Levels of N-terminal pro brain natriuretic peptide are enhanced in people with the uncomplicated metabolic syndrome: a nested case-control analysis of the population-based Casale Monferrato Study

Levels of N-terminal Pro Brain Natriuretic Peptide are enhanced in people with the uncomplicated metabolic syndrome: a case-cohort analysis of the population-based Casale Monferrato Study

Short title: NT-proBNP and metabolic syndrome

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ABSTRACT

Background: Both metabolic syndrome (MetS) and NT-proBNP confer increased risk of cardiovascular diseases (CVD). We assessed if NT-proBNP levels were greater in people with uncomplicated MetS, who had neither CVD/chronic kidney disease (CKD) nor diabetes, as compared to subjects who met none of the defining criteria of the MetS.

Methods: A case-cohort study from the non-diabetic population-based Casale Monferrato Study was performed, after exclusion of all subjects with established CVD, CKD (eGFR<60 ml/min/1.73m^2) and CRP values ≥ 3 mg/l. Cases (n=161) with MetS were compared to all subjects within the cohort (n=124) who were completely free of any component of the MetS. Serum NT-proBNP were centrally measured by immunoenzymatic assay.

Results: NT-proBNP levels were significantly higher in cases than in control subjects [35.4 (15.5-98.2) vs 24.4 (11.7-49.6) pg/ml, p=0.014]. In logistic regression analysis, compared to NT-proBNP values in the lower quartiles (≤49.64 pg/ml), higher values conferred OR 4.17 (1.30-13.44) of having the MetS, independently of age, sex, microalbuminuria, CRP, eGFR, and central obesity. This association was evident even after the exclusion of hypertensive subjects. Further adjustment for log-HOMA and diastolic blood pressure did not modify the strength of the association, while central obesity was a negative confounder.

Conclusions: Compared to people without any component of the MetS, those with uncomplicated MetS, who had neither CVD/CKD nor diabetes, had increased NT-proBNP values, even if they were normotensive and though absolute values were still in the low range. The insulin-resistance state did not mediate this association, while central obesity was a negative confounder.

Keywords: natriuretic peptides, metabolic syndrome, cardiovascular risk, survey, population-based study

Introduction

N-amino terminal fragment of the prohormone B-type natriuretic peptide (NT-proBNP), a well-established biomarker of both left ventricular hypertrophy and heart failure, is associated with an increased risk of mortality and cardiovascular disease (CVD) in the general population [1-3]. In addition, a recent prospective study has demonstrated that in non-diabetic people NT-proBNP levels are inversely and independently associated with an increased risk of having diabetes over a 11-years follow-up period, suggesting a direct role of BNP in the regulation of metabolic processes in humans [4]. In keeping with this hypothesis receptors for natriuretic peptides have been found in the adipose tissue [5] and higher natriuretic peptide levels have been associated with a favorable adipose tissue distribution profile [6]. Moreover, studies in experimental animals have shown that BNP transgenic mice fed with a high-fat diet exhibit less weight gain, ectopic fat accumulation, and insulin resistance [7]. Further, treatment with BNP increases energy expenditure and thermogenic activation in both brown and white adipose tissue [8]. The underlying mechanism is still unclear, however, it has been proposed that BNP binding to the adipocyte natriuretic peptide receptor (NPR)-A activates downstream signalling cascades leading to increased mitochondrial biogenesis and uncoupled respiration [7,8].

The metabolic syndrome (MetS), which confers an increased risk for both type 2 diabetes and CVD, comprises a cluster of metabolic abnormalities with visceral adiposity, insulin resistance, and low-grade inflammation as its central pathophysiological features [9]. Therefore, the newly discovered role of natriuretic peptides in the control of metabolic processes is of particular relevance in patients with MetS [10]. Previous data on NT-proBNP levels in subjects with the MetS are conflicting, with studies showing either lower or similar values compared to people without the MetS [11-13]. However, given the opposite effect of obesity and CVD on NT-proBNP levels, inconsistencies among previous studies might be related to the different prevalence of these conditions in the examined populations.

