

LETTER TO THE EDITOR

HEPATITIS C IS ASSOCIATED WITH HIGH LEVELS OF CIRCULATING N-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE AND INTERLEUKIN-6

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To our knowledge, no study has evaluated N-terminal pro-brain natriuretic peptide (NTproBNP) together with interleukin-6 (IL-6) and interferon (IFN)-gamma serum levels in a large series of patients with hepatitis C virus (HCV) as possible markers of cardiac dysfunction. NTproBNP and IL-6 serum levels were valued in 55 HCV-patients, and in 55 sex- and age-matched controls. HCV-patients showed significantly higher mean NTproBNP and IL-6 levels than controls ($P = 0.001$); no significant difference was observed for IFN-gamma. By defining high NTproBNP level as a value higher than 300 pg/mL (that is used to rule out heart failure in patients under 75 years of age), 12% (6/49) of HCV-patients and 0 of controls had NTproBNP (χ^2 ; $P = 0.012$). In conclusion, this study demonstrates high levels of circulating NTproBNP and IL-6 in HCV-patients. The increase of NTproBNP may indicate the presence of a subclinical cardiac dysfunction. Further prospective studies quantifying symptoms and correlating these with echocardiographic parameters are needed to confirm this association.

Several studies have suggested that hepatitis C virus (HCV) infection is frequently found in patients with dilated cardiomyopathy (1, 2), and moreover, it has been suggested that HCV infection may contribute to the development of this unusual form of myocarditis (3-6).

Furthermore, it has been shown that interleukin-6 (IL-6) and NTproBNP are independent predictors of long-term risk of death or heart failure (HF) (7, 8).

However, to date, to our knowledge, no study has evaluated NTproBNP together with IL-6 in HCV, while only few studies have evaluated the importance of IL-6 in HCV-patients, most of which showed high IL-6 levels in these patients (9, 10).

The aim of this study is to evaluate serum levels of NTproBNP and IL-6 in a series of HCV-patients, and to correlate these parameters with the clinical features of the disease.

Key words: N-terminal pro-brain natriuretic peptide, HCV chronic infection, cytokines, heart failure, cardiac dysfunction, IL-6, Interferon-gamma

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MATERIALS AND METHODS

Patients

Fifty-five HCV-patients (39 F and 16 M; mean age 56 ± 10 SD years), consecutively referred to the Department of Internal Medicine, University of Pisa, Italy, were enrolled in the study between 2001 and 2006. HCV-patients without cryoglobulinemic syndrome (without other liver infection) were selected from a larger cohort of 491 chronic hepatitis referrals, without liver cirrhosis (by histology, laboratory evidence of liver failure and/or ultrasound-proven portal hypertension) (11); exclusion criteria were cancer, clinical and laboratory evidence of thyroid disorders and/or autoimmune diseases, treatment with immunomodulators, arterial hypertension, renal disorders, or diabetes. Forty/55 (73%) HCV-patients underwent liver biopsy for diagnostic purposes; liver histology activity index (grade), or stage of liver fibrosis were evaluated according to Ishak et al. (12). The mean activity index (grade) in HCV-patients was 5.1 ± 1.2 , and the stage was 2.0 ± 0.2 . Routine blood chemistry was carried out by standard methods.

Controls

Each of the 55 HCV-patients eligible for the study was matched, by sex and age, one-to-one with a control group of healthy subjects of the general population from the same geographic area (North-West Tuscany). This control group was extracted from a larger sample of 1640 HCV-negative subjects in a population-based survey of thyroid disorders; exclusion criteria were the same as those of the HCV patients. When more than one age-match was available per case, the choice was made at random.

The study protocol was approved by the local Ethics Committee. All subjects gave their informed consent to enter the study.

Virological studies

Antibodies against HCV (Anti-HCV) and HCV-RNA were determined on serum clotted and centrifuged at 37°C and stored at -80°C . Anti-HCV and HCV-RNA (polymerase chain reaction -PCR- technique) in the serum were investigated as previously described (11).

