Role of Serum N-Terminal Pro-Brain Natriuretic Peptide Measurement in Diagnosis of Cardiac Involvement in Patients With Anderson-Fabry Disease

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Enzyme replacement therapy has the potential to delay or reverse adverse cardiac remodeling in Anderson-Fabry disease (AFD); however, the current indications for enzyme replacement therapy rely on detecting relatively advanced features of the disease. We aimed to determine the relation between the serum N-terminal pro-brain natriuretic peptide (NT-proBNP) concentration and cardiac abnormalities in patients with AFD. We hypothesized that it might help to detect early disease. NT-proBNP was measured under at rest conditions in 117 patients with AFD (age 48 ± 15 years, 46.2% men). All patients underwent clinical evaluation with electrocardiography and echocardiography. The median NT-proBNP concentration was 24 pmol/L (range <5 to 6,059). Of the 117 patients, 67 (57%) had elevated, age-corrected, NT-proBNP levels. In the 56 patients (48%) with normal echocardiographic findings, the NT-proBNP levels were greater than the age-predicted cutoffs in 10 of 25 patients with abnormal electrocardiographic findings and 3 of 31 patients with normal electrocardiographic findings ($p < 0.05$). On multiple regression analysis, age, creatinine, left atrial volume index, E/Ea, and the presence of abnormal electrocardiographic findings were independently associated with log NT-proBNP ($R^2 = 0.67$, $p < 0.05$). In conclusion, NT-proBNP concentrations were elevated in patients with AFD and early cardiac involvement, suggesting its measurement could assist in decisions regarding the timing of enzyme replacement therapy. © 2013 Elsevier Inc. All rights reserved. **(Am J Cardiol 2013;111:111−117)**

Anderson-Fabry disease (AFD) is an X-linked lysosomal storage disorder, caused by deficiency of α-galactosidase A. This leads to accumulation of glycosphingolipid in tissues throughout the body and subsequent organ failure. In the heart, this typically manifests as left ventricular hypertrophy, which can progress to systolic and diastolic heart failure.[1] Enzyme replacement therapy (ERT) has the potential to delay or reverse adverse cardiac remodeling.[2,3] However, the current indications for ERT rely on the detection of relatively advanced features of the disease, when irreversible organ damage may have occurred.[4,5] Brain natriuretic peptide is a cardiac neurohormone secreted from the ventricles of the heart in response to increased wall stress. Brain natriuretic peptide and the N-terminal fragment of its pro-hormone (NT-proBNP) have an established role in determining the diagnosis and prognosis of heart failure.[6−8] In a male mouse knockout model for Fabry's disease, the brain natriuretic peptide mRNA levels were significantly increased compared to that of wild-type controls, despite a mild cardiac phenotype.[9] NT-proBNP has recently been associated with overall disease severity in AFD[10]; however, its role as a marker of early cardiac involvement has not been examined. The primary aim of the present study was to determine the relation between NT-proBNP levels and conventional markers of cardiac involvement in AFD.

Methods

This was an observational, cross-sectional cohort study. Data were collected prospectively. The cohort consisted of consecutively evaluated patients with AFD, seen at a dedicated cardiomyopathy clinic from April 2008 to April 2011. The diagnosis of AFD was determined by measuring the plasma and/or leukocyte α-galactosidase A enzyme activity, followed by sequencing of the α-galactosidase A gene. All patients were aged ≥18 years at evaluation. The study conformed to the principles of the Helsinki declaration.

All patients were evaluated clinically using a 12-lead electrocardiogram at rest and echocardiography. The New York Heart Association class, current medication, and blood pressure were recorded. The Mainz severity score index was used as an index of overall disease severity.[11] Serum creatinine was used to estimate the glomerular filtration rate using the 4-variable Modification of Diet in Renal Disease

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equation.[12] NT-proBNP was measured from routine venous blood samples taken at rest.

The venous blood samples were drawn into serum separator tubes and sent to the laboratory for quantification the same day as routine sample analysis. Serum NT-proBNP was measured using a 2-site electrochemiluminescence immunoassay on a Roche E170 analyzer. The results are reported in pmol/L, with a lower limit of detection of 5 pmol/L. The manufacturer's guidelines, derived from 1,981 blood donors aged 18 to 65 years and 283 subjects with no known cardiac disease aged 50 to 90 years, were used to define normal values (Supplemental file S1). Values greater than the 97.5th percentile for age and gender were considered abnormal.

