

B-Type Natriuretic Peptide Assessment in the Diagnosis of Rejection after Pediatric Heart Transplant

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Summary

Background: Rejection is one of the major causes of mortality following pediatric heart transplant. B-type natriuretic peptide (BNP) has been studied as a method for the diagnosis of acute rejection, especially in adult patients undergoing heart transplant.

Objective: To correlate serum BNP levels with acute rejection as diagnosed by endomyocardial biopsy in patients of the pediatric heart transplant group.

Methods: A total of 50 BNP samples were collected from 33 children in the postoperative period of heart transplant, and data on age, gender, skin color, blood group, immune panel, follow-up time after transplant, functional class, immunosuppressive regimen used and number of rejections were analyzed.

Results: Thirty three children with median age of 10.13 years were analyzed; of these, 54% were females and 78% were Caucasians. BNP levels were determined at a mean time from transplant of 4.25 years. Nine episodes of rejection were diagnosed in eight patients (27%) by means of endomyocardial biopsy; of these, three were grade 3A, five were grade 2, and one had humoral rejection. At the moment of biopsy, most patients were asymptomatic. The mean serum BNP level was 77.18 pg/ml, with 144.22 pg/ml in the group with rejection and 62.46 pg/ml in the group without rejection, with $p = 0.02$.

Conclusion: Asymptomatic children can present acute rejection in the postoperative period of heart transplant. Serum BNP levels show a statistically significant difference in the group with rejection and thus can be an additional method in the diagnosis of cardiac rejection. (Arq Bras Cardiol 2009;92(3): 215-220)

Key words: Natriuretic peptide, B-type; graft rejection; heart transplantation; biopsy; children.

Introduction

Despite recent advances described in the treatment of congestive heart failure in children, heart transplant remains the treatment of choice in a large number of children with complex congenital heart diseases and cardiomyopathies refractory to conventional therapy^{1,2}.

The tenth official pediatric heart transplantation report of the International Society for Heart and Lung Transplantation (2007)³ reports that rejection remains a major cause of mortality after pediatric heart transplant despite the new immunosuppressive agents and the development of new therapeutic strategies.

Early diagnosis is essential and noninvasive methods⁴⁻⁶ have been investigated in children because, despite being considered the method of choice, endomyocardial biopsy

has limitations concerning technical difficulties, costs and complications when performed in children⁷⁻⁹.

Serum levels of B-type natriuretic peptide (BNP), a neurohormone produced by the myocardium in response to ventricular distension or increased wall stress¹⁰⁻¹², have been used in heart transplant in adult patients as a diagnostic method of acute rejection¹³ and as a predictor of coronary heart disease¹⁴⁻¹⁶. Few reports are available in the pediatric population, with small number of patients and controversial outcomes, and this encouraged the conduction of the present study^{17,18}.

This study consists of the assessment of serum BNP levels in comparison with pathological findings of endomyocardial biopsy, aiming at the diagnosis of acute rejection in children undergoing orthotopic heart transplant.

Methods

This is a cross-sectional study of 33 pediatric patients followed-up after heart transplant in *Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo*, between June 2005 and October

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2006; a total of 50 biopsies were performed.

The inclusion criteria for participation in the study were: patients with a longer than six-month follow-up after heart transplant, whose guardians agreed on the performance of cardiac catheterization and of the study protocol and gave their informed consent.

Patients not undergoing cardiac catheterization during the study period and cases with flaws in specimen collection or storage were excluded.

The clinical parameters assessed were: mood change (irritability, apathy, agitation, sleepiness) or presence of anorexia, fever, arrhythmia or signs of congestive heart failure.

Outcome data such as follow-up time after transplant, total number of rejection episodes, age at transplant, immunosuppressive regimen used, need for vasoactive agents and/or anticongestive medication, as well as the presence of coronary heart disease, renal failure or diabetes mellitus were collected.