Therefore, we designed a case-cohort study from the population-based Casale Monferrato Study to compare circulating NT-proBNP levels in people with MetS, uncomplicated by CVD and chronic kidney disease (CKD), and in subjects who did not meet any of the defining criteria of the MetS.

Materials and Methods

The Casale Monferrato study is an ongoing population-based study started in 1988 in the town of Casale Monferrato, North-West of Italy. A non-diabetic cohort (n=2,211) was recruited in 2005 from an age- and sex-stratified sample of 3,700 individuals, aged 45-74 years, randomly identified through the files of the resident population, after having excluded those with heart failure, neoplastic and other chronic diseases, as previously detailed [14]. Subjects were examined at the diabetes clinic and blood samples collected after overnight fasting, and stored at -80° C. Ethics committee approval was obtained. Informed consent was obtained from all patients for being included in the study. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. A cross-sectional case-control study was designed from the non-diabetic cohort of the Casale Monferrato study, after exclusion of subjects (n=659) with CVD, CKD (eGFR<60 ml/min/1.73m^2), and/or high sensitive C reactive protein (hs-CRP) levels ≥ 3mg/l. Cases were those who fulfilled the criteria of the updated National Cholesterol Education Program's Adult Treatment Panel III report for the diagnosis of MetS, therefore they had at least three risk factors to sustain the diagnosis, including central obesity, hypertension/antihypertensive medications, dyslipidemia (low HDL-cholesterol and high triglycerides), impaired fasting glucose. We then identified as control subjects all those subjects who were completely free of any component of the MetS. Applying these criteria, this yielded 161 cases and 125 controls with full data on complications and samples available for analysis. The sample size provides a power of 80%

(α=0.05) to detect a difference in log-NTproBNP within the cohort of at least one-third of standard deviation (SD).

Waist circumference was measured at the midpoint between the lower rib and the iliac crest. Smoking status was classified as current smoker, never smoker, ex-smoker (smoking cessation at least a month prior to the visit). Hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or treatment with antihypertensive drugs. CVD was defined as a positive medical history of a cardiovascular event, including myocardial infarction, angina pectoris, coronary artery bypass graft and stroke, and/or ischemic changes on a resting 12-lead electrocardiogram, classified according to the Minnesota Code. The WHO Rose questionnaire was also administered and people with symptoms suggestive of CVD underwent further investigations to confirm the diagnosis.

Fasting blood samples were taken in the morning visit from all recruited subjects and plasma glucose levels measured using the glucose-oxidase method. Triglycerides, total-cholesterol, HDL-cholesterol, apolipoprotein B, serum creatinine and insulin were measured by standard techniques, hs-CRP by immunoturbidimetry (Roche-Diagnostic), and albumin excretion rate (AER) by nephelometry on single overnight urine collections. Serum insulin was measured by radioimmunoassay in the non-diabetic cohort only. The degree of insulin sensitivity was determined using the HOMA-IR calculated as fasting plasma glucose (mmol/l) x fasting serum insulin(mU/L)/22.5. Glomerular filtration rate (GFR) was estimated using the four component abbreviated equation from the MDRD Study [15].

Serum NT-proBNP levels were measured by a two-site sandwich electrochemiluminescence immunoassay (Elecsys proBNP II, Roche Diagnostic, Mannheim, Germany), using a Modular Analytics Evo analyzer with a E170 module (Roche) as we have previously described [16]. The intra-assay variation was below 3.0% and total CV ranges between 2.2 and 5.8% in low and high ranges of NT-proBNP.

