choy et al., 1994] showed that in post-AMI patients plasma BNP is superior to all clinical indices of left ventricular systolic dysfunction (EF ¡40%), including signs and symptoms and a clinical score (Peel Index). Dokainish et al. [Dokainish et al., 2004] found that BNP assay and comprehensive Doppler echocardiography have similar diagnostic accuracy for congestive HF, although echo-Doppler trended toward higher specificity than BNP for congestive HF. Moreover, serial BNP measurements during the treatment of acute HF provide incremental prognostic information over clinical presentation and repetitive

echocardiographic examination [Gackowski et al., 2004].

Mak et al. suggested that both BNP assay and a complete echocardiographic examination do not have adequate discriminatory power to be used in isolation in the evaluation of left ventricular diastolic function. Therefore, all available information, including systolic function, chamber dimensions and all Doppler variables must be considered in the analysis of individual patients [Mak et al., 2004].

Steg et al. assessed the diagnostic performance of BNP testing and echocardiographic assessment of left ventricular systolic function, separately and combined, for the identification of congestive HF in 1,586 patients presenting to the emergency department with acute dyspnea. The proportions of patients who were correctly classified were 67% for BNP alone, 55% for EF alone, 82% for the two variables together, and 97.3% when clinical, ECG, and chest radiograph data were added. This study suggested that BNP measurement was superior to twodimensional echocardiographic determination of left ventricular EF in identifying congestive HF, regardless of the threshold value. The two methods combined have marked additive diagnostic value [Steg et al., 2005].

Some studies indicated that BNP/NT-proBNP levels do not accurately predict serial hemodynamic changes and consequently do not obviate the need for pulmonary artery catheterization in patients requiring invasive hemodynamic monitoring [Forfia et al., 2005] [O'Neill et al., 2005] [Parsonage et al., 2005].

In conclusion, BNP assay shows significantly higher predictive characteristics than ECG and chest radiography, and a cost-benefit value significantly greater than that of echocardiography [Nielsen et al., 2003] [Ogawa et al., 2002]. BNP measurement may exclude normal heart with high probability owing to its high degree of sensitivity and NPV when used in screening high-risk populations, therefore reducing the echocardiographic diagnostic burden; this is the rationale for consider-

ing the BNP assay in the first step of an algorithm for the differential diagnosis of heart failure [Shimizu et al., 2002] [Cowie and GF, 2002] [Remme and K, 2001] [bib, 2003] [Cardarelli and TG, 2003] [Struthers, 1993].

2.4 Use of CNH Assay as Prognostic Marker in Cardiovascular Diseases

Several well-designed and conducted studies suggested that the CNH assay may be useful as a prognostic marker mainly in two clinical conditions: HF and acute coronary artery syndromes (ACS), as also demonstrated by systematic reviews [Clerico and Emdin, 2004b] [Galvani et al., 2004] [Parsonage et al., 2005] [Rodeheffer, 2004].

2.4.1 Prognosis in HF

The prognostic role of CNH assay (especially NT-proANP, BNP and NT-proBNP) in patients with HF is well demonstrated by a huge number of studies [Ueda et al., 2003] [Valle et al., 2005] [Doust et al., 2005] [Benedict et al., 1996] [Wallen et al., 1997] [Tsutamoto et al., 1999] [Stanek et al., 2001] [Anand et al., 2003] [Gardner et al., 2003a] [Baruch et al., 2004] [Kellett, 2004] [Kirk et al., 2004] [Berger et al., 2005] [Hulsmann et al., 2005] [Segawa et al., 2005] [Stanton and andWijeysundera HC et al, 2005].