BNP sample collection

Blood sample for BNP determination was collected at the moment of endomyocardial biopsy of children undergoing heart transplant. The method used for BNP determination was the Triage™ test (Biosite Diagnostics), an immunofluorescence test for the quantitative BNP determination in blood or plasma preserved with EDTA as the anticoagulant. The sample is placed in the device, and then plasma is separated from blood by a filter. A predetermined amount of plasma is put to react with fluorescent antibodies. After an incubation period, the sample is sent to analysis for detection of the complexes formed with fluorescent antibodies.

In this study, the specimens used had been collected six months before at most, and kept frozen at minus 20°C.

Endomyocardial biopsy

Biopsies were performed consecutively during the annual catheterization and were indicated in the cases in which acute rejection was suspected.

For data analysis, patients were divided into two groups (with rejection and without rejection) and BNP levels were evaluated in both groups. Acute cellular rejection was considered when endomyocardial biopsy findings were consistent with cellular rejection equal to or greater than grade 2 or with the presence of humoral rejection, according to the International Society for Heart and Lung Transplantation (ISHLT)'s criteria¹⁹.

Echocardiography

Echocardiography was performed according to the American Society of Echocardiography's recommendations²⁰ and was aimed at analyzing the following parameters: diastolic septal and wall diameter, left ventricular systolic and diastolic diameters, degree of valve regurgitation, presence of pericardial effusion, and signs of diastolic dysfunction (with transmitral flow and tissue Doppler analysis made at the mitral annulus). Ejection fraction was calculated using the Teichholz method.

Diastolic function was assessed using transmitral flow as obtained by pulsed Doppler in the four-chamber apical view, and the velocity patterns of the mitral E wave (rapid filling phase) and A wave (atrial contraction) were observed. E/A ratios equal to or greater than 1.0 were considered normal; E/A ratios lower than 1.0 were considered as ventricular relaxation abnormality; and E/A ratios greater than 1.5 were considered as restrictive pattern.

Tissue Doppler, a technique that evaluates the velocity of myocardial motion, was assessed at the lateral mitral annulus in the four-chamber apical view. Inversion of the normal E' wave (early diastolic wave) and A' wave (late diastolic wave) pattern with E' < A' or reduction of E' wave velocity was considered abnormal. Whenever any change in wave morphology or amplitude was observed, the other left ventricular and right ventricular walls were also studied.

Statistical analysis

Descriptive analysis was conducted regarding demographic, clinical, and pre and post-transplant data of the 33 patients.

The quantitative variables were summarized by the measures of central tendency and dispersion (minimum, maximum, median, and mean values, as well as standard deviation). Qualitative data were analyzed using absolute and relative frequency tables. Graphs were plotted in order to illustrate the data.

The Mann-Whitney (non-parametric) test was used to analyze the differences between BNP concentrations in the groups with and without rejection. Also, sensitivity and specificity were calculated using the ROC curve to establish the cut-off point at the highest sensitivity value for the detection of patients with acute rejection, adopting biopsy findings as the reference standard.

Sensitivity and specificity indexes, positive predictive value and negative predictive value were calculated for the comparative analysis of echocardiogram and BNP levels (noninvasive tests) with the reference standard (endomyocardial biopsy).

The results were interpreted with the significance level (α) set at 5%^{21,22}.

Ethical procedures for conducting the research

The study was conducted according to the follow-up criteria for patients transplanted in *Instituto do Coração (InCor) do Hospital das Clínicas da FMUSP* and did not pose any additional risk to the study participants. BNP test, as a diagnostic and experimental method, did not show any contraindications or side effects, nor did it require any special patient preparation. Measures for specimen collection and patient protection were the same adopted in the institution for immunosuppressed patients according to standards of the commission on hospital infection control.

Results

A total of 33 children with mean age of 11 years (4 to 19 years), median age of 10.13 and standard deviation of 4.55 years were studied. There was a predominance

of the female gender (54.5%) and Caucasians (84.8%). Anthropometric data: weight from 12 to 78 kg, with median of 29.0 kg; height from 90 to 170 cm, with median of 136.0 cm; and median body mass index (BMI) of 16.35 and standard deviation of 3.78.

