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Association between plasma B-type natriuretic peptide and anaemia in heart failure with or without ischaemic heart disease: a retrospective study

Mitsutoshi Tominaga, Makoto Kawai, Kosuke Minai, Kazuo Ogawa, Yasunori Inoue, Satoshi Morimoto, Toshikazu Tanaka, Tomohisa Nagoshi, Takayuki Ogawa, Michihiro Yoshimura

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Division of Cardiology, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan

Correspondence to
Dr Makoto Kawai;
cadmk@jikei.ac.jp

ABSTRACT

Objectives Anaemia is a risk of worsening heart failure. However, anaemia sometimes remains undetected because the superficial cardiac function does not precisely reflect the adverse impact of anaemia. Plasma B-type natriuretic peptide (BNP) could be helpful in these cases. However, the direct anaemic effects on BNP remain unknown. Herein, we compared the direct effect of anaemia on BNP and left ventricular ejection fraction (LVEF) using an advanced statistical procedure.

Design A retrospective study.

Setting Secondary care (cardiology), single-centre study.

Participants The study consisted of 3756 inpatients, including 684 without ischaemic heart disease (IHD) and 3072 with IHD.

Primary and secondary outcome measures Relationship between plasma BNP levels and LVEF values.

Results A path model was constructed to simultaneously examine the adverse impact of anaemia on LVEF and plasma BNP, allowing for renal function. The path model revealed that LVEF increased in response to low haemoglobin (Hb), and the phenomenon was prominent in non-IHD (standardised regression coefficients (St.β): −0.264, p<0.001) rather than in IHD (St.β: 0.015, p=0.531). However, the response of BNP was commonly observed in both groups (non-IHD St.β: −0.238, IHD St.β: −0.398, p<0.001, respectively). Additionally, this study showed a direct link between low estimated glomerular filtration rate and high BNP independently of LVEF. Incrementally, Bayesian structural equation modelling in covariance structure analysis clearly supported this result. The scatter plots and simple regression analysis revealed that an adequate blood supply was approximately Hb 110 g/L and over in the non-IHD patients, whereas blood was not supplied in sufficient quantities even by Hb 130 g/L in patients with IHD.

Conclusion The current study demonstrated that anaemia was a substantial risk for worsening cardiac overload as estimated by plasma BNP. The anaemic response of LVEF likely changed depending on underlying cardiac disorders (IHD or not). However, the response of BNP was robustly observed.

Strengths and limitations of this study

- Covariance structure analysis, including Bayesian structural equation modelling, can be an effective method for mega-trials as a next-generation statistical procedure.
- This was a retrospective study, and in those patients consecutively admitted to our institution, there were different profiles and numbers of patients in the non-ischaemic heart disease (IHD) (18% of overall) group and IHD group.
- Our findings relied on cross-sectional data, and there were no serial changes in the echocardiography data; the treatment medications differed in the non-IHD and IHD groups.
- Further studies are warranted to prospectively determine the adverse effects of anaemia on patients with heart failure among a larger number of patients.

INTRODUCTION

Anaemia commonly contributes to cardiovascular alterations, including heart failure (HF).[1–5] To avoid delaying the treatment of anaemia and to enable timely action, the harmful effects of anaemia in individual patients must be precisely evaluated. However, the adverse effects of anaemia itself might frequently be missed or underestimated in clinical practice for several reasons.

The various responses of left ventricular (LV) function in anaemic individuals could also contribute to the potential underestimation of a harmful effect of anaemia on cardiac overload. Although the reaction of the heart to anaemia may differ among underlying cardiac disorders, the heart tends to respond to the anaemic state in a compensatory manner by increasing cardiac output. The underestimation of the anaemic effect may happen in hearts where activation of systolic function by the reserve capacity is promoted.

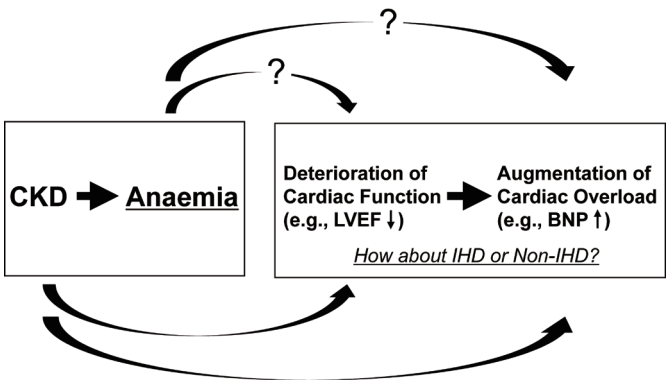

Figure 1 Graphical representation of the aim of this study. Anaemia can be induced by CKD, and both systolic function and cardiac overload can be affected. The aim of this study is shown in this figure. BNP, B-type natriuretic peptide; CKD; chronic kidney disease; IHD, ischaemic heart disease; LVEF, left ventricular ejection fraction.

When mention is made of ischaemic conditions, the adverse anaemic effect on the heart tends to be underestimated in the patients without ischaemic heart disease (IHD) rather than in those with IHD.

Recently, cardiac overload has been shown to be conveniently estimated by plasma B-type natriuretic peptide (BNP) levels. BNP is a cardiac hormone secreted particularly from the ventricle in patients with HF.[6–8] BNP is secreted mainly by mechanical stretching of the myocardium, while many neurohumoral factors can also induce its secretion,[9–11] and the plasma BNP level is considered a sensitive and reliable biomarker of the degree of HF in clinical practice.[12–15] It is, thus, commonly postulated that plasma BNP is a useful marker for the adverse effects of anaemia. Curiously enough, there were few reports clearly demonstrating the direct interaction of anaemia and plasma BNP levels in clinical practice. One possible reason may be that the plasma BNP levels are affected by many other factors than anaemia, and on the other hand, anaemia is regulated by many factors that may also affect plasma BNP levels. Thus, the direct effect of anaemia on plasma BNP levels is difficult to evaluate.

