Clinical update

Natriuretic peptides in cardiovascular diseases: current use and perspectives

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The natriuretic peptides (NPs) family, including atrial, B-type, and C-type NPs, is a group of hormones possessing relevant haemodynamic and anti-remodelling actions in the cardiovascular (CV) system. Due to their diuretic, natriuretic, vasorelaxant, anti-proliferative, and anti-hypertrophic effects, they are involved in the pathogenic mechanisms leading to major CV diseases, such as heart failure (HF), coronary artery disease, hypertension and left ventricular hypertrophy, and cerebrovascular accidents. Blood levels of NPs have established predictive value in the diagnosis of HF, as well as for its prognostic stratification. In addition, they provide useful clinical information in hypertension and in both stable and unstable coronary artery disease. Structural abnormalities of atrial natriuretic peptide gene (NPPA), as well as genetically induced changes in circulating levels of NPs, have a pathogenic causal link with CV diseases and represent emerging markers of CV risk. Novel NP-based therapeutic strategies are currently under advanced clinical development, as they are expected to contribute to the future management of hypertension and HF.

The present review provides a current appraisal of NPs’ clinical implications and a critical perspective of the potential therapeutic impact of pharmacological manipulation of this class of CV hormones.

Keywords
Natriuretic peptides • Cardiovascular diseases • Genetics • Natriuretic peptides analogues • NEP inhibitors • ARNi

Biology of the natriuretic peptides system

The natriuretic peptides (NPs) family includes three well-characterized hormones: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) that are intimately involved in the maintenance of cardio-renal homeostasis. Atrial natriuretic peptide and BNP are synthetized mainly in the heart, and to a lesser extent in other organs, whereas CNP is mainly produced by endothelial cells.¹² They are synthetized as pre-prohormones and subsequently cleaved to obtain a biological active α-carboxy terminal peptide along with the amino-terminal end³⁵ (Figure 1).

Soluble guanylyl cyclase (GC) receptors mediate NPs effects in target tissues. GC-A receptor is the main effector of both ANP and BNP actions, whereas GC-B receptor mediates CNP effects. An increase in cyclic guanylate monophosphate (cGMP) levels follows the activation of both GC-A and B receptors⁶ (Figure 2). An additional natriuretic peptide receptor (NPR-C) plays a fundamental role in NPs clearance. Moreover, NPR-C mediates vasoprotective properties of CNP and it has been also involved in cellular signalling pathways leading to antiproliferative and pro-apoptotic effects in specific circumstances.² Different from GC-A and B receptors, NPR-C contains a 37-amino acid cytoplasmic domain with a Gα inhibitory protein-activating sequence and it is devoid of kinase and GC activities. A decrease in cyclic adenylyl monophosphate levels follows its activation (Figure 2).⁷

Old and novel functional properties of natriuretic peptides

Natriuretic peptides have long been viewed as important cardio-renal hormones mostly because of their key role in the regulation of electrolytes and water balance homeostasis as well as of blood pressure (BP) levels through diuretic, natriuretic, and vasorelaxant effects, along with the ability to inhibit the renin–angiotensin–aldosterone system
(RAAS) and the sympathetic nervous system (SNS).\textsuperscript{1,2} They also contribute to modulate systemic vascular resistance mainly by inhibiting the contraction of vascular smooth muscle cells through cGMP-dependent kinases.\textsuperscript{2} Of note, it was shown that supraphysiologic concentrations of ANP did not produce vasodilation in the intestinal and skeletal muscle microcirculation, and that ANP did not counteract the vasoconstriction dependent from norepinephrine and arginine vasopressin in these vascular districts,\textsuperscript{8} supporting the existence of regional differences in vascular sensitivity to ANP.\textsuperscript{9}

A part from mechanical factors, i.e. increased volume overload and myocytes stress, hormonal stimuli are involved in the control of NPs release.\textsuperscript{1–3,10,11} Brain natriuretic peptide increases in a broad range of disease conditions, i.e. myocardial fibrosis and ischaemia, ventricular remodelling, and overload, all underlying chronic left ventricular (LV) dysfunction.