Data were expressed as mean (SD) or geometric means (inter-quartile range). Logistic regression analysis was used to estimate the odds ratios (ORs) of serum NT-proBNP levels for MetS. We estimated ORs of log-NT-proBNP and across its quartiles (model 1), after adjustment for age and sex (model 2) and after further adjustment for log-AER, log-CRP and eGFR (model 3), waist circumference (model 4) and log-HOMA (model 5). We also examined the role of other variables (treatment with ACE/ARB inhibitors, diuretics and other antihypertensive treatment, statins, BMI, and uric acid), which were retained in the final model if they added significantly to the likelihood of models or to the estimated coefficients of predictors. To assess pattern of ORs across increasing serum NT-proBNP levels, we constructed both models for log-NTproBNP values as a continuous measure - assessing the multiplicative increase of each increment of NTproBNP to risk of MetS - and models with quartiles of NT-proBNP, according to the values distribution among control subjects. We tested for linear trends across quartiles by entering a single ordinal term into the models. As ORs in the lower NT-proBNP quartiles were similar, they were aggregated as the reference category in the final analyses and compared with the upper quartile. P value of less than 0.05 was considered to indicate statistical significance. Analyses were performed with Stata (Stata Release 10.0, Stata Corporation, College Station, Texas).

Results

The study population (n=285) had a mean age of 58.1 years (SD 8.4). As shown in Table 1, cases were older than controls and had a greater proportion of men. As expected, mean waist circumference, BMI, systolic and diastolic blood pressure, blood glucose, insulin, HOMA and triglyceride levels were greater, while HDL-cholesterol levels lower in cases than in controls. Cases also had a more adverse cardiovascular risk profile with significantly higher values of LDL-cholesterol, apoB, hs-CRP, and AER.

As shown in Figure 1, NT-proBNP values showed a right skewed distribution and were significantly higher in cases than in control subjects (Table 1). In 8 (6.4%) control subjects and 36

(22.4%) cases NT-proBNP values were 100 pg/ml and over. Comparing subjects with and without each component of the MetS, we found that NT-proBNP values were significantly higher in subjects having IFG (39.02 vs 25.57 ng/ml, p=0.006), central obesity (37.90 vs 24.80, p=0.004), and hypertension (36.10 vs 23.69 ng/ml, p=0.005), whereas they were unaffected by the presence of dyslipidemia (30.44 vs 29.87 ng/ml, p=0.90). Examined subjects had either no component or two or more one components of the MetS and NTproBNP values were higher with increasing numbers of components of the MetS (Figure 2).

We then performed logistic regression analyses to assess whether higher NT-proBNP values conferred increased odds ratio of having the MetS, independently of main risk factors (Table 2). Models showed that higher levels of NT-proBNP conferred greater OR for the MetS (model 1). This association remained statistically significant after further adjustments for age and sex (model 2). With respect to NT-proBNP values ≤ 49.64 ng/ml, higher values conferred a 2.4 fold increased odds of having the MetS (OR=2.43, 95% CI 1.28-4.60). The strength of the association between NT-proBNP and MetS was virtually unmodified after further adjustment for AER, CRP and eGFR (model 3), while a negative confounding effect of waist circumference was evident (model 4). Indeed, after further adjustment for central obesity, OR increased to 4.17 (1.30-13.44). In contrast, further adjustment for log-HOMA did not modify the association (model 5). No effect on ORs of other examined variables, including drugs, was evident.

Models including either components of the MetS or systolic blood pressure could not be assessed due to overfitting of data, whereas the inclusion of diastolic blood pressure into model 3 reduced only slightly OR conferred by NT-proBNP >49.64 ng/ml (OR=3.28, 1.17-9.25). Logistic regression analysis was then performed in cases (n=15) and control subject (n=122) who were normotensive at the examination. Even in this subgroup of subjects, compared to NTproBNP values ≤49.64 ng/ml, higher values conferred an adjusted OR (model 3) of 6.64 (95% CI 1.14-38.14), which remained statistically significant even after adjustments for diastolic blood pressure.

