Background. Nowadays a number of diverse biochemical markers have been identified in patients with heart failure (HF) that could indicate the severity of the patients' illness. Among them, probably the most useful is brain natriuretic peptide (BNP) because it is easily obtained and because of its diagnostic and prognostic information. Our objective was to assess the association between BNP and other different associated variables previously known to be related to the evolution of HF, as well as its utility to distinguish systolic from diastolic HF.

Patients and method. We studied 114 patients admitted consecutively for symptomatic HF for all causes (age: 66 years, male: 60%). In all patients plasma BNP was measured, from the third day of admission, with a specific radioimmunoassay. Echocardiography was performed in 101 patients.

Results. BNP plasma levels increased in proportion to functional class (P = .01) and the degree of left ventricular dysfunction (P = .0001, r = .44). There was also an association between BNP and male sex (P = .008), higher plasma creatinine (P = .01, r = .25), larger ventricular diameters (P = .0001) and higher pulmonary systolic pressure (P = .0001, r = .44). In the multivariate analysis, BNP was independently related to the rest of variables with left systolic ventricular function (P = .0001). Despite this association, we did not find a satisfactory cut-off value in BNP, with a good sensitivity and specificity value from the total number of patients, of which specifically systolic dysfunction as a cause of HF was detected.

Conclusions. a) BNP increases proportionately to the left ventricular dysfunction and HF severity, and b) BNP is not a useful tool to distinguish systolic from diastolic HF.

Key words: Brain natriuretic peptide. Heart Failure. Diagnosis.

Péptido cerebral natriurético. Valor diagnóstico en la insuficiencia cardíaca

Introducción y objetivos. En la actualidad se han identificado diversos marcadores bioquímicos en la insuficiencia cardíaca (IC) que podrían indicarnos la gravedad del paciente. De entre ellos, el probablemente más útil por la facilidad de su obtención e información diagnóstico-prognóstica es el péptido cerebral natriurético (BNP). Nuestro objetivo fue analizar la asociación entre el BNP y diferentes variables asociadas a la IC, así como calcular su poder discriminante del tipo de IC (sístolica frente a diastólica).

Pacientes y método. Estudiamos a 114 pacientes ingresados de forma consecutiva por IC descompensada, cualquiera que fuera su etiología (edad media: 66 años, varones: 60%). En todos ellos se determinó la concentración de BNP, en muestras sanguíneas obtenidas a partir del tercer día de ingreso hospitalario, mediante ensayo radioinmunoassay. En 101 pacientes se realizó estudio ecoangiográfico.

Resultados. Las concentraciones plasmáticas de BNP fueron mayores en pacientes con grados avanzados de estadio funcional (p = 0,01), sexo masculino (p = 0,008), mayor creatinina plasmática (p = 0,01, r = 0,25), peor función sistólica del ventrículo izquierdo (p = 0,0001, r = 0,44), mayores diámetros ventriculares (p = 0,0001) y mayor presión sistólica de la arteria pulmonar (p = 0,001, r = 0,44). En el análisis multivariado, el BNP se asoció de forma independiente del resto de variables con la función sistólica del ventrículo izquierdo (P = 0,0001). A pesar de esta asociación, ningún valor de BNP fue capaz de discriminar con un adecuado valor de sensibilidad y especificidad, del total de pacientes con IC, cuáles presentaban disfunción sistólica.

Conclusiones. a) El BNP aumenta de forma proporcional a la disfunción ventricular izquierda y la gravedad de la insuficiencia cardíaca, y b) el BNP no puede emplearse en el diagnóstico diferencial del tipo de IC (disfunción sistólica frente a diastólica).

Palabras clave: Péptido cerebral natriurético. Insuficiencia cardíaca. Diagnóstico.
neurohumoral mechanisms involved in the pathophysiology of HF. Various studies have identified them as important prognostic markers in chronic HF and after acute myocardial infarction.

