Chapter 4

Anaemia and renal dysfunction are independently associated with B-type Natriuretic Peptide and N-terminal pro B-type Natriuretic Peptide in patients with heart failure

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Submitted
Abstract

**Aims:** Anaemia (by increasing plasma volume) and renal dysfunction (by decreasing clearance) may affect B-type Natriuretic Peptide (BNP) and N-terminal proBNP (NT-proBNP) levels, although this has not been well described in heart failure (HF) patients. We therefore aimed to study the influence of anaemia and renal function on BNP and NT-proBNP levels in hospitalised HF patients.

**Methods and Results:** We studied 541 patients hospitalised for HF, and BNP and NT-proBNP levels were measured before discharge. Of these patients (71 ± 11 years of age, 62% males, left ventricular ejection fraction 0.33 ± 0.14), 30% (n=159) was anaemic (Hb<7.5 mmol/L for women and Hb<8.1 mmol/L for men). Of the 159 anaemic patients, 73% had renal dysfunction (eGFR <60 ml/min/1.73m²) and of the non-anaemic patients, 57% had renal dysfunction. Multivariable analysis demonstrated that both plasma haemoglobin and eGFR were independently related to the level of both BNP and NT-proBNP (standardized beta’s of -0.20, -0.13 [BNP] and -0.26, -0.28 [NT-proBNP] respectively, P-values <0.01).

**Conclusion:** Anaemia and renal dysfunction are related to increased BNP and NT-proBNP levels, independent of the severity of HF. Thus, false positive values of elevated BNP and NT-proBNP levels can be anticipated in HF patients with anaemia and/or renal dysfunction.

Introduction

The diagnostic accuracy of B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) in patients who are presented at the emergency department for acute dyspnoea is well described. However, even with optimal cut-off values determined by receiver operating characteristic curves, approximately 10% of the patients have false negative outcomes, and 16%-24% of the patients have false positive outcomes. To further increase the diagnostic accuracy of these natriuretic peptides, it is important to identify other factors, beside the severity of HF, that influence BNP levels and NT-proBNP levels. We and others have previously described that, in addition to its correlation with severity of HF, both BNP and NT-proBNP levels are influenced by several other factors such as age, gender, obesity and renal function.

Another factor that might influence NT-proBNP levels and that is frequently found in patients with heart failure is anaemia. Since anaemia causes increased plasma volume independent of severity of HF, and because natriuretic peptides are released in reaction to ventricular plasma overload it is conceivable that natriuretic peptides are higher in anaemic HF patients compared to non-anaemic HF patients. In 209 patients without HF or renal disease Willis et al. recently demonstrated that NT-proBNP concentrations were significantly higher in patients with anaemia compared to patients without anaemia. In contrast, in a subgroup analysis of the Breathing Not Properly trial, no correlation was found between BNP levels and haemoglobin in 200 patients with systolic HF, and only a weak correlation was found between BNP levels and diastolic HF. In that study patients with renal failure were excluded. To our knowledge, the independent influence of anaemia on NT-proBNP levels has never been investigated in a HF population without exclusion of patients with renal failure. Moreover, we are not aware of any study investigating the influence of anaemia...
Anaemia, renal function, BNP and NT-proBNP. Since renal dysfunction is a well-known cause of anaemia\textsuperscript{16} and because it may influence both BNP levels and NT-proBNP levels by decreasing clearance,\textsuperscript{17} renal dysfunction should be taken into account when studying the effect of anaemia on BNP levels and NT-proBNP levels. Hence, the aim of the present study was to investigate the relationship between anaemia and renal function with BNP levels and NT-proBNP levels in HF patients.

