Modest Elevation in BNP in Asymptomatic Hypertensive Patients Reflects Sub-Clinical Cardiac Remodeling, Inflammation and Extracellular Matrix Changes

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Abstract

In asymptomatic subjects B-type natriuretic peptide (BNP) is associated with adverse cardiovascular outcomes even at levels well below contemporary thresholds used for the diagnosis of heart failure. The mechanisms behind these observations are unclear. We examined the hypothesis that in an asymptomatic hypertensive population BNP would be associated with sub-clinical evidence of cardiac remodeling, inflammation and extracellular matrix (ECM) alterations. We performed transthoracic echocardiography and sampled coronary sinus (CS) and peripheral serum from patients with low (n = 14) and high BNP (n = 27). Peripheral BNP was closely associated with CS levels (r = 0.92, p<0.001). CS BNP correlated significantly with CS levels of markers of collagen type I and III turnover including: PINP (r = 0.44, p = 0.008), CITP (r = 0.35, p = 0.03) and PIIINP (r = 0.35, p = 0.001), and with CS levels of inflammatory cytokines including: TNF-α (r = 0.49, p = 0.002), IL-6 (r = 0.35, p = 0.04), and IL-8 (r = 0.54, p<0.001). The high BNP group had greater CS expression of fibro-inflammatory biomarkers including: CITP (3.8±0.7 versus 5.1±1.9, p = 0.007), TNF-α (3.2±0.5 versus 3.7±1.1, p = 003), IL-6 (1.9±1.3 versus 3.4±2.7, p = 0.02) and hsCRP (1.2±1.1 versus 2.4±1.1, p = 0.04), and greater left ventricular mass index (97±20 versus 118±26 g/m^2, p = 0.03) and left atrial volume index (18±2 versus 21±4, p = 0.008). Our data provide insight into the mechanisms behind the observed negative prognostic impact of modest elevations in BNP and suggest that in an asymptomatic hypertensive cohort a peripheral BNP measurement may be a useful marker of an early, sub-clinical pathological process characterized by cardiac remodeling, inflammation and ECM alterations.

Introduction

B-type natriuretic peptide (BNP) is a cardioprotective hormone which is secreted by the left ventricle (LV) in response to wall stress and is involved in regulating volume homeostasis and cardiac remodeling [1,2]. BNP is utilized in the clinical setting as a diagnostic tool to identify heart failure (HF) and LV dysfunction in addition to providing prognostic information in patients with HF, hypertension, and coronary artery disease [3,4,5,6]. There is an emerging interest in its use in asymptomatic patients with cardiovascular risk factors [7,8]. Wang et. al. demonstrated that BNP levels above the 80th percentile (20 pg/ml for men and 23.3 pg/ml for women) in the Framingham Offspring Study was associated with a significant increased risk of major cardiovascular events including HF and death [7]. However, the underlying

mechanism of the negative prognostic impact of a modest elevation in BNP in asymptomatic patients is unclear.

Arterial hypertension is an important preventable cause of premature death that is estimated to account for 4.5% of the total global disease burden [9]. Hypertensive heart disease (HHD) describes a process of remodeling which occurs in the myocardium when exposed to sustained elevation in arterial blood pressure. The two most notable features are myocyte hypertrophy and excessive extracellular matrix (ECM) collagen deposition, also termed "reactive fibrosis". In physiological terms, increased fibrosis results in increased stiffness of the LV, diastolic dysfunction and if sufficiently severe, diastolic HF [10,11]. Inflammation appears to be an important upstream stimulus for the development of reactive fibrosis [12,13].

Several studies have shown a definite relationship between BNP and myocardial stiffness particularly in HHD [14,15]. Further-

more, there is evidence from experimental and animal studies which show that natriuretic peptides are involved in regulating myocardial fibrosis [16,17].

We hypothesized that a modest elevation in BNP levels in an asymptomatic hypertensive population would be associated with subclinical myocardial injury as determined by adverse cardiac remodeling and heightened expression of both inflammatory cytokines and serum markers of collagen metabolism. Coronary sinus serum was sampled as this localizes events to the myocardium and may be more sensitive to detect subtle changes in biomarker expression.