Discussion

The present analysis of the Casale Monferrato Study shows that people with uncomplicated MetS had greater NT-proBNP levels than people without any component of the MetS. Indeed, NT-proBNP values were two-fold higher in cases than in control subjects, though absolute values were still in the low range. After adjustment for central obesity, people with the MetS had a 4-fold higher odds of having NT-proBNP values in the highest quartile (>49.64 pg/ml) than those with lower values. This finding was independent of main risk factors and confounders, including age, sex, AER, CRP, eGFR, and waist circumference and it was evident even in normotensive subjects. The insulin-resistance state did not mediate this association as no changes in ORs were observed after inclusion of the variable HOMA in the fully adjusted multivariate model.

As we excluded from our analyses all subjects with low-grade inflammation (plasma CRP ≥ 3 g/l) and CKD, we reduced the likelihood of a potential confounding effect of both inflammatory processes and reduced renal function on NT-proBNP levels. Therefore, our finding likely reflects the underlying chronic cardiovascular stress due to the presence of the defining components of the MetS. In our cohort hypertension was highly prevalent, with 90% of cases with MetS being hypertensive, compared to only 2% of control subjects. Moreover, mean diastolic blood pressure values were quite high in cases, indicating an overall poor blood pressure control. However, our results were confirmed even limiting the analysis to normotensive subjects and after further adjustment for diastolic blood pressure, suggesting that other mechanisms are also involved in BNP increase. NPs have important physiological effects on cardiovascular system, body fluids, and electrolytic homeostasis [17]. Moreover, they have a well-known protective action on the vascular endothelium, reducing shear stress, modulating both coagulative and fibrinolytic cascades, and inhibiting platelet activation [18]. It is thus likely that the higher NT-proBNP values in uncomplicated patients with MetS reflect the presence of multiple risk factors, which define the cardiovascular risk status of individual subjects, and may help to predict the CV risk of asymptomatic people. Consistently, we have recently shown that in diabetic people of the Casale

Monferrato cohort, a slight increase in NT-proBNP levels was a strong independent predictor of CV mortality even in patients without pre-existing CVD [19]. Moreover, among patients at risk of heart failure, BNP-based screening and collaborative care allowed to reduce the combined rates of left ventricular systolic dysfunction, diastolic dysfunction, and heart failure [20].

As shown in table 3, previous studies assessing the relationship between NT-proBNP values and the MetS were conflicting [11-13]. Nonetheless, the literature generally refers to people with the MetS as having lower plasma NT-proBNP values compared to those without the MetS. However, inconsistencies among studies might be explained by the recruitment of cohorts with different prevalence of obese subjects due to the negative association between obesity and NT-proBNP. In our study, mean values of BMI and waist circumference were lower than in other Caucasians and higher than among Asians. As a consequence, the relative frequency of hypertension was higher than in other studies performed on people with the MetS. Finally, we recruited only subjects without CVD and we performed the analyses on subjects in the uncomplicated phase of the MetS. Therefore, our study adds to previous knowledge on this issue, providing evidence that in patients in the early phase of the MetS, including in those without hypertension, NT-proBNP is increased, thus representing an early marker of potential clinical utility.

Our study confirms previous data on the negative confounding effect of obesity on NT-proBNP values [21]. The cause of the relative NP deficiency seen in obese persons is poorly understood. It has been postulated that there is increased BNP clearance by the adipose tissue in obese subjects. However, there is evidence that NT-proBNP is predominantly cleared by the kidney [22].

