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The midregional portion of proadrenomedullin is an independent predictor of left ventricular mass index in hypertension

Sanjay S. Bhandari[a,*], Joan E. Davies[a], Joachim Struck[b], Leong L. Ng[a]

[a]*Departmet of Cardiovascular Sciences, University of Leicester, Leicester LE2 7LX, United Kingdom*
[b]*BRAHMS AG, Hennigsdorf, 16761 Berlin, Germany*

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Abstract

Left ventricular hypertrophy (LVH) is a risk factor for cardiovascular disease. Elevated natriuretic peptides in LVH have spurred interest that biomarkers may play a role in screening programs. Adrenomedullin (ADM) is a 52–amino acid peptide mediating vasorelaxation, natriuresis, and diuresis. The midregional portion of proADM (MRproADM) is secreted stoichiometrically with ADM; hence, it can be used as a surrogate marker of ADM. We compared the diagnostic performance of MRproADM for the detection of LVH with N-terminal pro–B-type natriuretic peptide (NTproBNP). Two hundred fifty-three hypertensive patients were derived from a local screening study. The MRproADM and NTproBNP levels were assayed using immunoluminometric assays. The MRproADM levels were significantly elevated in patients with LVH than those without (mean [SD]: 0.73 [0.25] vs 0.59 [0.18] nmol/L, $P < .001$). In multivariate analyses, male sex ($P < .001$) and log MRproADM ($P = .003$) retained significance for detecting LVH. Receiver operating characteristic curve for MRproADM yielded an area under the curve of 0.71; confidence interval, 0.62-0.81; $P < .001$, superior to NTproBNP. An optimal cutoff value for MRproADM as an indicator of LVH was 0.50 nmol/L, with a sensitivity, specificity, and negative predictive value of 90.5%, 36.5%, and 95.1%, respectively. The high negative predictive value of the MRproADM assay allows it to be used as a rule-out test for LVH when stratifying patients into high or low risk. Patients who test positive would necessitate echocardiography, enabling better resource allocation.

1. Introduction

Left ventricular hypertrophy (LVH) is a compensatory mechanism in response to left ventricular pressure overload in hypertension. It is associated with reactive fibrosis, which interrupts the electrical and mechanical performance of the myocardium leading to systolic and diastolic dysfunction [1,2]. Left ventricular hypertrophy is influenced by a number of variables such as blood pressure (BP) levels, sex, age obesity, diet and drug therapies and is an adverse sign of preclinical cardiovascular disease [3-5]. It is a risk factor for coronary heart disease, heart failure, sudden death, and ventricular arrhythmias [6,7]. Regression of LVH is associated with reduced cardiovascular risk in hypertensive patients independent of ambulatory BP [8]. Risk stratification using tools such as electrocardiogram (ECG) and echocardiography is limited. The standard voltage criteria of ECG have low sensitivity for the detection of anatomical LVH [9]. In contrast, limited resource implementation and poor reproducibility have impeded the use of echocardiography as a screening diagnostic tool [10]. Despite these uncertainties, the prognostic value of LVH is well established and justifies it as a marker of cardiovascular risk rather than BP. The prognostic use of novel biomarkers in hypertension has not been previously explored. Hypertension is associated with changes in plasma levels of vasoactive peptides; their relationship with LVH and cardiac function is not well known. The demonstration of elevated natriuretic peptides in LVH and the inexpensive assays have spurred interest that biomarkers may have a role to play in screening programs [11-13].

N-terminal pro–B-type natriuretic peptide (NTproBNP) is an emerging marker of cardiovascular disease and is associated with LVH. The natriuretic peptides are raised in

* Corresponding author. Department of Cardiovascular Sciences, Pharmacology and Therapeutics Group, University of Leicester, Leicester Royal Infirmary, Leicester LE2 7LX, United Kingdom. Tel.: +44 0116 2523125; fax: +44 0116 2423108.
E-mail address: sanjay1bhandari@hotmail.com (S.S. Bhandari).