Among humoral factors, the family of the natriuretic peptides, of which atrial natriuretic peptide (ANP) was the first discovered, are noteworthy. In 1988 the second component of the family, known as brain natriuretic peptide (BNP), was isolated in porcine brain. However, it soon was identified as a hormone synthesized and released by the heart, especially the ventricle. Both substances have a broad spectrum of biological functions: stimulation of natriuresis and diuresis, vasodilation and reduction of peripheral vascular resistance, and inhibition of the renin-angiotensin-aldosterone and sympathetic nervous systems. They also play an important role in fluid homeostasis and blood pressure. The exact mechanism of stimulation of the synthesis and release of BNP is not clearly defined, although high BNP values are seen in circumstances that course with increased pulmonary wedge pressure, systolic and diastolic dysfunction, left ventricular hypertrophy, and in acute coronary artery syndrome in myocardial infarction and unstable angina (although left ventricular dysfunction does not necessarily have to exist).

Plasma BNP determination has been identified in various studies as an excellent method for screening left ventricular dysfunction in the general population or in patients after myocardial infarction. In fact, it has been reported that a normal BNP value practically excludes the possibility of systolic dysfunction in a patient with dyspnea, or the evolution to heart failure after acute myocardial infarction. It has also been shown to be useful in diagnosing left ventricular hypertrophy in patients with high blood pressure.

BNP has been demonstrated to be an excellent biochemical marker in HF. Plasma BNP values have been associated with the patient’s functional class, degree of left ventricular dysfunction, and various hemodynamic parameters like left ventricular end-diastolic pressure or the tendency toward remodeling after infarction. This reflects the rise in plasma BNP concentration with clinical and hemodynamic deterioration of the patient. Finally, BNP has been shown to be a reliable prognostic indicator in HF, post-myocardial infarction (in the acute and chronic phases), and even the general population.

The first aim of this study was to assess the association between BNP and clinical, analytical, and echocardiographic variables associated with the evolution of HF. The second aim was to assess the effectiveness of BNP in identifying patients admitted for symptomatic HF who present a pathophysiological substrate of systolic dysfunction.

PATIENTS AND METHOD

Patients

The study group included 114 patients (46 women and 68 men, ranging in age from 40 to 90 years, mean 66 years) with heart failure of different causes admitted consecutively to our cardiology service for symptomatic HF. The recommendations of the HF working groups of the European and Spanish societies of cardiology were used to diagnose HF and HF caused by diastolic dysfunction.

The cause of HF was considered ischemic when at least one of the following circumstances existed: history of acute myocardial infarction, typical angina with evidence of ischemia in the baseline or exercise ECG and/or perfusion radionuclide scan, history of significant obstruction of a coronary artery, and previous coronary angioplasty or aortocoronary revascularization surgery. The existence of cardiac valve disease was defined by the presence of a hemodynamically significant valve abnormality. The cause was considered hypertensive in patients with a history of high blood pressure and ventricular hypertrophy confirmed by ECG or echocardiography. Finally, the cause was considered to be dilated cardiomyopathy when there was left ventricular systolic dysfunction with LVEF below 40%-45% and ventricular dilation not attributable to the causes mentioned above.

Consequently, the cause of heart failure was concluded to be ischemic heart disease in 43 patients (in 36 patients with previous myocardial infarction, more than 3 months had to pass from the acute phase), hypertensive heart disease in 20 patients, dilated cardiomyopathy in 22 patients, and HF secondary to cardiac valve disease in 29 patients. Fifty-eight percent of patients received an angiotensin-converting enzyme inhibitor drug: 85%, loop diuretics; 23%, spironolactone; 55%, digitalis; 7%, beta-blockers; 32%, antiplatelet aggregant agents and, finally, 36%, anticoagulant treatment. Routine laboratory tests, a chest radiograph, and electrocardiogram were performed in all patients. The mean hospital stay of patients was 12 days. The other clinical characteristics of the study population are shown in table 1.
Study protocol

Blood was drawn for BNP determinations after day 3 of admission. This moment was selected in view of results published in the literature, which indicate that BNP values vary in the acute phase of the disease, stabilizing around the second or third day. From this time on, hemodynamic variables tended to regularize and adjust to treatment. The blood samples were extracted by peripheral venipuncture after the patient has been laying down for at least 30 min. Various clinical, analytical, and echocardiographic variables linked to the evolution and prognosis of HF were analyzed to confirm their association with BNP concentration.

