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ORIGINAL ARTICLE

NT-proBNP/BNP ratio for prognostication in European Caucasian patients enrolled in a heart failure prevention programme

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Abstract

Aims Guidelines support the role of B-type natriuretic peptide (BNP) and amino-terminal pro-BNP (NT-proBNP) for risk stratification of patients in programmes to prevent heart failure (HF). Although biologically formed in a 1:1 ratio, the ratio of NT-proBNP to BNP exhibits wide inter-individual variability. A report on an Asian population suggests that molar NT-proBNP/BNP ratio is associated with incident HF. This study aims to determine whether routine, simultaneous evaluation of both BNP and NT-proBNP is warranted in a European, Caucasian population.

Methods and Results We determined BNP and NT-proBNP levels for 782 Stage A/B HF patients in the STOP-HF programme. The clinical, echocardiographic, and biochemical associates of molar NT-proBNP/BNP ratio were analysed. The primary endpoint was the adjusted association of baseline molar NT-proBNP/BNP ratio with new-onset HF and/or progression of left ventricular dysfunction (LVD). We estimated the C-statistic, integrated discrimination improvement, and the category-free net reclassification improvement metric for the addition of molar NT-proBNP/BNP ratio to adjusted models. The median age was 66.6 years [interquartile range (IQR) 59.5–73.1], 371 (47.4%) were female, and median molar NT-proBNP/BNP ratio was 1.91 (IQR 1.37–2.93). Estimated glomerular filtration rate, systolic blood pressure, left ventricular mass index, and heart rate were associated with NT-proBNP/BNP ratio in a linear regression model (all $P < 0.05$). Over a median follow-up period of 5 years (IQR 3.4–6.8), 247 (31.5%) patients developed HF or progression of LVD. Log-transformed NT-proBNP/BNP ratio is inversely associated with HF and LVD risk when adjusted for age, gender, diabetes, hypertension, vascular disease, obesity, heart rate, number of years of follow-up, estimated glomerular filtration rate, and baseline NT-proBNP (odds ratio 0.71, 95% confidence interval 0.55–0.91; $P = 0.008$). However, molar NT-proBNP/BNP ratio did not increase the C-statistic ($\Delta -0.01$) and net reclassification improvement (0.0035) for prediction of HF and LVD compared with NT-proBNP or BNP alone. Substitution of NT-proBNP for BNP in the multivariable model eliminated the association with HF and LVD risk.

Conclusions This study characterized, for the first time in a Caucasian Stage A/B HF population, the relationship between NT-proBNP/BNP ratio and biological factors and demonstrated an inverse relationship with the future development of HF and LVD. However, this study does not support routine simultaneous BNP and NT-proBNP measurement in HF prevention programmes amongst European, Caucasian patients.

Keywords Heart failure prevention; Left ventricular dysfunction; Natriuretic peptides; NT-proBNP/BNP ratio; Screening

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Introduction

Guidelines acknowledge and advocate the use of single natriuretic peptide biomarker measurements [B-type natriuretic peptide (BNP) or amino-terminal pro-BNP (NT-proBNP)] to risk stratify patients for intensive heart failure (HF) prevention strategies.[1,2] ProBNP is released from cardiomyocytes in a 1:1 molar ratio,[3,4] yet measurable concentrations of BNP and NT-proBNP exhibit significant inter-individual variability, being influenced to differing degrees by patient factors including phenotype and genotype, immunoassay characteristics, and pre-analytical issues.[5–9] Furthermore, in addition to renal excretion, biologically active BNP undergoes receptor-mediated and enzymatic clearance and has a shorter half-life than inactive NT-proBNP, which is cleared almost exclusively by renal filtration.[5,10] Accordingly, the measured molar ratio of circulating BNP and NT-proBNP varies widely[11–13] and was found to predict worsening renal function[14] but not all-cause mortality[15] in patients with stage C HF.

Recently, a Japanese study indicated that elevated NT-proBNP/BNP ratio in people with Stage A/B HF is associated with the development of new-onset HF.[16] This suggests that routine measurement of both peptides may be warranted in programmes to prevent HF such as the St Vincent's Screening TO Prevent Heart Failure (STOP-HF) and NT-proBNP Selected PreventiOn of cardiac eveNts in a populaTion of dIabetic patients without a history of Cardiac disease (PONTIAC) programmes, which currently use single natriuretic peptide measurements.[17,18] However, compared with the STOP-HF and PONTIAC programmes, the Japanese work utilized a different BNP assay, in an older, genetically distinct population. Thus, in this study, we explore the clinical, biochemical, and echocardiographic imaging associates of NT-proBNP/BNP ratio in the STOP-HF follow-up study. We aimed to determine the association of baseline molar NT-proBNP/BNP ratio with new-onset HF and/or progression of left ventricular dysfunction (LVD), adjusted for age, gender, diabetes, hypertension, vascular disease, obesity, heart rate (HR), estimated glomerular filtration rate (eGFR), number of years of follow-up, and NT-proBNP at baseline. Finally, we determined whether routine, simultaneous evaluation of both BNP and NT-proBNP is warranted and should be recommended in guidelines.

Methods

Study population

The study population consisted of 782 patients from the STOP-HF cohort for whom BNP, NT-proBNP and creatinine measurements and echocardiographic parameters were available at baseline. As described previously, STOP-HF is an ongoing, prospective, longitudinal study population in Ireland, consisting of predominantly Caucasian patients.[17] All patients included in this study were Caucasian, although this was not part of the inclusion criteria. Participants are ≥40 years of age and have one or more risk factors for the development of HF, including high blood pressure, high cholesterol, diabetes, coronary artery disease, arrhythmia, valvular disorders, peripheral vascular disease, angina, left ventricular hypertrophy, or previous myocardial infarction. At initial assessment and 1–3 yearly review (as determined by risk, classified by BNP at last study visit), patient history, clinical examination, HF risk factors, medications, hospitalizations, Doppler echocardiogram, and blood biochemistry were recorded. Further details are provided in supporting information, *Data* S1.