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hypertensive patients compared with normotensive patients, reflecting their potential role in LV remodeling in hypertension [14-16]. The diagnostic performance of NTproBNP for the detection of LVH in a study by Mouly-Betin et al [17] reported a receiver operating characteristic (ROC) curve with an area under the curve (AUC) of 81.6%. The diagnostic accuracy of NTproBNP was significantly higher for female than male subjects, with a sensitivity, specificity, and positive predictive value of 88%, 76%, and 95%, respectively [17]. In contrast, a study by Nakamura et al [18] showed that the ability for BNP to detect patients with LVH was weak, with an ROC curve yielding an AUC of 58.8%, a sensitivity of 50%, and a specificity of 69%. Hence, there is conflicting literature regarding the feasibility of the natriuretic peptides as diagnostic markers of LVH. The application of the natriuretic peptides is extensive, making the interpretation of elevated levels in the context of essential hypertension problematic because of potential confounders such as age, sex, renal function, diuretics, and β-blockade therapy [19].

Adrenomedullin (ADM) is a 52–amino acid peptide. It has been detected in various tissues, adrenal medulla, brain, lungs, heart, kidneys, and gastrointestinal organs [20,21]. Adrenomedullin messenger RNA is expressed in endothelial cells [22]. Adrenomedullin is synthesized from a precursor preproADM consisting of a 185–amino acid chain [23]. Concerns exist regarding the accuracy and reliability of ADM quantification largely in part because of its short half-life of 22 minutes and partial binding to complement factor H [24,25]. In comparison to ADM, the midregional portion of proADM (MRproADM; 45-92 amino acids of preproADM) is more stable. The stoichiometric generation of MRproADM allows it to be used as a surrogate marker for the ADM system.

Adrenomedullin has a number of cardiovascular actions similar to the natriuretic peptides, promoting vasorelaxation, natriuresis, and diuresis and increasing cardiac output [26-28]. Adrenomedullin may have paracrine/autocrine actions in cardiorenal homeostasis. Adrenomedullin is raised in patients with hypertension [29]. The diagnostic and prognostic utility of MRproADM has been documented in post–acute myocardial infarction (MI) and heart failure [30,31].

Because of the previously reported variable performance of the natriuretic peptides (NTproBNP and BNP) in the detection of LVH, the objective of this current study was to investigate the diagnostic performance of MRproADM for the detection of LVH in a hypertensive patient population with the biomarker NTproBNP.

2. Materials and methods

2.1. Study population

Hypertensive patients were derived from a screening study performed in the local community. From patient records, information regarding history of ischemic heart disease (MI or angina), hypertension, diabetes mellitus, smoking, and cardiovascular medication was sought. This study complied with the Declaration of Helsinki and was approved by the local ethics committee. All patients gave written informed consent for physical examination, echocardiography, and peripheral blood sampling. Patients with secondary causes of hypertension, history of heart failure, and echocardiographic abnormalities (valvular disease) were excluded from the present study. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured 3 times at 5-minute intervals. Patients with antihypertensive therapy were not required to withdraw their medication when they entered into the study. The estimated glomerular filtration rate (eGFR) of these subjects was derived using the Modification of Diet and Renal Disease formula [32].

2.2. ECG criteria for LVH

Left ventricular hypertrophy was defined as a Sokolow-Lyon voltage criteria of (SV1 + RV5 or RV6) greater than 3.5 mV [33].

2.3. Blood sampling

Venesection was performed in recumbent volunteers. Samples for measuring the serum concentrations of the propeptides were collected in prechilled tubes containing EDTA and aprotinin. Plasma was stored at −70°C until assay, and all analyses were done in a single batch. Samples for measuring plasma creatinine were collected in tubes containing lithium and heparin.

2.4. Echocardiography

Transthoracic echocardiography was performed in patients using a Sonos 5500 instrument (Philips Medical Systems, Reigate, Surrey, United Kingdom). A 16-segment left ventricular wall motion index based on the American Society of Echocardiography model was derived by scoring each LV segment (1 = normal, 2 = hypokinesis, 3 = akinesis) and dividing the total by the number of segments scored. Left ventricular ejection fraction was calculated using the biplane method of discs formula [34]. Left ventricular mass was calculated using the Devereux et al [35] formula and indexed for body surface area to obtain LV mass index (LVMI). Left ventricular hypertrophy was diagnosed when the LVMI was greater than 134 or 110 g/m^2 in male or female subjects, respectively [36]. One experienced physician performed the examination and reading of images.

