Natriuretic Peptide Processing in Patients with and Without Left Ventricular Dysfunction

Sofie Verstreken, MD, Leen Delrue, PhD, Marc Goethals, MD, Jozef Bartunek, MD and Marc Vanderheyden, MD

Summary
This study aimed to examine the relationship between corin expression and circulating brain natriuretic peptide in patients with left ventricular (LV) dysfunction.

Circulating levels of B-type natriuretic peptide (BNP) can be an indicator of LV dysfunction. The 32-amino-acid BNP is cleaved by corin, a cardiac serine protease, from its 108-amino-acid pro-brain natriuretic peptide (proBNP) precursor.

This study included 25 patients with idiopathic dilated cardiomyopathy (DCMP) and LV dysfunction and 44 heart transplant recipients with normal LV function who underwent diagnostic left and right heart catheterization. Blood samples were used to determine the ratio of plasma proBNP/BNP levels, and LV endomyocardial biopsies were used to determine the expression of NPPB, which encode BNP and corin, respectively, by quantitative reverse transcription-polymerase chain reaction.

Patients with DCMP revealed worse hemodynamic profiles and higher plasma proBNP and BNP levels than those of the transplant recipients. Myocardial NPPB expression was higher and CORIN expression was lower in the DCMP patients than in the transplant recipients. CORIN expression significantly correlated with NPPB expression (r = −0.585; P < 0.001), ejection fraction (EF; r = 0.694; P < 0.01), LV end-diastolic pressure (r = −0.373; P < 0.05), and indexed end-diastolic LV volume (r = −0.452; P < 0.001). In addition, the plasma proBNP/BNP levels inversely correlated with the CORIN expression (r = −0.362; P < 0.005).

Decreased myocardial CORIN expression and the corresponding higher levels of circulating unprocessed proBNP in DCMP may partly account for the relative BNP resistance observed in patients with LV dysfunction. (Int Heart J 2019; 60: 115-120)

Key words: Brain natriuretic peptide, BNP, Corin, Heart failure, Left ventricular function

B-type natriuretic peptide (BNP) is a vasoactive peptide hormone that is synthesized and secreted mainly from the heart ventricles. The human gene for BNP (NPPB) encodes a 134-amino-acid preproBNP precursor, which after removal of a 26-amino-acid signal peptide, gives rise to a 108-amino-acid proBNP polypeptide. proBNP is further processed by the type 2 transmembrane serine protease corin, resulting in an inactive 76-amino-acid N-terminal fragment BNP (N-proBNP) and a physiologically active 32-amino-acid carboxyl-terminal BNP molecule. The biological activity of the cleaved BNP is 8-fold greater than the unprocessed proBNP form.

Interestingly, as the concentration of BNP in the plasma increases, ventricular function declines, and thus, the BNP levels are useful for diagnosing left ventricular (LV) dysfunction and for stratifying and guiding therapy for patients with heart failure; however, patients with extreme heart failure and high circulating BNP levels (assessed with the standard clinical conventional assays) often display blunted BNP-mediated cardiovascular and renal effects. Furthermore, the infusion of recombinant BNP in patients with acute heart failure promptly leads to symptomatic improvement. The biological basis for this paradox has been attributed to a desensitization and down-regulation of the natriuretic peptide receptor type A and the upregulation of phosphodiesterase 5, leading to enhanced cGMP degradation and augmented neutral endopeptidase activity. Recently, it was postulated that abnormal proBNP processing, resulting in lower levels of circulating active BNP during episodes of overt heart failure, may at least partially account for such natriuretic peptide resistance. Thus, the abnormalities in corin-mediated natriuretic peptide processing may attenuate the in vivo biological activity of the natriuretic peptide system and contribute to heart failure progression; however, data concerning proBNP processing in humans are scarce. This study aimed to examine the expression of the gene encod-
ing corin (CORIN) and its relationship with circulating BNP forms in patients with LV dysfunction.

Methods

Study population: This study included 25 consecutive symptomatic NYHA III idiopathic dilated cardiomyopathy (DCMP) patients with depressed LV function and 44 heart transplant (Tx) recipients with normal LV systolic and diastolic function referred for elective diagnostic left-heart catheterization. Patients with ischemic cardiomyopathy, atrial fibrillation, or renal insufficiency, defined by an end-diastolic pressure of the left ventricle (LVEDP) ≥ −70 mmHg, were excluded. During investigation, five DCMP patients were treated with digoxin; all DCMP patients received angiotensin-converting-enzyme inhibitors, diuretics, and beta-blockers. All patients provided their oral informed consent, and the local ethical committee approved the study.