**Methods**

**Study population**

The present study complies with the Declaration of Helsinki, the local ethics committee has approved the research protocol and informed written consent has been obtained from the subjects. All patients in the present study were recently admitted for decompensated HF (NYHA II-IV), when they were included in a multicenter HF trial conducted in the Netherlands (COACH).\textsuperscript{18} All participating sites (n = 17) were experienced HF centre’s. Patients were at least 18 years of age with evidence of structural underlying heart disease. Detailed information on the study design has been published before.\textsuperscript{18} In short, COACH is a randomised controlled trial investigating the effect of education and counselling on readmission for HF and mortality. Of the 1049 patients included in the COACH study, 543 patients had NT-proBNP levels available at baseline, 601 patients had BNP levels available at baseline, and 541 patients had both BNP and NT-proBNP levels available at baseline. Main reasons for missing BNP data were: no Triage\textsuperscript{®} BNP meter available and the absence of a possibility to store plasma samples at -80°C (n = 177), unplanned hospital discharge (n = 75) or death during admission (n = 20). Main reasons for missing NT-proBNP data were: the start of the NT-proBNP sub study after already 272 patients were included in COACH, the absence of a possibility to store plasma samples at -80°C (in 1 out of 17 clinics; n = 71), unplanned hospital discharge or logistical problems (n = 155) and death during admission (n = 9).

**Measurement of BNP and NT-proBNP levels**

Blood was collected shortly before discharge between 8:00 AM and 4:00 PM, after patients had been clinically stabilised and were recovered well enough to go home. Ten millilitres of whole blood was taken from an antecubital vein and collected into tubes containing potassium ethylenediaminetetraacetic acid (EDTA; 1 mg/ml blood) when patients were in a supine position. The tubes were centrifuged for 10 minutes (2500 x g) and the plasma was separated and stored in polypropene tubes at -70°C to -80°C. The plasma samples were transported (on dry ice) to the Core Laboratory at the University Medical Centre Groningen, the Netherlands.

**BNP measurement**

In 364 out of the 541 patients, BNP levels were determined on site in whole blood within 4 hours after blood collection. In 177 out of the 541 patients BNP levels were determined in plasma at the Core Laboratory. All measurements were performed using a fluorescence immunoassay kit (Triage\textsuperscript{®}, Biosite Incorporated, San Diego, CA). Details on the system provided by the manufacturer indicated the analytical sensitivity of the assay is less than 5.0 pg/ml. The system has been validated before.\textsuperscript{19} The measurable range of the BNP assays was 5.0-5000.0 pg/ml.
NT-proBNP measurement: All measurements of NT-proBNP levels were performed in plasma at the Core laboratory on an Elecsys™ 2010 analyser, a commercially available electrochemiluminescent sandwich immunoassay (Elecsys proBNP, Roche Diagnostics, Mannheim, Germany). The intra-assay precision (coefficient of variation) is 1.2 – 1.5%, and the inter-assay precision (coefficient of variation) is 4.4 – 5.0%, with an analytical range of 5 – 35000 pg/ml.

Anaemia
The definition of anaemia according to the World Health Organisation was used; Hb < 7.5 mmol/l (12 g/dl) for women and Hb < 8.1 for men (13 g/dl).

Renal function
Serum creatinine was determined from a blood draw shortly before discharge, in the local laboratory at each centre. Estimated Glomerular Filtration Rates (eGFR’s) were calculated using the Levey – modified Modification of Diet in Renal Disease formula:

\[
eGFR (\text{ml/min/1.73m}^2) = 186 \times \text{SCr}^{-1.154} \times \text{age}^{-0.203} \times (0.742 \text{ if female}) \times (1.21 \text{ if black})
\]

Boston score
For 501 out of the 541 patients with BNP and NT-proBNP levels available, the Boston score, a quantification related to severity of HF, was calculated. This score was used in combination with left ventricular ejection fraction (LVEF) to adjust possible determinants of BNP levels and NT-proBNP levels for severity of HF in the multivariable regression analysis. In short, the score consists of a medical history sub score, a physical examination sub score and a chest radiography sub score. For each sub score, a maximum of 4 points is allowed. The diagnosis HF is classified definite for a total score of 8 – 12 points, possible for a total score of 5 – 7 points and unlikely for a total score of 4 points or less. The Boston score was missing in 13 cases, since no chest radiography data were available of these patients.