Methods

Participants and Protocol

We studied 41 Caucasian subjects with a history of hypertension who volunteered for the study. Patients were recruited prior to routine elective coronary catheterisation for suspected ischaemic chest pain. Patients had normal self-reported exercise capacity. At the time of recruitment patients were examined for signs of congestive cardiac failure/fluid overload which included assessment for: elevation in jugular venous pressure, basal pulmonary crepitations, a gallop rhythm (S3) or peripheral pitting oedema. Exclusion criteria included any findings suggestive of fluid overload, renal insufficiency (serum creatinine >1.5 mg/dl or estimated creatinine clearance (eCrCl) <45 ml/min as calculated using the Cockcroft-Gault formula), evidence of infection, conditions known to alter collagen turnover (including chronic liver disease, connective tissue disorders, metabolic bone diseases, and malignancy), conditions known to alter BNP levels (including HF, cardiomyopathy and recent ischemic events), and recent surgery or physical trauma (<6 months). were categorised into high and low BNP groups according to values above and below the 80th percentile described in the Framingham Offspring Study (BNP 20 pg/ml for men and 23.3 pg/ml for women) [7]. All patients had appropriate clinical and laboratory evaluation to identify suitability for the study and exclusion criteria.

Office blood pressure monitoring was performed in the sitting position using a standard sphygmomanometer. Subjects underwent transthoracic echocardiography (TTE) and right heart catheterization for coronary sinus blood sampling within one week of each other. All subjects gave written informed consent to participate in the study. The Ethics Committee at St Vincent's University Hospital approved the study protocol which conformed to the principles of the Helsinki Declaration.

Coronary Sinus Blood Sampling

Following puncture of the right femoral vein, a 6F sheath was introduced using a Seldinger technique. A 5F catheter was positioned under fluoroscopic guidance to the os of the CS as confirmed by contrast injection. Peripheral blood samples were taken from the side arm of the femoral sheath and further samples from the CS os. Samples were collected into vacutainer tubes using ethylenediaminetetraacetic acid tubes for BNP and gel separation and clot activator tubes for biomarker determination. Samples were immediately stored at 4°C. Within 1 hour the serum tubes were centrifuged at 1500 rpm for 5 minutes at 4°C to separate off the serum. Serum was then aliquoted out into small volume microcentrifuge tubes and stored in a −80°C freezer until use.

Echocardiography

TTE was performed using a Philips IE33 by a single experienced echocardiographer. Measurements were performed by a single reader blinded to BNP results. The following measurements were performed as per ASE guidelines [18]: ejection fraction (EF) using the biplane Simpson's method from apical 2- and 4- chamber windows, left atrial volume index (LAVI) using a biplane area-length formula, end-diastolic inter-ventricular septal (IVS) and posterior wall (PW) thickness and LV internal dimension in diastole (LVIDd). LV mass index (LVMI) was calculated based on modeling the LV as a prolate ellipse [18]. Diastolic parameters including peak early (E) and late (A) diastolic mitral inflow velocity and its ratio (E/A), deceleration time (DT) and average of the medial and lateral mitral annular diastolic velocities (e') were also measured according to ASE guidelines [19]. All echocardiography data were based on the mean of 3 measurements on consecutive cardiac cycles.

Serum Biomarker Assessment

BNP was quantified using a Triage meter BNP assay (assay sensitivity <5 ng/ml) (Biosite Inc.).Serum procollagen type I amino-terminal (assay sensitivity 13.0 ng/ml) (PINP), carboxy-terminal (assay sensitivity 2.0 ng/ml) (PICP), carboxy telopeptide of type I collagen (assay sensitivity 0.5 ng/ml) (CITP) and procollagen type III amino-terminal (assay sensitivity 1.9 ng/ml) (PIIINP) were quantified using radioimmunoassay (Orion Diagnostica). A custom made ultrasensitive Meso Scale Discovery (MSD) 4-Plex ELISA enabled simultaneous detection of interleukin (IL)-6, IL-8 and tumour necrosis factor alpha (TNFα) using an MSD Sector Imager 2400 reader. For each of these inflammatory markers, the assay sensitivity of was ≤0.7 pg/ml. Duplicate measurements were performed in all the above tests. High sensitivity CRP (hsCRP) serum concentrations were also determined using ELISA (MSD). All assays were carried out according to manufacturer's guidelines or as described previously [20].

