Serum N-Terminal Pro–Brain Natriuretic Peptide Is a Sensitive Marker of Myocardial Dysfunction in AL Amyloidosis

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Background—Brain natriuretic peptide (BNP) is a marker of ventricular dysfunction and can be used to assess prognosis in heart failure and after myocardial infarction. Heart involvement is the most important prognostic factor and causes death in almost all patients with light-chain amyloidosis (AL). We investigated the prognostic value of NT-proBNP and its utility in monitoring amyloid heart dysfunction.

Methods and Results—NT-proBNP was quantified at diagnosis in 152 consecutive patients seen at the coordinating center of the Italian Amyloidosis Study Group (Pavia) from 1999 throughout 2001. Heart involvement was estimated on the basis of clinical signs, electrocardiography, and echocardiography. NT-proBNP concentrations differed in patients with (n=90, 59%) and without (n=62, 41%) heart involvement (median: 507.8 pmol/L versus 22.1 pmol/L, $P=10^{-7}$). The best cutoff for heart involvement was at 152 pmol/L (sensitivity: 93.33%, specificity: 90.16%, accuracy: 92.05%) and distinguished two groups with different survival ($P<0.001$). The Cox multivariate model including NT-proBNP was better than models including echocardiographic and clinical signs of heart involvement. NT-proBNP appeared to be more sensitive than conventional echocardiographic parameters in detecting clinical improvement or worsening of amyloid cardiomyopathy during follow-up.

Conclusions—NT-proBNP appeared to be the most sensitive index of myocardial dysfunction and the most powerful prognostic determinant in AL amyloidosis. It adds prognostic information for newly diagnosed patients and can be useful in designing therapeutic strategies and monitoring response. NT-proBNP is a sensitive marker of heart toxicity caused by amyloidogenic light chains. **(*Circulation*. 2003;107:2440-2445.)**

Key Words: amyloid ■ cardiomyopathy ■ natriuretic peptides ■ prognosis ■ survival

Amyloidoses are disorders of protein conformation and metabolism that result in tissue deposition of insoluble fibrils, organ dysfunction, and death. In primary systemic amyloidosis (AL), fibrils are composed mostly by the N-terminus of a monoclonal immunoglobulin light chain.[1] AL is a systemic disease, and most patients have clinical involvement of more than one organ at diagnosis. Half of patients present with various degrees of cardiac amyloidosis at diagnosis, but virtually all eventually die of cardiac-related death.[2] Heart involvement is by far the major prognostic determinant[3] and conditions the therapeutic strategy.

No biochemical marker is presently available for the diagnosis of amyloid heart involvement and the monitoring of cardiac function after therapy. Currently used tools, such as low voltages on ECG, increased interventricular septum (IVS) thickness and "granular sparkling" at echocardiography, have unsatisfactory sensitivity and/or specificity.[2]

Natriuretic peptides, particularly brain (or type B) natriuretic peptide (BNP), have been reported to be very effective in diagnosing ventricular dysfunction[4] and in assessing prognosis of heart failure and after myocardial infarction.[5] Takemura and colleagues[6] demonstrated that the expression of atrial natriuretic peptide (ANP) and BNP was augmented in the ventricular myocytes of patients with cardiac amyloidosis, particularly in regions adjacent to amyloid deposits, and showed that the elevation of BNP was more pronounced than that of ANP.

BNP is initially formed as a prepro-polypeptide that undergoes consecutive proteolytic cleavage steps to produce the active hormone (amino acids 77 to 108) and the N-terminal inactive peptide (NT-proBNP) (amino acids 1 to 76) on release. The N-terminal peptide and the hormone are secreted on an equimolar basis.[7,8] We evaluated the level of NT-proBNP as a marker of cardiac involvement in a consec-

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utive series of 152 patients with AL amyloidosis and assessed its prognostic value.

Methods

Patient Evaluation

NT-proBNP was evaluated at diagnosis in all patients with AL amyloidosis seen between January 1999 and December 2001 at the coordinating center of the Italian Amyloidosis Study Group (Internal Medicine and Medical Oncology, Pavia, Italy). All patients had biopsy-proven amyloidosis in association with a clonal plasma cell disorder. Patients on dialysis were excluded because of altered metabolism of NT-proBNP.[9]

Patients gave consent for the use of their blood samples for experimental purposes, in accordance with Institutional Review Board guidelines.

