Blood haemoglobin is an independent predictor of B-type natriuretic peptide (BNP)

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ABSTRACT

BNP (B-type natriuretic peptide) and anaemia are both associated with adverse outcome in patients with chronic heart failure. Whether low haemoglobin levels are independently predictive of elevated BNP levels in subjects without heart failure is unknown. In the present study, we examined the relationship between haemoglobin and BNP levels in 234 patients with suspected coronary heart disease without a history of chronic heart failure, adjusting for known predictors of BNP levels. By univariate analysis, haemoglobin levels were inversely related to logarithmically transformed BNP values ($r = -0.30, P < 0.0001$). After adjustment for patient age, gender, body mass index, history of myocardial infarction, use of diuretics, angiotensin-converting enzyme inhibitors and β-blockers, estimated creatinine clearance rate, extent of coronary disease, left ventricular ejection fraction and left ventricular end-diastolic pressure, blood haemoglobin remained an independent predictor of plasma BNP (standardized β-coefficient = −0.253, P < 0.0001). A similar relationship was observed between haematocrit and BNP (standardized β-coefficient = 0.215, P < 0.0001). We conclude that haemoglobin levels are independently predictive of plasma BNP levels in patients with suspected coronary heart disease without heart failure. Anaemia may contribute to elevated BNP levels in the absence of heart failure, and may represent an important confounder of the relationship between BNP, cardiac function and prognosis.

INTRODUCTION

BNP (B-type natriuretic peptide) is a 32-amino-acid hormone derived predominantly from the ventricular myocardium [1]. The main stimulus for BNP secretion is stretch of cardiomyocytes [2]. Accordingly, circulating BNP levels are elevated in conditions characterized by volume overload and correlate with indices of haemodynamic status and ventricular function [3,4]. Over the past few years BNP has emerged as a reliable marker of heart failure [5], and fully automated biochemical assays have been developed for clinical use. BNP is also a powerful prognostic indicator in patients with acute coronary syndromes [6–9], in patients with stable coronary artery disease [10], in patients with heart failure [11,12] and in the general population [13]. A number of non-cardiac factors are associated with circulating BNP levels and may confound the relationship between BNP and indices of cardiac function, including age [14,15], gender [14,15], renal function [16] and BMI (body mass index) [17]. Whether anaemia is a confounding factor for BNP is unknown.

Anaemia of chronic disease is a common cause of low haemoglobin levels in patients with chronic heart failure and is particularly prevalent in advanced heart failure, where its presence is associated with an adverse prognosis [18–20]. Recently, an inverse association between haemoglobin levels and BNP has been described.

Key words: anaemia, B-type natriuretic peptide (BNP), haemoglobin, heart failure.

Abbreviations: ACE, angiotensin-converting enzyme; BMI, body mass index; BNP, B-type natriuretic peptide; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

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in patients with diastolic heart failure [21]. One contribut-
ing factor to the anaemia in heart failure may be haemodilution secondary to fluid retention, a mechanism that could explain an inverse association between BNP and anaemia in heart failure patients. However, factors other than haemodilution may potentially contribute to a relationship between BNP and haemoglobin levels. To test the hypothesis that haemoglobin is an independent predictor of BNP in subjects without heart failure, we examined the association between haemoglobin and BNP levels in a large cohort of patients with suspected coronary artery disease, adjusting for established predictors of BNP.

MATERIALS AND METHODS

Patients
A series of 263 patients, referred to diagnostic cardiac catheterization for suspected coronary heart disease, were included consecutively. Patients with a recent myocardial infarction (<2 weeks), significant valvular heart disease, significant cardiac arrhythmia (including atrial fibrillation), ongoing myocardial ischaemia as evidenced by ST-T segment depression, manifest renal or hepatic failure, or chronic symptomatic congestive heart failure [NYHA (New York Heart Association) class III and IV] were ineligible. Thirteen patients with mild exertional dyspnoea (NYHA function class II) were also excluded from the current analysis. Sixteen additional patients were excluded from the analysis because of cardiac arrhythmia during the investigation (three patients), pronounced vasovagal reaction requiring leg elevation during the investi-
gation (one patient), discovery of undiagnosed mitral valve prolapse (one patient), recent undiagnosed myocardial infarction (one patient), technical errors in blood sample handling (three patients), and insufficient material for analysis of BNP or haemoglobin (seven patients), leaving 234 patients for data analysis. The baseline data of this cohort have been published previously [6]. Prior to catheterization, all patients were interviewed and examined by two experienced physicians who followed a standardized procedure. The same morning a venous blood sample for determination of haemoglobin and BNP levels was drawn from the descending aorta into a pre-
chilled plastic tube containing EDTA and aprotinin. The test tube was immediately placed on ice and centrifuged at 4 °C within 15 min of blood collection. After blood sampling, the catheter was first placed in the ascending aorta for aortic blood pressure recording, then introduced retrogradely into the left ventricle through the aortic valve for intraventricular blood pressure recording. LVEF (left ventricular ejection fraction) was ascertained by single-plane contrast ventriculography in the 30° right oblique position during held inspiration, using the area–length method. Haemodynamic measurements were performed by a single investigator, who was blinded to the BNP data. Significant coronary artery disease was defined as a diameter stenosis of at least 50 % in any of the main epicar-
dial coronary arteries. Patients were classified according to the number of main vessels affected as no significant coronary artery disease, single-vessel disease, double-