Laboratory measurements

Serum BNP and NT-proBNP from the peripheral circulation were measured using the Triage® BNP test and an NT-proBNP assay on the Abbott Architect system. eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula. Chronic kidney disease (CKD) was classified by stage according to the National Kidney Foundation KDOQ1 Clinical Practice Guidelines 2012 (refer to *Data* S1). No patients in the cohort had CKD Stage 5 (eGFR $<$ 15 mL/min/1.73 m^2). As only seven patients had CKD Stage 4, we amalgamated these patients with Stage 3b to permit statistical analysis.

Echo Doppler studies

A Philips IE33 ultrasound scanner with standard adult probe was used for data acquisition Echocardiography was performed at each study visit by a designated, experienced echocardiographer for those with BNP $\geq$ 50 pg/mL. For those with BNP $<$ 50 pg/mL, echocardiography was not performed unless deemed clinically necessary by the cardiologist. Refer to *Data* S1 for further details.

Study outcomes

The primary outcome was the relationship between molar NT-proBNP/BNP ratio and development of HF or LVD over the follow-up period. We analysed this and its components: new-onset HF, progression of LVD [progression of left ventricular systolic dysfunction (LVSD), and/or left ventricular diastolic dysfunction (LVDD)]. We defined HF as per the 2016 European Society of Cardiology (ESC) guidelines (refer to *Data* S1),[19] and the diagnosis was made by an experienced cardiologist. Progression of LVDD was defined as a follow-up ratio of

transmitral Doppler early filling velocity to tissue Doppler early diastolic mitral annular velocity at the lateral wall (E/e′) > 13 and 2+ increase from baseline to follow-up, and/or follow-up lateral e′ < 9 and 2+ decrease from baseline to follow-up. Progression of LVSD was defined as a follow-up ejection fraction (EF) <50% and 5%+ decrease from baseline to follow-up. In addition, we examined the association between molar NT-proBNP/BNP ratio and major adverse cardiovascular events (MACEs). MACEs were defined as emergency hospitalizations for any one of the following: arrhythmias, transient ischaemic attack, stroke, myocardial infarction, peripheral or pulmonary thrombosis/embolus, or HF. We also evaluated demographic, clinical, biochemical, and Doppler echocardiographic associates of molar NT-proBNP/BNP ratio.

Sample size calculation

Sample size calculations were based on a previous study by Suzuki *et al*., where 6.9% of participants developed HF during the follow-up period, yielding a hazard ratio of 4.42 per unit change in log-transformed molar NT-proBNP/BNP ratio.[16] However, the confidence intervals in this study were wide. To ensure adequate power, we used new-onset HF and progression of LVD, assuming a population prevalence of 20%, a more conservative odds ratio of 1.35 in log-transformed molar NT-proBNP/BNP ratio, an α = 0.05 (two-tailed), and power level of 85%, giving a total required sample size of at least 632.

Statistical analysis

All statistical analyses were performed using STATA/IC 16.0. To calculate the molar ratio of NT-proBNP to BNP, we transformed BNP and NT-proBNP values from picograms per millilitre (pg/mL) to picomol per millilitre (pmol/mL) by dividing the observed value by the molecular weight of BNP and NT-proBNP (3.464 and 8.460, respectively). We calculated quartiles for the molar NT-proBNP/BNP ratio variable according to their distribution in the study population. All continuous variables were non-normally distributed and are thus presented as median, interquartile range (IQR). Between-group comparisons of these variables were performed using the Mann–Whitney *U* test (two categories) or Kruskall–Wallis test (≥3 categories). Categorical variables are presented as counts and percentages and comparisons between groups were made using the χ^2 test.

Multivariable linear regression using the enter method was used to evaluate the strongest determinants of log-transformed molar NT-proBNP/BNP ratio. Multivariable logistic regression analysis was performed to examine the association between molar log-transformed NT-proBNP/BNP ratio and the categorical variable HF or LVD, its individual components, and MACE. In addition to estimating the unadjusted effect for outcomes, we created models adjusting for (1) age, gender, diabetes mellitus, hypertension, obesity, vascular disease, HR, number of years of follow-up, and NT-proBNP at baseline; and (2) as per Model 1, plus use of renin–angiotensin–aldosterone system inhibitors (RAAS-Is), use of beta-blockers and use of other anti-hypertensives. For HF and MACE, due to the low number of events and risk of over-fitting the model, we employed a more parsimonious model, adjusting for age, gender, number of years of follow-up, and NT-proBNP at baseline. Because of the high correlation between NT-proBNP and BNP and the introduction of multicollinearity into the model when both were included, we did not include BNP in the model. Instead, we examined an alternative model where NT-proBNP was substituted by BNP.

We performed linear regression to explore the relationship between molar log-transformed NT-proBNP/BNP ratio and the log of left atrial volume indexed to body surface area (LAVI), left ventricular mass indexed to body surface area (LVMI), EF, and ratio of transmitral Doppler early filling velocity to tissue Doppler early diastolic mitral annular velocity (E/e′). The model was adjusted as per Models 1 and 2, with the exclusion of number of years of follow-up as these were baseline measures. We estimated the integrated discrimination improvement (IDI) and the category-free net reclassification improvement (NRI) metric for the addition of molar NT-proBNP/BNP ratio to adjusted models.

Results

Baseline characteristics

A total of 782 patients were included in the analysis. The main demographic, clinical, echocardiographic, and biochemical characteristics of the whole study population and within each quartile of molar NT-proBNP/BNP ratio are presented in *Table 1*. The median age of the patients was 66.6 years (IQR 59.5–73.1), and 47.4% of participants were female. Almost half of the patients had diabetes, and 72.9% had hypertension. The median molar NT-proBNP/BNP ratio was 1.91 (IQR 1.37–2.93). The distribution of molar NT-proBNP/BNP ratio by NT-proBNP in each ratio quartile is presented in *Figure 1*. Age, HR, prevalence of vascular disease, use of beta-blockers, LVMI, and LAVI were significantly different between Quartile 1 and Quartiles 2–4. BNP levels were highest in Quartile 1 and decreased linearly from Quartile 1 to Quartile 4. NT-proBNP decreased from Quartile 1 to Quartile 3, but the levels in Quartile 4 were higher than those observed in quartile 1 (111.4 pg/mL vs. 119.5 pg/mL; *P* = 0.005).