Besides the well-described haemodynamic functions, new properties of NPs have been discovered over the past two decades that relate to the interaction with cellular growth and proliferation at the vascular level.\textsuperscript{12} Natriuretic peptide increases in a broad range of disease conditions, i.e. myocardial fibrosis and ischaemia, ventricular remodelling, and overload, all underlying chronic left ventricular (LV) dysfunction.

Finally, a role of NPs in the control of lipid metabolism, in the promotion of mitochondria biogenesis in adipocytes, and in the process of ‘browning’ of white adipocytes to increase energy expenditure has been recently identified.\textsuperscript{17}

As a result of their complex cardiovascular (CV) properties, NPs are currently viewed as active players in the process of CV remodelling and in the natural history of heart failure (HF).

**Predictive role of cardiovascular risk in the general population**

The issue of a potential usefulness of NPs levels for stratifying cardiovascular risk (CVR) in asymptomatic individuals from the general population currently represents an active subject of investigation.\textsuperscript{18}
In the offspring study from the Framingham population, NPs levels significantly predicted mortality from CV causes in apparently healthy subjects. NT-proBNP predicted major adverse CV events (MACE) and death in a population-based study performed in the city of Copenhaghen. Cardiovascular risk, as estimated by two validated algorithms for risk stratification, i.e. the SCORE project chart and the Framingham algorithm, increased gradually across tertiles of NT-proANP in a cohort of adult males from Southern Italy. In a community-based cohort from the USA, free from HF, NT-proBNP levels emerged as a more robust cardiac biomarker able to predict CV mortality and morbidity, independently from conventional clinical risk factors and cardiac structural abnormalities.

Assessment of either BNP or NT-proBNP levels, in addition to measurement of conventional cardiovascular disease (CVD) risk factors, for improvement in risk discrimination for subsequent CVD has led to variable results, from only modest incremental improvement for CVD to a net reclassification improvement for major CV events. This controversial issue was recently addressed by a primary prevention study considering a cohort of hypercholesterolaemic middle-aged men at moderate CVR. In this study, NT-proBNP, once added to conventional risk factors, almost doubled the incremental gain for prediction of CVD compared with C-reactive protein, even when excluding subjects with minor as well as with major cardiac abnormalities.

The predictive role of NPs for CVR in apparently healthy individuals may serve as a useful tool for early identification of ‘at risk subjects’. A major issue that remains to be verified in future studies is whether reduction of NPs levels in this subjects category may truly contribute to decrease their CVR and therefore to effectively prevent CVDs.

Of note, studies supporting the suitability of NPs levels as markers of CVR in asymptomatic individuals parallel those showing the ability of NPs to detect subclinical damage, as it was shown in the context of hypertension and of chronic kidney disease.

Thus, based on the whole CV actions of NPs and on their tight relationships with the neuroendocrine regulation, NPs can be viewed as sensitive markers of preclinical haemodynamic and structural CV changes.

**Figure 2** Natriuretic peptides produced by cardiomyocytes (CM) upon haemodynamic and neuroendocrine regulation act on target cells through specific receptors, soluble guanylyl cyclase (sGC), type A and B (GC-A and GC-B). The type of biological effect mediated by each receptor through the cGMP pathway, involving protein kinase G (PKG) and phosphodiesterase (PDE), is described. C-type natriuretic peptide produced by endothelial cells (EC) acts through GC-B and also through natriuretic peptide receptor (NPR-C), a non-GC receptor able to inhibit adenylate cyclase (AC) and to reduce cAMP levels. The biological effects of NPR-C within the cardiovascular system are shown. NPR-C plays also a fundamental role in NPs clearance.
Diagnostic and prognostic role of natriuretic peptides circulating levels in cardiovascular diseases

