Technical Report

Ventricular natriuretic peptide (BNP) in heart transplantation: BNP correlation with endomyocardial biopsy, laboratory and hemodynamic measures

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A prospective study of 81 heart transplant (HT) patients was carried out in order to evaluate the evolution of brain natriuretic peptide (BNP) levels in HT patients and compare them with the degree of rejection as determined by endomyocardial biopsy. All patients were subjected to endomyocardial biopsy (532), and determination of BNP and creatinine levels as well as hemodynamic parameters. A control group of 36 volunteers was included. BNP values were significantly greater in HT patients than in healthy volunteers. In the first 3 months, BNP levels in patients with treatable rejection were significantly greater than in patients without graft rejection, although evident overlapping was observed in both distributions and discriminatory potential was low. After the third month, BNP values were similar in patients with and without rejection. Creatinine levels were observed to increase over time after transplantation, but no correlation was observed between the creatinine and BNP levels. A significant positive correlation was observed between BNP and right ventricle and pulmonary arterial pressures.

Heart transplantation (HT) improves the quality of life and prolongs survival among patients with end-stage myocardiopathy. The current gold standard for diagnosing graft rejection is an endomyocardial biopsy, which requires invasive cardiac catheterization. Despite their useful contributions, noninvasive techniques such as echocardiography,[1,2] Tl scintigraphy,[3] antimyosin monoclonal antibody scintigraphy,[4] and magnetic resonance imaging (MRI)[5] are unable to replace or serve as guides to biopsy performance.

Brain natriuretic peptide (BNP) was first identified in porcine brain by Sudoh *et al*[6] in 1988, and later isolated by Kambayashi *et al*[7] from the human atrium in 1990. It belongs to the group of natriuretic peptides. BNP is thought to participate in the normal homeostatic mechanisms that maintain the composition and volume of extracellular fluid.[8,9] BNP is known to have natriuretic, diuretic and vasorelaxant properties; may have antagonistic effects on the rennin–angiotensin–aldosterone system;[8,10,11] and plays an important role in fluid homeostasis and blood pressure.[11–14] Atrial natriuretic peptide (ANP) and BNP have similar properties, but the half-life of BNP is five- to six-fold longer, and BNP is mainly secreted from the ventricles in the heart, whereas ANP mainly derives from the atria.[15–17]

There is great interest in the diagnostic use of BNP for detection of ventricular dysfunction. Several authors have suggested that determination of BNP levels could be used as a screening technique for cardiac disease. Because patients with ventricular dysfunction and moderate heart disease could benefit from early treatment, and since clinical assessment of these patients is not a very reliable method for diagnosis, several authors have suggested that natriuretic peptides be used to predict ventricular dysfunction.[14,18,19] In addition, an increase in serum BNP concentrations proportional to

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the clinical severity of heart failure has been demonstrated.[20]

High BNP values have also been found in patients with high blood pressure, ventricular hypertrophy, ischemic heart disease and other heart diseases,[21–26] renal failure subjected to hemodialysis,[12,27] and in heart transplantation.[28–30] The utility of BNP in the diagnosis of heart transplant rejection has not been thoroughly investigated to date; the studies were generally limited to few cases with a short follow-up duration and sometimes contradictory results.[31,32] In this context, the simple laboratory assessment of BNP could be very useful in selecting patients for subsequent invasive techniques such as biopsy.

The aim of this study was to evaluate the evolution of BNP concentrations in heart transplant patients and its relationship to the degree of graft rejection, as determined by endomyocardial biopsy, and to study the correlation between BNP concentration, laboratory variables, and hemodynamic pressures.

Materials and methods

A prospective study was conducted on 81 consecutive patients (71 men, 10 women) subjected to orthotopic HT in our institution between January 1999 and January 2002. Retransplantations were excluded, as were those with combined kidney, liver or lung transplants; pediatric transplantations; and deaths before first biopsy performance. The mean patient age at the time of transplantation was 54 ± 10 years (range 15–66 years). The indication for HT was advanced ischemic heart disease in 61.3% of cases, idiopathic dilated myocardiopathy in 21.3%, myocarditis in 10%, and valve disease in 7.5%.