A recent study has shown that low NT-pro-BPN levels are a potent predictor of Type 2 diabetes onset [4]. However, in our study, subjects with uncomplicated MetS, a known risk factor for the development of type 2 diabetes, had greater NP-proBNP levels. The explanation of this finding is unclear, however, experimental studies have demonstrated a role of NP on adipose tissue

metabolism and on central control of energy balance. Indeed, NPs exert potent lipolytic effects mediated by the NP receptor type A/cGMP pathway in human fat cells and they contribute to lipid mobilization in vivo. A browning of white fat and thermogenesis has also been shown. Moreover, NPs also enhance oxidative capacity and fat oxidation in skeletal muscle of mice and humans, and cardiac NPs have emerged as potent metabolic hormones [7,8, 23]. It is thus temping to speculate that the rise in NT-proBNP levels in MetS is a compensatory mechanism and that among subjects with MetS the risk of type 2 diabetes is greater in those with insufficient compensatory ability. However, causal relationships cannot be inferred in a cross-sectional study and further studies are required to address this hypothesis.

Strengths of our study include the identification of both cases and control subjects within a population-based cohort, thus allowing to prevent selection bias; the standardized data collection, allowing to exclude detection bias; the exclusion criteria, allowing to provide data on apparently healthy subjects with the MetS.

There are certain limitations to our study. First, this is a cross-sectional study and this restricts our ability to assess temporal relationships between NT-proBNP and MetS and to identify causal biological mechanisms underlying this association. Second, the presence of CVD was assessed based on clinical data and resting ECG. Therefore, we cannot exclude the possibility that some patients with CVD and normal ECG were erroneously included. However, the WHO Rose questionnaire was administered and people with symptoms suggestive of CVD underwent further investigations before inclusion in our survey. Third, although the population-based Casale Monferrato cohort was quite large, only 161 cases and 125 controls could be compared applying exclusion criteria. The subgroup of normotensive subjects was even smaller. However, adjusted OR of NTproBNP in the upper quartile still remained significantly associated with the risk of having the MetS and this finding was unchanged after further adjustment for diastolic blood pressure. Moreover, the ratio of normotensive cases compared to control subjects was more than 1 to 7,

which allowed us to provide quite robuste data. We therefore believe that our results represent a starting point for studies based on larger numbers of subjects.

In conclusion, our case-cohort study within the population-based Casale Monferrato Study provided evidence that, compared to people without any component of the MetS, those in the uncomplicated phase of the MetS, who had neither CVD/CKD nor diabetes, had yet increased NT-proBNP values, even if they were normotensive. The insulin-resistance state did not mediate this association, while central obesity was a negative confounder. Further prospective studies of large cohorts are need to further explore the role of NTproBNP in the MetS.

Duality of interest:
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Guarantor: Graziella Bruno

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G.B is the guarantor of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Statement of Human Rights: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008

Statement of Informed Consent: Informed consent was obtained from all patients for being included in the study**.**

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Table 1. Physical and clinical characteristic of case and control subjects of the Casale Monferrato Study