To define heart involvement, the investigators, who were blind to the NT-proBNP results, relied on the presence of at least one of the following criteria: clinical symptoms of heart failure (severity of symptoms was graded according to the New York Heart Association classification class ≥II), unexplained low voltages on the resting ECG (ie, QRS voltage amplitude in the limb leads ≤0.5 mV, in the absence of pericardial effusion or myxedema), and at least one of the following echocardiographic features: increased IVS thickness in the absence of hypertension, valvular disease or ECG criteria for left ventricular hypertrophy and/or increased myocardial echogenicity (granular sparkling), and/or unexplained increased right ventricular free wall and/or unexplained increased posterior wall.[2] In our laboratory, the intraobserver and interobserver coefficient of variation of the measurements are, respectively, 3.2% and 3.9% for septal wall thickness and 3.4% and 3.9% for posterior wall thickness. Reference limits were ≤12 mm for IVS thickness, <7 mm for the right ventricular free wall, and ≤12 mm for the posterior wall. The ejection fraction was evaluated by the standard 2-dimensional area-length method in the 4-chamber echocardiographic view.

Follow-up visits were scheduled every 3 months. Urine and blood samples were collected for immunofixation and determination of serum creatinine and proteinuria, using routine laboratory methods. Serum and urine monoclonal proteins were detected by high-resolution immunofixation[10] and quantified according to the guidelines of the College of American Pathologists.[11]

Hematological response to treatment was defined as a ≥50% decrease in serum and urine monoclonal component.[12] The response was evaluated every 3 months and established at the nadir of serum and urine monoclonal protein.

Serum NT-proBNP Quantification

Serum samples were frozen immediately and kept at −20°C until NT-proBNP testing. NT-proBNP levels were measured with an electrochemiluminescence sandwich immunoassay (ECLIA, Roche) on an Elecsys System 2010. Serum NT-proBNP levels are typically higher in women and increase with age, therefore upper reference limits (the 97.5 percentiles of healthy subjects) in men and women are, respectively, 10.4 pmol/L and 18 pmol/L in subjects <50 years old; and 26.4 pmol/L and 39.8 pmol/L in individuals >50 years old (data generated by Roche from 712 normal subjects). Within-run precision was determined to be 3.5% at 23.9 pmol/L (22 samples) and 2.6% at 1318 pmol/L (22 samples); between-run precision was 3.9% at 25 pmol/L (22 samples) and 3.1% at 1403.3 pmol/L (22 samples). The detection limit reported by the manufacturer is 0.6 pmol/L.

Statistical Analysis

Differences in mean NT-proBNP concentrations between subgroups were tested for statistical significance by the nonparametric Mann-Whitney *U* test, because NT-proBNP was not normally distributed. Receiver operator characteristic (ROC) analysis was performed to determine the best cutoff values to detect cardiac involvement, defined as reported above, as well as to assess the discrimination ability (by calculating the area under the ROC curve) of NT-proBNP to recognize patients with heart involvement. A "heart involvement score" accounting for relevant echocardiographic (IVS thickness >12 mm and ejection fraction <50%) and clinical features (NYHA class ≥II) was computed on the basis of the presence of 0, 1, 2, or 3 of such features. Cumulative survival probability from diagnosis was calculated by means of the Kaplan-Meier estimator. To identify possible predictors of survival, a series of univariate Cox models were fitted. Subgroup analysis was performed to assess survival from time of response separately in patients with NT-proBNP below and above the cutoff value. Variables with probability values of <0.2 were introduced into multivariate Cox models. Model validation was performed by calculating Maddala-explained variation and LeCessie–van Houwelingen shrinkage coefficient for calibration. Discrimination was assessed graphically by calculating the linear predictor according to the Cox model and plotting Kaplan-Meier curves of the quartiles of its distribution. In patients with NT-proBNP above the cutoff value, NT-proBNP levels before and after treatment were compared by means of the Wilcoxon matched pairs test.