Moreover, anaemia is often induced by chronic kidney disease (CKD),[16–18] and CKD can exert influence on not only the anaemic condition but also on LV function and plasma BNP levels in diverse ways. Thus, CKD poses an impediment when attempting to grasp the direct effect of anaemia on LV function and plasma BNP levels.

Thus, there is an intricate web of connection among the confounding factors of anaemia, CKD, LV function (eg, left ventricular ejection fraction [LVEF]) and plasma BNP levels, making it difficult to reveal the direct effects of anaemia itself on LVEF and plasma BNP. We, thus, are often faced with a diagnostic dilemma for the adverse impact of anaemia. Ideally, this question could be solved using one equation model. However, the statistical analysis of such a model would be problematic. This type of analysis cannot be performed by commonly used statistical procedures, such as multiple regression analysis alone.

Herein, we propose that covariance structure analyses are suitable for this purpose. In many areas, covariance structure analysis plays an important role in understanding how the relationships between observed variables might be generated by interaction effects and/or hypothesised latent variables. Recently, we successfully introduced this statistical approach in the cardiovascular field.[19–24] The aim of this study is graphically represented in figure 1. In this study, we attempted to use covariance structure analyses to simultaneously examine the effects of anaemia on LVEF and plasma BNP in consideration of estimated glomerular filtration rate (eGFR).

METHODS

Study patients

A cross-sectional study was conducted using the cardiac catheterisation database in our institution. The study population consisted of 3756 subjects consecutively admitted to our institution between 2012 and 2017 for various heart disorders. Patients receiving haemodialysis (420 patients) were excluded from this study as haemodialysis has a marked effect on the levels of plasma BNP and eGFR, precluding their evaluation. Among the study population, there were 684 patients with non-IHD and 3072 patients with various types of IHD. As our data were deconsolidated anonymously, our ethics committee waived the requirement for patient consent.

Definition of diseases

IHD was diagnosed on the basis of symptoms, ECG findings, blood sampling results and coronary artery morphology. Organic stenosis was defined as 75% stenosis or more in the coronary arteries as determined by coronary angiography. Patients with coronary spastic angina were included in the IHD group of this study if the disease activity was stable, and the provocation test was planned during hospitalisation. Valvular diseases included all types of disorders, such as aortic stenosis and/or regurgitation and mitral stenosis and/or regurgitation. Arrhythmia included patients who needed catheter ablation, an implantable cardioverter-defibrillator, cardiac resynchronisation therapy and patients with a pacemaker or syncope. Cardiomyopathy was defined as patients who were diagnosed before admission and treatment or who were diagnosed after admission, excluding cases of ischaemic cardiomyopathy. The other category included basal heart disease and conditions not stated above. Hypertension, diabetes mellitus and dyslipidaemia were defined as previously described.[25]

Patients with renal dysfunction were defined as those having an eGFR of <60 mL/min/1.73 m^2 at admission according to the guidelines of the Japanese Society of Nephrology. We calculated the eGFR according to the Modification of Diet in Renal Disease Study equation[26] as shown below, with coefficients modified for Japanese

patients: eGFR (mL/min/1.73 m^2)=194×age$^{-0.287}$×creatinine$^{-1.094}$ (and ×0.739 for females).[27]

Blood sampling and biochemical measurements

We used blood sample and haemodynamic data during cardiac catheterisation in the analysis. Serum biochemical analyses and plasma BNP level measurements were performed in a central laboratory in our hospital during the study. Plasma BNP level was measured as described in previous reports.[14 15 19 20 23] In brief, whole blood (5 mL) was collected in tubes containing ethylenediaminetetraacetic acid disodium salt (1 mg/mL blood). The plasma BNP level was then measured with a rapid enzyme-linked immunosorbent assay (non-extracted) kit using an antibody to human BNP (Shionogi, Tokyo, Japan).

Left ventriculography during cardiac catheterisation

The LVEF was obtained from left ventriculography (LVG) traces during end-systolic and end-diastolic phases of cardiac catheterisation. The contrast LVG images were acquired at a frame rate of 30 frames per second in the right anterior oblique 30° projection. The LVEF was calculated from single-plane cineangiograms by means of the area-length formula using a semiautomated trace method with QAngio XA V.7.1 (Medis medical imaging systems bv, Leiden, The Netherlands). This technique for calculating LVEF might include methodological limitations.

Statistical analysis

Continuous variables are expressed as the mean±SD or median with IQR. Categorical variables are expressed as a percentage. Two groups or conditions were compared using the Mann-Whitney U test or χ^2 test as appropriate. Comparisons between two data sets of continuous variables were performed using simple regression analysis and/or Pearson's product-moment correlation coefficient analysis, where appropriate. Multiple regression analysis was performed when multiple values were compared. The Kolmogorov-Smirnov test was used to determine whether the BNP values were normally distributed. Subsequently, the BNP data were log-transformed (log-BNP) to achieve a normal distribution for the analysis. Statistical analyses were performed using IBM SPSS Statistics V.25 (IBM). A path model based on a covariance structure analysis was proposed to investigate the relationships among clinical factors in this study population and, in particular, to examine probable causal effects on the plasma BNP level. Path analysis was performed with IBM SPSS Amos V.25 (Amos Development, Wexford, Pennsylvania, USA). All statistical analyses were confirmed at a significance level of p values lower than 0.05 ($p<0.05$). The confirmatory factor model in this study, not an exploratory factor model, defines some hierarchical regression models between clinical factors and IHD. Paths between variables are drawn from independent to dependent variables with a directional arrow for every regression model, that is, an arrow with a head on one end only. For every regression, the total variance of the dependent variable is theorised to be caused either by independent variables of the model or by extraneous variables (e). Each path shows the standardised coefficient of regression of the independent variable versus the dependent variable of the relevant path. The indirect effect was determined by multiplying the path coefficients of the intervening variables. Recently, we reported our research by applying a covariance structure analysis.[19–24] In addition, as covariance structure analysis consists partly of the Bayesian estimation method and the Bayesian adaptive trial design, Bayesian structural equation modelling successfully gave a description of this result, and it is expected to be a next-generation statistical procedure for mega-trials.[28] In this study, Bayesian structural equation modelling shows that the statistical information makes it possible to get to know the visualised range of the relationship between the bivariate marginal posterior plots at a glance (a two-dimensional plot of the bivariate posterior density). The corresponding author had full access to all the data in the study and takes responsibility for its integrity and analysis.