Statistical Analysis

For continuous variables, summary statistics are presented as the mean ± standard deviation (SD) or median and 25–75th percentiles. Categorical variables are presented as frequencies and percentages (in parenthesis). Comparisons between the low and high BNP groups were made using independent t-test, Welsh test, or chi square test where appropriate. The relationships between BNP, markers of collagen turnover and markers of inflammation were assessed using Pearson's correlation coefficient for variables that were approximately normally distributed. In addition, Pearson's correlation coefficient was used with log-transformation of variables with non-normal distribution. A p value of <0.05 was considered statistically significant.

Results

Participant Characteristics

Baseline characteristics of the participants are presented in table 1. The mean age of participants was 65±9 years and 44% were male. While 71% were taking either an angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) and 54% were taking a β-blocker, blood pressure (BP) control remained sub-optimal (mean±SD systolic BP/ diastolic BP, 150±19/77±10 mmHg). No patient was taking both an ACEi and an ARB. 14 and 27 patients were categorised into low and high BNP groups respectively. The only significant difference between the two groups was in the usage of β-blockers (29% versus 66%, p = 0.03, low versus high BNP groups respectively). Of note, there was no significant difference between the two groups in terms of the number of patients taking both an ACEi/ARB and a β-blocker (29% versus 52%, p = 0.27, low versus high BNP groups respectively), or in terms of the number of

Table 1. Baseline characteristics of the study participants.

	Total Population (n = 41)	Low BNP (n = 14)	High BNP (n = 27)	P
Age, yr	65±9	63.2±9.4	66.6±9.4	0.28
Gender, male	18 (44)	7 (50)	11 (41)	0.81
BMI, kg/m^2	28±6	27.5±7.2	28.2±5.7	0.74
SBP, mmHg	150±19	146±18	151±19	0.38
DBP, mmHg	77±10	75±11	78±11	0.4
PP, mmHg	72±18	71±18	73±19	0.69
Diabetes	10 (24)	3 (21)	7 (26)	0.99
Biochemical parameters				
Sodium, mmol/l	138±4	138±4	139±3	0.35
Potassium, mmol/l	4.2±0.4	4.1±0.3	4.3±0.4	0.14
Urea, mmol/l	6.2±1.7	6.1±1.1	6.3±2.0	0.62
Creatinine, mg/dl	1.03±0.27	1.04±0.24	1.02±0.28	0.78
Hemoglobin, g/dl	13.1±1.9	12.6±2.1	13.4±1.7	0.21
eCrCl, ml/min	78±29	81±36	77±26	0.7
Calcium, mg/dl	9.1±0.5	9.0±0.6	9.2±0.5	0.42
Alkaline Phosphatase, U/L	77±30	80±3.2	76±28	0.72
Medications				
ACEi/ARB	29 (71)	9 (64)	20 (74)	0.72
β-blocker	22 (54)	4 (29)	18 (66)	0.03
Statin	25 (61)	9 (64)	16 (59)	0.99

Data are presented as mean ± SD or as number (percentage).
BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; eCrCl = estimated creatinine clearance; ACEi = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor antagonist.
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patients taking two or more medications (43% versus 67%, p = 0.15, low versus high BNP groups respectively).

Echocardiographic Characteristics

Table 2 shows the echocardiographic parameters of the study participants. Both LVMI (97±20 versus 118±26 g/m^2, p = 0.03) and LAVI (18±2 versus 21±4, p = 0.008) were significantly higher in the high BNP group when compared to the low BNP group. There were no other significant differences between the two groups.

Serum Biomarker Expression between Groups

In samples collected from the periphery, only hsCRP was significantly different between the two groups (1.1±1.4 versus 2.7±2.7, p = 0.4, low versus high BNP groups respectively) (table 3). CS samples revealed greater expression of a number of fibro-inflammatory biomarkers in the high BNP group including: CITP (3.8±0.7 versus 5.1±1.9, p = 0.007), TNF-α (3.2±0.5 versus 3.7±1.1, p = 003), IL-6 (1.9±1.3 versus 3.4±2.7, p = 0.02) and hsCRP (1.2±1.1 versus 2.4±1.1, p = 0.04).