TABLE 1. Main Characteristics of the Studied AL Population

No. of patients	152
Age, median (range)	61 (34–78)
Monoclonal protein in serum or urine	149 (98)
Serum κ/λ, biclonal	106/31 (71/21), 12 (8)
Urine κ/λ, biclonal	109/33 (73/22), 7 (5)
Amyloid organ involvement	
Single/2 or more	54/98 (36/64)
Dominant syndrome	
Kidney	59 (39)
Heart	58 (38)
Liver	11 (7)
Peripheral nervous system	6 (4)
Soft tissues	3 (2)
Skin	3 (2)
Lymph nodes	3 (2)
Lung	3 (2)
Other	6 (4)
Clinical evidence of kidney involvement	94 (62)
Nephrotic syndrome	47 (31)
Serum creatinine >106.1 μmol/L (1.2 mg/dL)	24 (16)
Clinical evidence of heart involvement	90 (59)
Heart failure (≥NYHA class II)	58 (38)
IVS thickness >15 mm	43 (29)
Ejection fraction <50%	35 (23)
Treatment for ≥6 mo	116 (76)
Oral melphalan plus prednisone	54
High-dose dexamethasone	34
Autologous stem cell transplantation	19
Various (thalidomide, iododoxorubicin, immunotherapy, other alkylating agents)	9

Values are n (%) or median (range).

Results

Patient Characteristics and Outcome

One hundred fifty-two patients with AL were included in the study, and their main characteristics are reported in Table 1.

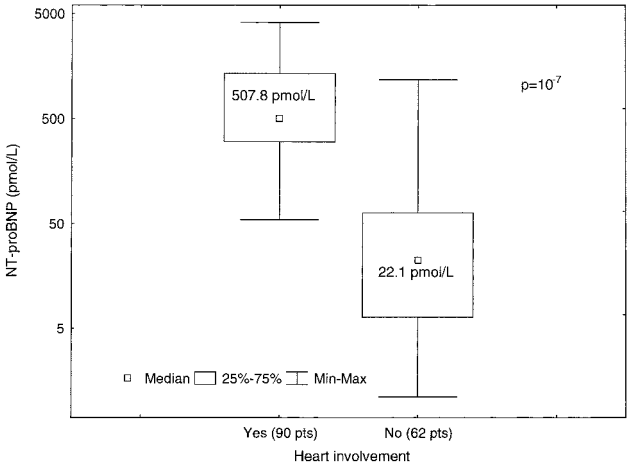

Figure 1. Level of NT-proBNP in patients with AL amyloidosis.

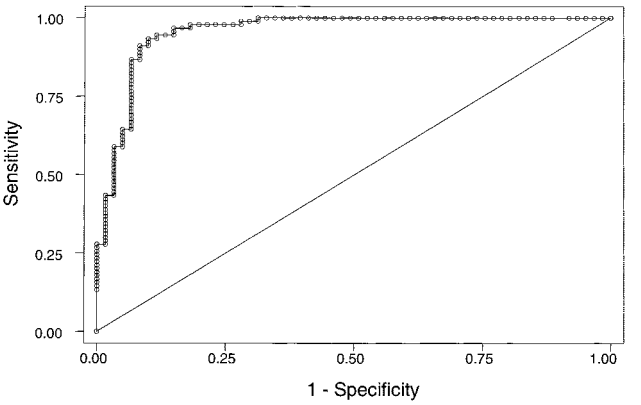

Figure 2. ROC curve based on ln(NT-proBNP). Area under the curve: 0.9561 (95% CI, 0.9209 to 0.9913).

Median age was 61 years, with only 17% of patients <50 years old.

A monoclonal component was found by high-resolution immunofixation in the serum and/or urine of 149 (98%) patients. In the remaining 3 patients, the diagnosis was confirmed by immunoelectronmicroscopy.[13] There was a clear predominance of λ over κ light chains both in serum and urine.

In 98 (64%) patients, more than one organ was involved at diagnosis. Evidence of heart involvement was found in 90 (59%), and heart failure (NYHA class ≥II) was the dominant clinical feature in 58 (38%). Forty-three patients (29%) had an IVS thickness >15 mm, and 35 (23%) had an ejection fraction <50%.

One-hundred 16 patients were treated with chemotherapy for 6 months or more, as reported in Table 1.

The median overall follow-up was 12 months (range, 0.3 to 44.8), 17 (range, 3 to 44) for living patients and 3.5 (range, 0.3 to 44.8) for those who had died. The cause of death was cardiac in 38 (79%) of the 48 patients in whom it was known: 26 patients died of heart failure and 12 of sudden death.