Echocardiographic study

Echocardiography was performed with a Hewlett Packard Sonos 2.500 instrument in 101 patients for which plasma BNP was obtained, at the indication of the attending cardiologist of each patient. The left ventricular ejection fraction (LVEF) was calculated with the Simpson method (using the two and four-chamber apical views). Ventricular diameters were calculated in M-mode, referred to the plane from the longitudinal parasternal 2D view. Systolic function was defined as conserved when LVEF was more than 55%, and as mildly, moderately, or severely impaired when LVEF was 45%-55%, 35%-45%, or less than 35%, respectively. HF was attributed to systolic dysfunction in patients with LVEF less than 45%.

Blood BNP determination

After drawing blood, samples were centrifuged for 30 min. The plasma was aspirated and stored in plastic tubes at –70°C until later analysis. In healthy subjects, the determination was made previously with different anticoagulants (EDTANa and EDTAK), without observing any differences in the results (coefficient of variation less than 1%). Plasma BNP concentrations were measured with a specific radioimmunometric assay (Shionora Kit). This assay consisted in duplicate BNP determinations using two monoclonal antibodies to recognize the carboxyterminal sequence and annular structure of human BNP. For this purpose, a solid-phase «sandwich» technique was used in which the first antibody is on the «ball» introduced in each test tube (solid phase), and the second antibody is marked with 125I. Excess unbound marker is easily eliminated in the washout phase, while the solid phase retains only the antibody/antigen/marking antibody combination.

According to the manufacturer, the assay sensitivity (minimum detectable amount) was 2 pmol/ml with a 95% probability. Cross-reactivity with ANP and CNP was less than 0.001% for both. The BNP values defined by the manufacturer as normal were less than 18.4 pg/ml.

Statistical analysis

Categorical variables are expressed in percentages and quantitative values as means ± standard deviation. The Student t test was used for comparison of means, and analysis of variance (ANOVA) for comparisons of multiple groups, with the Scheffe test (post hoc). Categorical variables were compared with the 2 test. A Pearson correlation was made for continuous quantitative variables. Multivariate analysis was carried out using multiple linear regression (stepwise method).

Analysis of ROC curves was used to confirm BNP capacity to discern all patients admitted for heart failure, those who presented systolic dysfunction as the fundamental pathophysiological finding. The optimal sensitivity and specificity were estimated by the position on the resulting curve of the minimum distance to the perfect sensitivity and specificity point (100%, 100%). The area under the curve indicated the degree of discrimination of the variable analyzed, ranging
from 0.5, or non-discriminative, to 1.0, fully discriminative.

RESULTS

The characteristics of the study population are summarized in Table 1. Twenty-nine percent of the patients had severely depressed systolic function. A total of 24 patients (21%) were NYHA FC I, while 90 patients were functional class II or III (52% and 31%, respectively). No patient was FC IV. About half of the patients studied had been admitted previously for HF. All the patients included in the study had high plasma BNP values in relation to the upper limit or normality defined by the manufacturer.

In Tables 2 and 3 and in Figures 1 and 2, the results of univariate analysis, in which we evaluated the association between BNP and the other variables, are summarized. We found a significant association between the cause of HF and BNP concentration, in such a way that the patients with ischemic heart disease had the highest BNP values. On the other hand, LV systolic function was worse in patients with ischemic disease or HF secondary to dilated cardiomyopathy, compared with patients with hypertensive and cardiac valve disease (LV shortening fractions 0.20 and 0.21 versus 0.27 and 0.34, respectively; \( P = .0001 \)). Likewise, when LVEF was evaluated for each cause (Figure 3), a significantly higher percentage of patients with ischemic heart disease and dilated cardiomyopathy presented moderate-to-severe depression of LVF (left ventricular function). High BNP values also were associated with advanced functional class (\( P = .01 \)) and male sex (\( P = .008 \)). Plasma creatinine correlated positively with plasma BNP. However, we found no significant association with the other clinical variables evaluated. Among the echocardiographic variables analyzed, BNP correlated positively with ventricular diameters and pulmonary artery systolic pressure, and inversely with the shortening fraction; patients with severely impaired systolic function had the highest BNP values.