All patients were subjected to endomyocardial biopsy via protocol-based percutaneous femoral right catheterization (two in the first month, monthly until the sixth month, and after 9, 12, and 15 months). Parallel blood tests (± 24 h) were performed for basic parameters with creatinine and BNP levels. At the time of biopsy, measurements were made of right ventricle systolic and diastolic pressures (RVS, RVD), and mean pulmonary arterial pressure (MPA).

The degree of graft rejection was defined according to the classification of the International Society for Heart and Lung Transplantation (ISHLT).[33] Treatable rejection was considered to be $\geqslant 2$ in the first 90 days, and $\geqslant 3$ thereafter. The sample was differentiated in time (before and after 3 months post-HT), since the treatment requirements differed in the two periods according to the biopsy results. Since the aim was to determine whether BNP can serve as a guide to biopsy performance, this time distinction appears advisable.

For comparison of BNP concentration in the HT patients with respect to the general population, 36 healthy individuals, matched by age, were used (mean 51 years; range 15–64 years). Control subjects were required to be free of heart, kidney, or thyroid disease.

BNP Determination

After drawing blood, the samples were centrifuged for 5 min. The plasma was aspirated and stored in plastic tubes at $-30°C$ until analysis. Plasma BNP concentrations were measured in duplicate with a specific solid-phase 'sandwich' immunoradiometric assay (Shionora BNP Cis®) with two monoclonal antibodies were prepared against sterically remote sites: the first was coated on the beads as solid phase, and the second was radiolabelled with ^{125}I and used as the tracer. BNP molecules are 'sandwiched' between the two antibodies. Excess unbound tracer is easily removed during the washing step, and the bead solid phase retains only the absorbed antibody/antigen/tracer antibody combination. The amount of radioactivity bound to the solid phase is proportional to the amount of BNP present at the beginning of the assay.

The detection limit (defined as the smallest concentration different from zero, with a probability of 95%) was 2 pg/ml. Crossreaction with ANP and CNP was less than 0.001% for both. The BNP values defined as normal by the manufacturer were less than 18.4 pg/ml.

Statistical Analysis

BNP levels are expressed as the mean with the standard deviation (s.d.) and the median. The Mann–Whitney *U*-test was used to analyze the differences in BNP in the groups with and without graft rejection. The time course of BNP concentration was graphically represented, with its relation to the existence or absence of rejection and its discriminatory potential, based on the plotting of receiving operator characteristic (ROC) curves.

Evaluations were likewise made of the correlation between BNP concentration, creatinine and pulmonary pressures (based on the Pearson's correlation coefficient), and of the relation between pulmonary pressures and the existence of graft rejection (Student's *t*-test). Statistical significance was considered to be $P < 0.05$.

Results

A total of 532 cardiac biopsies were performed on the 81 patients, and complete data (ie, assessable biopsy result (sufficient biopsy sample), pulmonary arterial pressure, creatinine levels, and BNP) were obtained for 410 samples. There were no major complications related with the procedure.

The BNP values were significantly greater in the HT patients (258 ± 316 pg/ml, median 153 pg/ml)

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than in the healthy controls (17 ± 16 pg/ml, median 9 pg/ml), with statistical significance ($P < 0.001$) observed at all time intervals. Figure 1 shows histograms for the control population.

On average, maximum BNP concentration was reached within the first post–transplantation month (first 2 weeks mean: 369.27 pg/ml, median: 267.5 pg/ml; first month 358 pg/ml, median 259 pg/ml), with the existence of extreme values a common observation. Subsequently, the concentrations decreased and stabilized towards the fifth month, with values in the range of 60–120 pg/ml (Figure 2).

The relationship between BNP levels and graft rejection, as determined by endomyocardial biopsy, is shown in Figure 2a and b. In the first 90 days, 89 biopsies showed evidence of rejection ≥ 2 according to the ISHLT classification, of which 52 were scored ≥ 3. Although BNP levels in patients with treatable rejection (494 ± 462, $n = 89$; median 310 pg/ml) were significantly greater than in patients without graft rejection (268 ± 245, $n = 96$; median 222 pg/ml) ($P < 0.0001$), evident overlapping was observed in both distributions. As can be seen from the ROC curve (area under the curve, AUC: 0.667; Figure 3 and Table 1), no BNP cutoff point presented sufficient discriminatory capacity, with acceptable sensitivity and specificity, for diagnosing graft rejection.