Statistical analyses
In order to study the independent relationship between anaemia haemoglobin (Hb) and renal function (as expressed in the eGFR) with BNP and NT-proBNP levels, univariable and multivariable linear regression analyses were performed in the patient population with both BNP and NT-proBNP levels available (n=541). Because the BNP and NT-proBNP levels had a skewed distribution the natural logarithm was used to get an optimal residual analysis. To study potential confounding factors, the following variables were used in univariable analyses (Pearson and Spearman correlation coefficients when appropriate) with BNP and NT-proBNP as the dependent variables: age, gender, LVEF, the Boston score, New York Heart Association (NYHA) functional class, HF aetiology, duration of HF symptoms, pulmonal congestion, rales, heart rate, systolic and diastolic blood pressure, presence of atrial fibrillation/flutter, presence of pacemaker, hematocrit, renal disease, body mass index (BMI), hypertension, pulmonary embolism, chronic obstructive pulmonary disease or asthma, diabetes mellitus (type 1 and 2), HF II medication at admission and at discharge (diuretics, ACE inhibitors/angiotensin receptor blocker, beta-blockers, spironolactone). In the multivariable linear regression analysis, a stepwise approach was used. Besides Hb and eGFR, a univariable P-value < 0.15 was required to enter a variable into the multivariable model, and if the P-value was > 0.05 a variable was removed from the model. NT-proBNP or BNP
levels were presented in bar charts stratified by quintiles of Hb. To get more insight in the
distribution of NT-proBNP and BNP across the ranges of Hb, ANOVA trend analyses were
performed for NT-proBNP or BNP after these were divided by quintiles of Hb. The associa-
tions of the anaemia and renal function with NT-proBNP and BNP were presented in bar
charts. Outcomes were considered significant when P<0.05. Values are presented as means
± SD except when stated otherwise. All analyses were performed with SPSS version 11.

Results

Study populations

Demographic and clinical characteristics of the 541 patients with both BNP and NT-proBNP available are presented in table 1. Mean age of these 541 patients was 71 (± 11) years, and more than half of the population was male (62%) and had a non-ischemic aetiology for HF (59%). On average, LVEF was 0.33 (±0.14), Hb was 8.4 (±1.2) mmol/l, eGFR was 54 (± 20) ml/min/1.73m² and BMI was 26 (± 5) kg/m². At discharge, patients were
classified as NYHA functional class II (52%), III (46%) or IV (2%), and were on medical
therapy including diuretics (88%), ACE inhibitors/ angiotensin II receptor blockers (71%),
beta-blockers (61%) (table 1). Characteristics were not significant different between pa-
tients with available BNP/NT-proBNP levels (n=541) and the total patient group included
in COACH (n=1049).

BNP and NT-proBNP levels

The median BNP value in the 541 patients with BNP and NT-proBNP available was 448 pg/ml, the interquartile range 209 – 916 pg/ml, the minimum value 14 pg/ml and the
maximum value 5000 pg/ml. The median NT-proBNP value was 2599 pg/ml, the inter-
quartile range 1314 – 5885 pg/ml, the minimum value 39 pg/ml and the maximum value
75361 pg/ml (table 1).

Anaemia

Hb levels were available in 528 of the 541 patients. BNP and NT-proBNP levels divided by
quintiles of haemoglobin are presented in figure 1. Anaemia was present in 30% (n=159)
of the 541 patients in which BNP and NT-proBNP levels were available. Of these 159 anaem-
ic patients 114 (73%) also had renal dysfunction (eGFR < 60 ml/min/1.73m²) and of the
369 non-anaemic patients 209 (57%) had renal dysfunction (figure 2).