Heart transplant was indicated for dilated cardiomyopathy in 22 (67%) patients, restrictive cardiomyopathy in two (6%), congenital heart disease in eight (24%), and rheumatic heart disease in one patient (3%).

Regarding patient distribution according to blood group, 15 (45.0%) were from group O; 12 (36.0%) from group A; three (9.0%) from group B; and three (9.0%) from group AB.

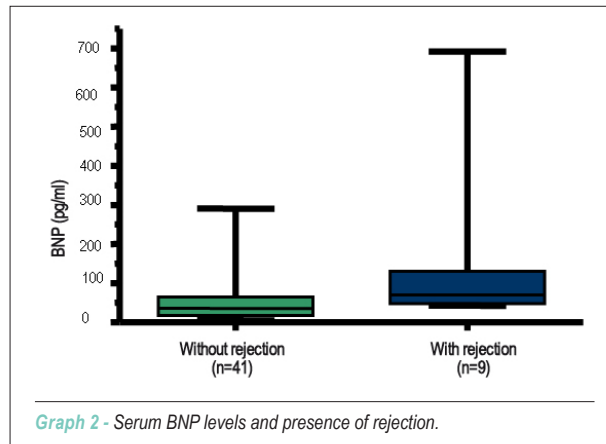
The median follow-up time after transplant at the moment of BNP determination was 4.25 years (from six months to 13.6 years). The mean age at the moment of transplant was 4.90 years (from four months to 15 years).

In relation to immunosuppression, calcineurin inhibitor and mycophenolate mofetil were the most frequently used agents (78.7% and 66.6%, respectively). A smaller proportion of patients were on chronic calcineurin inhibitor and azathioprine and corticosteroid. The mean number of rejections was 3 ± 1.4 rejections per patient.

By means of endomyocardial biopsy, rejection was diagnosed in nine patients (27%), of whom three had grade 3A, five had grade 2, and one had humoral rejection with no signs of cellular rejection (Graph 1).

In relation to the symptoms, most of the patients were asymptomatic at the moment of the test, and catheterization was performed during a routine procedure in 36 samples (72%). Rejection was clinically suspected (mood change, presence of arrhythmia or abnormal echocardiogram) in 10 patients (20%), and four samples (8%) were collected as controls after treatment of rejection.

The mean serum BNP level was 79.9 pg/mL, with 144.2 pg/mL in the group with rejection and 65.80 in the group without rejection, $p = 0.02$ (Graph 2). The analysis of the ROC curve showed 38 pg/mL as the value at a 100% sensitivity and



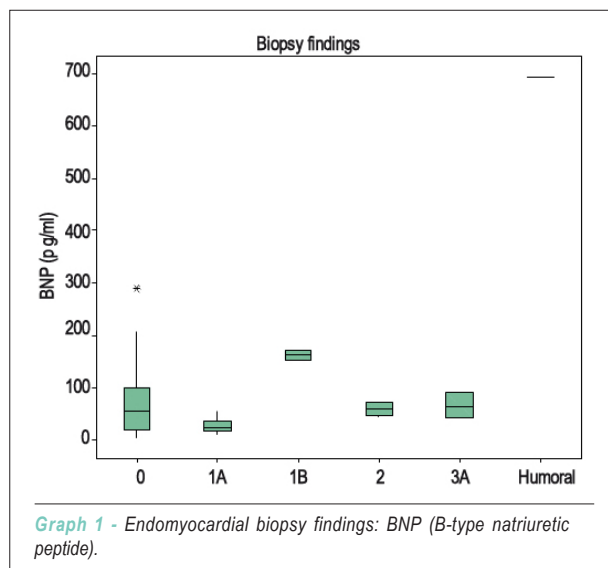
Graph 2 - Serum BNP levels and presence of rejection.

56% specificity for the diagnosis of cardiac rejection, with an area under the curve of 0.74 and $p = 0.01$ (Graph 3).