2.5. NTproBNP assay

Our NTproBNP assay was based on a noncompetitive assay [37]. Sheep antibodies were raised to the N-terminal of human NTproBNP, and monoclonal mouse antibodies were raised to the C-terminal. The N-terminal immunoglobulin G was affinity purified and biotinylated. Samples or NTproBNP standards were incubated in C-terminal immunoglobulin G–

coated wells with the biotinylated antibody for 24 hours at 4°C. Detection was with methyl-acridinium ester–labeled streptavidin. The lower limit of detection was 0.3 pmol/L. There was no cross-reactivity with atrial natriuretic peptide, BNP, or C-type natriuretic peptide.

2.6. MRproADM assay

The sandwich immunoluminometric assay used to determine MRproADM levels has been reported previously [38]. Tubes were coated with sheep polyclonal antisera directed against amino acid sequence 83 to 94 of preproADM as a solid-phase antibody. Sheep antibody raised against the amino acid sequence 68 to 86 of preproADM was used as a tracer labeled with methyl-acridinium ester. Dilution of peptide representing 45 to 92 of preproADM in normal horse serum was used as calibrators. The immunoassay was conducted by incubating 10 μL of sample/standard and 200 μL of tracer in the coated tubes for 2 hours at room temperature. Test tubes were washed 4 times with 1 mL of LUMI wash solution (BRAHMS, Berlin, Germany), and bound chemiluminescence was measured on an LB952T luminometer (Berthold, Germany).

2.7. Statistical analysis

Statistical analysis was performed using Statistics Package for Social Sciences version 12.0 (SPSS, Chicago, IL). Variables that did not follow a Gaussian distribution were log transformed before statistical analysis to satisfy modeling assumptions. Concentrations of MRproADM, NTproBNP, and plasma creatinine had a non-Gaussian distribution and were log transformed. For continuous variables in 2 independent groups, the Mann-Whitney *U* test was used. Spearman correlation coefficients were used to investigate the influence of patient characteristics on NTproBNP and MRproADM levels in univariate analyses. Scatter diagrams were constructed to illustrate the general trend between the 2 variables. Box plots were also constructed consisting of median boxes, which represent the interquartile ranges, and whiskers, which represent the 2.5th and 97.5th percentiles. To analyze the interaction of multiple independent variables on LVMI, the univariate linear regression model was used. To compare the predictive value of NTproBNP and MRproADM, ROC curves were generated and the area under the curves was calculated. A P value < .05 was deemed to be statistically significant.

3. Results

Baseline characteristics of the 253 patients are presented in Table 1. Eight patients had no previous antihypertensive therapy. One hundred thirty-five patients were on monotherapy, 86 patients were taking 2 drugs, 22 patients were taking 3 drugs, and 2 patients were on 4 antihypertensive medications. The antihypertensive therapies included angiotensin-converting enzyme (ACE) inhibitors (n = 84), calcium channel blockers (n = 89), β-blocker (n = 87), and thiazide diuretic (n = 122). Forty patients had ECG changes consistent with LVH as defined by the Sokolow-Lyon criteria.

Table 1
Baseline characteristics of study sample data expressed as mean (±SD)

Variables	Value
Clinical characteristics	
No. of patients	253
Age (y)	66.1 (8.0)
Sex distribution (men/women)	112/141
Diabetes mellitus (%)	10.3
MH of MI or angina (%)	12.3
Antihypertensive treatments	
ACE inhibitors (%)	33.2
β-Blocker (%)	34.4
Calcium channel antagonist (%)	35.2
Thiazide diuretic (%)	48.2
SBP (mm Hg)	142.4 (18.9)
DBP (mm Hg)	80.1 (12.5)
Heart rate (beat/min)	71.9 (14.6)
Sokolow-Lyon voltage ≥3.5 mV (%)	15.8
Biochemical measurements	
Creatinine (μmol/L)	93 (32.6)
eGFR (mL/[min 1.73 m^2])	68 (15.5)
MRproADM (nmol/L)	0.61(0.20)
NTproBNP (pmol/L)	140.8 (188.1)
Echocardiographic characteristics	
Interventricular septum (cm)	1.0 (0.24)
LV posterior wall (cm)	0.97 (0.20)
LV mass (g)	176.6 (61.4)
LVMI (g/m^2)	94.5 (30.0)

MH indicates medical history.