Table 1 Baseline characteristics according to baseline molar NT-proBNP/BNP ratio quartile

	All (n = 782)	Quartile 1 (n = 195)	Quartile 2 (n = 196)	Quartile 3 (n = 196)	Quartile 4 (n = 195)	P value (Q1 vs. Q2–4)	P value (Q1 vs. Q4)
Age, years, median (IQR)	66.6 (59.5–73.1)	68 (60.7–74.7)	65.6 (59.6–71.3)	65.6 (58.0–71.7)	70 (59.4–73.6)	0.029[a]	0.061[a]
Female, n (%)	371 (47.4)	92 (47.2)	93 (47.4)	95 (48.5)	91 (46.7)	0.987	0.919
BMI (kg/m^2), median (IQR)	28 (25–31)	28 (26–32)	28 (25–31)	28 (25–32)	27 (24–31)	0.181[a]	0.116[a]
SBP (mm/Hg), median (IQR)	138 (125–151)	139 (127–154)	135.5 (126–148)	139 (125–149)	137 (124–152)	0.289[a]	0.104[a]
DBP (mm/Hg), median (IQR)	81 (74–88)	81 (72–88)	80 (73–86)	82.5 (75–88)	81 (74–89)	0.166[a]	0.551[a]
Heart rate, median (IQR)	70 (61.8–79)	67 (60–76)	68 (60–77)	70 (63–79)	72 (63–82)	<0.0001[a]	<0.0001[a]
eGFR (mL/min/1.73 m^2)	71.4 (60.7–83)	73.0 (62.4–87.2)	71.5 (59.8–81.8)	70.2 (60.1–82)	71.4 (59.6–82)	0.510[a]	0.211[a]
Medical history, n (%)							
Diabetes mellitus	360 (46)	78 (40)	105 (53.6)	90 (45.9)	360 (46)	0.058	0.356
Hypertension	670 (72.9)	147 (75.4)	141 (71.9)	147 (75)	135 (49.2)	0.481	0.174
Myocardial infarction	82 (10.5)	29 (14.9)	13 (6.6)	21 (10.7)	19 (9.7)	0.065	0.123
Vascular disease	136 (17.4)	47 (24.1)	25 (12.8)	31 (15.8)	33 (16.9)	0.024	0.079
Atrial fibrillation	56 (7.2)	11 (5.6)	10 (5.1)	16 (8.2)	19 (9.7)	0.243	0.128
Anaemia	36 (4.6)	8 (4.1)	9 (4.6)	21 (6.1)	7 (3.6)	0.660	0.792
Dyslipidaemia	609 (77.9)	158 (81)	155 (79.1)	153 (78.1)	143 (73.3)	0.305	0.070
Stroke	24 (3.1)	7 (3.6)	4 (2)	5 (2.6)	8 (4.1)	0.626	0.792
Obesity	242 (30.9)	64 (32.8)	53 (27)	68 (34.7)	57 (29.2)	0.351	0.444
Medication, n (%)							
ACEI	228 (29.2)	53 (27.2)	58 (29.6)	56 (28.6)	61 (31.3)	0.838	0.472
ARB	187 (23.9)	53 (27.2)	45 (23)	50 (25.5)	39 (20)	0.368	0.132
Beta-blocker	249 (31.8)	77 (39.5)	68 (34.7)	56 (28.6)	48 (24.6)	0.009	0.001
Ca channel blocker	201 (25.7)	56 (28.7)	45 (23)	46 (23.5)	54 (27.7)	0.455	0.710
Statin	539 (68.9)	138 (70.8)	135 (68.9)	136 (69.4)	130 (66.7)	0.851	0.433
Diuretic	213 (27.2)	56 (28.7)	52 (26.5)	60 (30.6)	45 (23.1)	0.377	0.135
Echocardiographic parameters, median (IQR)							
Ejection fraction (%)	67 (62–72)	66 (61–72)	65 (61–72)	66 (61–71)	67 (61–72)	0.952[a]	0.755[a]
LAVI (mL/m^2)	25.1 (21.1–30.6)	27.1 (22.3–33.5)	25.1 (21.8–30.0)	24 (20.6–28.8)	24.6 (20.9–31.5)	0.006[a]	0.026[a]
E/e′	8.2 (6.6–10.3)	8.2 (6.6–10.4)	8.1 (6.6–10.0)	8.4 (6.8–10.6)	8 (6.2–10.6)	0.522[a]	0.327[a]
LVMI (g/m^2)	92.2 (79.6–108.4)	93.7 (79.3–109.5)	91.4 (79.6–108.6)	87.9 (76.6–105.6)	95.3 (82.6–111.6)	0.048[a]	0.435[a]
Natriuretic peptides, median (IQR)						P value (All)[b]	P value (Q1 vs. Q4)
BNP (pg/mL)	22.1 (9.5–54.1)	49.8 (22–80.6)	24.5 (12.5–50)	16.8 (8.4–35)	11.3 (5.6–27.1)	<0.0001	<0.0001
NT-proBNP (pg/mL)	103.2 (56.3–210.7)	111.4 (53.8–200)	97.8 (48.2–194.8)	93.7 (51.4–188.8)	119.5 (66.7–265.4)	0.002	0.005
BNP (pmol/mL)	6.4 (2.7–15.6)	14.4 (6.3–23.2)	7.1 (3.6–14.4)	4.8 (2.4–10.1)	3.3 (1.6–7.8)	<0.0001	<0.0001
NT-proBNP (pmol/mL)	12.20 (6.7–24.9)	13.2 (6.4–23.7)	11.6 (5.7–23)	11.1 (6.1–22.3)	14.1 (7.9–31.4)	0.002	0.005
NT-proBNP/BNP ratio	4.65 (3.33–7.13)	2.53 (2.02–2.96)	3.88 (3.59–4.24)	5.65 (5.13–6.30)	9.14 (7.75–11.9)	<0.0001	<0.0001
Molar NT-proBNP/BNP ratio	1.91 (1.37–2.93)	1.04 (0.83–1.21)	1.59 (1.47–1.74)	2.31 (2.10–2.58)	3.75 (3.18–4.88)	<0.0001	<0.0001