Cytokines, chemokines and analytical assays

Blood samples for analysis of plasma NTproBNP were collected and centrifuged, and plasma was stored at -80°C until analysis. Circulating concentrations of NTproBNP were measured by a sandwich immunoassay on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany).

Serum IL-6 and interferon (IFN)-gamma levels were assayed by a quantitative sandwich immunoassay using commercially available kits (R&D Systems, Minneapolis,

MN, USA), with a sensitivity of 0.7pg/mL , and 9pg/mL , respectively.

Serum IFN-gamma concentrations were measured using commercially available kits (R&D Systems). The mean minimum detectable dose was 9pg/mL for IFN-gamma; the intra- and inter-assay coefficients of variation were 2.8% and 6.1%, respectively. Samples were assayed in duplicate. Quality control pools of low, normal, or high concentration for all parameters were included in each assay.

Data analysis

Values are given as mean \pm SD for normally distributed variables, or as median \pm IQR for not normally distributed variables (NTproBNP, IL-6). Group values were compared by univariate ANOVA, for normally distributed variables; or by Kruskal-Wallis (≥ 3 groups) or Mann-Whitney U (2 groups) tests. Proportions were compared by the χ^2 test. *Post-hoc* comparisons on normally distributed variables were carried out using the Bonferroni-Dunn test. Univariate analysis was performed by simple regression. A Spearman rank correlation for NTproBNP and IL-6 was made. A multivariate logistic regression analysis considering age, gender, ALT, and presence or absence of high levels of NTproBNP or IL-6 as dependent variables was carried out on HCV-patients.

RESULTS

Plasma NTproBNP concentrations were significantly ($P < 0.001$) higher in HCV-patients (mean 120 ± 174 ng/L; median 38pg/mL , range 7-1891), than in controls (mean 17 ± 19 ng/L; median 2.7ng/L , range 1.2-121) (Fig. 1).

By defining high NTproBNP level as a value higher than 125pg/mL [the single cut-off point for outpatients under 75 years of age (13)] 20/35 HCV-patients and 5/50 controls had high NTproBNP (χ^2 ; $P < 0.001$).

With a cut-off point of 300pg/mL [that is used to rule out HF in patients under 75 years of age (13)] 6/49 HCV-patients and 0/55 controls had high NTproBNP (χ^2 ; $P = 0.012$).

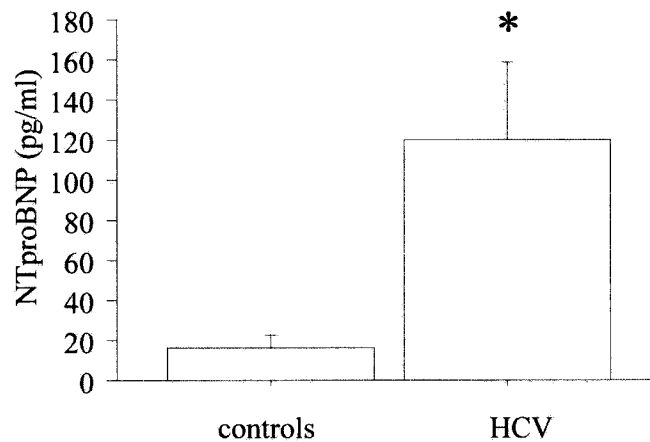
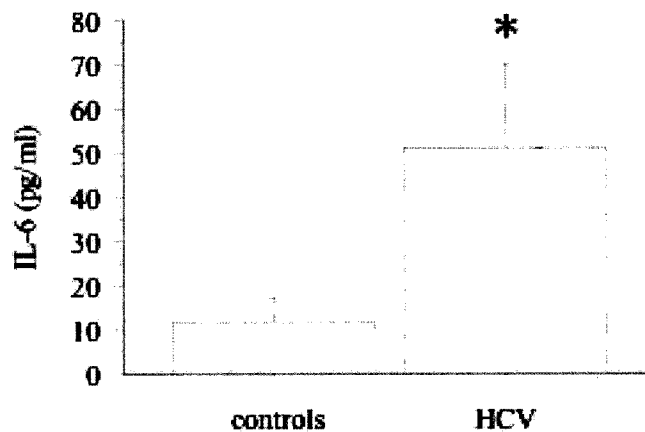
With a cut-off point of 900pg/mL [that should be used for ruling in HF in patients aged 50-75; such as the patients of our study (13)], 4/51 HCV-patients and 0/55 controls had high NTproBNP (χ^2 ; $P = 0.042$) (Table I).