To examine the cut-off value of plasma BNP for an adequate blood supply, we performed the scatter plots and simple regression analysis between haemoglobin (Hb) and log-BNP levels or Hb and LVEF values in each group (group I; all study patients, group II; non-IHD patients and group III; patients with IHD).

Patient and public involvement

Patients and other members of the public were not involved in the design and conception of this study.

RESULTS

Patient characteristics

Table 1 shows all the patient characteristics. Among the 3756 patients (group I), there were 684 in non-IHD group (group II) and 3072 patients with IHD (group III). These two groups were compared using the Mann-Whitney U test (for continuous variables) or χ^2 test (for categorical variables) as appropriate.

Results of a simple regression analysis of log-BNP and LVEF

We examined correlative relationships by simple regression analysis among the body mass index (BMI), eGFR, Hb, LVEF and log-BNP levels in the whole study population. The cross-linkage among them is shown in the left side of table 2. The results of all clinical factors were significantly correlated with LVEF and log-BNP levels. In addition, the regression coefficients of Hb predicting LVEF and log-BNP were both significantly negative, even though the relationship between LVEF and log-BNP was also a reciprocal relation.

Multiple regression analysis for determining the LVEF and plasma BNP levels

As shown on the left side of table 2, there were negative correlation coefficients between two values, Hb, LVEF

Table 1 The characteristics of all patients

Characteristics	Overall (n=3756)	Non-IHD (n=684)	IHD (n=3072)	P value*
	No (%) or mean±SD (median; IQR)			
Male gender	3102 (82.6)	452 (66.1)	2650 (86.3)	<0.001
Age (years old)	65.6±11.9	62.5±14.2	66.3±11.2	<0.001
BMI (kg/m^2)	24.4±3.9	23.3±4.3	24.7±3.8	<0.001
Current smoker	789 (21.0)	112 (16.4)	677 (22.0)	0.001
Family history of IHD	939 (25.0)	105 (15.4)	834 (27.1)	<0.001
Systolic blood pressure (mm Hg)	133±25	131±27	134±24	0.027
Diastolic blood pressure (mm Hg)	72±13	71±14	72±13	0.507
Heart rate (beats per minute)	73±14	76±18	72±13	<0.001
Hb (g/L)	135±19	135±21	134±18	0.425
	(137; 124–147)	(136; 121–151)	(137; 124–147)	–
Creatinine (mg/dL)	0.93±0.59	0.92±0.44	0.94±0.63	0.934
eGFR (mL/min/1.73 m^2)	68.3±19.5	66.7±19.9	68.7±19.3	0.004
CRP (mg/dL)	0.74±1.87	0.78±1.76	0.73±1.89	0.001
BNP (pg/mL)	117.7±244.7	190.6±279.4	101.1±232.9	<0.001
	(42.0; 17.2–121.0)	(90.9; 29.4–224.0)	(36.2; 15.5–99.2)	–
Left ventricular haemodynamic parameters				
LVEF (%)	56.8±11.9	52.3±15.2	57.9±10.6	<0.001
Underlying cardiovascular disease no				
IHD†	3072 (81.8)	–	3072 (100.0)	–
Acute coronary syndrome	233 (6.2)	–	233 (7.6)	–
Old myocardial infarction	949 (25.3)	–	949 (30.9)	
Angina pectoris	2666 (71.0)	–	2666 (86.8)	
Coronary spastic angina	203 (5.4)	–	203 (6.6)	
Cardiomyopathy	292 (7.8)	225 (32.9)	67 (2.2)	<0.001
Valvular disease	338 (9.0)	201 (29.4)	137 (4.5)	<0.001
Arrhythmia	439 (11.7)	201 (29.4)	238 (7.7)	<0.001
Atrial fibrillation	239 (6.4)	119 (17.4)	120 (3.9)	<0.001
Hypertension	2766 (73.6)	408 (59.7)	2358 (76.8)	<0.001
Type 2 diabetes mellitus	1467 (39.1)	146 (21.3)	1321 (43.0)	<0.001
Dyslipidaemia	2701 (71.9)	282 (41.2)	2419 (78.7)	<0.001
Medication				
ACE inhibitors	760 (20.2)	109 (15.9)	651 (21.2)	0.001
ARBs	1439 (38.3)	187 (27.3)	1252 (40.8)	<0.001
Beta-blockers	1551 (41.3)	203 (29.7)	1348 (43.9)	<0.001
Diuretics	772 (20.6)	236 (34.5)	536 (17.4)	<0.001
Statins	2231 (59.4)	161 (23.5)	2070 (67.4)	<0.001

*P values, the statistical significance for the comparison of clinical factors between the non-IHD and IHD groups.
†The aetiologies of these IHDs include overlapping cases.
ARB, angiotensin II type I-receptor blockers; BMI, body mass index; BNP, B-type natriuretic peptide; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; IHD, ischaemic heart disease; LVEF, left ventricular ejection fraction.

and the log-BNP levels. The anaemia raised LVEF and log-BNP levels, but the increase in LVEF suppressed the log-BNP levels intrinsically. To identify hidden mechanisms underlying this finding, the relationships between triplets, we performed a multiple regression analysis for factors contributing to LVEF and the plasma BNP levels among the variables such as male gender, BMI, eGFR and Hb. As shown in the upper right side of table 2, the Hb levels, gender and log-BNP levels were negatively correlated with the LVEF values (p<0.001, respectively),