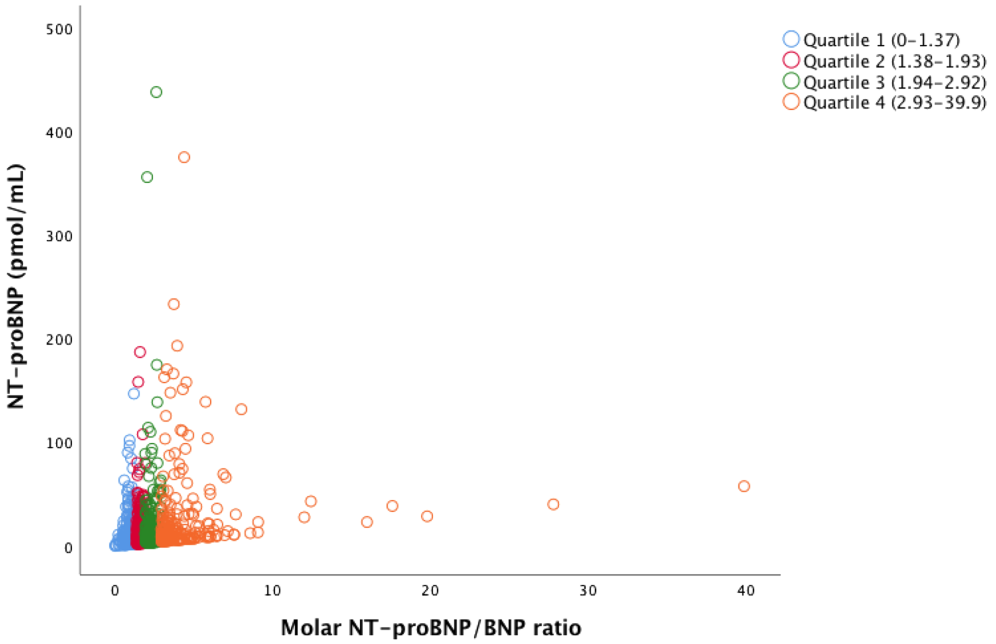
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BNP, B-type natriuretic peptide; Ca, calcium; E/e′, ratio of transmitral Doppler early filling velocity to tissue Doppler early diastolic mitral; eGFR, estimated glomerular filtration rate; LAVI, left atrial volume indexed to body surface area; LVMI, left ventricular mass indexed to body surface area; NT-proBNP, amino-terminal pro-B type natriuretic peptide; SBP, systolic blood pressure.

[a]Mann–Whitney U test.

[b]Kruskall–Wallis test, all others χ^2 test.

Q1 = 0–1.37, Q2–1.38 – 1.93, Q3 = 1.94–2.92, Q4 = 2.93–39.9.

Figure 1 Distribution of molar NT-proBNP/BNP ratio by NT-proBNP in each ratio quartile. BNP, B-type natriuretic peptide; NT-proBNP, amino-terminal pro-B-type natriuretic peptide.



Predictors of NT-proBNP/BNP ratio, BNP, and NT-proBNP

In multivariable linear regression analysis, four variables were significantly associated with molar NT-proBNP/BNP ratio, and the explanatory power of the model was low (r^2 = 0.032) (*Table 2*). Higher eGFR and higher systolic blood pressure (SBP) were associated with lower log-transformed molar NT-proBNP/BNP ratio (P = 0.006 and P = 0.033 respectively). Conversely, higher HR and higher LVMI were associated with higher ratio (P = 0.005 and P = 0.014, respectively). Mild renal impairment (CKD Stage 2) did not significantly influence molar NT-proBNP/BNP ratio. CKD Stages 3a, 3b, and 4 were associated with higher ratio and higher NT-proBNP, while only CKD Stages 3b and 4 significantly influenced BNP levels.

Relationship between echocardiographic parameters and log-transformed molar NT-proBNP/BNP ratio

Multivariable linear regression analysis showed that in a model adjusted for NT-proBNP and other covariates, for every 1% increase in log-transformed molar NT-proBNP/BNP ratio, there was a 0.049% decrease in log-transformed LAVI (P = 0.001) (*Table 3*). However, when BNP was substituted for NT-proBNP as a covariate, this relationship was no longer significant (P = 0.161). Molar NT-proBNP/BNP ratio was not significantly associated with LVMI, EF or E/e′.

Relationship between molar NT-proBNP/BNP ratio and HF-related outcomes

During the median follow-up period of 5 years (IQR 3.4–6.8), 48 patients (6.1%) developed HF, 21 (2.7%) developed LVSD, and LVDD was present in 207 (26.5%) (*Figure 2*). In the multivariable logistic regression model adjusted for NT-proBNP, a one-standard deviation (SD) unit increase in log-transformed molar NT-proBNP/BNP ratio was associated with a 29% decrease in the risk of developing HF or LVD (95% CI 0.55–0.91; P = 0.008) (*Table 4*). Higher log-transformed molar NT-proBNP/BNP ratio was also associated with a lower risk of HF, while the association with LVD did not reach significance when adjusted for use of medications. When BNP was substituted for NT-proBNP in the model, the relationship between log-transformed molar NT-proBNP/BNP ratio and HF or LVD, LVD and HF was rendered non-significant (P = 0.842, P = 0.819, and P = 0.465 respectively).