Univariable determinants of BNP levels

Hb (P<0.001) and eGFR (P<0.01) were univariably related to the natural logarithm of
BNP. Additionally, the following variables were potential confounders to these relationships (P<0.15): age (P=0.11), LVEF (P<0.001), NYHA functional class (P=0.01), Boston
score (P=0.14), ischemic/ non-ischemic aetiology of HF (P=0.04), pulmonary congestion
(P=0.05), myocardial infarction before admission (P=0.12), systolic (P<0.01) and dia-
stolic blood pressure (P<0.01), atrial fibrillation/ flutter (P=0.11), hematocrit (P<0.001),
BMI (P<0.001), prescribed diuretics at admission (P=0.06).
Table 1: Patient characteristics (n=541)

<table>
<thead>
<tr>
<th>Demographics</th>
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<tbody>
<tr>
<td>Age (yrs)</td>
<td>71 ± 11</td>
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<td>Gender (m)</td>
<td>62%</td>
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<table>
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<tr>
<th>Heart failure/ physical examinations</th>
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<tbody>
<tr>
<td>BNP (median [IQR], pg/ml)</td>
<td>448 (209 – 916)</td>
</tr>
<tr>
<td>NT-proBNP (median [IQR], pg/ml)</td>
<td>2599 (1314 – 5885)</td>
</tr>
<tr>
<td>LVEF</td>
<td>0.33 ± 0.14</td>
</tr>
<tr>
<td>NYHA at discharge (II, III, IV)</td>
<td>48%, 49%, 3%</td>
</tr>
<tr>
<td>Ischemic/ non-ischemic HF</td>
<td>41% / 59%</td>
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<tr>
<td>Duration HF symptoms (yrs)</td>
<td>2.7 ± 4.5</td>
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<tr>
<td>Pulmonal congestion (X-ray)</td>
<td>65%</td>
</tr>
<tr>
<td>Rales during admission</td>
<td>89%</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>74 ± 13</td>
</tr>
<tr>
<td>Systolic BP (mm/Hg)</td>
<td>118 ± 21</td>
</tr>
<tr>
<td>Diastolic BP (mm/Hg)</td>
<td>69 ± 12</td>
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<tr>
<td>Sinus rhythm</td>
<td>55%</td>
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<td>Atrial fibrillation/ flutter</td>
<td>38%</td>
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<table>
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<th>Medical History/ Co-morbidities</th>
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<tr>
<td>Myocardial infarction</td>
<td>41%</td>
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<tr>
<td>Hypertension</td>
<td>43%</td>
</tr>
<tr>
<td>COPD/ Asthma</td>
<td>31%</td>
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<tr>
<td>Diabetes (type1 or 2)</td>
<td>29%</td>
</tr>
<tr>
<td>Renal diseases</td>
<td>8%</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m^2)</td>
<td>54 ± 20</td>
</tr>
<tr>
<td>Haemoglobin (mmol/l)</td>
<td>8.4 ± 12</td>
</tr>
<tr>
<td>Hematocrit (1/l)</td>
<td>0.41 ± 0.06</td>
</tr>
<tr>
<td>Body Mass Index (kg/m^2)</td>
<td>26 ± 5</td>
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</table>

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<th>Medication at admission</th>
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<td>Diuretics</td>
<td>65%</td>
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<tr>
<td>ACE/ARB</td>
<td>45%</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>40%</td>
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<tr>
<th>Medication at discharge</th>
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<tbody>
<tr>
<td>Diuretics</td>
<td>88%</td>
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<tr>
<td>ACE/ARB</td>
<td>71%</td>
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<td>Beta-blockers</td>
<td>61%</td>
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</table>

ACE/ARB = ACE inhibitor or Angiotensin Receptor Blocker, BNP= B-type Natriuretic Peptide, BP = Blood Pressure, COPD= Chronic Obstructive Pulmonary Disease, eGFR= estimated Glomerular Filtration Rate, HF= Heart Failure, LVEF= Left Ventricular Ejection Fraction, NT-proBNP = N-terminal pro B-type Natriuretic Peptide, NYHA= New York Heart Association functional class.
Anaemia, renal function, BNP and NT-proBNP

Figure 1: BNP (A) and NT-proBNP (B) (95% CI) divided by quintiles of Haemoglobin.