After the first 90 days, 18 biopsies were observed as rejection ≥ 3 (ISHLT classification). The BNP values were similar in the patients with and without rejection (163 ± 289, $n = 18$; median 59 pg/ml vs 137 ± 199, $n = 207$; median 76 pg/ml) ($P = \mathrm{NS}$). The area under the ROC curve (0.45, Figur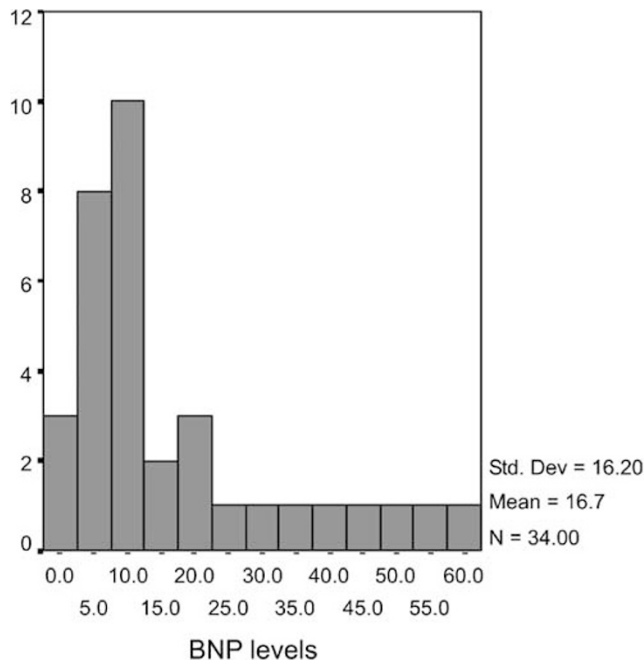e 4 and Table 1), likewise reflects the incapacity of BNP to discriminate the existence of graft rejection. A BNP cutoff point of 400 pg/ml, in this period, would only detect two of the 18 cases of rejection (sensitivity 11%), with a likewise very low positive predictive value (three out of 18: 17%). Upon lowering the cutoff point to 100 pg/ml, sensitivity increases yet remains low (28%), with an important loss of specificity.

The creatinine levels were shown to increase over time after transplantation (Figure 5). In the first 4 months, the creatinine concentrations were 1.0 ± 0.5 mg/dl; this was followed by additional increases, reaching 1.5 ± 0.6 mg/dl at the end of follow-up ($P < 0.001$). No correlation was observed between the creatinine and BNP levels ($r = 0.15$, $P = 0.7$).

The RVS, RVD and MPA were similar in the patients with and without rejection. Although the differences in RVS and MPA were statistically significant, they were clinically irrelevant (RVS-rejection ≥ 3: 40 ± 8 mmHg, $n = 70$, rejection < 3: 37 ± 9 mmHg, $n = 340$; $P = 0.01$; MPA-rejection ≥ 3: 25 ± 5 mmHg, $n = 70$, rejection < 3: 22 ± 5 mmHg, $n = 340$; $P = 0.01$). For this analysis, no sample time division was used; 52 of the 70 biopsies indicating rejection ≥ 3 were in the first 90 days, and 18 of the 70 were after 90 days. When biopsies, RVS, RVD and MPA were analyzed on the basis of time after transplantation (rejection ≥ 2; 89 in the first 90 days and 62 after 90 days), similar clinically irrelevant results were obtained.