Table 2 The results of the simple and multiple regression analyses to identify the clinical factors influencing LVEF and logarithmic BNP levels (in upper and bottom tables, respectively)

Dependent variable: LVEF	Simple regression analysis				Multiple regression analysis (R^2=0.276)				
	β	St. β	95% CI	P value	β	St. β	95% CI	P value	VIF
Male gender	–	–	–	–	−3.495	−0.112	−4.667 to −2.322	<0.001	1.159
BMI	0.147	0.049	0.025 to 0.269	0.018	0.024	0.008	−0.088 to 0.135	0.680	1.136
eGFR	0.083	0.120	0.055 to 0.112	<0.001	0.000	0.001	−0.025 to 0.026	0.971	1.099
Hb	−0.041	−0.062	−0.068 to −0.014	0.003	−0.143	−0.216	−0.170 to −0.117	<0.001	1.297
Log BNP	−9.770	−0.460	−10.540 to −8.999	<0.001	−11.734	−0.553	−12.560 to −10.907	<0.001	1.250
Constant	–	–	–	–	97.656	–	92.646 to 102.666	<0.001	–

Dependent variable: Log BNP	Simple regression analysis				Multiple regression analysis (R^2=0.401)				
	β	St. β	95% CI	P value	β	St. β	95% CI	P value	VIF
Male gender	–	–	–	–	−0.113	−0.077	−0.163 to −0.062	<0.001	1.167
BMI	−0.031	−0.212	−0.036 to −0.026	<0.001	−0.015	−0.103	−0.019 to −0.010	<0.001	1.119
eGFR	−0.011	−0.358	−0.012 to −0.010	<0.001	−0.006	−0.183	−0.007 to −0.005	<0.001	1.043
Hb	−0.012	−0.389	−0.013 to −0.011	<0.001	−0.009	−0.291	−0.010 to −0.008	<0.001	1.220
LVEF	−0.022	−0.460	−0.023 to −0.020	<0.001	−0.022	−0.458	−0.023 to −0.020	<0.001	1.034
Constant	–	–	–	–	4.969	–	4.785 to 5.154	<0.001	–

β,regression coefficient; BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; LVEF, left ventricular ejection fraction; St. β, standardised regression coefficient; R^2, adjusted coefficient of determination; VIF, variance inflation factor.

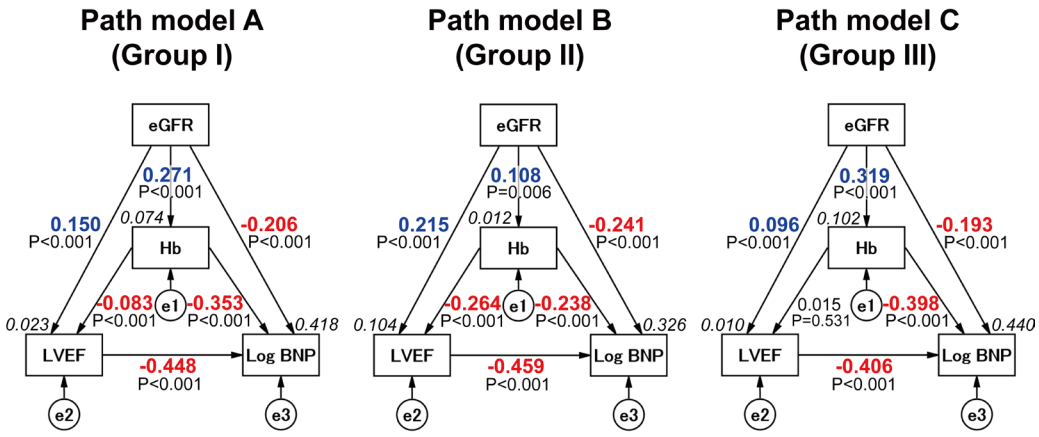

Figure 2 Explanatory drawing of possible cascade as path model A (all study population: group I), path model B (non-IHD patients: group II) and path model C (patients with IHD: group III). Each path shows the standardised coefficient of regression of independent variable versus the dependent variable of the relevant path. The standardised regression coefficients (direct effect) (bold characters mean statistically significant, blue coloured characters mean positive correlation, and red coloured characters mean negative correlation) and squared coefficients of multiple correlations (italic characters) are shown. BNP, B-type natriuretic peptide; e, extraneous variable; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; IHD, ischaemic heart disease; LVEF, left ventricular ejection fraction.

while BMI and eGFR were not significantly correlated with the LVEF values. In addition, as shown in the lower right side of table 2, the Hb levels, male gender, BMI, eGFR and LVEF also negatively correlated with the log-BNP levels (p<0.001). These results did not provide a full account of the relationships among the Hb, LVEF and log-BNP levels. Consequently, we performed the covariance structure analyses to provide a detailed account of these relationships.

Concept of the proposed path model A (group I)

As shown in simple and multiple regression analyses, we confirmed that eGFR, LVEF and the values of Hb and log-BNP were cofounding between most of the pairs. To examine the direct effect of anaemia itself on cardiac function and cardiac overload, we applied covariance structure analyses. The theoretical path model was proposed as shown in figure 2. We assumed the principle of cause and effect among the factors. Following logic, the theoretical path model was proposed by positioning eGFR at the top of the diagram. The LVEF, Hb and log-BNP levels were placed at levels below eGFR. Paths were drawn from independent to dependent variables with a directional arrow for every regression model, that is, from eGFR to Hb, LVEF and log-BNP levels, from Hb to LVEF and log-BNP levels, and finally from LVEF to log-BNP levels.