BNP, fibro-inflammatory biomarkers and cardiac remodeling. Peripheral BNP was very closely associated with CS levels (r = 0.92, p<0.001).

CS BNP correlated significantly with CS levels of markers of collagen type I and III turnover including: PINP (r = 0.44, p = 0.008), CITP (r = 0.35, p = 0.03) and PIIINP (r = 0.35, p = 0.001). Furthermore, peripheral BNP also correlated significantly with CS PINP (r = 0.47, p = 0.005), CITP (r = 0.55, p<0.001) and PIIINP (r = 0.34, p = 0.03) (Figure 1).

Both CS and peripheral BNP correlated significantly with CS levels of inflammatory cytokines including: TNF-α (r = 0.49, p = 0.002 and r = 0.43, p = 0.006 for CS and peripheral BNP respectively), IL-6 (r = 0.35, p = 0.04 and r = 0.49, p = 0003), and IL-8 (r = 0.54, p<0.001 and r = 48, p = 0.003) (Figure 2).

In this population CS BNP was significantly associated with LAVI (r = 0.59, p<0.001) and LVMI (r = 0.37, p = 0.003)(Figure 3).

Discussion

The present cross-sectional study demonstrated that in an asymptomatic hypertensive population a modest elevation in BNP was associated with increased expression of fibro-inflammatory biomarkers in serum sampled from the coronary sinus. A modest elevation in BNP is associated with increased LVMI and LAVI. Furthermore, significant correlations exist between markers of inflammation, collagen turnover and BNP.

Hypertension, Reactive Fibrosis and BNP

It is estimated that between 25 and 30% of the adult population in the USA have hypertension [21]. It plays a major etiological role in the development of HF and ischaemic heart disease, with even modest increases in blood pressure contributing to the long term risk [9,22]. The relationship between reactive fibrosis and hypertension is well described. Studies examining the collagen volume fraction of post-mortem hearts and endomyocardial biopsies demonstrate excessive accumulation of ECM collagen in patients with a history of hypertension and left ventricular hypertrophy (LVH) [23,24,25]. Indeed, evidence of excessive interstitial fibrosis has been demonstrated even in the early phases

Table 2. Echocardiographic measurements.

	Total Population (n = 41)	Low BNP n = 14	High BNP n = 27	P
Ejection Fraction, %	65±9	67±11	64±9	0.41
IVS, mm	11.5±2.6	11.1±1.7	11.7±2.9	0.62
PW, mm	9.3±2	9.0±1.1	9.5±2.3	0.52
LVIDd, mm	52±5	51±6	53±4	0.29
LVMI, g/m^2	114±28	97±20	118±26	0.03
Peak E, cm/s	75±20	70±17	78±22	0.23
Peak A, cm/s	81±25	76±23	83±26	0.42
EA	0.96±0.3	0.97±0.3	0.95±0.3	0.91
DT, ms	238±55	238±45	238±59	0.99
e', cm/s	11±3	11±4	10±2	0.33
E/e'	7.2±2.3	6.7±2.4	7.4±2.2	0.34
LAVI, ml/m^2	20±4	18±2	21±4	0.008

Data are presented as mean ± SD.
LVMI = left ventricular mass index; IVS = intraventricular septum; PW = posterior wall; LVIDd = left ventricular internal dimension in diastole; DT = E-wave deceleration time; IVRT = isovolumic relaxation time; LAVI = LA volume index.
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of hypertension in patients with only mild LVH [24,26]. These observations have been directly linked to abnormalities in diastolic and systolic function [25,27]. Furthermore, regression of the degree of fibrosis results in improvement in LV function [28,29]. Therefore, the diagnosis of early changes in myocardial collagen content is of clinical relevance. However, taking endomyocardial biopsies in asymptomatic patients is unjustifiable and imaging techniques may be inadequate due to lack of sensitivity, technical and interpretive difficulties, dependence on loading conditions, and limitations in the presence of conditions such as atrial fibrillation [30,31]. To address this issue, circulating terminal peptides which are cleaved and released into the serum during collagen type I synthesis (PICP, PINP)and degradation (CITP) and collagen type III synthesis and degradation (PIIINP) have been examined and validated as markers of myocardial collagen content and function [20,27,32,33]. However, there are a number of difficulties with the practical utility of these biomarkers. First,

they are not specific to the myocardium. Second, their validation excluded subjects with rheumatological, hepatic or malignant disease and finally they are not routinely measured in clinical practice. In contrast, BNP is cardiac specific, extensively validated in unrestricted populations and widely available. The correlations observed in our study suggest that peripheral BNP may be a useful surrogate of markers of collagen turnover as detected in the coronary sinus. This is consistent with data showing BNP's association with cardiovascular stiffening [14,15].