Relationship between molar NT-proBNP/BNP ratio and MACE

During the follow-up period, 45 MACE occurred. The association between molar NT-proBNP/BNP ratio and MACE was not significant in the multivariable models including NT-proBNP or BNP (P = 0.296 and 0.287, respectively) (*Table 4*).

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Table 2 Linear regression of determinants of log-transformed molar NT-proBNP/BNP ratio (n = 782)

	Molar NT-proBNP/BNP ratio		BNP		NT-proBNP	
	ß (SE)	*P* value	ß (SE)	*P* value	ß (SE)	*P* value
Age	−0.004 (0.004)	0.153	0.024 (0.004)	<0.0001	0.021 (0.003)	<0.0001
Male	0.011 (0.048)	0.866	−0.309 (0.060)	<0.0001	−0.298 (0.059)	<0.0001
Body mass index	−0.008 (0.005)	0.085	−0.009 (0.006)	0.137	−0.016 (0.006)	0.004
BMI < 25	*Reference*		*Reference*		*Reference*	
BMI 25–29.9	−0.117 (0.063)	0.063	−0.049 (0.079)	0.538	−0.162 (0.077)	0.037
BMI 30–34.9	−0.108 (0.073)	0.109	−0.123 (0.091)	0.180	−0.240 (0.089)	0.008
BMI 35+	−0.152 (0.086)	0.078	−0.166 (0.109)	0.127	−0.322 (0.106)	0.002
Diabetes mellitus	−0.021 (0.049)	0.813	−0.083 (0.061)	0.198	−0.181 (0.078)	0.020
Vascular disease	−0.065 (0.063)	0.907	0.245 (0.079)	0.002	0.195 (0.078)	0.013
Atrial fibrillation	0.070 (0.097)	0.472	0.713 (0.121)	<0.0001	0.783 (0.119)	<0.0001
Anaemia	0.004 (0.111)	0.974	0.145 (0.139)	0.296	0.149 (0.137)	0.267
Dyslipidaemia	−0.069 (0.056)	0.210	−0.043 (0.070)	0.542	−0.112 (0.069)	0.103
Treatment effect	−0.049 (0.050)	0.328	0.170 (0.063)	0.007	0.121 (0.062)	0.051
eGFR	−0.003 (0.001)	0.006	−0.004 (0.001)	0.004	−0.007 (0.001)	<0.0001
CKD Stage 1	*Reference*		*Reference*		*Reference*	
CKD Stage 2	0.102 (0.085)	0.118	−0.035 (0.081)	0.663	0.052 (0.079)	0.510
CKD Stage 3a	0.252 (0.085)	0.003	0.184 (0.106)	0.083	0.421 (0.103)	<0.0001
CKD Stage 3b + 4	0.263 (0.107)	0.014	0.351 (0.134)	0.009	0.596 (0.131)	<0.0001
SBP	−0.003 (0.002)	0.033	0.003 (0.002)	0.087	0.0003 (0.002)	0.980
DBP	0.002 (0.003)	0.551	−0.003 (0.003)	0.395	−0.001 (0.003)	0.971
Heart rate	0.006 (0.002)	0.005	−0.013 (0.003)	<0.0001	−0.008 (0.002)	0.002
LAVI	−0.005 (0.003)	0.103	0.037 (0.004)	<0.0001	0.032 (0.004)	<0.0001
LVMI	0.003 (0.001)	0.014	0.001 (0.001)	0.365	0.004 (0.001)	0.004

ß, unstandardized ß coefficient; BNP, B-type natriuretic peptide; CKD, chronic kidney disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; LAVI, left atrial volume indexed to body surface area; LVMI, left ventricular mass indexed to body surface area; NT-proBNP, amino-terminal pro-B type natriuretic peptide; SBP, systolic blood pressure; SE, standard error.

Table 3 Linear regression of association between log-transformed molar NT-proBNP/BNP ratio and echocardiographic variables at baseline (n = 782)

	Model	ß (SE)	*P* value
LAVI			
	Unadjusted	−0.038 (0.016)	0.021
	Model 1 + NT-proBNP	−0.049 (0.014)	0.001
	Model 1 + NT-proBNP, use of RAAS-I, BB, and other anti-hypertensives	−0.043 (0.014)	0.003
	Model 1 + BNP	0.005 (0.004)	0.161
LVMI			
	Unadjusted	0.005 (0.013)	0.697
	Model 1	−0.005 (0.013)	0.967
	Model 1 + NT-proBNP, use of RAAS-I, BB, and other anti-hypertensives	0.003 (0.013)	0.805
	Model 1 + BNP	0.006 (0.004)	0.072
EF			
	Unadjusted	−0.001 (0.007)	0.849
	Model 1 + NT-proBNP	−0.001 (0.007)	0.881
	Model 1 + NT-proBNP, use of RAAS-I, BB, and other anti-hypertensives	−0.0003 (0.007)	0.961
	Model 1 + BNP	−0.001 (0.002)	0.606
E/e′			
	Unadjusted	−0.007 (0.018)	0.696
	Model 1 + NT-proBNP	0.003 (0.018)	0.882
	Model 1 + NT-proBNP, use of RAAS-I, BB, and other anti-hypertensives	0.008 (0.018)	0.672
	Model 1 + BNP	−0.001 (0.005)	0.774

ß, unstandardized ß coefficient; BB, beta-blocker; BNP, B-type natriuretic peptide; E/e′, ratio of transmitral Doppler early filling velocity to tissue Doppler early diastolic mitral; EF, ejection fraction; LAVI, left atrial volume indexed to body surface area; LVMI, left ventricular mass indexed to body surface area; NT-proBNP, amino-terminal pro-B type natriuretic peptide; RAAS-I: Renin-angiotensin-aldosterone system inhibitor; SE, standard error.
Model 1 adjusted for age, gender, diabetes, hypertension, vascular disease, obesity, heart rate, eGFR, and NT-proBNP.