Various multiple linear regression models were used for multivariate analysis. When all the clinical variables studied were included, BNP showed a significant association with NYHA FC (\( P = .008 \)), male sex (\( P = .002 \)), and plasma creatinine (\( P = .0001 \)). However, non-significant associations were seen with the cause of HF (\( P = .1 \)), age (\( P = .09 \)), underlying heart disease (\( P = .1 \)), ECG rhythm (\( P = .9 \)), previous admissions for HF and their number (\( P = .7 \) and 0.6, respectively), systolic blood pressure, and heart rate at admission (\( P = .3 \) and 0.6, respectively). A second model included all echocardiographic variables. A significant association was found between BNP concentration and the LV end-systolic diameter (\( P = .002 \)), LVEF (grouped as a categorical variable as described above; \( P = .02 \)), and PASP (\( P = .01 \)). A non-significant association was found between BNP and LV end-diastolic diameter (\( P = .1 \)). Finally, in a third model we included LV shortening fraction (and the rest of the clinical, analytical, and echocardiographic variables) and excluded ventricular diameters (given the colinearity between them and LV shortening fraction). The only variable associated significantly and independently between the rest and BNP concentration was the LV shortening fraction (\( P = .0001 \)).

Diagnostic value of BNP in systolic dysfunction responsible for heart failure

The degree of left ventricular dysfunction was associated with BNP concentration in univariate analysis (LVEF and shortening fraction) and multivariate analysis (shortening fraction). Patients with severe degrees of left ventricular dysfunction, LVEF < 35%,
presented the highest BNP values (467 ± 401 versus 197 ± 342 for patients with conserved systolic function; \( P < .01 \)). It should be emphasized that patients admitted for HF who had normal systolic function presented BNP values clearly higher than those described as normal by the manufacturer. BNP concentrations also were higher in patients with HF due to systolic rather than diastolic failure (413 ± 380 versus 198 ± 320; \( P = .03 \)).

The analysis of ROC curves is shown in Figure 4, in which BNP concentration was used to identify patients admitted for HF due to systolic dysfunction. Although the area under the curve was 0.76 (\( P = .001 \)), no BNP value was sufficiently sensitive or specific. The optimal BNP value found was 143 pg/ml, with a sensitivity of 70% and specificity of 65% for detecting systolic dysfunction. In spite of this, extreme BNP values (over 350 pg/ml) identified systolic dysfunction as the cause of HF with a specificity of more than 90%.

**DISCUSSION**

Our study confirmed the existence of a close relation between BNP concentration and other findings intimately related with the evolution of HF (ventricular function or NYHA FC). These results provide additional information because they included an unselected population of patients with HF due to any cause. However, in spite of the correlation found between BNP and systolic function, no BNP value had discriminant value in differentiating between systolic and diastolic dysfunction as the pathophysiological substrate of HF.

Ischemic heart disease was the most frequent cause of HF in the study population, as has been communicated in most current series. \(^{33-36}\) Ischemic heart disease occurred in 38% of patients. These were the patients with the highest BNP values, although this association was non-significant in multivariate analysis. There is little information about the influence of the cause of HF on BNP concentration. Talwar et al.\(^{33}\) found a grea-
ter concentration of N-proBNP (N-terminal fragment of the prohormone, which increases with BNP) in patients with ischemic heart disease than in patients with arterial hypertension. Earlier experience suggests that in ischemic heart disease BNP concentration increases proportionately to infarction severity or size and that BNP would be a sensitive marker of ventricular remodeling. This could explain in part the higher BNP concentration seen in patients with HF secondary to ischemic heart disease because most of the patients had previous myocardial infarction. Patients with ischemic heart disease had the worst systolic function, which would explain for the most part the higher BNP concentration found.