NT-proBNP and Amyloid Heart Involvement

Median NT-proBNP in patients with heart involvement was 507.8 pmol/L (range, 54.5 to 4130 pmol/L) and in the 35 patients with ejection fraction <50% it was 940.1 pmol/L (range, 167 to 4130 pmol/L). Remarkably, in no case was NT-proBNP lower than the 97.5 percentile for normal subjects, indicating 100% sensitivity. By contrast, patients without apparent cardiac amyloidosis had significantly ($P=10^{-7}$) lower NT-proBNP levels (median, 22.1 pmol/L; range, 1.1 to 1175 pmol/L), although there was an overlap with the other group (Figure 1). Because the distribution of NT-proBNP levels within the population was skewed, the logarithm (ln) of its value was used for statistical analysis.

The area under the ROC curve for the detection of heart involvement was high: 0.96 (95% CI, 0.92 to 0.99) (Figure 2). The best cutoff for the diagnosis of heart involvement was 152 pmol/L. With this cutoff, sensitivity was 93.33%, specificity was 90.16%, and accuracy was 92.05%. Using the prevalence (59%) of heart involvement observed in our cohort, the positive and negative predictive values were 93.33% and 90.32%, respectively. Eight patients with normal echocardiograms had NT-proBNP above the cutoff value. However, 4 had clinical signs of heart failure (exertional dyspnea and peripheral edema in all cases plus hepatomegaly in 2 patients), therefore being classified as patients with amyloid heart involvement. The cutoff discriminated two groups with highly significant survival difference: 9 months versus not reached ($P<0.0001$) (Figure 3).

Prognostic Relevance of NT-proBNP

The death rate for patients with NT-proBNP level below and above 152 pmol/L was 7.6 per 100 person-years (95% CI, 3.6 to 15.7) and 72.2 per 100 person-years (95% CI, 54.2 to 86.1), respectively. The Cox univariate model is reported in Table 2. It includes, in addition to the single variables, also the "heart involvement score" as defined in the Methods section. Because ln(NT-proBNP), evidence of heart involvement, the score, IVS thickness, and NT-proBNP cutoff (152 pmol/L) were correlated to each other, 5 competitive Cox multivariate models were generated. The model including ln(NT-proBNP) (Table 2) had the highest prognostic power, according to model validation statistics, even in respect to the model including the "heart involvement score" (data not shown).

Response to treatment resulted in survival benefit both in the group of patients with NT-proBNP level above the cutoff value ($P=0.0002$) (Figure 4A) and in patients with NT-proBNP level below the cutoff value ($P=0.02$) (Figure 4B). Interestingly, none of the patients with NT-proBNP below the cutoff value died after the achievement of hematological response.

Four patients with NT-proBNP serum concentrations above the cutoff value did not have amyloid heart involvement. Their characteristics are reported in Table 3. Two of them, with high serum creatinine, probably had subclinical heart involvement that was not detectable by means of conventional echocardiography and clinical evaluation: One of them died suddenly 6 months later and the other died of heart failure 11 months later. The two remaining patients do not have renal failure or evidence of heart involvement 12 and 10 months after diagnosis, and at present we do not have a plausible explanation for this discrepancy.

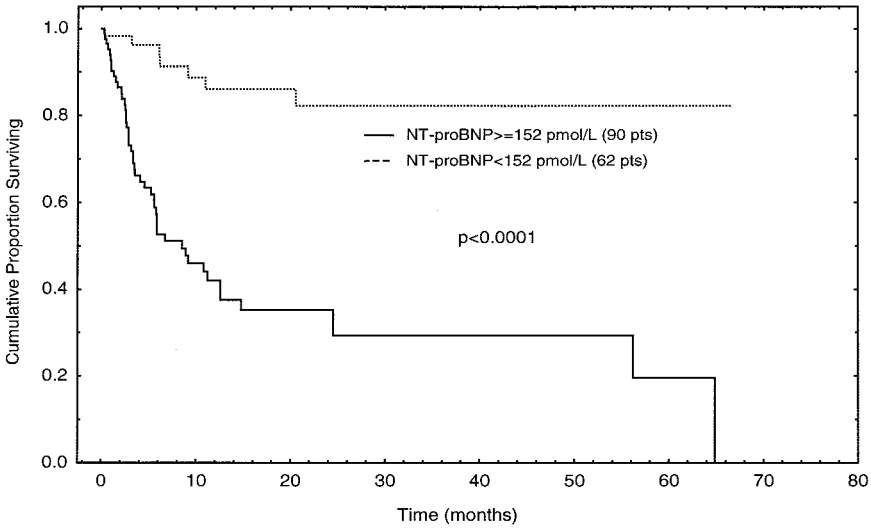

Figure 3. Survival of patients with AL amyloidosis according to NT-proBNP cutoff value (152 pmol/L).