A

B

P-trend <0.001
Chapter 4

Multivariable determinants of BNP levels

Hb and eGFR were independently related to the natural logarithm of BNP, and these relationships were confounded by BMI, LVEF and the Boston score (P-values ≤ 0.02). The interaction term of LVEF*eGFR added significant value to the multivariate regression model (standardised Beta = 0.11, P = 0.03). The R-square of the multivariable model was 0.21 (table 2).

Table 2: Multivariable linear regression analyses for the relationships of anaemia and renal function with lnNT-proBNP and lnBNP

<table>
<thead>
<tr>
<th>Multivariable related</th>
<th>lnNT-proBNP R² = 0.35</th>
<th>lnBNP R² = 0.21</th>
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<tbody>
<tr>
<td></td>
<td>St. Beta</td>
<td>P-value</td>
</tr>
<tr>
<td>eGFR*</td>
<td>-0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Haemoglobin*</td>
<td>-0.26</td>
<td>&lt;0.001</td>
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* Corrected for body mass index, left ventricular ejection fraction and the Boston score.

eGFR = Estimated Glomerular Filtration Rate, LnBNP = natural logarithm of B-type natriuretic Peptide plasma levels, LnNT-proBNP = natural logarithm of N-terminal pro-B-type natriuretic Peptide plasma levels, St. = standardised. The interaction term between Boston score and LVEF added significant value to the multivariable model of lnNT-proBNP (Standardized Beta 0.11, P=0.02) and the interaction term between eGFR and LVEF added significant value to the multivariable model of lnBNP (Standardized Beta 0.11, P=0.03).

Multivariable determinants of BNP levels

Hb and eGFR were independently related to the natural logarithm of BNP, and these relationships were confounded by BMI, LVEF and the Boston score (P-values ≤ 0.02). The interaction term of LVEF*eGFR added significant value to the multivariate regression model (standardised Beta = 0.11, P = 0.03). The R-square of the multivariable model was 0.21 (table 2).

Univariable determinants of NT-proBNP levels

Hb (P<0.001) and eGFR (P<0.001) were univariably related to the natural logarithm of NT-proBNP. Additionally, the following variables were potential confounders to these relations (P<0.15): age (P<0.01), LVEF (P<0.001), NYHA functional class (P<0.01), Boston score (P=0.10), pulmonary congestion (P=0.01), systolic (P=0.03) and diastolic blood pressure (P=0.01), hematocrit (P<0.001), BMI (P<0.001), hypertension (P=0.14), prescribed diuretics at admission (P<0.01) and prescribed diuretics at discharge (P=0.13).

Multivariable determinants of NT-proBNP levels

Hb and eGFR were independently related to the natural logarithm of NT-proBNP levels, and these relations were confounded by BMI, LVEF and the Boston score (P-values ≤ 0.01). The interaction term of Boston score*LVEF added significant value to the multivariate regression model (standardised Beta = 0.11, P = 0.02). The R-square of the multivariable model was 0.35 (table 2).
Figure 2: BNP levels (A) and NT-proBNP levels (B) (95% CI) divided by anaemia status and renal function

A

<table>
<thead>
<tr>
<th>Condition</th>
<th>N</th>
<th>(%)</th>
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<tbody>
<tr>
<td>No anaemia, no renal dysfunction</td>
<td>160</td>
<td>(30%)</td>
</tr>
<tr>
<td>No anaemia, renal dysfunction</td>
<td>209</td>
<td>(40%)</td>
</tr>
<tr>
<td>Anaemia, no renal dysfunction</td>
<td>45</td>
<td>(8%)</td>
</tr>
<tr>
<td>Anaemia, renal dysfunction</td>
<td>114</td>
<td>(22%)</td>
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B