BNP values rose proportionately to the severity of HF assessed by functional class. Likewise, patients with NYHA FC III had the highest BNP concentration. This association also was significant in the multivariate model, which included the clinical variables evaluated. Men had higher BNP concentrations than women did, a difference that remained significant after adjusting for the rest of the clinical variables (including NYHA FC). However, when echocardiographic variables were introduced in the multivariate analysis, this significance disappeared. The probable cause lay in the differences in left ventricular function between male and female patients, men having a more impaired systolic function (mean LV shortening fraction 0.2 versus 0.3, respectively; P = .0001).

Although atrial fibrillation has been associated with poor prognosis of HF, its presence was not accompanied by a greater BNP concentration. The age of patients was not influential, therefore, in spite of the correlation reported between age and BNP values. In the study population, the hemodynamic circumstances of each patient had more influence on plasma BNP than age.

Previous studies have demonstrated that BNP concentration increases in kidney failure, this circumstance being the second most important after myocardial processes accompanied by high levels of natriuretic peptides. In our study we found a weak, but significant correlation between BNP and plasma creatinine (r = .25), which remained significant after adjusting for other clinical variables and confirmed this claim. Despite this, of all the natriuretic peptides, BNP is probably the least influenced by renal function (as reported by Omland et al), which means that it is capable of providing more exact diagnostic and prognostic information. In fact, the introduction of plasma creatinine in the multivariate models did not obscure the relation between BNP and left ventricular function.

An echocardiographic study was made in 101 patients. We found no difference between the clinical characteristics of this subgroup and the overall group of patients. Of the variables analyzed, BNP correlated significantly with ventricular diameters, PASP, and LV shortening fraction. These results confirm those of other studies in which BNP was correlated with invasive pulmonary artery, left ventricular, and right atrial pressures obtained invasively, in this case PASP using an echocardiographic approach, thus confirming that BNP is a noninvasive hemodynamic marker. When stratifying patients into four groups of different LVEF, we found that BNP concentration was higher with more severe left ventricular dysfunction. The main difference was between patients with HF and conserved LVEF and those with HF and severely depressed LVEF. In the multivariate analysis that included echocardiographic variables, every variable except LV end-diastolic diameter was significantly associated with BNP concentration. The results revealed a close relation between ventricular diameters (especially end-systolic diameters) and BNP, n HF of any cause, not only HF after myocardial infarction as has been reported. When all the variables (clinical, analytical, and echocardiographic variables) were introduced in the model, including LV shortening fraction in place of ventricular diameters, the only one that remained significant was the shortening fraction. In spite of the existence of numerous variables of confusion (such as plasma creatinine), BNP concentration was associated independently with left ventricular function, confirming the close relation between them. Other studies have shown that BNP is the most important independent predictor of left ventricular dysfunction.

The BNP concentration in the study population was clearly higher than the value given as normal by the manufacturer. It was striking that the patients with
conserved or slightly depressed systolic function also had clearly elevated BNP values (197 ± 342 and 199 ± 227 pg/ml). These results provide new evidence of the role that BNP could play in the sometimes complicated diagnosis of diastolic heart failure, in which BNP values are clearly elevated.\textsuperscript{13,39}