Heart failure

The diagnostic role of NPs in both acute and chronic HF is well established. Both ANP and BNP levels increase in parallel with the degree of LV dysfunction and haemodynamic stress, although they are not useful to discriminate between systolic and diastolic HF. Furthermore, the value of NPs as reliable markers for long-term prognostic stratification both in acute and chronic HF conditions is well documented. In a study of patients presenting with acute HF, measurement of BNP, NT-proBNP, and mid regional (MR)-proANP levels showed significant diagnostic performance, although only MR-proANP had long-term prognostic value. In a large collection of patients hospitalized for acutely destabilized HF (ADHF), the prognostic performance of both NT-proBNP and MR-proANP levels was confirmed by evidence of incremental prognostic value to clinical risk factors for predicting mortality at 1 year. In chronic HF, subsequent measurements of either BNP or NT-proBNP levels provide independent information regarding the risk for disease progression across a wide spectrum of adverse outcomes: ventricular remodelling, malignant ventricular arrhythmias, hospitalization for HF, need for transplantation, and death. In one of the longest available follow-up study of patients with chronic HF, evaluating the prognostic power of multiple biomarkers, plasma ANP levels turned out to be the strongest long-term predictor of death in all disease stages. Prognostic value of NPs has been shown both in HF with reduced and with preserved EF. Even a preventive role of BNP-based screening towards HF development has been recently documented. Among patients with CV risk factors, at risk of HF, BNP assessment, associated with collaborative care, reduced the combined rates of LV systolic dysfunction, diastolic dysfunction, and HF.

Natriuretic peptides guidance in heart failure therapy

The important clinical implications of NPs in HF indicate that NPs level measurement is one of the most effective tools for a HF hormonal-guided therapy. In ADHF, BNP measurement led to better accuracy in diagnosis, reduced rate of hospitalizations and of admissions in intensive care units, and had favourable effects on treatment costs and mortality rates. In fact, a useful algorithm for BNP-guided treatment of ADHF has been developed. Also in chronic HF, NPs level represents a useful marker to monitor the course of the disease in relation to the benefits of therapeutic strategies. However, due to uncertainties produced by different studies, a low level of recommendation for NP-guided therapy in chronic HF has been assigned by AHA-ACC-HF guidelines. A recent meta-analysis, performed in the attempt to overcome existing controversies and including 12 randomized clinical trials, has shown that in patients with chronic HF due to systolic dysfunction NP-guided therapy reduced all-cause mortality and HF-related hospitalizations. It was confirmed that individuals older than 75 years are those who benefit less, likely as a result of increased rate of comorbidities in this age range.

Hypertension

A diagnostic independent role of NPs levels has not been assessed in hypertension. On the other hand, few studies examined the significance of NPs in terms of prediction of CVR in hypertensive patients. Higher NT-proBNP levels predicted mortality in hypertensive patients. MR-proANP levels were significantly associated with lower ankle-brachial index, higher urinary albumin-creatinine ratio, greater LV mass index, independently of age, gender, body mass index, systolic BP, glomerular filtration rate, smoking, diabetes, cholesterol levels, medication use, previous acute myocardial infarction (AMI), or stroke, particularly in African-Americans.

Stable and unstable atherosclerotic disease

Natriuretic peptides are sensitive markers of both clinically evident and subclinical myocardial ischaemia, as a consequence of the ischaemia-dependent LV dysfunction. In addition, a growing body of evidence indicates that NPs may play a direct contributory role into atherosclerotic disease pathogenesis. It is known that NPs affect vascular remodelling by exerting a significant impact on vitality, proliferation, apoptosis, and necrosis of both endothelial and vascular smooth muscle cells. Natriuretic peptides are significantly more expressed in human coronary explants of advanced atherosclerotic lesions in comparison with early atherosclerotic lesions. Furthermore, well-defined stages of atherosclerosis are characterized by progressively more pronounced increases in NT-proANP and NT-proBNP levels, from endothelial dysfunction to moderate and severe coronary atherosclerosis, irrespective of the underlying myocardial disease.

As a consequence, clinical usefulness of NT-proNPs appears well established in both stable and unstable atherosclerotic disease, with particular regard to prognostic implications. In subjects experiencing AMI, a Kaplan–Meyer curve, based on either NT-proANP or NT-proBNP or both being above the respective median value, led to a significant prediction of future MACE. Similar data were obtained in patients experiencing either AMI or stroke.