The NTproBNP concentrations were significantly higher in female than in male hypertensive patients (mean [SD]: 155.2 [185.9] vs 122.7 [190.1] pmol/L, $P = .007$] as has been reported in healthy populations [39]. The MRproADM levels were also significantly elevated in female compared with male hypertensive patients (0.65 [0.2] vs 0.57 [0.2] nmol/L, $P < .001$]. Sex-specific definitions of LVH were used because of the significant differences in LVMI between women and men (88.5 [29.6] vs 101.96 [28.9] g/m^2, $P < .001$, respectively). Plasma NTproBNP was elevated in hypertensive patients with LVH than those without LVH (249.30 [279.71] vs 119.25 [156.10] pmol/L, $P = .001$, Fig. 1). Plasma levels of MRproADM were significantly higher in hypertensive patients with LVH than those hypertensive patients without LVH defined by echocardiographic examination (0.73 [0.25] vs 0.59 [0.18] nmol/L, $P < .001$, Fig. 2).

3.1. Univariate analysis

In univariate analyses, LVMI was correlated with male sex ($r_s = 0.258$, $P < .001$, Table 2). A positive relationship was observed between SBP and LVMI ($r_s = 0.190$, $P = .003$). Both biomarkers NTproBNP and MRproADM were significantly correlated with LVMI ($r_s = 0.151$, $P = .016$ and

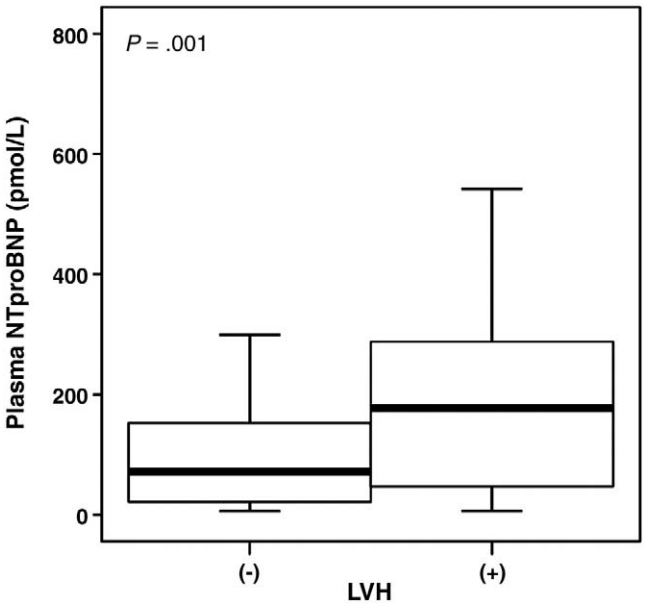

Fig. 1. Box plot demonstrating plasma NTproBNP levels in hypertensive patients with (+) and without (−) LVH.

r_s = 0.141, P = .025, respectively). No significant correlations were observed between LVMI and age, diabetes mellitus, ACE inhibitor therapy, DBP, or eGFR.

3.2. Multivariate analyses

The significant variables in univariate analyses were used as factors in the linear regression model. Multivariate analyses revealed that male sex (P < .001), history of MI or angina (P = .026), SBP (P = .048), β-blocker therapy (P = .033), LVH on ECG (P = .029), and $\log_{10}$ MRproADM (P = .003) were independent predictors of LVMI.

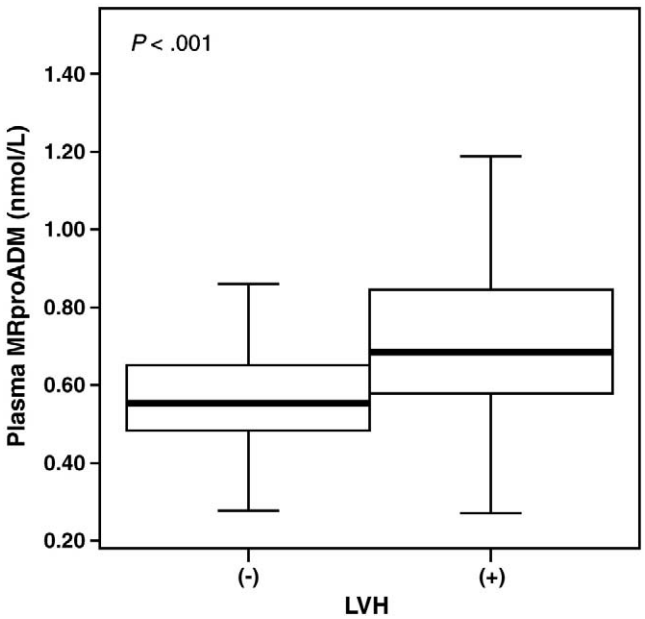

Fig. 2. Box plot demonstrating plasma MRproADM levels in hypertensive patients with (+) and without (−) LVH.