Measurements of natriuretic peptide levels in plasma:
Whole blood samples (5 mL) were collected from the femoral veins of patients under stable hemodynamic conditions and were subsequently frozen and maintained at −80°C. Levels of BNP from plasma samples were quantitatively determined with a fluorescence (Alere Diagnostics GmbH, Mannheim, Germany) as outlined in prior publications. Notably, the BNP and NT-proBNP bioassays do not use specific antibodies and thus, also recognize the precursor (proBNP) protein. ProBNP levels were measured using the Bioplex 2000 proBNP assay (Bio-Rad Laboratories, Hercules, CA). In this two-step sandwich fluorescence immunoassay, a proBNP-specific monoclonal antibody against the hinge region of the prohormone (residues 75–80 that are absent from processed natriuretic peptides) is used as the capture antibody and an anti-BNP monoclonal phycoerythrin-conjugated antibody is used for detection. The assay reveals no cross-reactivity with circulating BNP and NT-proBNP, enabling stringent determination of the prohormone only. The detection limit, calculated as the mean ± 3 standard deviations, was computed as 4 ng/L with an intra-assay coefficient of variation < 5%. The ratio of proBNP/BNP levels was used to estimate the degree of unprocessed BNP and reflects the natriuretic peptide processing efficiency.

Left-right heart catheterization: The left and right sides of patients’ hearts were catheterized from the femoral arteries and veins. Pulmonary capillary wedge pressures were measured using a Swan-Ganz catheter, whereas LV pressures were recorded with catheters positioned in the patients’ left ventricular cavities. LV angiograms were obtained with patients in the right and left anterior oblique positions. LV volumes and ejection fractions (EFs) were derived from single-plane angiograms using the area-length method. An impaired preload reserve was defined by an end-diastolic pressure of the left ventricle (LVEDP) of ≥ 16 mmHg.

Gene expression in endomyocardial biopsies: LV endomyocardial biopsies were obtained from DCMP patients using a long guiding sheath and a disposable transfemoral biopette (Cordis Corp., Hialeah, FL) at the level of the distal interventricular septum. In Tx recipients, ventricular endomyocardial biopsies were procured during routine annual check-ups. None of the Tx patients was suffering from acute rejection during biopsy. Biopsy samples were snap frozen in liquid nitrogen and stored at −80°C.

Highly sensitive reverse transcription-polymerase chain reaction (RT-PCR) was used for quantifying the gene expression as previously described. Briefly, total RNA was isolated from the ventricular endomyocardial biopsies using the RNeasy fibrous tissue mini kit with DNase digestion (Qiagen, Hilden, Germany). RNA was reverse transcribed with random primers using the high-capacity cDNA archive kit (Applied Biosystems, Foster City, CA). RT-PCR was performed in 96-well plates on an ABI Prism 7000 sequence detection system (Applied Biosystems) using TaqMan universal PCR master mix with final reaction volumes of 25 μL. PCR primers and FAM probes for all of the target genes were purchased as Assays-On-Demand (Applied Biosystems): Hs00198141_m1 for CORIN and Hs00173590_m1 for NPPB. The relative expression of the target genes was normalized to the level of GAPDH in the same cDNA sample. All reactions were performed in triplicates.

Statistical analysis: Data were analyzed using SPSS (v 16.0; SPSS Inc., Chicago, IL) and are presented as the means ± the standard errors for normally distributed variables and as medians [twenty-fifth-seventy-fifth quartiles] when non-Gaussian distributed. Mann-Whitney tests and Pearson’s correlation coefficients were used for appropriate comparisons of nonparametric data; student’s t-tests and Spearman’s correlation coefficients were used for appropriate comparisons of parametric data. Statistical significance was set at a two-tailed probability level of less than 0.05.

Results

Clinical characteristics and plasma levels of natriuretic peptides: The Table summarizes the baseline clinical and hemodynamic data of all patients included in the study. As assumed, DCMP patients were characterized by a worse hemodynamic profile, with lower EF values and higher EDVI and pulmonary capillary wedge, and mean pulmonary artery pressures, than in the Tx recipients. Collectively, the plasma BNP levels ranged from 21 to 3036 pg/mL (median, 235 [64–602] pg/mL), and proBNP levels ranged from 14 to 3132 pg/mL (median, 366 [95–1128] pg/mL). Plasma natriuretic peptide levels were significantly higher in the DCMP patients than in the Tx recipients (P < 0.001; Table). The proBNP/BNP ratio, which represents the degree of unprocessed BNP, was significantly higher in the DCMP group and correlated inversely to LV EF (r = −0.492; P < 0.001).