In the high BNP group we observed a significant increase in CITP expression when compared to the low BNP group suggesting a predominance of collagen type I catabolism. These data may appear counterintuitive in the context of increased LVMI observed in this group. However, previous studies have demonstrated an increase in CITP in HHD and have suggested that this may be an early indication of degradation in the ECM scaffolding with resultant progression to chamber dilatation

Table 3. Comparison of biomarker levels from peripheral and coronary sinus samples in low and high BNP groups.

	Peripheral Serum Samples (n = 41)			Coronary Sinus Serum Samples (n = 41)		
	Low BNP (n = 14) n = 14	High BNP (n = 27) n = 27	P	Low BNP (n = 14) n = 14	High BNP (n = 27) n = 27	P
BNP,pg/mL	10.4 (9.4,14.1)	58 (32,129)	<0.001	24 (9.8,42.3)	115 (59,403)	<0.001
PICP,ng/mL	272±43	267±4	0.74	331±107	297±10	0.39
PINP,mg/mL	27.5±14.3	35.3±12.9	0.266	27.5±7.8	32.4±12.8	0.179
CITP,μg/L	3.3±1.1	4.9±3.7	0.06	3.8±0.7	5.1±1.9	0.007
PIIINP,μg/mL	2.7±1.4	2.2±0.8	0.30	2.4±0.5	2.8±1.0	0.11
TNFα,pg/mL	4.0±2.0	3.7±1.1	0.63	3.2±0.5	3.7±1.1	0.03
IL-6,pg/mL	1.2±1.0	1.9±1.6	0.13	1.9±1.3	3.4±2.7	0.02
IL-8, pg/mL	6.7±2.7	6.5±3.2	0.86	5.7±1.7	6.4±3.6	0.46
hsCRP,mg/L	1.4±1.1	2.7±2.7	0.04	1.2±1.1	2.4±1.1	0.04

Data are presented mean ± standard deviation or as median and 25th and 75th percentiles in parenthesis. BNP = B-type natriuretic peptide; IL = interleukin; hsCRP high sensitivity C-reactive protein; TNFα = tumor necrosis factor-α; PICP = carboxy-terminal; PINP = amino-terminal; CITP = carboxy-terminal telopeptide of procollagen type I; PIIINP = amino-terminal propeptide of procollagen type III.
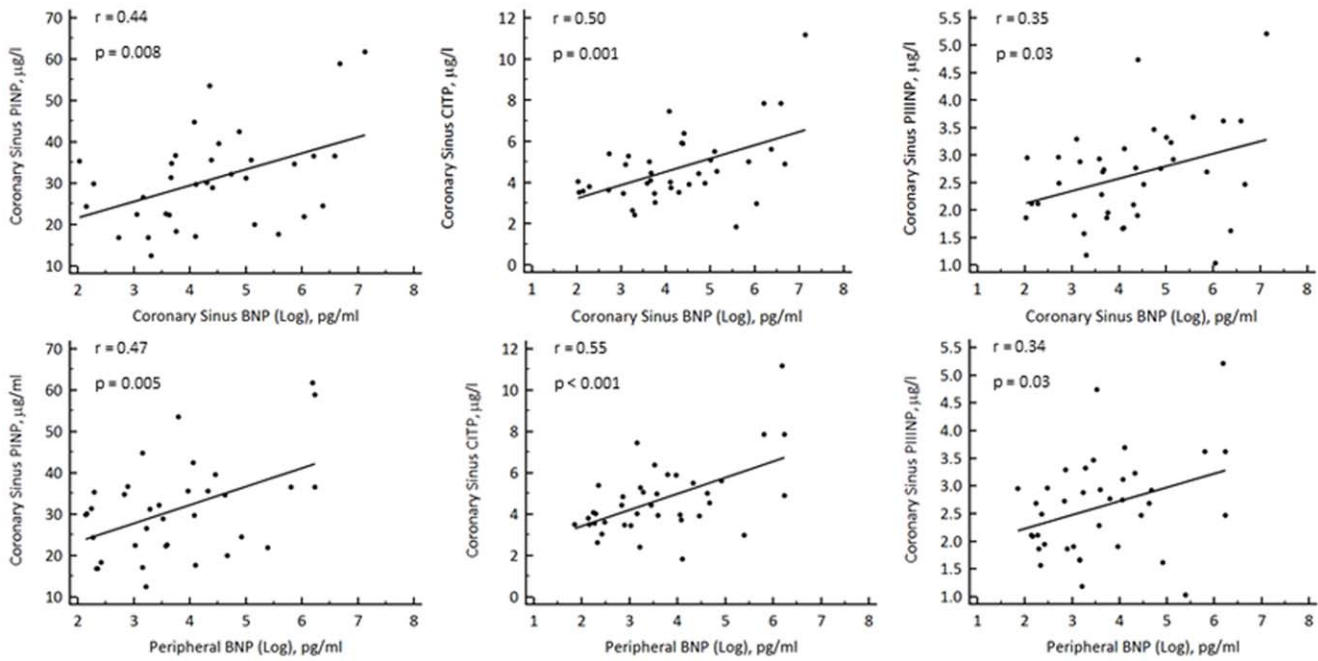doi:10.1371/journal.pone.0049259.t003