<table>
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<tr>
<th>Condition</th>
<th>N</th>
<th>(%)</th>
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<tr>
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<td>(22%)</td>
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Anaemia was defined as Hb < 7.5 for women and Hb < 8.1 for men. Renal dysfunction was defined as eGFR < 60 ml/min/1.73m².
Discussion

The major finding of the present study is that both BNP levels and NT-proBNP levels are associated with anaemia and renal dysfunction, independent of the severity of heart failure.

**Anaemia and BNP/NT-proBNP levels**

Anaemia is a common phenomenon in HF, and is related to the severity of disease. Although BNP and NT-proBNP are also related to severity of HF, the negative association between both BNP and NT-proBNP and Hb, as found in the present study, cannot only be explained by the severity of HF. Hb was related to BNP and NT-proBNP levels independent of severity of HF as measured by both LVEF and the Boston score. The present findings are in agreement with the results of Willis et al. on 209 patients without HF or renal failure. They found that Hb added significant value to the multivariable linear regression model of NT-proBNP determinants. Another study in patients with suspected coronary artery disease (n=234) also showed an independent association between Hb and BNP levels. However, Wu et al. found no correlation between BNP levels and Hb in 200 patients with systolic HF, and only a small correlation was found between BNP and diastolic HF (r=0.047; p<0.05). The differences compared to our results might be explained by the exclusion of patients with renal dysfunction in this study by Wu et al. Since anaemia is often caused by renal insufficiency, a subgroup of HF patients without renal insufficiency does not fully represent HF patients in clinical practice and one might argue whether such a subgroup is the best to investigate the effect of anaemia on BNP levels.

The most obvious explanation for the independent associations of anaemia with BNP/NT-proBNP levels is that anaemia results in elevated plasma volume independent of severity of HF. Since BNP and NT-proBNP are released in reaction to ventricular plasma overload, it is conceivable that BNP and NT-proBNP levels are higher in anaemic HF patients compared to non-anaemic HF patients. Additionally, patients with anaemia and renal dysfunction showed higher BNP and NT-proBNP levels, compared to anaemic patients without renal dysfunction (figure 1, 2). This finding can be explained by previous findings, where renal dysfunction was found to be a major cause of anaemia in HF patients, mediated by a erythropoietin production deficiency in the kidneys.

**Renal function and BNP/NT-proBNP levels**

Elevated levels of BNP were also independently related to renal dysfunction and, although not directly compared, this relation seemed less powerful than the relation between renal dysfunction and NT-proBNP (-0.13 vs. -0.28 respectively). This difference may be explained by differences in clearance. NT-proBNP is probably mainly cleared from the blood by the kidneys, while BNP is most likely mainly cleared by neutral endopeptidases and natriuretic peptide clearance receptors. This implies that the influence of renal dysfunction should be more pronounced on NT-proBNP levels compared to BNP levels.

**Implications for clinical practice**

Our data indicate that haemoglobin and renal function should be taken into account when interpreting elevated levels of BNP and NT-proBNP. Although elevated levels naturally
could be related to worsening of heart failure, they can also be caused by anaemia or renal
dysfunction, while the severity of heart failure remains unchanged.

Conclusions
In this large group of hospitalised heart failure patients, lower haemoglobin levels and
worse renal function were independently associated with elevated BNP levels and elevated
NT-proBNP levels. These results indicate that in HF diagnosis BNP levels but especially NT-
proBNP levels might be overestimated in patients with renal dysfunction and/or anaemia.

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Diego, CA) for providing BNP assay kits, and to Novartis (Arnhem, the Netherlands) for an
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References