Results of path model A (group I)

The precise results of path model A in the study population (group I) are shown in table 3. The exploratory factor analysis revealed that eGFR played a causative role in Hb level (standardised regression coefficient (St. β): 0.271, p<0.001). In addition, eGFR was causatively linked to LVEF (St. β: 0.150, p<0.001) and log-BNP (St. β: −0.206, p<0.001) and Hb played a causative role in LVEF (St. β: −0.083, p<0.001) and log-BNP (St. β: −0.353, p<0.001). Finally, LVEF played a causative role in log-BNP (St. β:

Table 3 The results of path model A (group I)

			Regression coefficients				
				Standardised			
Clinical factor			Estimated	Direct effect	Indirect effect	Total effect	P value
Hb (R^2=0.074)	←	eGFR	0.262	0.271	0	0.271	<0.001
LVEF (R^2=0.023)	←	eGFR	0.092	0.150	−0.022	0.128	<0.001
	←	Hb	−0.052	−0.083	0	−0.083	<0.001
Log BNP (R^2=0.418)	←	eGFR	−0.006	−0.206	−0.153	−0.359	<0.001
	←	Hb	−0.011	−0.353	0.037	−0.316	<0.001
	←	LVEF	−0.022	−0.448	0	−0.448	<0.001

The results (direct, indirect and total effects) of the theoretically proposed path model analysis to identify the clinical factors influencing each other (see figure 2, path model A).
BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; LVEF, left ventricular ejection fraction; R^2, squared multiple correlations.

Table 4 The results of path model B (group II: non-IHD)

Clinical factor			Regression coefficients: Estimated	Standardised: Direct effect	Standardised: Indirect effect	Standardised: Total effect	P value
Hb (R^2=0.012)	←	eGFR	0.117	0.108	0	0.108	0.006
LVEF (R^2=0.104)	←	eGFR	0.164	0.215	−0.029	0.186	<0.001
	←	Hb	−0.188	−0.264	0	−0.264	<0.001
Log BNP (R^2=0.326)	←	eGFR	−0.007	−0.241	−0.111	−0.352	<0.001
	←	Hb	−0.007	−0.238	0.121	−0.117	<0.001
	←	LVEF	−0.018	−0.459	0	−0.459	<0.001

The results (direct, indirect and total effects) of the theoretically proposed path model analysis to identify the clinical factors influencing each other (see figure 3, path model B).
BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; LVEF, left ventricular ejection fraction; R^2, squared multiple correlations.

−0.448, p<0.001). The estimated direct, indirect and total effects are summarised in table 3.

We confirmed this result with additional analyses of the same path model in the two sets of groups divided by gender and age (younger and elder groups). The results demonstrated that Hb levels and eGFR values had a distinctly negative correlation to log-BNP levels but did not have a consistent relationship to LVEF values.

In addition, we used the same path model between the anaemia and renal dysfunction group (eGFR <60 mL/min/1.73 m^2 and Hb <130 g/L) and the healthy condition group (eGFR ≥60 mL/min/1.73 m^2 and Hb ≥130 g/L). The results showed a similar trend, but the negative correlation of Hb levels and eGFR values with log-BNP levels in the CKD with anaemia group was slightly stronger than that in the healthy condition group.

Concept of proposed path models B and C (groups II and III)

The functional cardiac reactions to anaemia may differ among various underlying cardiac disorders. We postulated that systolic function affected by anaemia would widely vary between non-IHD and IHD patients. To obtain further information, path models B and C were proposed for the non-IHD and IHD groups in the same manner as path model A was proposed for the whole study population. Path models B and C are shown in figure 2.

Results of path models B and C (groups II and III)

The precise results of path model B of patients with non-IHD (group II) are shown in table 4. The exploratory factor analysis revealed that eGFR played a causative role in Hb level (St. β: 0.108, p=0.006). In addition, eGFR was causatively linked to LVEF (St. β: 0.215, p<0.001) and log-BNP (St. β: −0.241, p<0.001), and Hb played a causative role in LVEF (St. β: −0.264, p<0.001) and log-BNP (St. β: −0.238, p<0.001). Finally, LVEF played a causative role in log-BNP (St. β: −0.459, p<0.001).

The precise results of path model C in the patients with IHD (group III) are shown in table 5. The exploratory factor analysis revealed that eGFR played a causative role in Hb level (St. β: 0.319, p<0.001). In addition, eGFR was causatively linked to LVEF (St. β: 0.096, p<0.001) and log-BNP (St. β: −0.193, p<0.001), and Hb did not play a causative role in LVEF (St. β: 0.015, p=0.531

Table 5 The results of path model C (group III: IHD)

Clinical factor			Regression coefficients: Estimated	Standardised: Direct effect	Standardised: Indirect effect	Standardised: Total effect	P value
Hb (R^2=0.102)	←	eGFR	0.299	0.319	0	0.319	<0.001
LVEF (R^2=0.010)	←	eGFR	0.053	0.096	0.005	0.101	<0.001
	←	Hb	0.009	0.015	0	0.015	0.531
Log BNP (R^2=0.440)	←	eGFR	−0.006	−0.193	−0.168	−0.361	<0.001
	←	Hb	−0.012	−0.398	−0.006	−0.404	<0.001
	←	LVEF	−0.021	−0.406	0	−0.406	<0.001

The results (direct, indirect and total effects) of the theoretically proposed path model analysis to identify the clinical factors influencing each other (see figure 3, path model C).
BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; LVEF, left ventricular ejection fraction; R^2, squared multiple correlations.

(not significant)) but played a significant causative role in log-BNP (St. β: −0.398, p<0.001). Finally, LVEF played a causative role in log-BNP (St. β: −0.406, p<0.001). The estimated direct, indirect and total effects are summarised in tables 4 and 5.