Figure 1. Scatter plots showing significant correlations between levels of BNP (log transformed) in serum from both the coronary sinus and periphery and levels of markers of collagen type I and type III turnover including PINP, CITP and PIIINP in serum from the coronary sinus.
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[27,33,34]. Despite LAVI measuring within normal limits in both groups, we noted a significant increase in the high BNP group (18 ± 2 versus 21 ± 4 ml/m^2, p = 0.008). It is possible that the observed elevation in CITP is as a result of this early LA remodeling.

It is interesting to note that the BNP cut-off identified previously and used in this study divides our population into LAVI groups similar to those used by Ristow *et. al.*, where an increased risk of HF in subjects with LAVI measurements of 20–30 ml/m^2 versus <20 ml/m^2 was demonstrated. Consistent with the negative
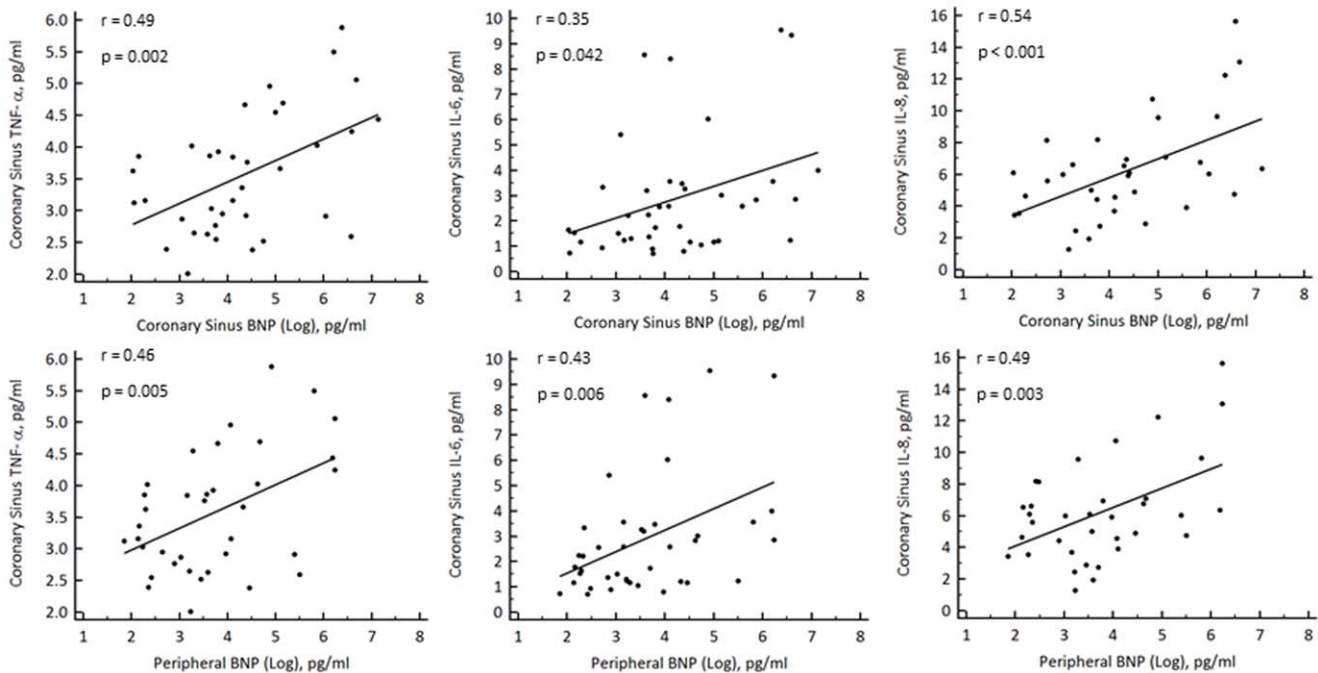