Standard 12-lead electrocardiograms were recorded at 25 mm/s and 10 mm/mV. The rhythm, heart rate, PR interval duration, and QRS complex duration were measured in milliseconds. A QRS duration >120 ms, PR interval <120 ms, or PR interval >200 ms was considered abnormal. Left ventricular (LV) hypertrophy was assessed using the Sokolow-Lyon and Cornell criteria.[13,14] Patients were considered to have electrocardiographic (ECG) evidence of LV hypertrophy if they met 1 or both criteria. Repolarization changes were assessed in 3 regions: inferior (leads II, II, and aVF), anterior (leads V_1 to V_4), and lateral (leads V_5 to V_6, I, and aVL). Patients with T-wave inversion in $\geq$1 of these regions were classified as having repolarization abnormalities. Abnormal ECG findings were defined as $\geq$1 of the following: conduction disease (abnormal QRS duration or PR interval); repolarization changes; and LV hypertrophy according to the voltage criteria.

Patients with a paced rhythm were grouped according to ECG abnormalities present before pacemaker implantation.

Transthoracic echocardiography was performed with the patient in the left lateral decubitus position with commercially available equipment (M3S Probe, Vivid i or Vivid 7; GE-Vingmed, Horten, Norway). The images were stored digitally for off-line analysis (EchoPac, version 108.1.5; GE-Vingmed). Complete 2-dimensional, color, pulsed, and continuous wave Doppler images were acquired using standard techniques. The LV ejection fraction was calculated using Simpson's biplane method.[15] The left atrial volumes were calculated using the ellipsoid model and indexed the to body surface area (left atrial volume index).[16] The relative wall thickness was calculated as (interventricular septal thickness at diastole + posterior wall thickness in diastole)/LV end-diastolic diameter and is expressed as a percentage. The LV mass was calculated using the Devereux cubed formula ($0.8 \times \{1.04 \times [(\text{LV end-diastolic diameter} + \text{posterior wall thickness in diastole} + \text{interventricular septal thickness at diastole})^3 - (\text{LV end-diastolic diameter})^3]\} + 0.6$ g) and indexed to the body surface area to obtain the LV mass index.[17] Patients with a normal LV mass index were classified as having concentric remodeling in the presence of a relative wall thickness >42% or normal geometry if the relative wall thickness was $\leq$42%. Patients with an increased LV mass index were classified as having concentric hypertrophy if the relative wall thickness was >42% or eccentric hypertrophy if the relative wall

Table 1
Clinical and echocardiographic characteristics of study population (n = 117)

Clinical Characteristics	Value
Male gender	54 (46%)
Age (yrs)	
Mean ± SD	48 ± 15
Range	19–79
Mainz severity score index	
Mean ± SD	20 ± 11
Range	1–53
Creatinine (μmol/L)	
Mean ± SD	91 ± 76
Range	43–692
Glomerular filtration rate (ml/min/1.73 m^2)	
Mean ± SD	87 ± 27
Range	7–158
Hemoglobin (g/dl)	
Mean ± SD	13.2 ± 1.4
Range	8.9–16.7
Systolic blood pressure (mm Hg)	
Mean ± SD	118 ± 16
Range	84–165
Diastolic blood pressure (mm Hg)	
Mean ± SD	72 ± 10
Range	40–91
Heart rate (beats/min)	
Mean ± SD	64 ± 12
Range	38–93
Medications	
Enzyme replacement therapy	97 (83%)
β Blocker	23 (20%)
Angiotensin-converting enzyme inhibitor	37 (32%)
Angiotensin II receptor blocker	15 (13%)
New York Heart Association class	
I	75 (64%)
II	33 (28%)
III	9 (8%)
Rhythm	
Sinus rhythm	102 (87%)
Atrial fibrillation	4 (3%)
Paced rhythm	11 (9%)
Atrial fibrillation	
Paroxysmal	13 (11%)
Persistent	4 (3%)
Echocardiographic findings	
Ejection fraction (%)	
Mean ± SD	61 ± 6
Range	31–74
Left ventricular end-diastolic diameter (mm)	
Mean ± SD	48 ± 5
Range	36–61
Left ventricular mass index (g/m^2)	
Mean ± SD	113 ± 45
Range	49–332
Left atrial size (mm)	
Mean ± SD	39 ± 7
Range	27–58
Left atrial volume index (ml/m^2)	
Mean ± SD	25 ± 10
Range	12–60
Left ventricular outflow tract obstruction	3 (3%)
Remodeling	
Normal	47 (40%)
Concentric	16 (13%)
Concentric hypertrophy	45 (39%)
Eccentric hypertrophy	9 (8%)

Data are presented as n (%) or mean ± SD and range.