	Case subjects	Control subjects	P
N	161	124	
Age (years)	61.0 ± 8.0	54.5 ± 6.5	<0.0001
Males (%)	98 (60.9%)	24 (19.3%)	<0.0001
BMI (kg/m^2)	29.4 ± 4.5	22.3 ± 2.7	<0.0001
<25	24 (13.3%)	115 (84.6%)	<0.001
25-29	84 (46.7%)	19 (14.0%)	
>29	72 (40.0%)	2 (1.5%)	
Waist circumference (cm)	100.6 ± 9.8	76.4± 8.9	<0.0001
Systolic blood pressure (mmHg)	153.7 ± 18.7	118.4 ± 10.3	<0.0001
Diastolic blood pressure (mmHg)	94.6 ± 10.8	77.1 ± 6.7	<0.0001
Hypertension (%)	146 (90.7%)	2 (1.6%)	<0.0001
Total cholesterol (mmol/l)	5.77 ± 1.05	5.45 ± 0.83	0.006
LDL-cholesterol (mmol/l)	3.34 ± 0.93	3.05 ± 0.70	0.004
HDL-cholesterol (mmol/l)	1.51 ± 0.36	2.00 ± 0.43	<0.0001
Triglycerides (mmol/l)	1.90 (1.46-2.44)	0.84 (0.69-1.01)	<0.0001
ApoB (mg/dl)	106.8 ± 26.4	87.8 ± 18.9	<0.0001
Glucose (mg/dl)	102.0 ± 10.9	86.1 ± 7.7	<0.0001
HOMA	3.34 (2.34-4.74)	1.27 (0.95-1.89)	<0.0001
Insulin (ng/ml)	13.4 (10.2-19.0)	6.0 (4.7-8.4)	<0.0001
AER µg/min	51.1 (25.8-78.1)	32.3 (19.0-47.8)	<0.0001
CRP (mg/l)	1.3 (0.9-2.0)	0.79 (0.5-1.2)	<0.0001
eGFR (60 ml/min/1.73m^2)	84.5 (76.0-102.3)	90.7 (84.0-100.0)	<0.0001
Smokers no	87 (54.8%)	68 (54.8%)	0.19
ex	45 (20.2%)	25 (20.2%)	
yes	29 (18.0%)	31 (25.0%)	
NT-proBNP (pg/ml)	35.4 (15.5-98.2)	24.4 (11.7-49.6)	0.014
<11.73	37 (23.0%)	31 (25%)	0.012
11.73-27.62	30 (18.6%)	31(25%)	
27.63-49.64	25 (15.5%)	31(25%)	
>49.64	69 (42.9%)	31(25%)	

Table 2: Odds ratios for the metabolic syndrome by NT-proBNP values in the case-cohort study of the Casale Monferrato Study

	Model 1	Model 2	Model 3	Model 4	Model 5
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Log-NT-proBNP	1.27 (1.05-1.54)	1.29 (1.00-1.66)	1.22 (0.93-1.61)	1.47 (0.92-2.34)	1.56 (0.91-2.68)
NT-proBNP (ng/ml)					
<11.73	1.00	1.00	1.00	1.00	1.00
11.73-27.62	0.81 (0.41-1.62)	0.97 (0.42-2.22)	1.02 (0.43-2.44)	0.55 (0.14-2.11)	0.65 (0.13-3.34)
27.63-49.64	0.68 (0.33-1.38)	0.66 (0.27-1.60)	0.46 (0.18-1.18)	0.51 (0.10-2.57)	0.48 (0.06-3.83)
>49.64	1.86 (0.99-3.53)	2.11 (0.94-4.73)	1.97 (0.82-4.72)	2.85 (0.71-11.38)	3.39 (0.67-17.3)
P for trend	*0.05*	*0.08*	*0.22*	*0.12*	*0.15*
NT-proBNP (ng/ml) ≤49.64	1.00	1.00	1.00	1.00	1.00
>49.64	2.25 (1.35-3.76)	2.43 (1.28-4.60)	2.51 (1.26-5.00)	4.17 (1.30-13.44)	4.62 (1.17-18.32)