Figure 2. Scatter plots showing significant correlations between levels of BNP (log transformed) in serum from both the coronary sinus and periphery and the inflammatory cytokines TNF-α, IL-6 and IL-8 in serum from the coronary sinus.
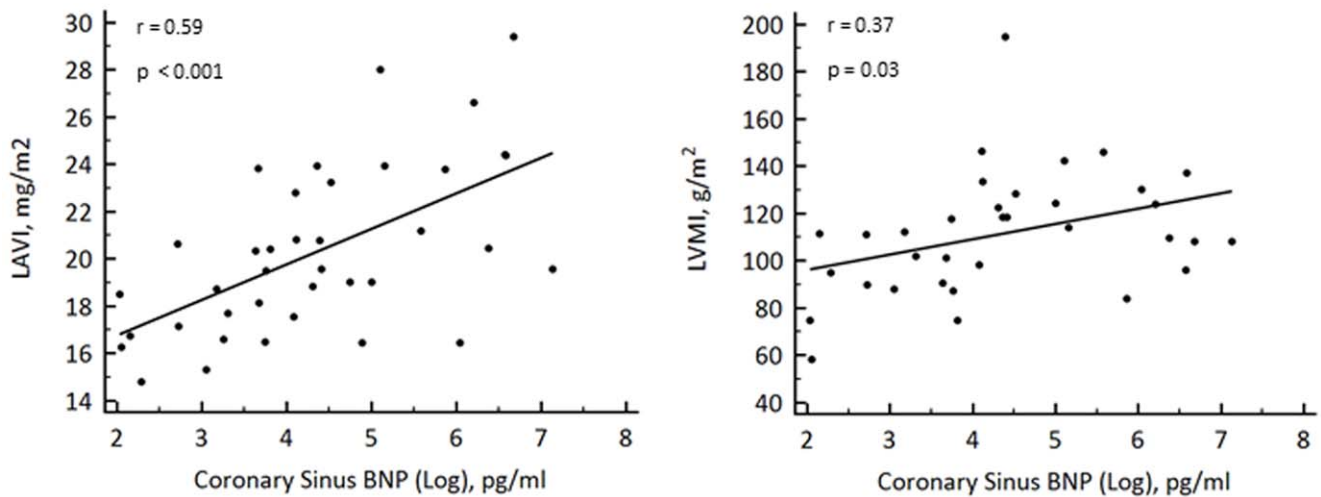doi:10.1371/journal.pone.0049259.g002

Figure 3. Scatter plots showing significant correlations between CS levels of BNP (log transformed) and structural changes in the heart including LAVI and LVMI.
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prognostic attributes of both BNP and LAVI, CITP has also been associated with adverse cardiac events [35].