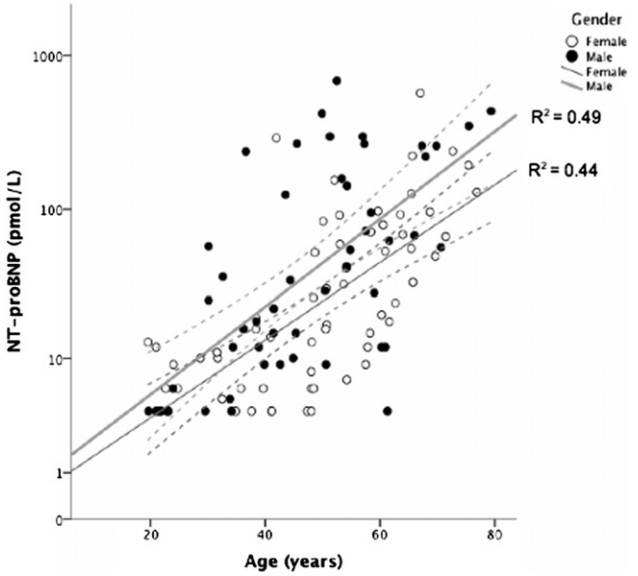

Figure 1. NT-proBNP increases with age in men and women (n = 114).

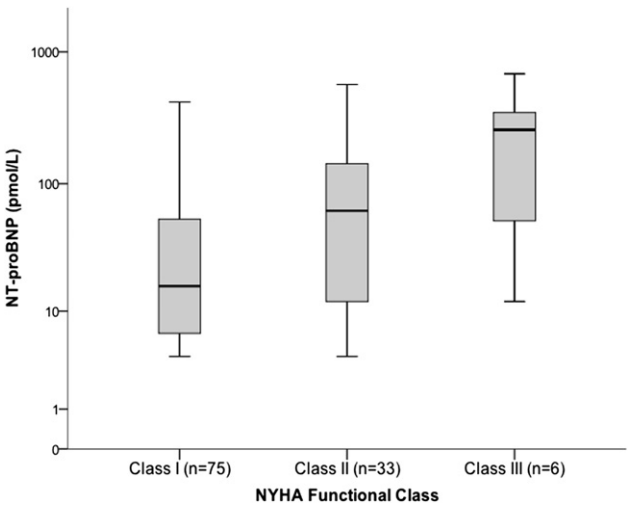

Figure 2. NT-proBNP increases with New York Heart Association functional class (n = 114).

thickness was ≤42%.[14] Diastolic dysfunction was graded according to the mitral inflow pattern, pulmonary vein flow, and tissue Doppler indexes at the lateral mitral annulus and classified as normal, mild (impaired relaxation), moderate (pseudonormal), or severe (restrictive).[18] An estimate of LV filling pressure was made using the ratio between transmitral peak E velocity and the peak Ea velocity measured at the lateral wall (E/Ea).[19] The Tei LV performance index was calculated using pulsed wave spectral Doppler traces of the LV outflow tract and transmitral inflow.[20] The LV outflow tract gradients were measured at rest and after provocation with the Valsalva maneuver. LV outflow tract obstruction was defined as a gradient of ≥30 mm Hg. In the present study, abnormal echocardiographic findings were defined as either interventricular septal thickness at diastole or posterior wall thickness in diastole of ≥13 mm or LV mass index of ≥95 g/m^2 in women and ≥115 g/m^2 in men.[16]

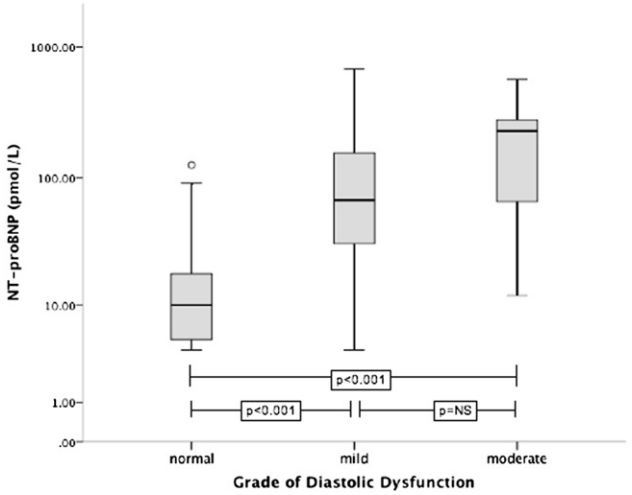

Figure 3. NT-proBNP is a marker of diastolic dysfunction (n = 109). Diastolic function was unable to be accurately classified in 5 patients because of heart rhythm or missing values.