Results of the Bayesian estimation method

A two-dimensional plot of the bivariate posterior density showing the relationship between the bivariate marginal posterior plots using Bayesian structural equation modelling is shown in figure 3. The abscissa means that Hb was causatively linked to LVEF, and the ordinate means that Hb was causatively linked to log-BNP levels. The centre value of circular plots on the abscissa and ordinate is in complete accord with data that were analysed with a path model based on the previous covariance structure analyses. Neither two-dimensional plots for the overall group nor non-IHD group crossed both horizontal and vertical axes. Only two-dimensional plots for the IHD group crossed the vertical axis. Hb played a causative role in log-BNP in all groups (groups I, II and III), and it also played a causative role in LVEF in the overall group (group I) and non-IHD group (group II) but not the IHD group (group III).

Results of scatter plots and simple regression analysis between Hb and log-BNP levels or Hb and LVEF values in each group (groups I, II and III).

Scatter plots in each group (groups I, II and III) showed the relationship between Hb and log-BNP levels (A-1, A-2 and A-3) or Hb and LVEF values (B-1, B-2 and B-3) among the dichotomous groups divided by Hb level in figure 4. There was a significant linear regression between Hb and log-BNP levels among the higher Hb plots in each group of less than Hb 110 g/L in A-2. On the other hand, there was a significant linear relation between Hb and log-BNP among the higher Hb plots in every group in A-3. There was a significant linear linkage even in Hb 130 g/L. These results suggest that the adequate blood supply was attained by Hb 110 g/L and over in non-IHD patients, whereas blood was not supplied with sufficient quantities even by Hb 130 g/L in patients with IHD.

There was a significant linear regression between Hb and LVEF values among the higher Hb plots in every group in B-1 and B-2 (groups I and II). There was a significant linear relation even at Hb 130 g/L, but this significant linear regression was observed only at Hb higher than 130 g/L in B-3 (group III). The similar results were already shown as the results of path models or Bayesian structural equation modelling.

DISCUSSION

Direct effects of anaemia itself on the heart revealed by covariance structure analyses

This study was designed to clarify the direct effects of anaemia on cardiac function, particularly on cardiac overload, with covariance structure analyses using LVEF as a marker of systolic function and plasma BNP level as a marker of cardiac overload, considering renal dysfunction in patients with HF. To the best of our knowledge, this is the first report of a theoretical path model created to clarify the adverse effects of anaemia on cardiac overload. The current analyses successfully revealed that both CKD and anaemia can separately induce cardiac overload, as estimated by plasma BNP, whereas CKD reduced systolic function while anaemia apparently increased systolic function in a compensatory manner. The increase in systolic function due to anaemia contributed to the decrease in cardiac overload. Nevertheless, the plasma BNP levels were increased under the influence of anaemia. This phenomenon was clearly observed in the non-IHD group. This study answered a series of questions about why the adverse effects of anaemia itself would frequently be missed or underestimated in clinical practice. The conceptual diagram is shown in figure 5.

Plasma BNP level as a sensitive marker of cardiac overload induced by anaemia

This study clearly showed that anaemia is a substantial factor for increased plasma BNP level. The molecular

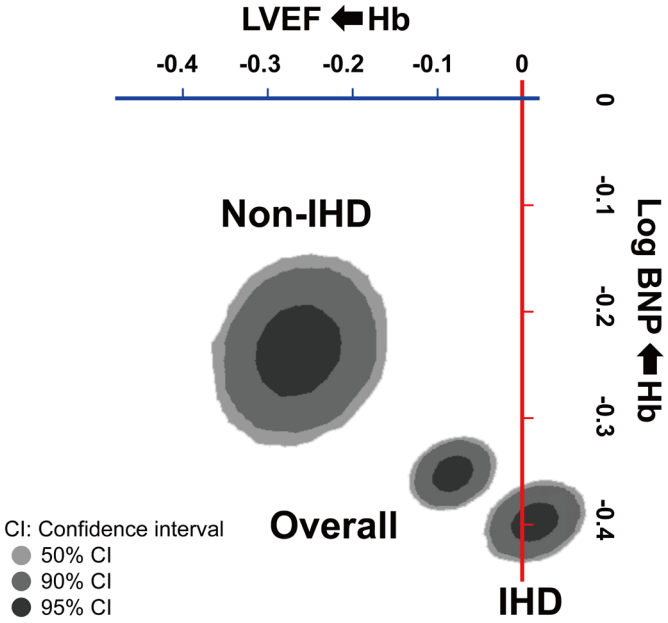

Figure 3 Bivariate marginal posterior plots using Bayesian structural equation modelling among the groups (groups I, II and III). A two-dimensional plot of the bivariate posterior density shows the relationship between the bivariate marginal posterior plots. Ranging from light to dark, the three shades of grey represent 50%, 90% and 95% credible regions, respectively. A credible region is conceptually similar to a bivariate confidence region that is familiar to most data analysts acquainted with classical statistical inference methods. The abscissa (blue coloured axis) and ordinate (red coloured axis) mean the Bayesian estimated posterior means for each standardised direct effect from Hb to LVEF (abscissa: blue axis) or from Hb to log-BNP (ordinate: red axis). BNP, B-type natriuretic peptide; Hb, haemoglobin; IHD, ischaemic heart disease; LVEF, left ventricular ejection fraction.