Hypertension, Inflammation and BNP

Increasing evidence suggests that inflammation may play a key role in the pathophysiology of hypertension [36]. A common event in many models of hypertension and cardiac fibrosis is heightened myocardial expression of pro-inflammatory cytokines [37,38]. Melendez *et. al.* recently demonstrated a significant increase in myocardial collagen volume fraction, ventricular stiffness and concentric LVH in rats infused with IL-6 [37]. TNF-α has been directly implicated in the development of myocardial fibrosis [38,39]. IL-8 is a potent neutrophil chemokine that is involved in the early inflammatory responses prior to replacement fibrosis [40]. Finally CRP, the most investigated cytokine in hypertension, has been repeatedly implicated in both the initiation and progression of the disorder [36]. Previous work has demonstrated an association between BNP and inflammatory cytokines. Mechanistically, BNP has immunomodulatory activity on macrophages and directly regulates the production of major inflammatory cytokines including TNF- α [41]. A positive feedback mechanism may exist, as TNF-α has been shown to induce secretion of BNP from cardiomyocytes *in vitro* [42,43]. While these data are relatively new, taken together, they suggest an interdependent relationship between BNP and inflammatory cytokines in the progression of cardiac interstitial fibrosis. In our data, the correlations observed between BNP and measured cytokines support this concept and suggest that peripheral BNP may be a useful indicator of myocardial injury.

Clinical Implications and limitations

The precise clinical implications of these observations are uncertain and it is not yet clear how best to manage asymptomatic hypertensive patients with a modestly elevated BNP. The prognostic evidence of elevated cardiovascular risk associated with such patients at a minimum underline the need for close attention to risk factor management. The observation that elevated BNP in hypertensive patients is associated with abnormal nocturnal blood pressure patterns underlines the need to completely assess blood pressure control [44]. There may also be a strong argument for the use of therapies which have been shown to regulate markers of myocardial interstitial disease such as agents that modulate the renin angiotensin aldosterone system [28,29]. Furthermore, the Jupiter trial has shown a potential impact of anti-inflammatory effects of statin therapy in improving outcome in a similar patient population as the high BNP group [45]. It is possible that some of these benefits were mediated by the impact of rosuvastatin on myocardial fibrosis, a downstream result of inflammation.

In interpreting these data certain limitations need to be considered. First, we have used serum markers of collagen turnover taken from the CS as a surrogate of myocardial interstitial disease without endomyocardial biopsy evidence of fibrosis. Second, although BNP is a robust predictor of cardiovascular outcome in an unselected patient population [7,8]_EN-REF_4 our population excluded patients with diseases that alter collagen and inflammatory markers. Third, we note the increased usage of beta-blockers in the elevated BNP cohort. There is some evidence that beta-blocker therapy can modulate BNP levels, although variable effects are seen dependent upon the patient cohort and the duration of therapy [46,47]. In addition, as there is clinical and experimental evidence that some beta-blocker therapies have anti-inflammatory [48,49] and anti-fibrotic [49,50] effects we postulate that the levels of markers of inflammation and fibrosis may have been even higher in elevated BNP group had they not been taking beta-blockers. However, the true relationship between beta-blocker therapy, BNP expression, inflammation and fibrosis warrants further detailed study. Finally, blood pressure readings were taken from a single reading and by multiple operators which may have resulted in an inaccurate representation of the overall blood pressure control. For this reason no correlations were performed between blood pressure reading s and any of the biomarkers measured.

Conclusions

Risk stratification allows the physician to identify those patients who will benefit most from more aggressive surveillance and management. For asymptomatic hypertensive patients a means of risk stratification which is rapid, readily available, relatively inexpensive and capable of providing information beyond a history and physical examination is of particular importance. In this regard, BNP is a useful marker of adverse outcomes in

asymptomatic patients even at levels well below the contemporary thresholds used in the diagnosis of HF. Our data provide insight into the mechanisms behind these observations and suggest that in an asymptomatic hypertensive cohort a peripheral BNP measurement may be a useful marker of an early, sub-clinical pathological process characterized by cardiac remodeling, inflammation and extracellular matrix turnover.

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Conceived and designed the experiments: DP CW RM SD ML JB KMD. Performed the experiments: DP RM CW PC AP. Analyzed the data: DP CW PC JB ML KMD. Contributed reagents/materials/analysis tools: KMD. Wrote the paper: DP CW JB KMD.

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