In spite of the correlation found between LVF and BNP, and the large difference in BNP concentration between patients with systolic versus diastolic failure, we found that no value offered an adequate sensitivity and specificity when we analyzed the effectiveness of BNP in discriminating HF due to systolic dysfunction. The explanation was that with BNP values between 100 and 300 pg/ml, patients had a highly variable systolic function, ranging from conserved to severely depressed. The existence of confusion factors (principally renal function) did not obscure the association between BNP and left ventricular function, but they did limit the discriminant capacity of BNP in differentiating systolic dysfunction in the overall group of patients. Other studies have found a wide range of LVEF values for intermediate BNP concentrations.\textsuperscript{20,29} In the study by McClure et al.\textsuperscript{40} BNP was incapable of differentiating between patients who, after myocardial infarction, presented mild and moderate left ventricular dysfunction and those with conserved systolic function. Since BNP not only increases in systolic but also diastolic failure, various degrees of diastolic dysfunction for a similar systolic function would produce a variable BNP concentration, limiting the capacity of BNP for identifying systolic failure in patients with HF. Therefore, and although BNP values over 350 pg/ml identified systolic dysfunction with a specificity superior to 90%, it must be concluded that the available information does not support the use of BNP for differentiating between HF due to diastolic failure and HF due to systolic failure.

This association between BNP, HF, and other clinical and echocardiographic variables associated with HF is a probable consequence of its site of synthesis and the mechanisms involved in its release. Thus, although the atrium participates in BNP secretion, its contribution is small because BNP is released fundamentally by the ventricles in proportion to the degree of left ventricular dysfunction.\textsuperscript{21} Sumida et al\textsuperscript{22} found that BNP secretion increased in both the infarcted and non-infarcted areas in patients with previous myocardial infarction. For these authors, the increase in parietal tension or stretching forces that appear around the necrosis or throughout the ventricle as a result of dilation and remodeling stimulates BNP secretion by the left ventricle. Hame et al\textsuperscript{23} found that the expression of BNP mRNA was maximal in the region bordering the infarction area and surrounding tissue. Nagaya et al\textsuperscript{24,25} found that the persistence of high BNP values after the acute phase of infarction predicted the evolution toward progressive ventricular remodeling, speculating that sustained parietal tension leads to the expansion of the infarction area, with subsequent ventricular dilation, and would trigger an increase in BNP values. In our study, BNP was strongly associated with left ventricular diameter, particularly end-diastolic diameter, which indicates that the increase in parietal stress secondary to dilation increased BNP release.

These findings reveal that BNP can act as a non-invasive biochemical marker of myocardial damage. This, together with the small influence of renal function on plasma BNP concentration (in our study renal function had less effect than left ventricular function), probably contributed to the direct association between BNP and other variables related with HF.

\textbf{Study limitations}

In the present study, not all patients underwent echocardiographic study. It is unlikely that this influenced the results because the clinical characteristics of this subgroup did not differ from those of the overall group of patients.

Previous studies have reported that the administration of ACE inhibitors,\textsuperscript{42} digitalis,\textsuperscript{43} or beta-blockers\textsuperscript{44} can modify plasma BNP values, indicating that the potential of BNP as a marker of left ventricular dysfunction or greater mortality is limited in patients treated with these agents. Treatments were not assessed in this study. It does not seem plausible that the inclusion of treatments would have limited the diagnostic value of BNP in multivariate analysis, given previous results that show that the association between BNP and LVEF was not modified by including treatment in the multivariate models.\textsuperscript{22,27}

None of the patients included in the study was NYHA FC IV. This was not due to deliberate exclusion of the most seriously ill patients, but was circumstantial because of the consecutive inclusion of patients.

\textbf{Conclusions and clinical implications}

In view of the data obtained from our study, we conclude that: \textit{a}) \textit{BNP} increased in proportion to the left ventricular dysfunction and severity of the heart failure, and \textit{b}) \textit{BNP} cannot be used for the differential diagnosis of the type of HF (systolic versus diastolic dysfunction).

The strong independent association of plasma BNP with LVEF, lower influence of confusion factors, stability \textit{in vitro}, and ease of analysis (which has increased with new quantification techniques that make low-cost results available in less than 15 min)\textsuperscript{28} suggest that plasma BNP could become a routine test. BNP determinations would complement the information provided by other variables used in the diagnosis of HF.
so it could be included as an important factor in clinical and therapeutic decision-making.

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