Angiography and blood sampling procedures
After rest for at least 15 min, the femoral artery and vein were cannulated and a pigtail catheter introduced into the aorta. Before contrast ventriculography, a 10 ml blood sample was drawn from the descending aorta into a pre-

Biochemical analyses
The plasma samples were stored for a maximum of 12 months at −70 °C pending analysis of BNP. BNP in plasma was determined using RIA after prior extraction with Vycor glass (Crown Crossing, Liverpool, New South Wales, Australia) [22]. The intra- and inter-assay coefficients of variation were 7 % and 10 % respectively. The blood concentration of haemoglobin, haematocrit and the concentration of creatinine in serum were deter-
mind by routine laboratory methods. The creatinine clearance rate (in ml/min) was estimated using the Cock-
roft-Gault formula [[(140−age) × weight (kg)/serum crea-
tinine (µmol/l)] multiplied by a constant of 1.25 in men
and 1.03 in women.

Statistical analysis
We present categorical variables as counts and percentages of total and continuous variables as median and inter-

### RESULTS

The characteristics of the patients are presented in Table 1. Using the World Health Organization definition (haemoglobin < 12 g/dl for women and < 13 g/dl for men), 17 patients (7.3%) were diagnosed with anaemia. The characteristics of patients subdivided according to the absence or presence of anaemia are also shown in Table 1. Patients with anaemia had borderline significantly higher plasma BNP levels than those without anaemia.

Logarithmically transformed BNP concentrations correlated significantly both with LVEF ($r = -0.33$, $P < 0.0001$; $n = 234$) and LVEDP ($r = 0.39$, $P < 0.0001$; $n = 234$), and were correlated inversely with blood haemoglobin ($r = -0.30$, $P < 0.0001$; $n = 194$) and haematocrit ($r = -0.294$, $P < 0.0001$; $n = 194$). The relationship with haemoglobin was evident both in patients with ($r = -0.301$, $P < 0.0001$; $n = 198$) and without ($r = -0.442$, $P = 0.010$; $n = 36$) angiographically significant coronary artery disease. In a multivariate linear regression model, adjusting for patient age, gender, BMI, history of myocardial infarction, pulmonary disease, use of diuretics, ACE (angiotensin-converting enzyme) inhibitors and $\beta$-blockers, estimated creatinine clearance, triple-vessel disease, LVEF and LVEDP, blood haemoglobin remained an independent predictor of plasma BNP (standardized
Figure 1 Scatter plot of the relationship between blood haemoglobin and logarithmically transformed values of plasma BNP

Table 2 Predictors of plasma BNP using a multivariable model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardized β-coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender</td>
<td>-0.018</td>
<td>0.774</td>
</tr>
<tr>
<td>Age</td>
<td>0.259</td>
<td>0.000</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.079</td>
<td>0.206</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>0.071</td>
<td>0.488</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>0.028</td>
<td>0.568</td>
</tr>
<tr>
<td>Diuretic use</td>
<td>-0.027</td>
<td>0.632</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>0.073</td>
<td>0.171</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>0.135</td>
<td>0.007</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>-0.014</td>
<td>0.872</td>
</tr>
<tr>
<td>Triple-vessel disease</td>
<td>0.120</td>
<td>0.021</td>
</tr>
<tr>
<td>LVEDP</td>
<td>0.309</td>
<td>0.000</td>
</tr>
<tr>
<td>LVEF</td>
<td>-0.210</td>
<td>0.000</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>-0.253</td>
<td>0.000</td>
</tr>
</tbody>
</table>

β-coefficient = -0.253, P < 0.001; Table 2). These variables explained 50% of the variability of BNP (adjusted $R^2 = 0.497$). Similar results were obtained for haematocrit (standardized β-coefficient = -0.215, $P < 0.0001$). Predictors of haemoglobin levels are presented in Table 3. These variables explained 30% of the variability of haemoglobin (adjusted $R^2 = 0.296$).