The data were analyzed using PAWS statistical software, version 18.0. Continuous variables are presented as the mean ± SD and categorical variables as frequencies and percentages. Logarithmic transformation allowed the NT-proBNP concentration to be treated as a normally distributed variable. Bivariate correlation analysis was performed using linear regression. To identify independent correlates of logNT-proBNP, all variables with $p < 0.05$ on univariate analysis were included in a forward elimination multivariate regression analysis. Receiver operating characteristics curve analysis was performed to test the ability of NT-proBNP to detect cardiac involvement, defined by either abnormal ECG or echocardiographic findings. Fisher's exact test was used to calculate the significance of the observed data. The 95% confidence intervals were 2-sided with an α level of 5%.

Results

During a 3-year period, 117 patients were studied: 54 (46.2%) were men, with a mean age of 48 ± 15 years. The clinical and echocardiographic characteristics of the study population are listed in Table 1. The median NT-proBNP concentration was 24 pmol/L (range <5 to 6,059). Of the 117 patients, 67 (57%) had elevated, age-corrected, NT-proBNP levels. Three patients had an NT-proBNP concentration >1,000 pmol/L, two with end-stage renal failure, who required hemodialysis (6,063 and 4,139 pmol/L), and one with advanced mesothelioma (1,487 pmol/L). These patients were considered outliers and were excluded from the subsequent correlation analysis.

The NT-proBNP concentrations were greater in the men and correlated with age (Figure 1). The values in patients not receiving ERT (n = 20) ranged from <5 to 1,487 pmol/L. The NT-proBNP concentration also increased according to the New York Heart Association class (Figure 2) and grade of diastolic dysfunction (Figure 3). The LogNT-proBNP correlated with the Mainz severity score index score, systolic blood pressure, serum creatinine, and echocardiographic indexes of LV function (E/Ea

Table 2
Relation between log N-terminal pro-brain natriuretic peptide (NT-proBNP) and echocardiographic and clinical parameters (n = 114)

Parameter	Univariate		Multivariate	
	β Coefficient	p Value	β Coefficient	p Value
Age (yrs)	0.656	<0.0001	0.265	0.001
Male gender	0.129	0.171		
Mainz severity score index	0.514	<0.0001	—	NS
Systolic blood pressure (mm Hg)	0.170	0.07		
Glomerular filtration rate (ml/min/1.73 m^2)	−0.585	<0.0001	−0.188	0.012
Abnormal electrocardiographic findings	0.518	<0.0001	0.155	0.024
Ejection fraction (%)	0.013	0.887		
Left ventricular end-diastolic diameter (mm)	−0.118	0.213		
Left atrial volume index (mls/m^2)	0.610	<0.0001	0.272	<0.001
Left ventricular mass index (g/m^2)	0.616	<0.0001	—	NS
Right ventricular hypertrophy	0.437	<0.0001	—	NS
E/Ea	0.642	<0.0001	0.236	0.002
E/A ratio	−0.288	<0.0001	—	NS
Tei index	0.292	0.002	—	NS
Mitral regurgitation	0.365	<0.0001	—	NS
Left ventricular outflow tract obstruction	0.179	0.056		