Monitoring the Response to Therapy With NT-proBNP

Variations in NT-proBNP concentration were monitored in 41 patients with a long follow-up. Of these, 21 had a concentration above the cutoff at diagnosis and achieved a hematological response. Among these 21 patients, the reduction of the amyloidogenic protein >50% was accompanied by a significant reduction of NT-proBNP (median, 402.5 pmol/L versus 165.8 pmol/L; $P=0.0001$) in 19 patients (90.5%), whereas the echocardiographic parameters improved (≥3 mm reduction of IVS thickness in all cases and of left ventricular posterior wall in 3 and normalization of ejection fraction in the two patients in whom it was reduced) only in 6 (28.6%) patients. In 9 of the 21 patients, the NT-proBNP dropped below 152 pmol/L. Of the 2 cases without NT-proBNP reduction, 1 had refractory congestive heart failure and died 4 weeks later and the other died of heart failure after 29 months. The modifications of NT-proBNP were closely related to clinical heart manifestations and

TABLE 2. Cox Survival Analysis

Variable	Hazard Ratio	95% CI	*P*
Univariate model			
Male gender	2.25	1.16–4.37	0.010
Age (y)	1.02	1.00–1.05	0.087
ln(NT-proBNP) (pmol/L)	1.68	1.40–2.00	<0.001
NT-proBNP (≥152 pmol/L)	6.80	3.05–15.17	<0.001
Heart involvement (clinical evidence)	7.21	3.24–16.06	<0.001
IVS thickness (mm)	1.14	1.06–1.23	<0.001
NYHA class ≥II	4.28	2.42–7.59	<0.001
Heart involvement score	1.81	1.43–2.3	<0.001
No. of organs involved	1.76	1.38–2.26	<0.001
Weight loss (kg/mo)	2.17	1.27–3.85	0.005
ln(creatinine) (μmol/L)	1.48	0.99–2.22	0.072
Multivariate model			
ln(NT-proBNP) (pmol/L)	1.62	1.33–1.98	<0.001
Male gender	1.93	0.99–3.76	0.054
Age (y)	1.01	0.97–1.04	0.718
Weight loss (kg/mo)	1.56	0.87–2.79	0.137
ln(creatinine) (μmol/L)	0.85	0.54–1.36	0.508

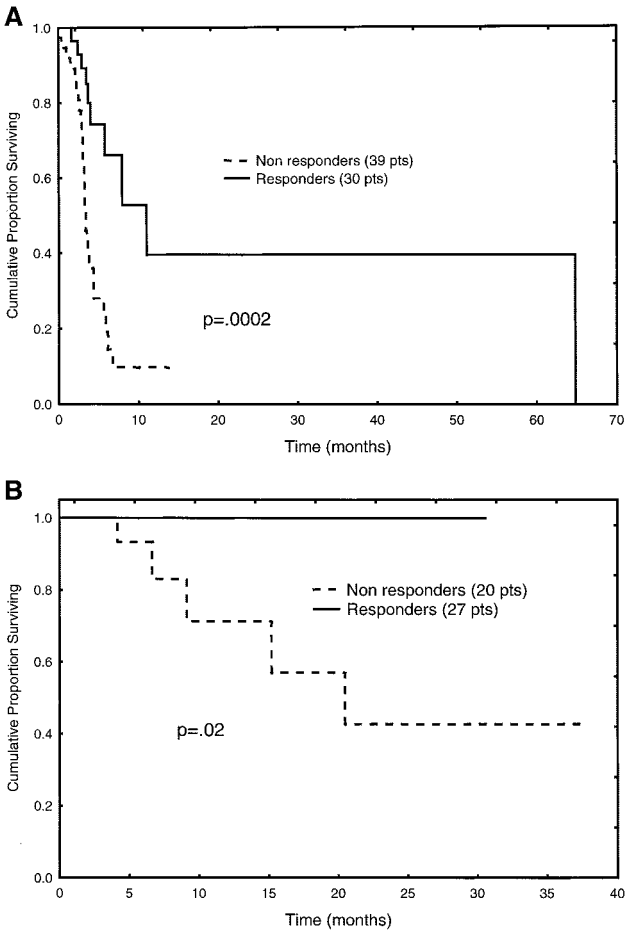

Figure 4. Effect of response to therapy on survival: A, Patients with NT-proBNP ≥152 pmol/L; B, patients with NT-proBNP <152 pmol/L. Hematologic response to treatment was defined as ≥50% decrease in serum and urine monoclonal component.