In a 10-year follow-up study performed in patients with stable CAD, NT-proBNP levels within the highest quartile were associated to 50% survival rate. Moreover, NT-proBNP levels in the 75–100 percentile were significantly associated with AMI, CV death, and all-cause death in the Claricor trial. Similar prognostic value has been reported for NT-proANP levels in patients with stable angina. In a multimarker model, involving MR-proANP, midregional pro-adrenomedullin (MR-proADM), C-terminal pro-endothelin-1 (CT proET1) and copeptin, adjusted for clinical factors, MR-proANP, and MR-proADM proved to be the strongest biomarkers in identifying patients with stable ischemic heart disease at higher risk of CV death and HF. In the latter study, elevated levels of MR-proANP and MR-proADM identified patients in whom, despite apparently low clinical risk, therapy with ACEI resulted in a reduction in the risk of CV death or HF. This evidence represents an excellent example of the potential additive prognostic role of NPs in CAD.
patients. Future studies are required to confirm and expand this observation.

As mentioned above, MR-proANP is emerging in CVD diagnosis and prognosis. Identification of the mid-regional portion of the NT prosegment of proANP derives from a technical improvement in the hormonal assay detection. Due to its higher resistance to degradation, MR-proANP appears to behave as a more reliable marker for diagnostic and prognostic purposes in CVD patients, being often superior to BNP and to other biomarkers. Further confirmation of this role may reveal useful to improve CVD management.

Valve disease

Both diagnostic and prognostic power of NPs levels have been investigated in valve heart disease. In aortic stenosis, NPs correlate extensively with the severity of stenosis, LV chamber size, wall thickness and stress, EF, left atrial size, and right ventricular pressure. Since NT-proBNP correlates better to severity of aortic stenosis and LV remodelling, whereas ANP correlates more closely with atrial pressure, measurement of both peptides may reveal more useful for diagnostic purposes. Although BNP cannot discriminate between asymptomatic and mildly symptomatic patients with aortic stenosis, it may help reveal the transition from compensated hypertrophy to early decompensation in patients with preserved EF. Both peptides are related to the severity of mitral regurgitation. However, BNP is superior to ANP in reflecting the cardiac consequences of mitral regurgitation including adverse clinical outcomes.

Although evidence is still insufficient, it is reasonable to expect that increased NPs levels may integrate the standard indications for surgery in order to identify patients who need a prompt operation. In addition, NPs level may reveal helpful to monitor the post-operative outcome.

Natriuretic peptides genetic variants and cardiovascular risk

Common genetic variants of NPPA (ANP) and NPPB (BNP), able to modulate NPs levels, contribute significantly to hypertension risk. In fact, in genome-wide association studies performed in both Caucasian and Asian populations genetic variants at the MTHFR-NPPB locus (which contains both NPPA and NPPB) act through increased ANP/BNP production to lower BP and, consequently, to influence susceptibility to hypertension development. Among others, the minor allele at rs5068 NPPA variant is associated to higher ANP levels and plays a fundamental role in hypertension risk.

Notably, CVR can also be influenced by genetic variations of NPs in a BP-independent manner. Available evidence indicates that NPPA promoter variants associate directly to higher degree of LV hypertrophy in hypertension. Moreover, the common T2238C ANP coding variant induced endothelial-dependent impaired vasorelaxation in young healthy subjects without affecting BP. It increased the risk of acute CV events (stroke and AMI) in different cohorts; it exerted a significant prognostic role on CV outcomes in patients with stable ischaemic heart disease, and finally, it showed a significant gene-by-treatment interaction in a large cohort of hypertensive patients. This variant, resulting from a T-to-C nucleotide substitution at position 2238 of the gene, leads to a change from a stop codon to an arginine amino acid. The mechanisms of vascular disease promotion, resulting from the structurally altered ANP molecule, have been dissected out providing a good basis for a molecular to clinical level translation. In fact, it was discovered that C2238/ANP does not differently activate NPR-A, compared with wild-type ANP. In contrast, it activates NPR-C with a significantly higher affinity than wild-type ANP, and, by doing so, it promotes endothelial cell damage in vitro and endothelial dysfunction in vivo, ultimately leading to increased CVR. Selective blockade of NPR-C in C2238/ANP carriers may represent a suitable strategy to reduce their CVR.