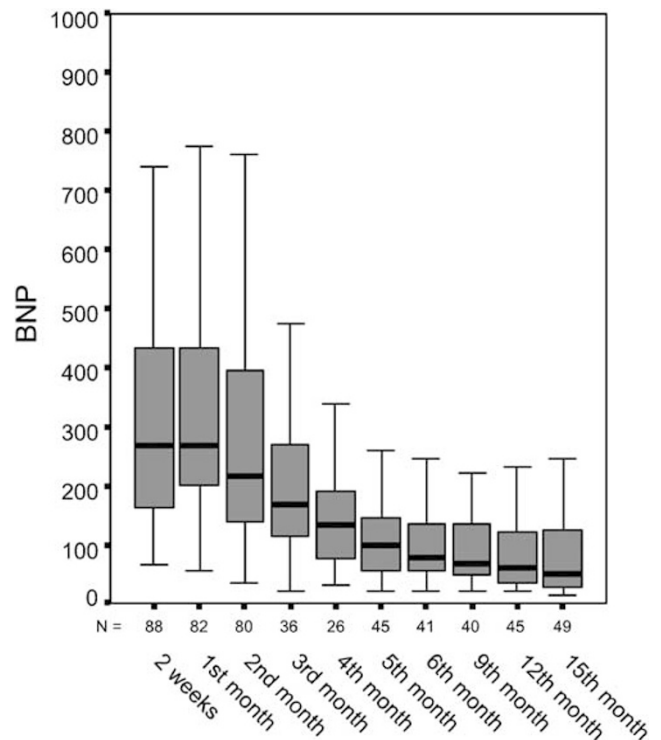**Figure 1** Histograms for the control population.

Figure 2 Time course of BNP concentration in heart transplant patients. The BNP levels were significantly higher than in the control group in all periods analyzed.
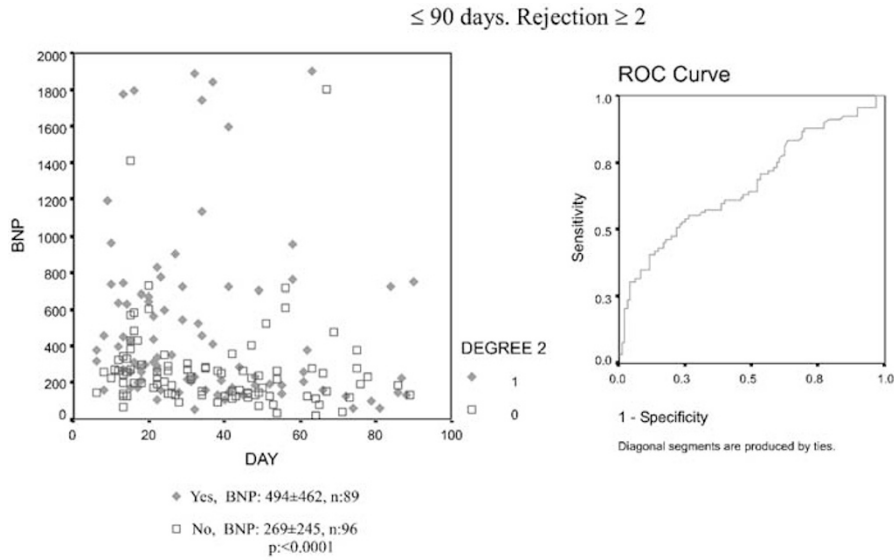
≤ 90 days. Rejection ≥ 2

◆ Yes, BNP: 494±462, n:89

□ No, BNP: 269±245, n:96
p:<0.0001

Figure 3 BNP levels and rejection at endomyocardial biopsy (ISHLT classification) in the first 90 days after transplantation. In this period, treatable rejection was defined as ≥2. The BNP values were greater in the patients with graft rejection, although without discriminatory capacity, as shown by the ROC curve.

Table 1 Sensitivity and specificity values in diagnosing graft rejection for the different BNP cutoff points

| BNP | ≤90 days: Rejection ≥2 | | >90 days: Rejection ≥3 | |
	Sensitivity	Specificity	Sensitivity	Specificity
100	94	9	28	61
200	71	44	17	85
300	52	77	11	91
400	42	85	11	94
600	30	94	11	96
800	15	98	6	97
1000	11	98	6	99

A significant positive correlation was observed between BNP and the right ventricle and pulmonary arterial pressures (BNP-MPA, $r = +0.54$, $P < 0.0001$; BNP-RVS, $r = +0.46$, $P < 0.0001$; BNP-RVD, $r = +0.20$, $P < 0.0001$).