TABLE 3. Patients Without Clinical Evidence of Heart Involvement and With NT-proBNP ≥152 pmol/L

Patient	Gender	Age, y	Organs Involved	NT-proBNP, pmol/L	IVS Thickness, mm	Ejection Fraction, %	Creatinine, μmol/L	Follow-Up
1	M	62	Kidney	1175.0	11	51	442.0	Sudden death at 6 mo
2	F	67	Kidney	351.3	10	68	238.7	Heart failure and death at 11 mo
3	M	71	Liver	402.5	10	73	75.1	Alive at 12 mo
4	F	66	Kidney	160.9	9	56	68.9	Alive at 10 mo

M indicates male; F, female.

response to therapy. This report gives detailed data of two representative cases.

Patient 1 (Figure 5A) was a 60-year-old woman, diagnosed in July 1998 with multiple myeloma and cardiac amyloidosis (IVS thickness, 14 mm; NYHA class IV). She achieved a hematological response after 8 courses of high-dose dexamethasone with marked improvement of heart dysfunction (NYHA class from IV to II). She relapsed in January 2001 with an increase of monoclonal component and eventually died of heart failure in October 2001. NT-proBNP level paralleled the initial decrease of the serum monoclonal component (NT-proBNP, from 1138 to 423 pmol/L; monoclonal component, from 25 to 6 g/L) and its subsequent progression (NT-proBNP, 1663 pmol/L; monoclonal component, 48 g/L at the time of patient's death). However, the IVS thickness did not change significantly throughout the course of the disease until the last observation 1 month before the patient's death (15 mm).

Patient 2 (Figure 5B) is a 40-year-old man, diagnosed with cardiac amyloidosis in May 1997. He had exertional dyspnea and ventricular arrhythmias (IVS thickness, 20 mm; NYHA class III). He achieved a complete hematological remission after two autologous stem cell transplantations, with improvement of heart function and return to full working activity. NT-proBNP dropped immediately after treatment (from 249 to 89 pmol/L) and is now within the reference range (25.8 pmol/L), whereas IVS thickness, after an initial increase (up to 22 mm), remained stable (18 to 17 mm) throughout the follow-up.

The parallelism between monoclonal component concentration and NT-proBNP observed in the majority of patients suggests a cause-effect relation.

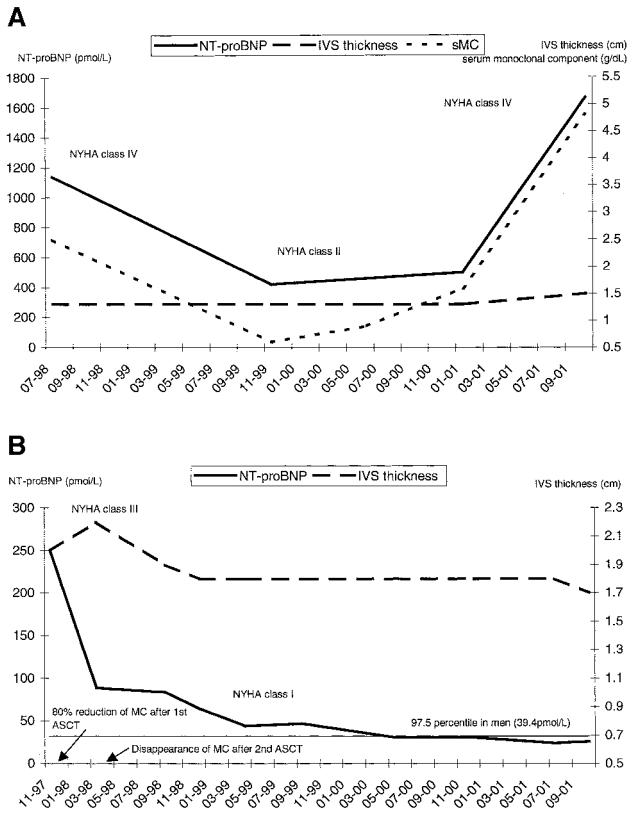

Figure 5. A, Patient 1; B, patient 2. IVS indicates interventricular septum; sMC, serum monoclonal component; NYHA, New York Heart Association; ASCT, autologous stem cell transplantation. See text for patients' clinical history.