CORIN and NPPB expression: In DCMP patients, myocardial NPPB expression was significantly higher (170 [38–369] versus 2 [1–25] relative units; P < 0.001), whereas CORIN expression was significantly lower (6 [2–15] versus 19 [14–31] relative units; P = 0.005) than in the Tx recipients (Figure 1). Strong inverse correlations were observed between CORIN and NPPB expression (r =
In the heart, natriuretic peptide processing and CORIN expression are related to left ventricular volume and blood pressure. The results from this study offer several new mechanistic insights into the regulation of BNP in advanced heart failure. We found that both myocardial corin and BNP are related to left ventricular stretch, with BNP upregulated and corin downregulated in the failing myocardium. The downregulation of corin is associated with less efficient BNP processing, as evidenced by a higher proBNP/BNP ratio. The higher ratio may partly explain the relative BNP resistance observed during heart failure.

**ProBNP regulation in heart failure:** The overly simplistic paradigm of natriuretic peptide processing, involving two forms of circulating BNP, has been recently challenged. Low- and high-molecular-weight forms of BNP are both detected by immunohistology in the healthy subjects and heart failure patients.\(^1\,2\,11\,22\) We were able to detect the intact, unprocessed proBNP form in patients both with and without LV dysfunction. These results are consistent with those of Hawkridge, et al.,\(^23\) who reported that the elevated levels of BNP, detected in the heart failure patients in clinical assays, comprised primarily of proBNP, with only a minor contribution from active BNP. In addition, we further demonstrated that the ratio of unprocessed proBNP over BNP increases when filling pressures are higher, indicating that natriuretic peptide processing in advanced heart failure becomes rate limiting as 

\(-0.585; P < 0.001\), plasma ProBNP levels \( (r = -0.517; P < 0.001) \), and proBNP/BNP ratios \( (r = -0.362; P < 0.005) \).

**Relationship between myocardial gene expression and LV hemodynamics:** In general, positive relationships were observed between NPPB expression and LVEDP \( (r = 0.548; P < 0.001) \) and EDVI \( (r = 0.309; P < 0.05) \), whereas NPPB expression was inversely correlated with EF \( (r = -0.630; P < 0.001; \text{Figure } 2) \). Likewise, a positive correlation was found between CORIN expression and EF \( (r = 0.694; P < 0.001; \text{Figure } 2) \), whereas CORIN expression was inversely correlated with LVEDP \( (r = -0.373; P < 0.05) \) and indexed end-diastolic volume of the left ventricle \( (LVEDVI; L \text{V} \text{EDV}I) \) \( (r = -0.452; P < 0.001) \).

Patients with an impaired preload reserve revealed higher BNP \((556[327–822] \text{ versus } 128 [60–265] \text{ pg/mL}; P < 0.001) \) and proBNP \((1015 [626–1570] \text{ versus } 229 [74–593] \text{ pg/mL}; P < 0.001) \) plasma levels, as well as higher NPPB expression \((190 [35–346] \text{ versus } 6 [1–170] \text{ relative units}; P < 0.05) \), than those with a preserved preload reserve. In addition, CORIN expression was lower \((8 [4–13] \text{ versus } 15 [6–31] \text{ relative units}; P = 0.02) \) and proBNP/BNP ratios were higher \((1.68 [1.43–1.90] \text{ versus } 1.43 [1.11–1.74]; P < 0.05) \) in patients with impaired preload reserve than in those with preserved preloads. Finally, patients with CORIN expression levels above the median had lower proBNP/BNP ratios than those below the median \((1.50 [1.21–1.73] \text{ versus } 1.76 [1.43–2.14]; P < 0.05; \text{Figure } 3) \).

**Discussion**

The BNP-mediated pathway plays an important physiological role in maintaining the normal intravascular volume and blood pressure. The results from this study offer several new mechanistic insights into the regulation of BNP in advanced heart failure. We found that both myocardial corin and BNP are related to left ventricular stretch, with BNP upregulated and corin downregulated in the failing myocardium. The downregulation of corin is associated with less efficient BNP processing, as evidenced by a higher proBNP/BNP ratio. The higher ratio may partly explain the relative BNP resistance observed during heart failure.

**Natriuretic Peptide Processing and CORIN**

**Table.** Clinical Characteristics, Hemodynamics, and Natriuretic Peptide Levels in Patients

<table>
<thead>
<tr>
<th>Category</th>
<th>DCMP (n = 25)</th>
<th>Tx (n = 44)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical characteristic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>56 ± 13</td>
<td>61 ± 11</td>
<td>0.014</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>82</td>
<td>89</td>
<td>NS</td>
</tr>
<tr>
<td>Serum creatinine (mg %)</td>
<td>1.33 ± 0.6</td>
<td>1.24 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Medication (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE-I</td>
<td>100</td>
<td>40</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>100</td>
<td>15</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Aldosterone antagonist</td>
<td>90</td>
<td>0</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Diuretic</td>
<td>100</td>
<td>30</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Digoxin</td>
<td>20</td>
<td>0</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Hemodynamics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean PA (mm Hg)</td>
<td>28 ± 1</td>
<td>23 ± 1</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>PCWP (mm Hg)</td>
<td>18 ± 1</td>
<td>14 ± 1</td>
<td>0.03</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>22 ± 3</td>
<td>14 ± 1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LVEDVI (mL/m²)</td>
<td>123 ± 7</td>
<td>66 ± 4</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>36 ± 3</td>
<td>75 ± 3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BNP (pg/mL)</td>
<td>367 [117–698]</td>
<td>87 [54–249]</td>
<td>0.001</td>
</tr>
<tr>
<td>NT-proBNP (pg/mL)</td>
<td>1423 [522–4452]</td>
<td>276 [191–1099]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ProBNP (pg/mL)</td>
<td>644 [199–1459]</td>
<td>97 [64–376]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ProBNP/BNP ratio</td>
<td>1.7 [1.4–2.1]</td>
<td>1.4 [1.1–1.7]</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