Other NPPA variants, including G664A within exon 1, have been associated to CVD phenotypes with controversial results. The minor allele at NPPA rs5068 was reported to lower cardiometabolic risk, thus supporting the protective role of higher NP levels.

Natriuretic peptides as an emerging target for cardiovascular diseases

Renin–angiotensin–aldosterone system and SNS represent established targets in the therapeutic management of major CVDs. In fact, the well-defined involvement of both systems in the control of cardiac output, systemic vascular resistance, blood volume, and BP, as well as of CV remodelling, has provided solid pathophysiological basis for the significant benefits obtained with both RAAS-blocking agents and beta-blockers in the treatment of hypertension, CAD, and HF. However, in spite of the optimal currently available treatment strategies, hypertension and CAD are still far from being considered under control and CVDs are still the leading cause of death and morbidity in Western countries. For these reasons, novel therapeutic tools for the care of these conditions are actively searched. Among the potential targets, the NPs family has long represented an attractive candidate. In fact, based on its wide range of CV functions, NPs-derived therapies are expected to improve CVD treatment. Evidence of the protective effects of NP-based therapeutic approaches is already available with regard to major CVDs, as it will be summarized below. However, on the basis of previous negative findings, uncertainties necessarily exist about the theoretical benefits of the developing NPs-based therapies.

Hypertension

The use of ANP as a therapeutic anti-hypertensive agent has been explored extensively. Both short- and long-term infusions of ANP have been tested in essential hypertension as well as in secondary forms of hypertension, producing reduction in BP, along with natriuresis and diuresis. Due to the lack of peptide stability following oral administration, an alternative approach was attempted by a strategy based on pharmacologically increased circulating ANP levels. For this purpose, a new class of drugs was introduced: selective inhibitors of neutral endopeptidase (NEP). However, candesartan revealed no substantial benefits in hypertension partly due to simultaneous increases of both vasodilators (NPs, bradikinin) and vasoconstrictors (endothelin-1, angiotensin II). To circumvent the limitations of
selective NEP inhibition, vasopeptidase inhibitors (VPI) were developed. By simultaneously blocking NEP and ACE, VPI led to reduced degradation of NPs and bradykinin with a concomitant inhibition of angiotensin II generation. In fact, use of omapatrilat resulted in a BP lowering effect greater than that achieved by ACEI alone. However, a three-fold greater risk of angioedema, particularly in black individuals, prevented a safe introduction of NEP/ACEI inhibitors into clinical practice.

Recently, a novel class of drugs (ARNi), which combine the action of both Angiotensin II receptor blocker (ARB) and NEP inhibition, was introduced. Among them, the LCZ696 compound provides a 1:1 ratio blockade of Angiotensin type 1 receptor with a valsartan moiety and NEP inhibition with a prodrug moiety (AHU377) metabolized in the final LBQ657 molecule. Its greater efficacy in controlling both systolic and diastolic BP, when compared with valsartan, was reported in a randomized double-blind Phase II trial. LCZ696 was well tolerated and no cases of angioedema were reported, not even in black subjects. These findings need to be confirmed in larger studies. Moreover, the efficacy of these drugs on target organ protection and on major CV endpoints prevention needs to be verified.

**Heart failure**

Efficacy of VPI was investigated in chronic HF. Two major trials provided no definitive information on the effects of dual ACE/NEP inhibition. In fact, omapatrilat was not superior to enalapril with respect to the primary endpoint in HF. Furthermore, occurrence of hypotension and dizziness were more frequently observed with the use of omapatrilat rather than with that of ACEI in HF patients. No symptomatic benefits were observed in chronic HF with the use of sinorphan.