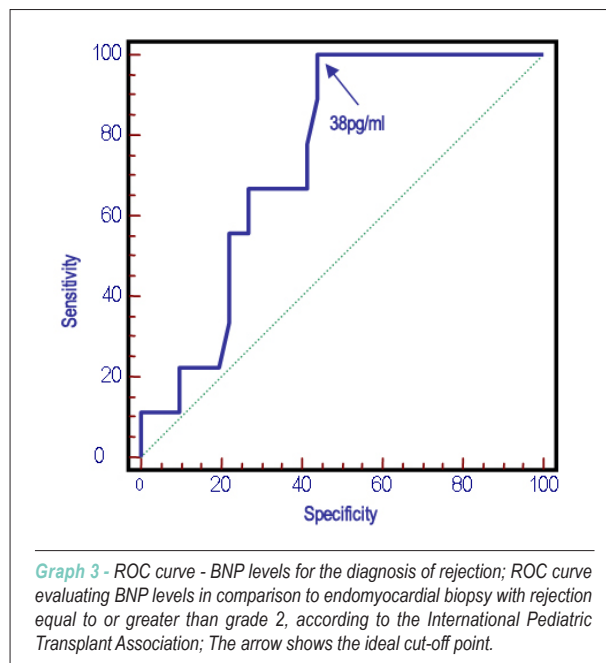
Echocardiography

Echocardiographic analysis (Table 1) found abnormalities in four out of the nine samples with rejection (44%), and in four of the 41 biopsies without rejection (10%), with a 50% positive predictive value and 88% negative predictive value. The sensitivity of the method was 44% and the specificity was 90% ($p = 0.02$).

The abnormalities found in patients with rejection were: moderate biventricular systolic dysfunction in a patient with humoral rejection; mild left ventricular systolic dysfunction in a patient with grade-2 rejection; and moderate mitral regurgitation and diastolic dysfunction with relaxation abnormality with E' and A' wave pattern inversion on tissue Doppler in two patients with grade-3 rejection, respectively.



Graph 1 - Endomyocardial biopsy findings: BNP (B-type natriuretic peptide).



Graph 3 - ROC curve - BNP levels for the diagnosis of rejection; ROC curve evaluating BNP levels in comparison to endomyocardial biopsy with rejection equal to or greater than grade 2, according to the International Pediatric Transplant Association; The arrow shows the ideal cut-off point.

Table 1 – Echocardiographic analysis in the diagnosis of rejection

	With rejection (n=9)	Without rejection (n=41)	p
LVDD (mm)	40.0 (4.0)	37 (5)	0.76
LVSD (mm)	25.0 (4.6)	23.0 (5.6)	0.20
septum/wall (mm)	7.0/7.0	7.0/7.0	0.80
RVSP (tricuspid regurgitation) (mmHg)	43 (7.2)	35 (7.6)	0.20
Pericardial effusion	0	1	}
Moderate/severe* heart valve regurgitation	1	2	
E/A mitral <1	1	0	
Tissue- Doppler abnormality	1	1	
Biventricular systolic dysfunction	1	0	

LVDD - left ventricular diastolic diameter; LVSD - left ventricular systolic diameter; RVSP - right ventricular systolic pressure; * change in relation to previous study

Hemodynamics

In the comparison of the groups with and without rejection (Table 2), no difference was observed in relation to pulmonary systolic pressure, pulmonary capillary pressure, cardiac output, or pulmonary or systemic vascular resistance.

In relation to the presence or absence of coronary heart disease and abnormal BNP values, greater than 100 pg/mL levels were observed in three of the four patients with coronary heart disease (p = 0.01).

BNP and renal failure

Increased BNP levels not related to rejection were observed in two patients with abnormal renal function, with BNP= 208 pg/mL and 291 pg/mL, (p=0.02).