Discussion

Investigators have long searched for a noninvasive marker in HT to detect onset of allograft dysfunction, but most efforts have focused on a marker for diagnosis of rejection.[2,34,35] However, allograft

> 90 days. Rejection ≥ 3

◆ Yes, BNP: 163±289, n:18
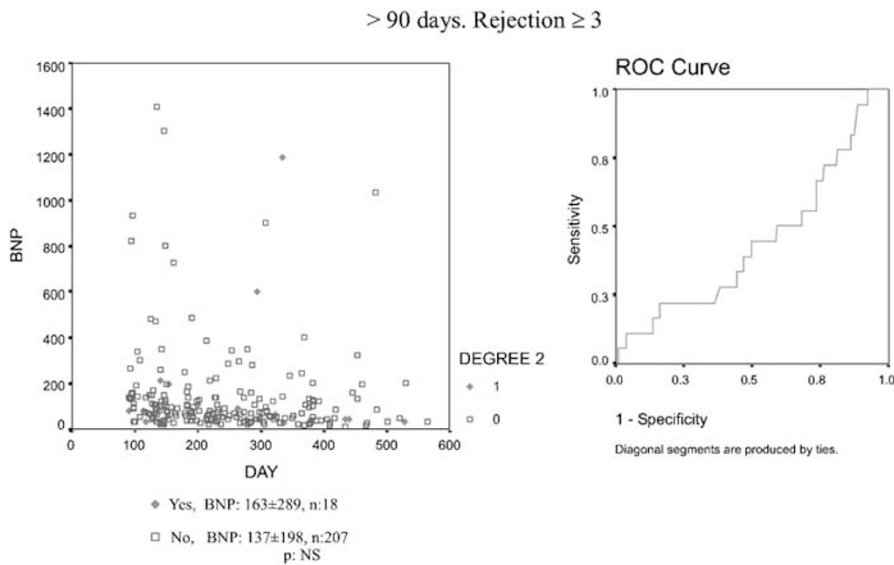
□ No, BNP: 137±198, n:207
p: NS

Figure 4 BNP levels and rejection at endomyocardial biopsy (ISHLT classification) after the third month of HT. Treatable rejection was considered for ≥3. In this period, no significant differences were observed in BNP levels between patients with and without rejection.
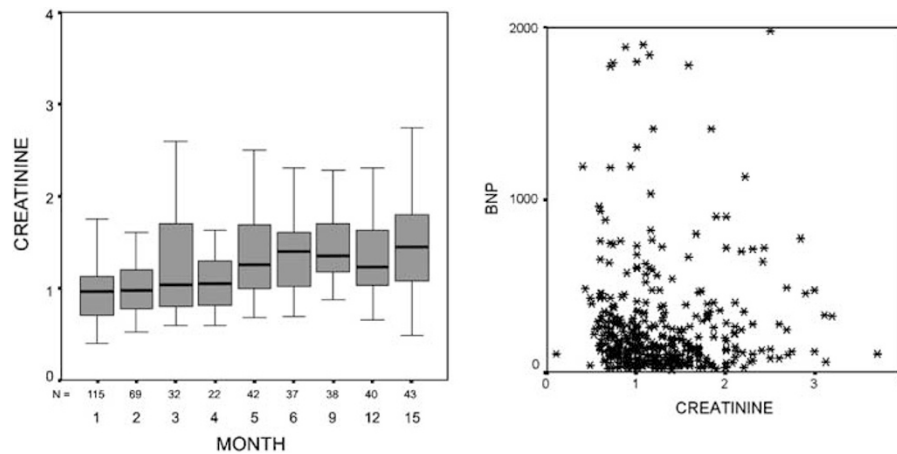
Figure 5 Time course of creatinine concentration in HT (left). Relation between BNP and creatinine concentrations (right).

dysfunction can also result from restrictive physiology of the denervated heart, which causes diastolic dysfunction in the absence of rejection[36] and right ventricular dysfunction with tricuspid regurgitation, both a common occurrence after transplantation.[37] BNP has the potential to serve as a useful screening measure for the presence of cardiac allograft function, independent of the underlying structural abnormality.[38] Previous studies of BNP in HT patients have shown baseline elevations compared with BNP levels in nontransplanted controls.