In order to better define the role of increased serum NTproBNP in HCV-patients, mean levels of NTproBNP were separately evaluated (by Mann-Whitney U test) among HCV-patient subgroups

Table I. The prevalence of HCV-patients with high circulating NTproBNP levels was higher than in controls.

NTproBNP cut-off point	HCV+ n=55	Controls n=55	χ^2 P value
> 125 pg/mL	20/35	5/50	< 0.001
> 300 pg/mL	6/49	0/55	0.012
> 900 pg/mL	4/51	0/55	0.042

> 125 pg/mL the single cut-off point for outpatients under 75 years of age; or > 300 pg/mL, that is used to rule out HF in patients under 75 years of age; or > 900 pg/mL, that should be used for ruling in HF in patients aged 50-75.

**Fig. 1.** Plasma NTproBNP concentrations in HCV-patients and controls. HCV-patients showed significantly higher mean (\pm SE) NTproBNP serum levels than controls ($P < 0.001$; Mann-Whitney U test).**Fig. 2.** IL-6 serum levels in HCV-patients and controls. HCV-patients showed significantly higher mean (\pm SE) IL-6 serum levels than controls ($P = 0.005$; Mann-Whitney U test).

defined according to main demographic and clinical features (age > 55 years; gender; disease duration >10 years; aminotransferase elevation and/or histologic activity in the liver), but no significance was found. No significant correlations were observed between NTproBNP and serological findings of HCV-patients or previous/ongoing treatments.

Patients with HCV showed significantly ($P = 0.005$; Mann-Whitney U test) higher mean IL-6 serum levels (mean 54 ± 16 ng/L; median 10ng/L, range 0.6-452), than controls (mean 11 ± 6 ng/L; median 0.9ng/L, range 0.6-43) (Fig. 2). By defining high IL-6 level as a value higher than the 95th percentile of the control group (> 23 pg/mL), 79% of HCV-patients and 6% of the control subjects had a high IL-6 level ($P < 0.001$; χ^2).

In order to better define the role of increased serum IL-6 in HCV-patients, mean levels of this chemokine were separately evaluated (by Mann-Whitney U test) among HCV-patient subgroups defined according to the main demographic and clinical features (age > 55 years; gender; disease duration >10 years; aminotransferase elevation and/or histologic activity in the liver), but no significance was found. No significant correlations were observed between IL-6 and serological findings of HCV-patients or previous/ongoing treatments.

HCV-patients had, obviously, raised ALT enzymes ($P < 0.0001$), in comparison with controls. Elevated levels of NTproBNP or IL-6 were not associated with serum HCV-RNA level or HCV genotype. No association was observed between NTproBNP or IL-6 and ALT levels in HCV-patients.

Spearman rank correlation for NTproBNP and IL-6 was made showing not significant results (Rho 0.133; Z-value 1.01; $P = 0.31$).

IFN-gamma was detectable in the serum of 9% of controls, 16% of HCV+ patients; IFN-gamma levels were not significantly different in the two groups. No relation was found between IFN-gamma and NTproBNP or IL-6.

DISCUSSION

Our study demonstrates significantly high serum levels of NTproBNP together with IL-6 in patients with HCV-patients compared to healthy controls. Some authors have recently stated that NTproBNP

appears superior to BNP for the evaluation of suspected acute HF in patients with preserved left ventricular ejection fraction (13). The International Collaborative for NTproBNP Study (ICON) helped to define appropriate cut-off points for NTproBNP in the emergency department (13): 300pg/mL should be used to rule out HF, while 450pg/mL, 900pg/mL, or 1800pg/mL - depending on age (< 50, 50-75, or > 75 years; respectively) - should be applied for ruling in HF. For outpatient evaluation, the manufacturer suggested a single cut-off point of 125pg/mL for patients under 75 years of age and 450pg/mL for patients over 75 years of age (13).