Figure 2 Incidence of HF outcomes over follow-up period by molar NT-proBNP/BNP ratio quartile. BNP, B-type natriuretic peptide; HF, heart failure; LVD, left ventricular dysfunction; LVDD, left ventricular diastolic dysfunction; LVSD, left ventricular systolic dysfunction; NT-proBNP, amino-terminal pro-B-type natriuretic peptide.

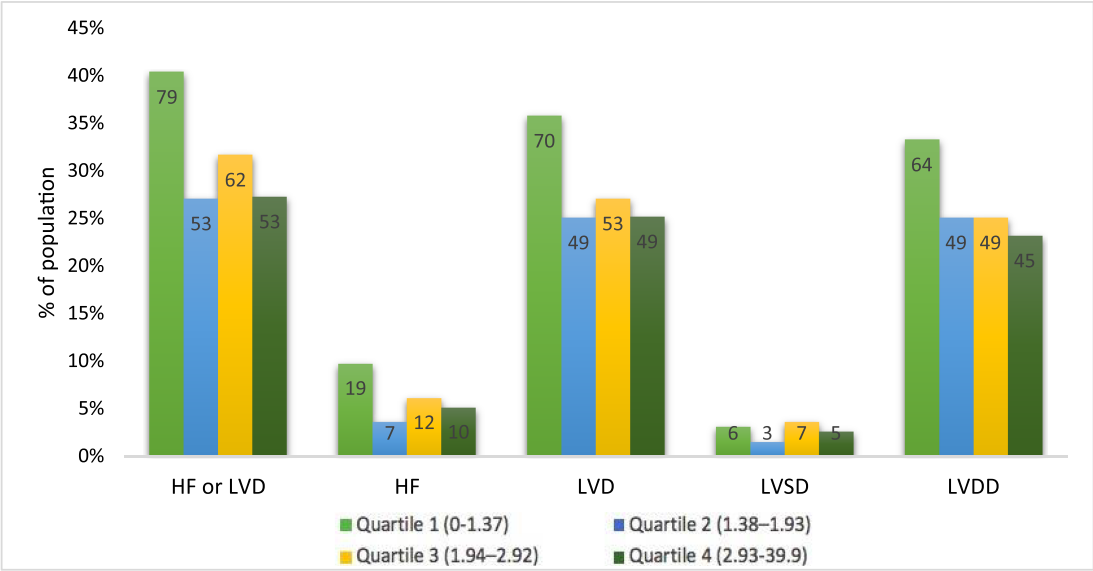

Table 4 Association between log-transformed molar NT-proBNP/BNP ratio and clinical outcomes (n = 782)

Outcome	Model	ß (SE)	OR (95% CI)	P value
HF or LVD (n = 247)				
	Unadjusted	−0.244 (0.120)	0.78 (0.62–0.99)	0.043
	Model 1 + NT-proBNP	−0.343 (0.129)	0.71 (0.55–0.91)	0.008
	Model 1 + NT-proBNP, use of RAAS-I, BB, and other anti-hypertensives	−0.324 (0.129)	0.72 (0.56–0.93)	0.012
	Model 1 + BNP	−0.027 (0.133)	0.97 (0.75–1.26)	0.842
HF (n = 48)				
	Unadjusted	−0.351 (0.217)	0.70 (0.46–1.08)	0.106
	Model 2 + NT-proBNP	−0.522 (0.227)	0.59 (0.38–0.92)	0.021
	Model 2 + BNP	−0.055 (0.239)	1.06 (0.66–1.69)	0.819
LVD (n = 221)				
	Unadjusted	−0.216 (0.126)	0.81 (0.63–1.03)	0.085
	Model 1 + NT-proBNP	−0.256 (0.128)	0.78 (0.60–0.99)	0.046
	Model 1 + NT-proBNP, use of RAAS-I, B, and other anti-hypertensives	−0.237 (0.129)	0.79 (0.61–1.02)	0.066
	Model 1 + BNP	−0.141 (0.194)	0.87 (0.59–1.27)	0.465
MACE (n = 45)				
	Unadjusted	−0.284 (0.226)	0.75 (0.48–1.17)	0.210
	Model 2 + NT-proBNP	−0.249 (0.239)	0.78 (0.49–1.24)	0.296
	Model 2 + BNP	−0.258 (0.243)	0.77 (0.48–1.24)	0.287
Performance metrics of molar NT-proBNP/BNP ratio in HF or LVD risk prediction models				
		C-statistic (95% CI)	IDI (95% CI)	NRI (95% CI)
	Model 1	0.71 (0.66–0.76)		
	Model 1 + molar NT-proBNP/BNP ratio	0.70 (0.65–0.75)	0.002 (−0.002–0.019)	0.035 (−0.203–0.224)

ß, unstandardized ß coefficient; BB, beta-blocker; BNP, B-type natriuretic peptide; CI, confidence interval; HF, heart failure; IDI, integrated discrimination improvement; LVD, left ventricular dysfunction; MACE, major adverse cardiovascular event; NRI, net reclassification index; NT-proBNP, amino-terminal pro-B type natriuretic peptide; OR, odds ratio; RAAS-I, renin–angiotensin–aldosterone system inhibitors; SE, standard error.
Model 1: Adjusted for age, gender, diabetes, hypertension, vascular disease, obesity, eGFR, and number of years of follow-up.
Model 2: Adjusted for age, gender, and number of years of follow-up (parsimonious model used due to low event numbers).