In all these studies, CNH concentrations were always found to be independent risk markers for morbidity (increased future major cardiovascular events and/or hospitalization) and/or mortality in patients with acute or chronic HF [Clerico and Emdin, 2004b]. In some studies CNH levels were stronger predictors of mortality and/or major cardiovascular events than left ventricular EF, NYHA class, and/or presence

of diabetes or hypertension, as well as sex and age in patients with chronic HF [Wallen et al., 1997] [Tsutamoto et al., 1999] [Gardner et al., 2003a] [Kellett, 2004] [Kirk et al., 2004] [Berger et al., 2005] [Segawa et al., 2005] [Stanton and andWijeysundera HC et al, 2005]. A continuous relationship was generally found between BNP levels and mortality [Kellett, 2004], thus suggesting that it is not possible to observe a threshold for the risk. On average, a systematic analysis of the most important studies suggested an odds ratio of about 2 for the risk of mortality in patients with BNP values above the cut-off [Clerico and Emdin, 2004b].

In general, BNP assay was found to be the most powerful indicator for poor outcome compared to other neuro effectors and hormones, at least in the largest studies [Clerico and Emdin, 2004b] [Latini et al., 2004] [Berger et al., 2005]. However, more data on the clinical relevance of combination of two or more neuro-hormones in risk stratification of HF are necessary.

2.4.2 Prognosis in ACS

Acute coronary artery syndrome encompasses a continuum of cardiac ischemic events, ranging from unstable angina pectoris, with no biochemical evidence of myocardial necrosis, to ST-elevation AMI [bib, 2000] [bib, 2002]. The prognosis of patients with ACS varies widely, and several clinical, electrocardiographic, and biochemical markers have been used to identify high-risk individuals in need of aggressive intervention [bib, 2002].

Several studies reported that CNH assay (in particular BNP and NT-proBNP) provides valuable prognostic information in patients with ACS [Richards et al., 1998] [Omland et al., 1996a] [Omland et al., 2002] [Arakawa et al., 1996] [Darbar et al., 1996] [Crilley and M, 2001] [McDonagh et al., 2001] [Richards et al., 2001] [Inoue et al., 2002]

[Sabatine et al., 2002] [Jernberg et al., 2002]. A meta-analysis confirmed the powerful prognostic value of BNP/NT-proBNP in patients with ACS for death both in the short term (¡50 days, mean odds ratio 3.38, CI 95% 2.44-4.68) and long term (¿10 months, mean odds ratio 4.31, 3.77-4.94) [Galvani et al., 2004].

Some studies suggest that BNP levels improve a simple risk score in patients with unstable angina and non-ST-elevation myocardial infarction [Wylie et al., 2004] as well as the risk assessment performance obtained with the cTnI and hs-CRP assays in patients with STsegment elevation myocardial infarction [Jernberg et al., 2004a]. These data suggest the utility of a "multimarker strategy" or biomarker profile to characterize patients with ACS [Wiviott et al., 2004].

A study reported that there may be clear differences among the profiles of individual natriuretic peptide levels in the 2 years after AMI. Similar profiles were found with BNP and NT-proBNP plasma concetrations, while those of NT-proANP and CNP differed significantly in a cohort of 236 patients with AMI complicated by clinical, radiologic or echocardiographic evidence of left ventricular dysfunction. Moreover, a single measurement of plasma natriuretic peptide levels during the hospital admission provides limited prognostic information, while NT-proBNP measured by an ELISA method in the 30 days after AMI identifies a cohort of patients at increased risk of adverse outcome thereafter [Squire et al., 2005].

Two studies reported that in patients with clinically stable, angiographically documented coronary artery disease, plasma BNP [Omland et al., 2005] or NT-proBNP [Kragelund et al., 2005] levels are independently related to long term survival in a multivariate model. These studies suggested that BNP and NT-proBNP are markers of long term mortality even in patients with stable coronary disease and add prognostic information above and beyond that provided by conventional cardiovascular risk factors and the degree of left ventricular systolic dysfunction [Omland et al., 2005] [Kragelund et al., 2005].

In order to explain these clinical findings, it is important to note that experimental studies in animals reported that myocardial ischemia or even hypoxia per se could induce the synthesis/secretion of CNH (in particular BNP) from the intact heart in vivo as well as ventricular cells in culture [Hama et al., 1995] [Toth et al., 1994]. Furthermore, these experimental data are also in accordance with clinical studies indicating that transient myocardial ischemia in patients with stable coronary artery disease is associated with an immediate rise in circulating BNP levels, and that the magnitude of rise is proportional to the severity of ischemia [Foote et al., 2004] [Sabatine et al., 2004] [Marumoto et al., 1995].