The time course of BNP concentration after HT was investigated by Ationu *et al*[28] in 14 patients subjected to 68 biopsies between 1 and 74 weeks after transplantation (although after the sixth month, only eight biopsies were analyzed). These authors observed a significant positive correlation between the time elapsed from transplantation and BNP concentration ($r = 0.65$, $P < 0.05$), which is inconsistent with our observation that the BNP concentration tended to stabilize after the fourth or fifth month. The same authors also published a study outlining the evolution of BNP in seven pediatric patients, reporting that the values measured between 2.5 and 3 years post-transplantation were significantly lower than those recorded in the first year.[39] This observation is more in line with our findings. In agreement with our study, most authors have found that BNP concentration remained high after transplantation, and were significantly higher than in healthy controls.[28–30]

Ationu *et al*[28] and El Gamel *et al*[29] likewise found no relation between BNP plasma levels and the existence of heart rejection as determined by biopsy. Masters *et al*[31] analyzed 77 biopsies in 10 patients between 4 and 40 weeks post-HT. They defined the existence of rejection as grade $\geqslant 2$ according to the biopsy evidence and, in the first 3 months, found patients with rejection to have significantly higher BNP values (544 ± 116) than subjects without rejection (198 ± 12). These results are in good agreement with our study. Since none of the six patients

without rejection presented BNP levels of $\geqslant 400$ pg/ml at any time in the course of monitoring; as a result, the authors arbitrarily chose this cutoff value, believing it would entail few false-positive results. However, despite the fact that the BNP levels among the patients without rejection episodes were under 400 pg/ml, the data presented by the authors showed that BNP concentrations above 400 pg/ml were relatively frequent and coincided with biopsies reported as rejection 0, 1A or 1B.

In our study, during the period comparable to that described in the study by Masters *et al* (first 3 months, with rejection defined as $\geqslant 2$), 14 biopsies in 14 different patients revealing no rejection occurred BNP values above 400 pg/ml. Moreover, six of these patients never presented rejection according to the serial biopsy results, despite the fact that on some occasions BNP concentrations were $\geqslant 400$ pg/ml. The sensitivity and specificity values reflected in Table 1, for different BNP cutoff points in the diagnosis of rejection, suggest that isolated or individual BNP determination lacks the discriminatory capacity to either replace or serve as a guide to endomyocardial biopsy performance. We also observed that BNP levels immediately following transplantation were frequently low, suggesting some undersecretion by the failing heart immediately prior to transplantation. Unfortunately our protocol, as described in 'Materials and methods', only included endomyocardial biopsy and BNP determination 15 days and 1 month post-HT, and we could not corroborate all the findings described by Masters *et al*, in particular, serum BNP values immediately following transplantation.

In this prospective study, BNP concentrations increased following HT, reaching peak values in the first 2 months. The levels subsequently decreased and stabilized after the fifth post-HT month, with concentrations in the range of 60–100 pg/ml, which is high, compared to the general population. Despite the fact that the BNP levels in the first 3 post-HT months were higher in the graft rejection

group on average, no BNP cutoff point afforded sufficient predictive or discriminatory capacity to diagnose or serve as a screening tool for rejection. This was particularly manifest after the third month. In contrast, a significant positive correlation was identified between BNP and the right ventricle and pulmonary arterial pressure values.