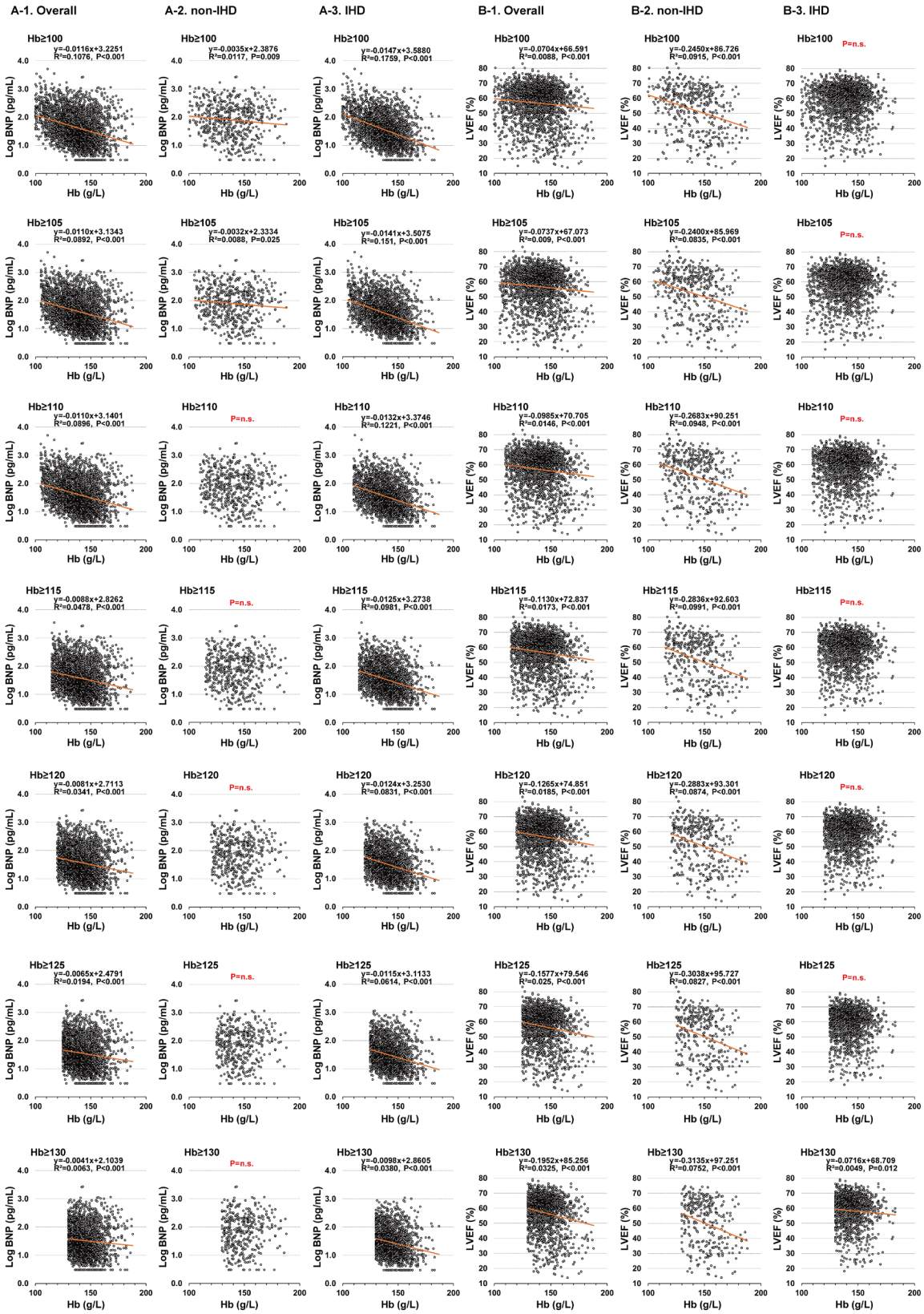

Figure 4 Scatter plots and simple regression analysis between Hb and log-BNP levels or Hb and LVEF values among the groups (groups I, II and III). Each scatter plot in each group (group I; A-1, B-1, group II; A-2, B-2 and group III; A-3, B-3) shows the relationship between Hb and log-BNP levels (A-1, A-2 and A-3) or Hb and LVEF values (B-1, B-2 and B-3) among the dichotomous groups divided by Hb level. The cut-off Hb values for these dichotomous groups were set from 100 g/L to 130 g/L in 5 g/L increments. The regression line (orange line) was drawn only at the $p<0.05$ level of significance, and others were not significant (p=n.s.). The regression equation and the squared coefficient of determination are shown in the upper part of each diagram. BNP, B-type natriuretic peptide; Hb, haemoglobin; IHD, ischaemic heart disease; LVEF, left ventricular ejection fraction.

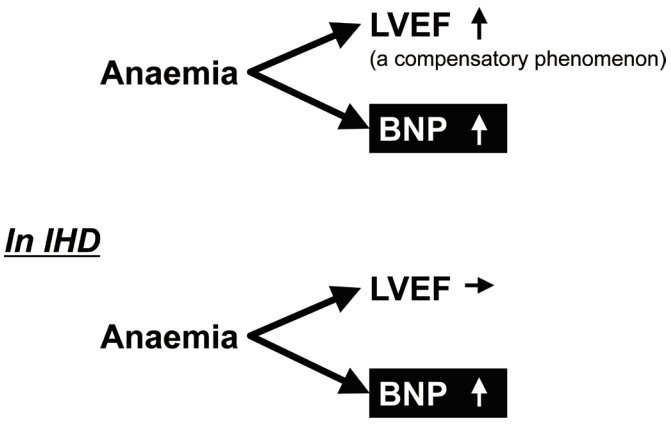

Figure 5 Graphical representation of the results of this study. This study focused on the significant effects of anaemia on LVEF and plasma BNP in patients with IHD and those without IHD. In patients without IHD, plasma BNP was a sensitive measured and recommended over LVEF for an evaluation of the harmful effects of anaemia. BNP, B-type natriuretic peptide; IHD, ischaemic heart disease; LVEF, left ventricular ejection fraction.

mechanism is interesting and should be discussed. The plasma BNP level is a suitable marker of the degree of ischaemic damage to the heart, as has been demonstrated in previous studies. Hypoxia-inducible factor (HIF)-1 is a dimeric protein complex that plays an integral role in the body's response to low oxygen concentrations or hypoxia. Of interest, HIF-1 is among the primary genes involved in the homoeostatic process, which can increase vascularisation in hypoxic areas, such as localised ischaemia and tumours. The production of BNP has been shown to sensitively react to the activation of HIF-1α in cardiomyocytes.[29–31] Thus, plasma BNP could be capable of serving as a marker of cardiac overload induced by anaemia. Our current results reinforced the results of these studies showing that the secretion of BNP is sensitive to ischaemia, which is an independent stimulus from that of myocardial stretch.