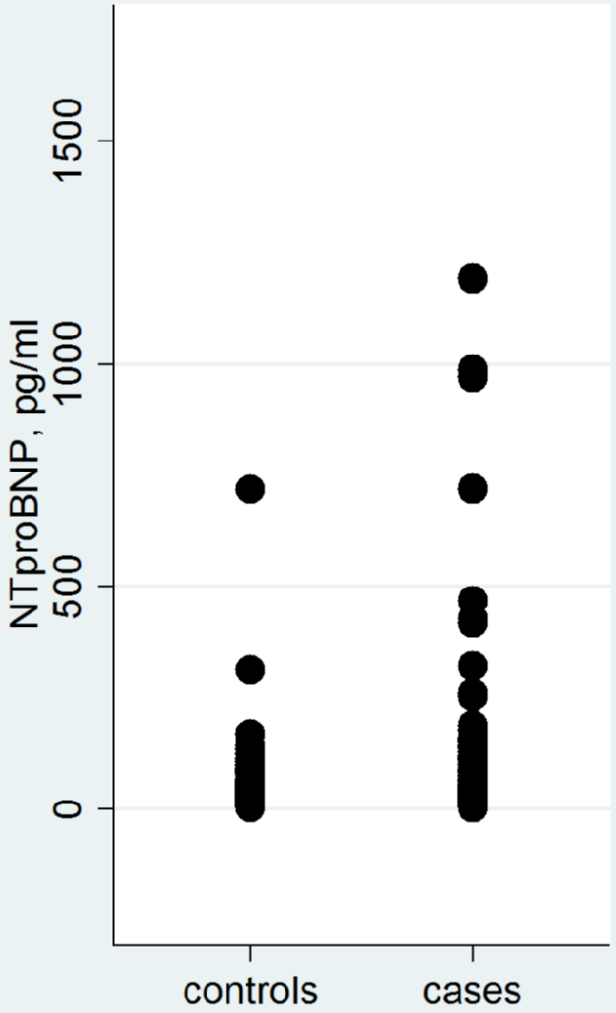
Model 1: unadjusted.
Model 2: adjusted for age and sex.
Model 3: adjusted for age, sex, AER, eGFR, CRP
Model 4: adjusted for age, sex, AER, eGFR, CRP and waist circumference
Model 5: adjusted for age, sex, AER, eGFR, CRP, waist circumference and log-HOMA-IR

Table 3: Comparison of study designs and main results of studies examining the association between NT-proBNP levels and the MetS

Study	Study design	Variables	Main findings
Olsen MH & al, 2005 (13)	Population-based study in Denmark, subjects without CVD (n=2070) recruited in 1992-93, MetS (n=318) defined by EGIR criteria	Age, gender, daily exercise, alcohol consumption, smoking status, BMI, waist circumference, lipids, FBG, insulin, heart rate, SBP, DBP	Nt-proBNP levels were lower in people with the MetS, attributable to the inverse relationships between serum NT-proBNP and BMI, serum insulin, and cholesterol. The MetS shifted the positive relationship between pulse pressure and Nt-proBNP to the right (ie, higher blood pressure for a given level of Nt-proBNP)
Bao Y & al, 2011 (11)	Case-control study in China, cases (n=230) and control subjects (n=239) recruited at an endocrinology clinic	Age, gender, BMI, SBP, DBP, FBG, lipids, LDLc, LVMI, LVEF	NT-proBNP levels were lower in people with the MetS compared with those without the MetS
Li W-Y & al, 2011 (12)	Case-control study in Taiwan, cases (n. 270) and control subjects (n=270) recruited among those referred to a clinic, matched by their propensity score	BMI, waist circumference, SBP, DBP, FBG, lipids, insulin	No difference in NT-proBNP levels between cases and control subjects
Present study	Case-cohort study within a population-based study in Italy, uncomplicated cases of MetS (n= 161) and control subjects with no component of the MetS (n=124)	Age, gender, waist circumference, eGFR, SBP, DBP, lipids, AER, CRP, HOMA-IR	NT-proBNP levels were higher in people with the MetS than in those without it, even in the subgroup of normotensive subjects

NT-proBNP: N-terminal pro-B-type natriuretic peptide; MetS: metabolic syndrome; EGIR: European Group for the Study of Insulin resistance; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBG: fasting blood glucose; LVMI: left ventricular mass index, LVEF: left ventricular ejection fraction, CVD: Cardiovascular Disease; eGFR: estimated glomerular filtration rate; AER: albumin excretion rate; CRP: C-reactive protein