2.4.3 Prognostic Relevance of CNH Assay in the General Population

While measurement of plasma BNP concentration has been shown to be useful in the diagnosis of HF (especially acute HF), its role as a screening test for detection of preclinical ventricular remodeling or dysfunction in the general population has not been established [Clerico and Emdin, 2004b] [Marumoto et al., 1995] [Dyrbye and MM, 2003]. However, some studies evaluated the prognostic relevance of CNH assay in the general population, especially in high-risk populations, such as elderly people [Ueda et al., 2003] [Benedict et al., 1996] [Arakawa et al., 1996] [Toth et al., 1994] [Goetze et al., 2004a] [Marumoto et al., 1995] [Dyrbye and MM, 2003] [andVasan RS, 2003]. These studies demonstrated that CNH (and especially BNP/NT-proBNP) levels are a sensitive and accurate biochemical marker of an increased risk of cardiac morbidity and total mortality in very elderly persons [Ueda et al., 2003] [Valle et al., 2005] [Wallen et al., 1997] [McDonagh et al., 2001] [andVasan RS, 2003] [Davis et al., 1992]. However, in a community-based study (mean age 56 years) there were no significant trends of increasing incidence of hypertension across BNP categories in men or women, while higher plasma BNP levels were associated with increased

risk of BP progression in men but not women [Knight et al., 1999]. The differences between the results of this study [Knight et al., 1999] and those of others [Ueda et al., 2003] [Rodeheffer, 2004] [McDonagh et al., 2001] [andVasan RS, 2003] [Davis et al., 1992] may be due to the different age and gender of subjects studied. Probably, the prognostic power of BNP assay increases progressively with age and may be gender-dependent.

2.5 CNH Assay in Management of Patients with HF

2.5.1 Clinical Relevance of CNH Assay in Tailoring the Therapy of HF

Medical therapy for HF is based on improving the symptoms and signs of fluid retention (change in dyspnea, edemas, and body weight are the usual markers of response to treatment) and titrating the dosage of drugs (such as diuretics, ACE inhibitors, $\beta-blockers, and spironolactone) following t$
$point for treating patients with HF that can be used to fine-tune therapy [Cowie and GF$

Many authors have suggested that the results of CNH assay (especially BNP/NTproBNP assay) may be useful in monitoring and tailoring the medical therapy in HF patients, and in providing a practical objective indicator of optimal therapy [Richards et al., 2002] [Latini et al., 2002a] [Richards and RW, 2004] [Bettencourt, 2004] [Cowie and GF, 2002] [bib, 2001] [Remme and K, 2001] [bib, 2003] [McCullough et al., 2004a] [Takeda et al., 2004] [Cardarelli and TG, 2003] [Murdoch et al., 1999] [Troughton and andYandle TG et al, 2000] [Kawai et al., 2001] [Maisel, 2001] [Nicholls et al., 2001] [Troughton et al., 2001] [McGeoch et al., 2002] [Mueller and P, 2002] [Hobbs, 2003] [Richards, 2003] [Maisel et al., 2004] [Morimoto et al., 2004] [Wu et al., 2004a] [Mueller et al., 2004], including patients subjected to cardiac transplantation

[Kirchhoff et al., 2004].

CNH usually respond to effective treatment with drugs [Clerico and Emdin, 2004b] [Richards et al., 2002] [Latini et al., 2002a] [Richards and RW, 2004] [Bettencourt, 2004] [Cowie and GF, 2002] or left ventricular assist device [Kemperman et al., 2004] [Thompson et al., 2005] with a prompt reduction of their circulating levels. Indeed, ACE inhibitors, valsartan, diuretics, nitrates, and endothelin receptor antagonists have been shown to reduce plasma CNH levels in parallel with hemodynamic and clinical improvement [Latini et al., 2002a] [Richards and RW, 2004] [Murdoch et al., 1999] [McGeoch et al., 2002] [Davidson et al., 1996b] [Nishikimi et al., 1996] [Missouris et al., 1998] [Hara et al., 2000] [Tsutamoto et al., 2001] [Johnson et al., 2002] [Cotter et al., 2004].