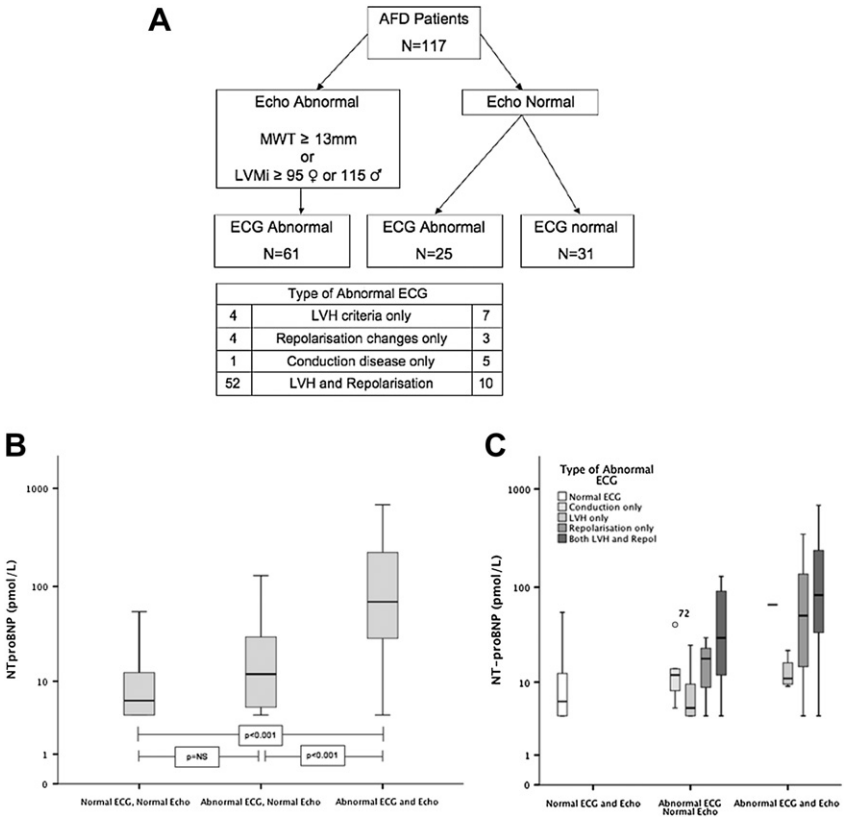

Figure 4. *(A)* Population stratified according to ECG and echocardiographic findings (n = 117). *(B)* NT-proBNP stratified according to ECG and echocardiographic abnormalities (n = 114). *(C)* Subgroup analysis indicated NT-proBNP levels were greater in those with both LV hypertrophy and repolarization changes (n = 114).

and Tei index; Table 2). No relation was seen between LogNT-proBNP and LV size, ejection fraction, or LV outflow tract obstruction. In a multivariate linear regression model, LogNT-proBNP independently correlated with age, serum creatinine, left atrial volume index, the E/Ea ratio, and abnormal ECG findings ($R^2 = 0.67$, $p < 0.05$).

Of the 117 patients, 61 (53%) had abnormal ECG and echocardiographic findings, 25 had abnormal ECG findings but a normal wall thickness and LV mass index on the echocardiogram, and 31 had normal ECG and echocardiographic findings (Figure 4). In the 56 patients (47.9%) with normal echocardiographic findings, the NT-proBNP levels

Table 3
Operating characteristics of N-terminal pro-brain natriuretic peptide (NT-proBNP) thresholds in Anderson-Fabry disease (AFD) (n = 117)

NT-proBNP (pmol/L)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Accuracy (%)
Ability to predict cardiac involvement*					
≥5	90.7	29.0	81.9	46.8	77.1
≥20	68.6	93.5	99.7	9.2	69.4
≥50	53.5	96.8	99.9	4.4	54.4
≥100	31.4	100.0	100.0	0.0	31.4
Ability to predict abnormal echocardiographic findings					
≥5	96.7	26.8	65.5	84.9	68.0
≥20	83.6	82.1	96.0	49.5	83.4
≥50	67.2	89.3	97.7	29.0	70.1
≥100	42.6	98.2	99.8	6.4	44.7

* Echocardiographic or electrocardiographic findings.

were greater than the age-predicted cutoffs in 10 of the 25 patients with abnormal ECG findings (range 12 to 129 pmol/L) and in 3 of the 31 patients with normal ECG findings (range 15 to 55 pmol/L; $p < 0.05$). All these patients were normotensive with systolic blood pressure of ≤130 mm Hg. No patient had normal ECG findings with abnormal echocardiographic findings.

Patients with abnormal ECG and echocardiographic findings had significantly greater NT-proBNP concentrations (median 72 pmol/L, range <5 to 6,063) than those with normal findings (median 12 pmol/L, range <5 to 129) or abnormal ECG findings alone (median 72 pmol/L, range <5 to 55; Figure 4). A subgroup analysis of ECG abnormalities showed a nonsignificant trend toward greater NT-proBNP levels in patients with repolarization changes than in those with ECG evidence of LV hypertrophy alone (Figure 4). The area under the receiver operating characteristics curve was 0.85 (95% confidence interval 0.79 to 0.92). An NT-proBNP level of ≥20 pmol/L had a positive predictive value of 99.7% and negative predictive value of 9.2% to identify abnormal ECG or echocardiographic findings (Table 3).