We found no relationship between BNP levels and creatinine concentration, with the latter increasing over time. El Gamel et al[29] obtained similar results in a group of 40 HT patients. Earlier studies had shown that patients with chronic renal failure enrolled in a hemodialysis program exhibited increased BNP levels.[12,27] Akiba et al[40] analyzed the relation between BNP and creatinine in a group of healthy controls (creatinine 0.5–1.2 mg/dl; BNP 12 ± 22 pg/ml), in patients with isolated chronic glomerulonephritis (creatinine >1.2 mg/dl; BNP 17 ± 23 pg/ml), and in patients on dialysis (BNP 91 ± 93 pg/ml). These authors concluded that BNP is not directly related to renal failure but probably to the hemodynamic stress of dialysis. The results of Cataliotti et al[41] and Mallamaci et al[42] in heart failure patients on hemodialysis showed BNP to increase almost exclusively in the presence of ventricular hypertrophy or systolic dysfunction. In the subgroup of patients on dialysis without ventricular hypertrophy or associated cardiovascular disease, the BNP concentration was similar to that recorded in the controls. The authors thus concluded that BNP does not increase because of renal dysfunction or dialysis considered isolatedly. Since in our patients renal damage fundamentally occurred as a result of the nephrotoxic action of cyclosporine, the mentioned lack of a BNP–creatinine correlation appears logical.

The relationship between BNP concentration and hemodynamic parameters has been controversial. Ationu et al[28] found no correlation between ventricular BNP (closely related to plasma BNP) and the right ventricular pressures or any other hemodynamic variable, while in contrast El Gamel et al[29] obtained a good positive correlation to the transpulmonary gradient—although in both cases the number of patients involved was relatively small.

Park et al[38] recently published a prospective analysis of 87 HT patients with the evaluation of clinical and echocardiographic data, biopsies and BNP levels (237 determinations). Their results are practically analogous to our own, and curiously the mean BNP values are nearly identical (258 ± 276 pg/ ml, median 153 mg/dl). Based on the median, they decided to divide the series into two groups (low BNP: <150 pg/ml and high BNP: $\geqslant 150$ pg/ml). The degree of rejection as evidenced by endomyocardial biopsy and creatinine concentration was similar in both groups—the mean time elapsed from transplantation being significantly less in the high BNP group, thus reflecting the mentioned tendency of BNP concentration to decrease over time. The high BNP group presented significantly greater right

atrial, pulmonary arterial, and pulmonary capillary pressures—this observation is similar to our own identified positive correlation between BNP levels and right pressure values.

Natriuretic peptides possess diuretic, natriuretic, and vasodilatory properties (by inhibiting endothelin and antagonizing the angiotensin–aldosterone system), as well as neuromodulatory effects (by reducing sympathetic tone).[12,14,43] These properties underlie their release and increase in plasma in situations of heart failure of any cause and secondary to either systolic or diastolic failure.[20,44,45] Such peptides are therefore of great diagnostic and prognostic value in non-transplantation settings.

In contrast, in HT, situations of increased pulmonary pressure (prior to transplantation) may be observed, along with restrictive physiology[36] (due to cardiac denervation or cyclosporine). This may lead to diastolic dysfunction or a degree of right ventricle failure and a variable degree of tricuspid valve insufficiency, and thereby producing increased BNP *in the absence of rejection*. Likewise, it is common to identify treatable rejection at biopsy without aberrant clinical, echocardiographic, or hemodynamic features. Thus, normal-range BNP concentrations may occur in these HT patients *in the presence of rejection*. On the other hand, patients sometimes present clinical and echocardiographic criteria of rejection where high BNP values are often seen, which moreover improve after immunosuppressive therapy, where 'true' rejection can be assumed and in which the biopsy (logically performed on a patchy basis) is unable to identify rejection. This situation could be regarded as a false-negative biopsy result, despite the fact that biopsy is considered the gold standard for diagnosing rejection. All these considerations may in part justify the lack of a relationship between BNP concentration and the degree of graft rejection as determined by endomyocardial biopsy. The lack of discriminatory capacity in diagnosing rejection need not lessen the value of monitoring BNP in the follow-up of these patients.

In conclusion, BNP levels remain high at all times after HT with respect to the general population, and tend to stabilize at around 60–120 pg/ml after the fourth month. BNP levels are discretely higher among patients with treatable graft rejection, particularly in the first 90 days, although they lack discriminatory capacity to serve as a guide to endomyocardial biopsy. A direct correlation exists between BNP and the right ventricle and pulmonary arterial pressure values.

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