Discussion

No biochemical marker is presently available for assessing prognosis and monitoring the effect of therapy on amyloid cardiac dysfunction. The results obtained in this cohort of patients show that (1) NT-proBNP is a sensitive marker of heart dysfunction in amyloid patients, (2) it is the most powerful prognostic factor in AL; (3) increased NT-proBNP levels can detect inapparent heart involvement; and (4) NT-proBNP is useful in monitoring heart dysfunction caused by the amyloidogenic light chains and response to therapy.

NT-proBNP in Diagnosing Amyloid Heart Dysfunction and in Defining Prognosis

The diagnosis of amyloid heart involvement relies on evidence from ECGs, echocardiograms, clinical examination, and the patient's history. The judgment of this evidence requires experienced physicians and is expensive. Moreover, interoperator variability might affect the evaluation of follow-up echocardiograms. The sensitivity (93.33%) and specificity (90.16%) of NT-proBNP quantification are superior to those achievable by measurement of IVS thickness or by the detection of granular sparkling of myocardial texture at echocardiography.[2] Very recently, Koyama and coworkers[14] showed that the quantification of cycle-dependent variations of myocardial integrated backscatter may at least in part overcome the limitation of the somewhat subjective evaluation of indexes of increased myocardial echogenicity. However, the availability of this analysis is presently very limited. Our group reported that ^{99m}Tc-aprotinin scintigraphy has both high sensitivity (95%) and specificity (97%) in detecting

cardiac amyloidosis[15] but scintigraphy is cumbersome, expensive, and not readily available.

NT-proBNP measurement is widely available, extremely robust with an excellent reproducibility, and it is less expensive than echocardiography. As cardiac involvement is an adverse factor for autologous stem cell transplantation,[16] the value of this marker has potential for deciding the initiation of chemotherapy and designing optimal treatment.

According to our data, NT-proBNP is the most powerful tool in prognostic assessment of patients with AL. The multivariate model including ln(NT-proBNP) could discriminate prognostic groups better than the models including IVS thickness, or clinical judgment of heart involvement, or even a score accounting for relevant echocardiographic and clinical evidence of heart involvement. Its extremely wide dynamic range (from 1.1 to 4130 pmol/L) observed in our patient population and excellent reproducibility make NT-proBNP quantification a useful marker for monitoring the effect of treatment on heart involvement. The availability of this biochemical marker should also ease the comparison between the outcomes of different populations of patients with amyloidosis.

NT-proBNP in Follow-Up and Monitoring Response to Therapy

In our study, we observed a tight time correlation between clinical status, modification of monoclonal component, and NT-proBNP level during follow-up. The hematologic response to certain chemotherapy regimens can be achieved in a relatively short period of time; this is not, however, always reflected in the echocardiography pattern, which improves slowly, if ever. In fact, in 90% of patients with NT-proBNP value above the cutoff who achieved hematologic response, the NT-proBNP level markedly decreased, whereas echocardiographic findings did not significantly alter in 71% of them. This indicates that monitoring NT-proBNP significantly contributes to better estimate the effect of therapy.

In addition, these findings provide clues to the pathogenesis of the disease: The NT-proBNP increase in many of the AL amyloidosis patients before treatment may actually reflect the cardiotoxicity of the amyloidogenic light chains. This is concordant with the observation that patients with amyloidosis caused by transthyretin (who do not have pathogenic circulating light chains) may have severe myocardial infiltration but minimal heart failure[17] and with the observation that the infusion of light chains from patients with cardiac amyloidosis rapidly causes diastolic dysfunction in isolated mouse hearts.[18] Thus, heart dysfunction in amyloidosis might not only depend on the extent of amyloid deposition but could be partly related to a toxic effect of amyloidogenic light chains (oligomers?) exerted directly on myocardial cells. This underlines the importance of achieving the disappearance of the monoclonal component with therapies directed against the amyloidogenic plasma cell clone. Our data indicate that NT-proBNP measurement represents a novel powerful tool for assessing the prognosis of patients with AL, for designing optimal treatment and for evaluating response to treatment.

Acknowledgments

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