Table 2
Spearman ρ correlations

Variable	LVMI
Age	r_s = 0.067 P = .286
Male sex	r_s = 0.258 P < .001
SBP	r_s = 0.190 P = .003
DBP	r_s = 0.075 P = .236
Heart rate	r_s = −0.135 P = .032
MH of MI or angina	r_s = 0.147 P = .020
Diabetes mellitus	r_s = 0.061 P = .333
LVH on ECG	r_s = 0.199 P = .001
ACE inhibitors	r_s = 0.083 P = .189
Thiazide diuretic	r_s = −0.154 P = .014
β-Blocker	r_s = 0.175 P = .005
Calcium channel blockers	r_s = 0.151 P = .017
eGFR MDRD	r_s = 0.048 P = .449
Log NTproBNP	r_s = 0.151 P = .016
Log MRproADM	r_s = 0.141 P = .025

MDRD indicates Modification of Diet and Renal Disease.

3.3. Performance characteristics of NTproBNP and MRproADM for detecting LVH

Both ROC curves derived from NTproBNP and MRproADM for the detection of LVH were significantly different from the diagonal: MRproADM (AUC, 0.71; confidence interval [CI], 0.62-0.81; P <.001) and NTproBNP (AUC, 0.66; CI, 0.56-0.76; P = .001) (Fig. 3). On the basis of ROC analysis, the optimal cutoff value of MRproADM as an indicator of LVH was 0.50 nmol/L, with a sensitivity of 90.5%, specificity of 36.5%, positive predictive value of 22.1%, and negative predictive value (NPV) of 95.1%. A plasma level of 20.38 pmol/L for NTproBNP corresponded to a sensitivity of 88.1% for detecting LVH, with a specificity of 24.6% and an NPV of 91.2%.

4. Discussion

Left ventricular hypertrophy is a risk factor for cardiovascular disease. Risk stratification using tools such as ECG and echocardiography is limited. Echocardiographic

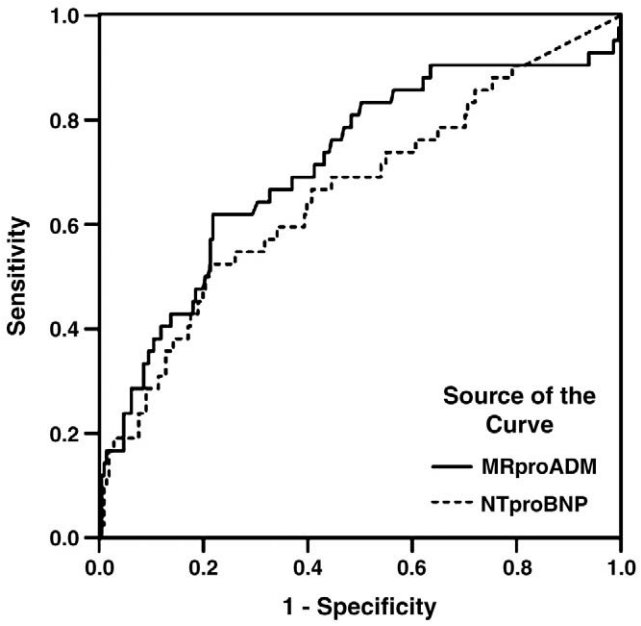

Fig. 3. Receiver operating characteristic curves demonstrating the ability of NTproBNP and MRproADM to predict LVH.

determination of LVH is deemed as a prognostic utility that integrates the effects of multiple risk factors for cardiovascular disease [40]. Hence, increased LVMI is a cardinal sign predictive of cardiovascular morbidity and mortality. Although echocardiography has been shown to be a sensitive tool for detecting LVH, there are concerns regarding its cost-effectiveness [10]. Interest has focused on the diagnostic and prognostic utility of novel biomarkers in hypertension. In this study, we compared the diagnostic performance of MRproADM for the detection of LVH with reference marker NTproBNP.