Quartile subanalysis based on NT-proBNP above and below 125 pg/mL

The ESC defines the upper limit of normal in the non-acute setting for NT-proBNP as 125 pg/mL.[19] We subdivided Quartiles 1 and 4 into those with NT-proBNP above 125 pg/mL (Quartiles 1a and 4a) and below 125 pg/mL (Quartiles 1b and 4b), to identify if these cohorts were uniform or heterogeneous. The clinical characteristics of these subquartiles are presented in *Table* S2. This categorization highlights the markedly heterogeneous phenotype of these patients. Those in Subquartile 1a were older and more likely to be female, have hypertension and vascular disease, and have higher BMI compared with Subquartile 1b, despite both groups having similar molar NT-proBNP/BNP ratios. Subquartiles 4a and 4b were also highly diverse.

We also compared the number of events across all quartiles and subquartiles (*Table* S3). Event rates were highest in those in those in Quartile 1a and lowest in those in Quartile 4b. Those in Quartile 1b had a similar risk of developing HF or LVD to those in Quartiles 2 or 3 (P = 0.581 and 0.767, respectively, analysis not presented). Thus, the increased risk of developing HF or LVD in Quartile 1 is driven by those patients in Quartile 1a who have higher NT-proBNP and BNP levels than those in Quartile 1b, despite having similar molar NT-proBNP/BNP ratios.

Discussion

In this study of stage A/B HF patients in the STOP-HF programme, we observed a wide distribution of molar NT-proBNP/BNP ratios associated with eGFR, SBP, LVMI and HR. Lower baseline molar NT-proBNP/BNP ratio was associated with elevated risk of HF or LVD over a 5-year follow-up period, independent of NT-proBNP and other covariates. However, molar NT-proBNP/BNP ratio did not significantly improve the C-statistic, IDI, or NRI, beyond NT-proBNP alone. Furthermore, when BNP was substituted for NT-proBNP in multivariable models, the relationship between molar NT-proBNP/BNP ratio and HF or LVD was no longer significant. While further work may be needed to confirm these data in other populations, our study suggests that routine, simultaneous measurement of BNP and NT-proBNP is not warranted in programmes designed to prevent the development of HF in European, Caucasian patients.

Our study is the first to characterize molar NT-proBNP/BNP ratios in a European Caucasian population with cardiovascular risk factors, yet builds upon previous studies conducted in HF patients[20,21] and one previous study in a cohort of Japanese patients with cardiovascular risk factors.[16] Interestingly, we found the opposite result to the Japanese study of Suzuki and Sugiyama, in terms of the relationship between NT-proBNP/BNP ratio and new-onset HF. Comparison of the two cohorts reveals numerous sources of heterogeneity between these two populations, which may account for the opposing findings. Patient phenotype varied between these populations, and different BNP assays, with different epitope specificities were used. Genetic differences between Japanese and European populations in the processing and metabolism of BNP and NT-proBNP may have also influenced results.

The median molar NT-proBNP/BNP ratio in our cohort of Stage A/B HF patients was 1.91, lower than ratios of 3 to 4 observed in Stage C HF patients[20,21] but similar to that found in other Stage A/B patients with cardiovascular risk factors.[16] BNP and NT-proBNP are cleaved in equimolar amounts in response to stimuli such as ventricular stretch, wall tension, and fibro-inflammation, but are found in markedly different concentrations in the circulation, due to the longer half-life of NT-proBNP.[3] The causal mechanism underpinning the divergence in BNP and NT-proBNP concentrations following synthesis most likely lies in differences in the processing and clearance of these two peptides. BNP binds to natriuretic peptide receptor A to exert its biological activity and is cleared by the natriuretic peptide clearance receptor C. BNP is also removed by the kidneys and can undergo degradation by enzymes such as neprilysin and dipeptidyl peptidase-4. In contrast, the sole fate of NT-proBNP is the clearance by the kidneys, making it more sensitive to changes in renal function than BNP.[22]

In accordance with previous studies, renal function was inversely related to molar NT-proBNP/BNP ratio and the association with renal function was stronger for NT-proBNP than BNP.[5,10,16] Factors such as unequal rates of sample degradation, cross-reactivity of BNP and NT-proBNP assays, and glycosylation of BNP and NT-proBNP may also contribute towards inter-individual variability.[23,24] The conflicting results obtained in the STOP-HF population, relative to the Japanese study may be related to several factors, including the younger age, and the lower baseline BNP and NT-proBNP levels of the European cohort. Notably, the STOP-HF population also had higher median BMI and a higher prevalence of diabetes than the Japanese cohort. Higher BMI, and possibly diabetes, is associated with increased glycosylation of pro-$BNP_{1–108}$ at the threonine 71 position.[25] The presence of the glycosidic residue sterically hinders corin and furin from accessing the pro-$BNP_{1–108}$ cleavage site, and impairs processing to BNP and NT-proBNP, resulting in lower levels of these two biomarkers.[4] Glycosylation of pro-$BNP_{1–108}$ may have also confounded immunoassay sensitivity in both of these studies and contributed to differences in NT-proBNP/BNP ratios. The Triage BNP assay has high affinity for glycosylated pro-$BNP_{1–108}$ while the Shionogi assay has significantly lower affinity for the glycosylated form (~5%).[8,26] This may have resulted in over-estimation of BNP relative to NT-proBNP, due to cross-reactivity with glycosylated pro-$BNP_{1–108}$, yielding a

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spuriously low NT-proBNP/BNP ratio in some patients. Taken together, our findings highlight the importance of performing studies in different populations, utilizing more than one type of BNP assay.

The explanatory power of our multivariable model, which included the known clinical and biochemical influencing factors, was low (adjusted r^2 = 3.2%), which may suggest that gene variants of enzymes, which influence the formation of BNP and NT-proBNP (e.g. corin and furin) as well as the biological activity (natriuretic peptide receptor A) and clearance of BNP (natriuretic peptide clearance receptor C, neprilysin, etc.), may also contribute towards the variation in the molar ratio of NT-proBNP to BNP. This could also explain the difference in results observed in genetically distinct populations such as the European, Caucasian STOP-HF, and Japanese stage A/B HF populations.