Different responses of systolic function to anaemia between the non-IHD and IHD groups

We expected that systolic function affected by anaemia would vary widely between non-IHD and IHD patients. Systolic function may apparently be augmented as a compensatory response to anaemia in non-IHD patients. On the other hand, in some patients with IHD, systolic function estimated by LVEF may be reduced simply because of chronic cardiac ischaemia due to a low oxygen supply, which would be referred to as myocardial hibernation.[32 33] We divided the study population into two groups, non-IHD and IHD, in this study. Consequently, a clear picture of the effect of anaemia on LVEF was gained; systolic function was apparently augmented as a compensatory response to anaemia in the non-IHD group. However, the compensatory response of LVEF to anaemia was insignificant in the IHD group. Cardiomyocytes could potentially respond to ischaemia with a large amount of standby capacity, and cardiac output could be increased to deliver a sufficient oxygen supply to the whole body in the non-IHD group, but patients with IHD with anaemia lacked this standby capacity in the heart, increasing the likelihood of cardiac hibernation.

The aetiologies of these IHDs include overlapping cases. However, even in the data group excluding overlapping cases (n=2081), the analysis result did not change. Unfortunately, the non-IHD group and the IHD group in this study had differences in medications such as renin–angiotensin inhibitors, beta blockers, diuretics and statins. In this respect, we could not make a postadjustment comparison between the two groups; therefore, these medication differences cannot negate the possibility of affecting the results. These issues are stated as the study limitations.

Possible mechanisms of the compensatory response of systolic function to anaemia and the eventual consequences of exposing the heart to anaemia

Understanding the mechanism of the compensatory response of systolic function would be interesting, especially in non-IHD patients. Possible mechanisms are described as follows. The tissue hypoxia induced by anaemia may cause a decrease in blood pressure via peripheral vasodilation, leading to an increased activation of the sympathetic nervous system, which in turn leads to tachycardia, increased stroke volume, renal vasoconstriction and reduced renal blood flow, causing salt and water retention. The reduced renal blood flow may also cause increased renin–angiotensin–aldosterone system activity, further augmenting salt and water retention and further increasing the circulating fluid volume. These compensatory responses would transiently augment the apparent systolic function estimated by LVEF. However, it is quite natural that cardiac myocytes should become greatly overloaded due to ischaemia. All these findings are also consistent with animal studies on the cardiac effects of anaemia. Anaemia in rats has been demonstrated to result in eccentric cardiac hypertrophy associated with increased capillary proliferation, abnormal diastolic wall stress, interstitial fibrosis, increased LV end-diastolic pressure, increased LV mass, LV dilation and decreased systolic functional reserve.[34 35] Thus, the heart could be expected to become damaged over time because of anaemia-induced workload, and systolic function would be reduced, even if the heart did not originally have significant coronary artery disease.

Clinical implications of this study

Anaemia, HF and CKD interact as a vicious circle, causing or exacerbating each other, which is the cardiorenal–anaemia syndrome.[36 37] As previously reported, anaemia itself may further worsen cardiac and renal function and render patients resistant to standard HF therapies.[38] In this study, we clearly demonstrated the adverse effects of anaemia on cardiomyocytes using plasma BNP with a robust statistical procedure and described the potential

risk of anaemia. Although CKD is a substantial risk for cardiac overload, anaemia should be considered an independent risk factor. Additionally, when LVEF is sufficiently preserved or augmented but the plasma BNP level remains relatively high, the adverse effects of anaemia on cardiac overload should not be underestimated simply by the superficial LV function. Plasma BNP would be helpful for resolving our dilemma for diagnosis of the adverse effects of anaemia. Treatment for anaemia should be initiated early before systolic function is truly reduced and HF advances. From the current study, the adequate blood supply would be attained by an Hb 110 g/L and over in non-IHD patients, whereas blood was not supplied at sufficient quantities even by Hb 130 g/L in patients with IHD. Ideally, Hb should remain 110 g/L at the lowest in patients with HF regardless of underlying cardiac disorders.

CONCLUSION

The current study demonstrated that anaemia itself was a substantial risk factor for worsening cardiac overload as estimated by plasma BNP. However, the adverse effects of anaemia may remain undetected and underestimated simply by looking at LV systolic function because the compensatory augmentation of systolic function due to anaemia occurring there. When increased plasma BNP levels are observed in patients with HF, anaemia should be always considered a substantial risk factor for the progression of cardiac overload, and early treatment is recommended.

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Contributors MT: Conceptualisation (Equal) Data curation (Equal) Methodology (Lead) Project administration (Equal) Validation (Equal) Writing—original draft (Equal) Writing—review and editing (Equal). MK: Conceptualisation (Equal) Formal analysis (Lead) Methodology (Equal) Project administration (Equal) Supervision (Equal) Validation (Lead) Writing—original draft (Equal) Writing—review and editing (Equal). KM: Conceptualisation (Equal) Data curation (Equal) Formal analysis (Supporting) Validation (Equal) Writing—review and editing (Equal). KO: Conceptualisation (Equal) Data curation (Equal) Formal analysis (Supporting) Writing—review and editing (Equal). YI: Conceptualisation (Equal) Data curation (Equal) Writing—review and editing (Equal). SM: Conceptualisation (Equal) Data curation (Equal) Writing—review and editing (Equal). TT: Conceptualisation (Equal) Data curation (Equal) Writing—review and editing (Equal). TN: Conceptualisation (Equal) Data curation (Equal) Writing—review and editing (Equal). TO: Conceptualisation (Equal) Data curation (Equal) Methodology (Supporting) Writing—review and editing (Equal). MY: Conceptualisation (Lead) Methodology (Equal) Project administration (Lead) Supervision (Lead) Writing—original draft (Lead) Writing—review and editing (Equal). All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

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