DISCUSSION

The new important finding of the present study is that blood haemoglobin (and haematocrit) is an independent predictor of circulating levels of BNP in subjects without heart failure. The association remained significant after adjustment for a number of cardiac and non-cardiac determinants of BNP levels, including indices of systolic (LVEF) and late diastolic (LVEDP) function, extent of coronary artery disease (triple-vessel disease), demographic factors (age and gender), historical factors (prior myocardial infarction, use of diuretics, ACE inhibitors and β-blockers) and renal function (estimated creatinine clearance).

Following the recent development of rapid fully automated assays, BNP measurement has been widely adopted for the diagnosis of heart failure. However, BNP elevation is not specific for heart failure, but is associated with a variety of factors, including advanced patient age and female gender [14,15], decreased BMI [17], decreased renal function [16] and increased left ventricular mass [4]. The present findings suggest that the presence of anaemia is another important confounder of the relationship between BNP levels and cardiac function and prognosis. Blood haemoglobin appeared to be a stronger determinant of BNP levels than factors such as BMI and renal function, and the association was of comparable strength with that between BNP and LVEDP and LVEF, factors traditionally considered to be major determinants of BNP production.

In heart failure, BNP elevation has been associated previously with anaemia and the severity of disease. In a recent study of 74 patients with chronic heart failure, haemoglobin and erythropoietin levels were associated with the severity of heart failure, BNP levels and prognosis, and in a multivariable model BNP did not provide independent prognostic information after adjustment for haemoglobin and erythropoietin [20]. In another study of 137 patients with heart failure and a normal ejection fraction, anaemia was associated with greater elevation of BNP, the severity of diastolic dysfunction and prognosis [21]. In heart failure, the classic assumption is that
plasma volume is expanded and can be monitored by assessing degree of oedema [23]. On the other hand, in heart failure patients treated with diuretics, plasma volume may be decreased [24]. Accordingly, the haemoglobin concentration in heart failure patients may differ considerably depending on volume state (pseudo-anaemia secondary to haemodilution, increased haemoglobin concentration due to diuretic-induced hypervolaemia, or true erythrocyte depletion).

In the present study, we found an independent association between haemoglobin concentration and BNP levels in patients without a history of heart failure. The exact mechanism cannot be deduced from the present data. Our data do not suggest that variability of BMI, diuretic use, pulmonary disease or renal dysfunction can explain this association. It is well known that patients with severe chronic anaemia often retain salt and water [25]. Potential mechanisms include reduction of renal blood flow and glomerular filtration rate and neurohormonal activation. However, unlike patients with myocardial disease, patients with anaemia have increased cardiac output and low systemic vascular resistance [25]. It is conceivable that the natriuretic peptide system is activated to counteract the haemodynamic and renal effects of vasoconstrictor neurohormones that may occur even in mild forms of anaemia [26]. Moreover, many of these vasoconstrictor neurohormones are powerful stimuli for BNP production [2]. To elucidate the association between BNP and haemoglobin further, investigations concerning the acute effects of blood transfusion and the chronic effect of iron or erythropoietin treatment on circulating levels of BNP and vasoconstrictor neurohormones, including noradrenaline, angiotensin II and endothelin, may be required.

The limitations of our present study include the generalizability of our results. The majority of our patients had coronary artery disease and were using antianginal medication. We therefore cannot automatically extrapolate the results to healthy individuals. However, our observation that the association between haemoglobin and BNP tended to be stronger in subjects without significant coronary artery disease than in those with significant disease suggests that a similar relationship exists in healthy individuals. Nevertheless, our results need to be confirmed in larger populations of healthy subjects.

Although the association between BNP and haemoglobin was statistically significant and of the same order of magnitude as the associations between BNP and LVEF and BNP and LVEDP, the variability in BNP explained by the variability of haemoglobin was modest. It should be emphasized therefore that haemoglobin is only one out of many determinants of circulating BNP levels.

In conclusion, blood haemoglobin levels are independently associated with plasma BNP levels in subjects without heart failure. Haemoglobin appears to be a strong and important confounder of the relationship between BNP, cardiac function and prognosis, and should be adjusted for in future studies of the diagnostic and prognostic value of BNP.

ACKNOWLEDGMENTS

C. W. K. is a recipient of a grant from the Research Foundation of Health and Rehabilitation in Norway. We are indebted to Dr Timothy G. Yandle in the Christchurch Cardioendocrine Research Group, Christchurch, New Zealand, for performing the BNP analyses.

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Received 10 December 2004/9 February 2005; accepted 8 March 2005
Published as Immediate Publication 8 March 2005, DOI 10.1042/CS20040349

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