ACE-I indicates angiotensin converting enzyme inhibitor; BNP, B-type natriuretic peptide; DCMP, idiopathic dilated cardiomyopathy; LVEDVI, indexed end-diastolic volume of the left ventricle; LVEF, ejection fraction of the left ventricle; LVEDP, end-diastolic pressure of the left ventricle; NT-proBNP, N-terminal fragment of pro brain natriuretic peptide; NS, not significant; PA, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; proBNP, pro brain natriuretic peptide; and Tx, heart transplant.
the disease progresses. It was proposed that the underlying mechanism for this is the exocytosis of proBNP from cardiomyocytes and the subsequent exposure to and cleavage by corin.24 Indeed, corin deficiency in the mouse models and in humans with specific polymorphic variants25 results in systemic hypertension and cardiac hypertrophy.25-27)

Data concerning the regulation of corin expression during heart failure are contradictory. On the one hand, corin expression is induced in cultured cardiomyocytes upon hypertrophic stimuli28 and in experimental models of myocardial infarction or doxorubicin-induced cardiomyopathy.28 On the other hand, the protein levels of corin in the atrium as well as its enzymatic activity29 are similar in nonfailing and failing human hearts, although higher amounts of protein have been found in the ventricles of failing hearts than in nonfailing or hypertrophic hearts. Moreover, Dong, et al. reported that corin levels in plasma are lower in patients with heart failure than in those with acute myocardial infarction. Importantly, we found that the decrease in CORIN expression was proportional to the extent of LV dilatation and the rise in filling pressures. In addition, this decrease was inversely related to NPPB expression. Collectively, these observations suggest that corin availability/production is blunted in the volume-overloaded heart and that an insufficient amount of corin is available to produce physiologically active BNP. These data indicate that diastolic stretch and LV dilatation are important hemodynamic factors hampering the conversion of proBNP to BNP.

Limitations: This study was limited by the fact that only pts with chronic heart failure on optimal medical therapy were included and therefore, corin expression and proBNP processing were not evaluated in a setting of acute heart failure; however, Ihebuogu, et al.30 found a similar relationship between high levels of uncleaved proatrial natriuretic peptide and low circulating corin levels in patients with acute heart failure, which suggests that a similar mechanistic relationship exists in acute and chronic heart failure. Furthermore, we did not measure the corin activity in endomyocardial biopsies nor plasma corin levels, although previous studies have similarly reported that lower soluble plasma corin levels in heart failure correlate with disease severity.30

Clinical implications: Patients with extreme heart failure and high circulating BNP levels often display blunted BNP-mediated cardiovascular and renal effects, resulting

![Figure 1](image1.png)
Figure 1. Endomyocardial expression of B-type natriuretic peptide (BNP) and corin mRNAs in idiopathic dilated cardiomyopathy (DCMP) and heart transplant (Tx) patients.

![Figure 2](image2.png)
Figure 2. Correlations between left ventricular ejection fraction (LVEF) and expression of B-type natriuretic peptide (BNP) and corin mRNAs. Open circles represent idiopathic dilated cardiomyopathy (DCMP) patients. Solid circles represent heart transplant (Tx) patients.
in fluid and salt retention. Our data identify a role of reduced corin expression in abnormal proBNP processing in this process. As commercial assays can cross-react with BNP and Nt-proBNP, they may not optimally reflect endogenous natriuretic peptide bioactivity. Accordingly, the monitoring of the different BNP forms, in particular, plasma levels of proBNP, may be helpful to more accurately diagnose heart failure and predict its severity. In addition, further research, determining the physiological contributions of each of these BNP forms to the cardiac function, may lead to novel therapies for patients with heart failure, such as strategies to enhance corin activation to promote vasorelaxation, natriuresis, and diuresis.

**Disclosures**

**Conflicts of interest:** None.

**References**

30. Ibebuogu UN, Gladysheva IP, Houng AK, Reed GL. Decompen sated heart failure is associated with reduced corin levels and decreased cleavage of pro-atrial natriuretic peptide. Circ Heart Fail 2011; 4: 114-20.