The concept of measuring the biologically inactive precursor peptide rather than its active peptide is well documented. The quantification of ADM is technically difficult in part because of its short half-life and immediate receptor binding in proximity to its generation for paracrine and autocrine actions [24,25]. The stoichiometric generation of MRproADM with ADM in a 1:1 ratio enables MRproADM to be used as a surrogate marker for the ADM system [38].

In this study, MRproADM levels were significantly elevated in the cohort with LVH on echocardiography than those without, which corroborates similar findings by Sumimoto et al [41]. The previously reported levels (mean [SD], 7.87 [2.70] fmol/L) are much lower than the MRproADM levels established in this study, which may relate to the improved design of our assay that measures the prohormone rather than the rapidly cleared bioactive peptide [41].

In the multivariate analyses, male sex ($P < .001$) and log MRproADM ($P = .003$) retained independent statistical significance for the detection of LVH. The performance of MRproADM as a diagnostic biomarker for LVH was assessed using ROC curves. The curve for MRproADM yielded an AUC, 0.71; CI, 0.62-0.81; $P < .001$, superior to that conferred by the natriuretic peptide NTproBNP. A cutoff value of 0.50 nmol/L was chosen because it maximized sensitivity without much loss of specificity. The sensitivity, specificity, and NPV for MRproADM in detecting LVH were 90.5%, 36.5%, and 95.1%, respectively. The very high NPV of the assay makes LVH very unlikely with concentrations less than the cutoff, suggesting that the most appropriate use of the assay would be as a rule-out test when risk stratifying patients into high- or low-risk groups. This may then enable the patients who tested positive for the biomarker to be further investigated using echocardiography, hence enabling better use of resources.

The NTproBNP levels were raised in patients with LVH compared with those without, possibly reflecting its potential role in LV remodeling in hypertension [15,16]. However, in multivariate analyses, NTproBNP no longer retained its significance as an independent predictor of LVMI ($P = .704$). This confirms findings by Belluardo et al [42] who reported that in mild hypertension the natriuretic peptides initially demonstrated a hyporesponsiveness with later activation with increasing severity of hypertension independent of LVH. Disappointingly, in this present study, the ROC plot for NTproBNP for the detection of LVH estimated an AUC of 0.66. This contrasts with a study by Mouly-Bertin et al [17] who documented that the performance of NTproBNP for the detection of LVH in hypertensive patients was far superior, with an AUC of 81.6%.

A study by Jougasaki et al [43] reported that there was a significant rise in the plasma concentrations of ADM between the aorta and the anterior interventricular vein and between the aorta and coronary sinus in patients with congestive heart failure. A further study by the same group reported increased ADM expression in ventricular myocytes of failing hearts when compared with healthy hearts [44]. Hence, the myocardium (in particular, hypertrophic myocardium in LVH) may represent the source of elevated MRproADM levels in hypertension. However, the extensive distribution of ADM messenger RNA infers the possibility of a complex interaction between the myocardium and other organs in hypertension [20,21]. The vasodilator actions of ADM mediated via its downstream generation of cyclic adenosine monophosphate may be protective in hypertension, counteracting the deleterious arteriolar vasoconstriction [29].

The limitations of this study include not establishing causality between MRproADM and LVH. In this study, men and women were combined; hence, the sex-specific differences in NTproBNP and MRproADM response to LVH may have influenced the performance characteristics of these peptides and their respective cutoff limits. Further studies are necessary to validate our findings. Regression of LVH during antihypertensive therapy is associated with an improved survival benefit [8]. Hence, further studies on

measurement of MRproADM before and during treatment to identify if such a regression in LVMI is accompanied by reduced activation of the ADM system should be performed.

This study demonstrates that plasma MRproADM is raised in hypertensive patients with LVH compared with those without. Plasma MRproADM is a strong independent predictor of LVMI and hence LVH. In contrast, NTproBNP was not independently predictive of LVMI in the hypertensive patients. The performance utility of MRproADM in detecting LVH is superior to that conferred by the natriuretic peptides. Its high sensitivity and NPV enable MRproADM to be used as a rule-out test for LVH in risk stratifying hypertensive patients.

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