More variable effects on plasma CNH levels have been reported after therapy with $\beta-blockers[Takedaet\ al.,\ 2004][Bouissouet\ al.,\ 1989][Colantonioet\ al$ $blockers[Latiniet\ al.,\ 2002a].Ohtaetal.[Ohtaet\ al.,\ 2000]reportedthatbothhighandlo$ $regulationofNPR-CmRNAexpressionintheheart[Ohtaet\ al.,\ 2000].Accordingtot$ $blockerscausesanearlyriseinplasmaCNH,whilesustainedtreatment,significantly$

Despite this huge number of studies suggesting the clinical relevance of monitoring patients with HF by means of CNH assay, two studies [Murdoch et al., 1999] [Troughton and andYandle TG et al, 2000] have been designed specifically to evaluate the clinical use of CNH assay in monitoring and tailoring the medical therapy in patients with HF. Murdoch et al. [Murdoch et al., 1999] studied 20 patients with mild to moderate chronic HF and receiving stable conventional therapy, who were randomly assigned to titration of ACE inhibitor dosage, according to serial measurement of plasma BNP or to optimal empirical ACE-inhibitor therapy for 8 weeks. Only the BNP-driven approach was associated with a significant reduction in plasma BNP concentration throughout the duration of the study and a significantly greater suppression when compared with empiric therapy after 4 weeks (mean reduction in BNP group -42.1%, 95% CI -58.2, -19.7; mean reduc-

tion in empiric therapy group 12.0%, 95% CI CI -31.8, 13.8; P= 0.03) [Murdoch et al., 1999].

Troughton et al. studied 69 patients with impaired systolic function (EF ¡40%) and symptomatic HF (NYHA class II-IV), who were randomized to receive treatment guided by either plasma NT proBNP concentration or standardized clinical assessment. During the follow-up (minimum 6 months, median 9.5 months), there were fewer total cardiovascular events (death, hospital admission, or HF decompensation) in the NTproBNP-guided group than in the clinical group (19 vs 54, p = 0.02). At 6 months, 27% of patients in the NT-proBNP-guided group and 53% in the clinical group had experienced a first cardiovascular event (p = 0.034). Changes in left ventricular function, quality of life, renal function, and adverse events were similar in both groups [Troughton and andYandle TG et al, 2000].

Morimoto et al. conducted a cost-effectiveness analysis of regular BNP measurement in the outpatient setting. The target population was symptomatic CHF patients aged 35-85 years, discharged from the hospital. Intervention was BNP measurement once every 3 months (BNP group) or no BNP measurement (clinical group). The baseline analysis during the 9-month period after hospitalization suggested that the introduction of BNP measurement in heart failure management is not only cost-effective by reducing hospitalization, but also improves the outcome of patients, as assessed by (quality-adjusted life year) analysis [Morimoto et al., 2004].

A randomized clinical trial compared the titration of $\beta-blockertherapywithbisoprolo$ $onepatientswithheartfailurewererandomizedintoaclinicaltrial.Theclinicalgrouph$ $blockerdosageincreasedaccordingtostandardcare, whereastheBNPgrouphad\beta-$ $blockerdosageup-titratedaccordingtoplasmaBNPlevelsplusstandardcare.Theprim$ $blockerdoseachievedafter3months.BNP-guidedup-titrationof\beta-$ $blockerinambulatorypatientswithheartfailuredidnotresultsignificantlydifferent$ $blockerattheendof3months.However, 45\%ofpatientsintheclinicalgroupwereonthen$ $blockervs.only19\%ofpatientsintheBNPgroup, althoughleftventricularejectionfra$