Currently, NPs analogues may offer a valid alternative for the treatment of ADHF compared with the original molecules. Carperitide is a 28-amino acid molecule synthesized by genetic recombination. Its main effects are vasodilation, natriuresis, and RAAS inhibition, thus reducing both preload and afterload, causing hypotension and increasing cardiac output. Carperitide was approved for the treatment of ADHF in Japan in 1995. It is currently used in patients with either normotensive or hypertensive ADHF leading to systolic BP improvement, filling pressures, and enhanced diuresis. Brain natriuretic peptide itself can be administered as a treatment for HF with beneficial actions on myocardial structure and function. In 2001, the FDA approved the use of nesiritide, which is a recombinant form of human BNP, for the treatment of ADHF. However, it was observed that nesiritide may worsen renal function and increase the risk of short-term mortality when given to patients with ADHF. The latter evidence was not confirmed in patients with decompensated HF and preserved EF. It is noteworthy that no studies, including the ATTEND Investigators trial, have shown a significant impact on major CV endpoints in HF under administration of either ANP or BNP recombinant, thus leaving still open the question of the true beneficial effects of NPs recombinants on CVD outcomes.

The new dual Angiotensin receptor and nephrinsin inhibitor LCZ696 have been tested in HF patients with preserved EF. It reduced NT-proBNP levels, as a consequence of improvement in LV wall stress, more than valsartan alone. Its impact on chronic
systolic HF, as well as on morbidity and mortality in HF, is currently being tested in a large prospective trial in comparison with ACEI.84

**Acute myocardial infarction**

Atrial natriuretic peptide can inhibit early activation of neurohormonal factors and inflammation after reperfusion therapy. It can also reverse arrhythmias, apoptosis of cardiac myocytes and endothelial cells, and limit infarct size and LV remodelling, thereby improving LV function in animal models with ischaemia/reperfusion injury.85

The ability of ANP to prevent LV dilation and remodelling after primary coronary angioplasty in patients with AMI has been proven.86 Patients received either ANP infusion or isosorbide dinitrate administration immediately after primary coronary angioplasty. The outcome after ANP infusion was superior to that obtained with isosorbide dinitrate. Findings from a multicentre study also suggest that ANP can reduce subsequent re-hospitalization or death due to HF. These cardioprotective effects of ANP were observed at a low dose of ANP without a large effect on systemic BP in patients with AMI.87

Nesiritide, given soon after AMI, induced increments in plasma cGMP and CNP and decrements in other endogenous cardiac peptides with a neutral effect on renal function and a trend towards favourable LV remodelling.88

Of note, few studies have shown that CNP also acts as an efficient anti-remodelling agent after AMI. In fact, serial infusions of CNP in rats after AMI were associated with reduction in LV dilatation, increase in systolic and diastolic performance, and reduction in ventricular fibrosis.12

All mentioned studies reinforce the therapeutic potential of NPs in the treatment of AML, and their beneficial effects on morbidity and mortality in post-MI patients. The contributory role of the dual angiotensin receptor and nephrin inhibitor in ischaemic heart disease remains a matter of future investigation.

**Cardiac surgery**

A promising field of therapeutic application for NPs is represented by cardiac surgery. In this regard, continuous intravenous administration of either ANP or BNP has been performed in cardiac surgery resulting in cardiac and reno-protective effects.89 In the NAPA trial, use of BNP led to lower creatinine values, higher glomerular filtration rate, and higher survival rate.90 A trial of BNP in patients with preoperative renal dysfunction showed better postoperative results in the BNP patients.91 A recent pilot study evaluated the cardioprotective effects of ANP shot, simultaneously to the induction of cardioplegia during CABG surgery, and reported evidence of lower operative mortality and complications, lower incidence of arrhythmias and of HF.92

**Conclusions**

Three decades of research have outlined an important contribution of NPs in CV physiology and pathology. Following assessment of their diagnostic role, particularly in HF, a significant prognostic implication has emerged towards CV outcome in HF, hypertension, and CAD. Furthermore, NPs have shown predictive power towards CVD occurrence in the general population. Based on the wide range of CV implications of these hormones, NP-derived therapeutic approaches are currently viewed as a reasonable tool to fight CVDs. Available evidence obtained with NPs-based therapeutic approaches is generating active clinical research. The next years will assess whether these compounds will contribute to improve CV therapeutic management.

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NPs and cardiovascular diseases