The association of molar NT-proBNP/BNP ratio with HF or LVD could have important implications for the implementation of screening and management programmes for HF and associated guidelines. However, when we added molar NT-proBNP/BNP ratio to risk prediction models for HF or LVD, the C-statistic did not increase, and the impact on the IDI and NRI was negligible, confirming that NT-proBNP/BNP ratio does not improve risk prediction relative to NT-proBNP or BNP alone. Moreover, multivariable models, which included BNP as a covariate mitigated the significance of the relationship between molar NT-proBNP/BNP ratio and HF or LVD. To explore this further, we examined the low and high molar NT-proBNP/BNP ratio quartiles (Quartiles 1 and 4) according to those with NT-proBNP above and below 125 pg/mL. This revealed that patients in these groups have highly heterogeneous phenotypes. In Quartile 1, the majority of those with elevated NT-proBNP and elevated risk of HF or LVD also had elevated BNP levels. Accordingly, these at-risk individuals would be identified by programmes measuring a single natriuretic peptide biomarker. While NT-proBNP/BNP ratio did not improve risk prediction in our cohort of patients with Stage A/B HF, it is worth noting that this metric has been shown to be of value in predicting cardiorenal syndrome in patients with acute HF.[14] Further research is required to determine the utility of NT-proBNP/BNP ratio in different patient cohorts, and in different cardiovascular outcomes.

We acknowledge that there are several limitations in this study. First, this is a retrospective, observational study and we cannot exclude the possibility that unmeasured variables have introduced bias or confounding into our study. Second, the results from this study are from a single centre in the east of Ireland and thus are not generalizable to other populations. Third, although samples were stored in a −80°C freezer, we cannot exclude that some sample degradation may have occurred. Fourth, the lower limit of detection for the Triage® BNP test is 5 pg/mL, and thus, there may be a subset of patients with BNP levels whose calculated molar NT-proBNP/BNP ratios are not wholly accurate. Fifth, the assays that we used in our study to measure BNP and NT-proBNP are known to cross-react with pro-BNP and BNP and NT-proBNP degradation fragments. Thus, we cannot be sure that the measured quantities pertain exclusively to $BNP_{1–32}$ and NT-$proBNP_{1–76}$. Sixth, the number of MACE during the follow-up period was low, and the study was not adequately powered to truly estimate the effect of molar NT-proBNP/BNP ratio on these outcomes.

Seventh, we did not perform echocardiography at follow-up in those with BNP $<$ 50 pg/mL unless deemed clinically necessary by a cardiologist (i.e. in response to clinical presentation). Therefore, we cannot exclude the possibility that we may have missed occurrences of asymptomatic LVD progression in those with BNP $<$ 50 pg/mL. Furthermore, our definition of progression of LVD did not consider certain aspects of diastolic function such as LA-size, e-wave deceleration time, or pulmonary vein flow, and this may have also resulted in underestimation of the number of cases of progression of LVD. Finally, the link between lower NT-proBNP/BNP ratio and HF or LVD may be mediated by related conditions, such as atrial fibrillation. We have not fully explored this in the present analysis, although NT-proBNP/BNP ratio was also associated with higher LAVI, and this in turn is associated with a higher risk of atrial fibrillation and HF.[27,28]

Conclusion

In conclusion, while molar NT-proBNP/BNP ratios are inversely and independently associated with the future development of HF and LVD, this study does not support routine simultaneous measurement of BNP and NT-proBNP in programmes to prevent HF. ACC/AHA and Canadian guidelines, which recommend natriuretic peptide biomarker-based screening followed by intensive team-based care, to prevent the development of LVD or new-onset HF do not need to be amended based on the present analysis. A significant proportion of the variability in NT-proBNP/BNP ratio remains unexplained by traditional covariates. Future work should evaluate the contribution of variants in the genes responsible for the processing, degradation and clearance of BNP, and NT-proBNP to the variability in the relative amounts of these peptides, and indeed to the risk of incident HF and progression of LVD.

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Conflict of interest

Prof Ken McDonald is a named inventor on a patent relating to novel biomarkers of cardiovascular disease. He is a co-principal investigator in the PARABLE study, which has received an unrestricted research grant from Novartis. He is also funded by the Health Research Board of Ireland. He had received honoraria and research grants from Pfizer, Alere, Menarini, Novartis, Servier, Abbott Diagnostics, Gen, Fire 1, and Bayer. Prof Ledwidge reports board membership and shares in Solvotrin Therapeutics and is a named inventor on several patents relating to formulations of iron, anti-inflammatory antiplatelet isosorbide prodrugs, and therapies for epigenetic regulation of cardiomyopathies. He is a co-principal investigator in the PARABLE study, which has received an unrestricted research grant from Novartis. He has also received honoraria and research grants from A. Menarini, Servier, Abbott Diagnostics, Genuity Science Ireland, and Bayer.

No other conflicts of interest were reported.

Author contributions

C. S., K. M., and M. L. developed the hypothesis, obtained the data, analysed the data, and wrote the manuscript. R. B., B. K., F. R., L. C., C. H., M. B., and C. J. W. obtained the data and contributed to the writing of the manuscript. C. R. contributed to the analysis of the data and the writing of the manuscript. M. L., C. J. W., and K. M. D. are co-principal investigators of the STOP-HF follow-up study.

Ethics approval, consent to participate, and consent to publication

The STOP-HF follow-up programme was approved by the St Vincent's University Hospital Ethics Committee and conformed to the principles of the Declaration of Helsinki, and all patients enrolled provided written, informed consent for participation and permission to publish. The corresponding author confirms that the consent of all authors has been obtained to publish this manuscript.

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Data availability statement

Participants in the STOP-HF follow-up programme have not consented to the publication of individual patient-level data.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. STOP-HF programme.
Table S1. Chronic kidney disease stage by eGFR.
Table S2. Clinical characteristics of those in molar NT-proBNP/BNP subquartiles 1 and 4.
Table S3. Chi-square analysis for difference in event rates between Quartile 1 and Quartile 4 subquartiles.

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