Accurate diagnosis of clinical deterioration in heart failure can be difficult [bib, 2001] [Remme and K, 2001]. To prevent development into overt congestion, which often requires hospitalization, early diagnosis is of paramount importance. There is a need for objective measurements to aid early diagnosis in a setting where symptoms may be non-specific and abnormalities on physical examination often subtle and minor [Lewin et al., 2005]. Heart failure guidelines recommend the use of weight gain monitoring to help in this task, with the added advantage that patient self-care is encouraged [bib, 2001] [Remme and K, 2001]. It is advised that an increase of 2 Kg over stable body weight over a period of 48-72 h should initiate contact with medical or nursing personnel [bib, 2001] [Remme and K, 2001] [Lewin et al., 2005].

However, Lewin et al. suggested that neither weight gain nor increase in BNP are adequately sensitive as a screen for clinical deterioration in patients with established heart failure (34 clinically stable and other 43 with clinical deterioration of heart failure status). In particular, weight gain is very insensitive, though an increase of 2 Kg demonstrates high specificity for clinical deterioration. On the other hand, BNP change appears to provide better sensitivity than weight change, but it has poor specificity in an established heart failure population [Lewin et al., 2005].

2.5.2 Cost-Effectiveness of CNH Assay in Management of Patients with Acute or Chronic HF

Nielsen et al. [Nielsen et al., 2003] sought to assess the cost-effectiveness of using plasma BNP as a pre-echocardiographic screening test for left ventricular systolic dysfunction in the general population. Screening high-risk subjects by BNP before echocardiography could reduce the cost per detected case of left ventricular systolic dysfunction by 26% for the cost ratio of 1/20 (BNP/echocardiogram). Greater reduced costs (up to 50%) can be predicted for the group of low-risk subjects

[Nielsen et al., 2003]. Other studies reported similar results [Sim et al., 2003] [Heidenreich et al., 2004], including a study on old people living in nursing homes [Valle et al., 2005].

Mueller et al. conducted a prospective, randomized, controlled study of 452 patients who presented to the emergency department with acute dyspnea: 225 patients were randomly assigned to a diagnostic strategy involving the measurement of BNP, and 227 were assessed in a standard manner. This study indicated that BNP assay improved the evaluation and treatment of patients with acute dyspnea and thereby reduced the time to discharge and the total cost of treatment in the emergency department [Mueller et al., 2004].

Other studies suggested that BNP testing can also reduce the cost for hospitalization in patients with heart failure [Morimoto et al., 2004] [Wu et al., 2004a], including old people living in nursing homes [Valle et al., 2005]. However, the cost-effectiveness analysis strongly depends on the relative cost of the BNP test compared to that of echocardiograms and/or hospitalization, as well as on the prevalence of HF in the population screened. Unfortunately, these parameters can vary considerably among departments, countries, and health-care systems; so that each laboratory/clinical department should analyze the cost-effectiveness in its own economical framework. Furthermore, cost-effectiveness analysis is also dependent on the sensitivity of BNP assay for detecting HF. Cost-effectiveness will improve if more specific assays are used: this would decrease the number of subjects with false-positive results, and consequently the number of further useless investigations. However, further larger and randomized clinical trials are necessary to confirm the clinical relevance and cost-effectiveness of BNP assay in the follow-up of patients with HF.

In conclusion, several experimental and clinical studies strongly suggest that the circulating CNH should be better considered as an index of activation of the neuro-endocrine system, rather than a marker of myocardial dysfunction [Clerico et al., 2006a]. The activation or deacti-

vation of the CNH system is almost always the resultant of one or more physiological or pathological changes. For this reason, the results of CNH assays must be interpreted by taking into account clinical history and examination, as well as all laboratory and instrumental tests. Of course, the great number of pathophysiological mechanisms that can affect the CNH system render sometimes difficult for clinicians to recognize the cause(s) of variations in its activity. CNH assays should be considered as an intellectual spur for the search of pathophysiological mechanisms that can satisfactorily explain the measured variations in hormone concentrations. On the other hand, CNH measurements add a complementary information to other instrumental and investigative tests [Emdin et al., 2005].

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