Table 2 – Hemodynamic evaluation

	With rejection (n=9)	Without rejection (n=41)	P
CO (L/min)	2.60 (0.35)	3.35 (0.98)	0.07
PAP(S) (mmHg)	22.0 (6.24)	22.0 (5.20)	0.87
PAP(D) (mmHg)	10.0 (4.42)	10.0 (4.42)	0.81
PCP (mmHg)	9.0 (1.83)	10.0 (3.41)	0.33
PVR (Woods)	1.30 (0.60)	1.40 (3.02)	0.94
SVR (Woods)	17.45 (6.29)	17.20 (5.05)	0.77
BP(S) (mmHg)	80.0 (12.04)	90.0 (19.15)	0.37
RA (mmHg)	6.0 (1.30)	7.0 (2.57)	0.85

CO - cardiac output; PAP(S) - systolic pulmonary artery pressure; PAP(D) - diastolic pulmonary artery pressure; PCP - pulmonary capillary pressure; PVR - pulmonary vascular resistance; SVR - systemic vascular resistance; BP(S) - systolic blood pressure; RA - right atrial pressure.

Discussion

In light of significant manifestations of rejection in asymptomatic patients that could lead to sudden death, non-invasive, high-sensitivity and specificity methods that could be repeated rapidly and in short time intervals have been the focus of a great part of the literature on heart transplant worldwide.

Studies have demonstrated BNP and NT-proBNP as reliable markers in heart failure and transplant, guiding treatment and helping in the diagnosis and prognosis^{23,24}.

Assessment of gene expression of post-transplant patients in the chronic phase with no hemodynamic changes and free from rejection correlates increased serum BNP levels with genes present in initial inflammatory processes, cardiac remodeling, immune activation and vascular changes²⁵, permitting an early prediction of the development of coronary heart disease or rejection .

Lan et al²⁶ studied children in the post-operative period of heart transplant and observed that BNP levels were high in the first post-transplant month and declined gradually after this period, returning to normal values (< 100 pg/mL) in 14 weeks.

As demonstrated in the present study, acute rejection may be asymptomatic in children in the postoperative period of heart transplant, and the evaluation of serum BNP levels may be an early indicator of this rejection, especially in the most severe forms such as humoral rejection²⁷⁻³¹.

The test is easy to perform, and can be done in the medical office with immediate result, so it can be repeated in cases in which rejection is suspected or even in routine outpatient visits. This permits cardiac catheterization to be postponed, which reduces morbidity rates among these children.

The cut-off point found in this study for the diagnosis of acute cardiac rejection using the ROC curve with 100% sensitivity was 38 pg/mL. This can indicate that lower values could be considered, prioritizing the sensitivity of the method.

Despite its low sensitivity for the diagnosis of cardiac rejection, echocardiography is easy to perform and has a high specificity, findings that are consistent with those reported by Akioka et al³². It should be used comparatively and along with all the resources currently available, including tissue-Doppler analysis. Further studies are necessary to increase the sensitivity of the method.

The assessment of hemodynamic measurements, except for reduced cardiac output, did not correlate with biopsy findings in this study, in corroboration with other studies of the literature³³⁻³⁵. The correlation between increased BNP levels and the presence of coronary heart disease was significant in this sample, thus showing the importance of the method in the screening of graft vascular disease as well.

Study limitations

The small number of patients, and consequently the small group with rejection, made analysis of subgroups difficult.

Clinical implications

BNP proved to be an important ancillary test in the diagnosis of cardiac rejection, especially in the most severe cases, such as

those with humoral rejection.

This test is easy to perform, only slightly invasive, allows immediate result, and can be repeated serially in order to postpone endomyocardial biopsy.

Its correlation with coronary heart disease also points out the importance of the method in the long-term follow-up of children in the late postoperative period of heart transplant, considering that this is an important cause of mortality after five years of transplant, and is frequently manifested as sudden death in outpatients.

Conclusion

Acute rejection can be asymptomatic in the late postoperative period of heart transplant children, and serial serum BNP level

determination may help the diagnosis of this rejection.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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