Discussion

The results of the present study have demonstrated that plasma NT-proBNP is elevated in patients with cardiac manifestations of AFD and correlates with symptom class and echocardiographic surrogates of elevated LV filling pressure. Increased NT-proBNP concentrations were found in patients without echocardiographic evidence of LV hypertrophy, suggesting that measurement of natriuretic peptides could be a useful adjunctive test in identifying patients with early subclinical cardiac disease.

The cardinal histologic feature of AFD-related cardiac disease is the accumulation of globotriaosylceramide in cardiomyocytes, conduction system cells, valvular fibroblasts, endothelial cells, and vascular smooth muscle cells.[1,21] Electron microscopy of cardiomyocytes typically shows a paucity of myofibrillar material and membrane-lined vacuoles filled with lamellar electron-dense inclusions.[22] In addition to the distortion of normal cellular architecture, it has been postulated that globotriaosylceramide accumulation triggers intracellular signaling pathways that lead to cardiomyocyte hypertrophy, apoptosis, and necrosis and interstitial fibrosis.[23] Microvascular ischemia and mitochondrial dysfunction might also play a role in disease progression.[24,25]

ERT is recommended for patients with obvious manifestations of AFD to mitigate the risk of future cardiac, cerebrovascular, and neurologic complications.[26] In most studies, clinically significant cardiac disease has been defined by the presence of myocardial hypertrophy on echocardiograms; however, numerous reports have suggested that this is a relatively late phenomenon (particularly in women) often associated with the presence of irreversible myocardial scarring on cardiac magnetic resonance imaging.[27] A number of studies have examined the ability of more sensitive echocardiographic methods such as tissue Doppler imaging to detect early disease; however, as with all Doppler techniques, these have been only modestly reproducible and lack specificity.[28]

Compared against conventional electrocardiography and 2-dimensional echocardiography, NT-proBNP was highly predictive for the presence of cardiac involvement. Although a positive correlation was seen with the LV mass, this was not significant on multivariate analysis. In contrast, markers of diastolic impairment (left atrial size and E/Ea, reflecting the LV filling pressure) were independent predictors of NT-proBNP.[29] Diastolic dysfunction in AFD has been attributed to myocardial fibrosis and Gb3 accumulation and is recognized to precede hypertrophy.[28] Even in subclinical disease, evidence has been found of impaired contractility of cardiomyocytes and greater tension of the myofilament at rest.[30] Therefore, a biomarker that correlates with tension-related remodeling would be potentially useful to identify early cardiac involvement in AFD.

At present, ERT for AFD is only licensed for use in patients with clear evidence of tissue damage. LV hypertrophy is the most widely used clinical marker of cardiac involvement in patients with AFD; however, the results of the present study have suggested that elevated NT-proBNP is a useful surrogate for early cardiac disease and could be used to justify earlier use of ERT. As a biomarker, it is readily available to all physicians and can be measured more frequently and economically than performing electrcardiography and echocardiography. We

suggest ≥20 pmol/L is likely to be associated with cardiac involvement in AFD and should prompt additional investigation. Additional work is needed to determine whether NT-proBNP can be used to monitor disease progression and determine the prognosis.

The NT-proBNP concentrations found in the present study were lower than those reported by Torralba-Cabeza et al,[10] in which the mean NT-proBNP level was 1,012 ± 3,469 pg/ml (range 5 to 27,161), equivalent to 120 pmol/L. However, their study included 7 patients who required hemodialysis, potentially biasing their data.[10] In our study, exceptionally high levels were also seen in those with advanced renal failure; however, we did not identify any patients in whom severe renal dysfunction was present without evidence of some degree of cardiac involvement. The study population represented a cross-section of adult patients with AFD, and although it was performed at a cardiac center, many patients were seen for routine cardiac screening rather than management of established cardiac disease.

The NT-proBNP concentrations are increased in patients with AFD and correlate with noninvasive markers of diastolic dysfunction. Increased NT-proBNP levels were present in patients without echocardiographic evidence of LV hypertrophy. These findings suggest that measurement of the NT-proBNP levels might assist in decisions on the timing of ERT. Its role as a surrogate marker for the response to therapy requires additional study.

Supplementary material

Supplementary data related to this report can be found at http://dx.doi.org/10.1016/j.amjcard.2012.08.055.

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