Formattato: Destro 2,5 cm, Superiore: 2 cm, Larghezza 29,7 cm, Altezza: 21 cm


NTproBNP, pg/ml
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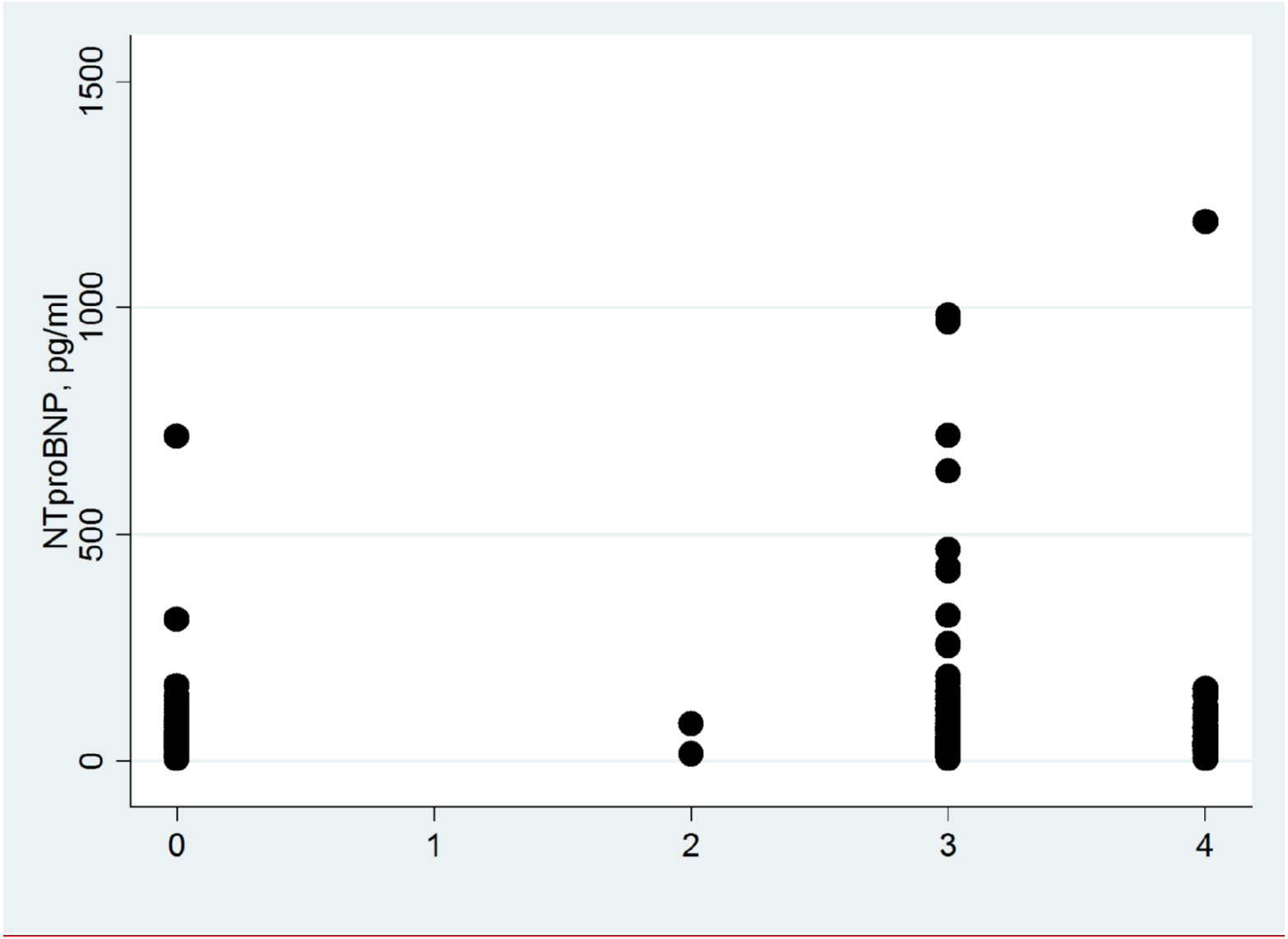
500
1000
1500
controls
cases


NTproBNP, pg/ml
0
500
1000
1500
0
1
2
3
4