The levels of NTproBNP found in HCV-patients are comprised in a gray zone (125-900 pg/mL), that is not necessarily associated with HF, however in 4/55 (7%) of HCV-patients the NTproBNP levels were higher than 900pg/mL, which is the cut-off value for ruling HF in patients aged 50-75, such as the patients in our study. Since, NTproBNP seems to correlate with HF severity, the values in the gray zone may be suggestive of a subclinical cardiac impairment.

Our data agree with other studies in that HCV infection may contribute to the development of this unusual form of myocarditis, frequently associated with myocardial antibodies and responsive to immunosuppressive therapy (3-6). In fact, the present study suggests that HCV may be an important agent in causing dilated cardiomyopathy and chronic HF and that elevated plasma NTproBNP levels may correlate with the clinical features of the disease. Other studies have suggested that HCV infection is frequently found in patients with dilated cardiomyopathy (1, 2). However, other studies were not able to find an association between HCV infection and dilated cardiomyopathy (14, 15). In a retrospective study, it was shown that NTproBNP is a sensitive marker of myocardial injury in patients with HF from HCV myocarditis (4).

Furthermore, elevated levels of NTproBNP during and after interferon-based antiviral therapy of HCV have been found, suggesting the presence of cardiac dysfunction (16). However, no conclusion could be drawn in this study regarding baseline NTproBNP levels in HCV-patients lacking an internal control (16). Interestingly, the median NTproBNP levels in our HCV-patients are quite similar to those of the baseline value of the patients of that study (36 vs

37 pg/mL; respectively), and another study of our group (17). Furthermore, NTproBNP levels in HCV patients were similar to those observed in HCV-associated cryoglobulinemia (18).

The findings of the present study may have important implications for patients with HCV. Most patients complain about fatigue, dyspnea and reduced physical capacity. The pathogenesis of these symptoms is not well understood and is sometimes attributed to the liver injury. However, it seems possible that these patients experience cardiac impairment at least contributing to these symptoms. Testing of NTproBNP may serve as a screening marker for cardiac insufficiency in the differential diagnosis of fatigue and dyspnea and may alleviate the decision for further diagnostic testing of cardiac function as it has been described for other groups of patients (19, 20). Besides diagnostic consequences, evaluation of NTproBNP may have therapeutic consequences for patients with HCV. In patients with known congestive HF, elevated plasma BNP concentrations could be reduced by treatment with ACE inhibitors (21), and angiotensin II receptor antagonists (22) as well as treatment with diuretics and vasodilators (23). Cytokines play an important role in chronic HF. High levels of IL-6 have been shown in HF by several studies (7, 8). Our results showing high levels of circulating IL-6 in HCV-patients are in agreement with other studies present in literature on HCV-patients, that have demonstrated high circulating levels in these patients (9, 10). However, few studies reported no increase of IL-6 levels in HCV (24).

It has been hypothesized that IL-6, as a potent proinflammatory cytokine, by itself may exacerbate the damage resulting from minor myocardial necrosis/injury or may itself stimulate muscle atrophy and myocardial failure during the development of HF. IL-6 and NTproBNP have been shown to be independent predictors of long-term risk of death for HF (7). This finding is in agreement with the results of our study; in fact, no relationship was observed between IL-6 and NTproBNP.

It has been shown that the combining measurements of IL-6 and NTproBNP seems to be a promising tool in the prognostic assessment of HF patients (8). However, even if we have shown that NTproBNP and IL-6 are both high in the circulation

of HCV-patients, we cannot exclude that different pathways may be differently involved in the increase of each of them.

In conclusion, this is the first study reporting elevated levels of NTproBNP in HCV-patients in association with IL-6. This may indicate the presence of cardiac dysfunction and explain, at least in part, some of the clinical symptoms of patients with HCV (1, 2, 4, 25). Further prospective studies quantifying the symptoms and correlating them with echocardiographic parameters are needed to confirm this association.

REFERENCES

1. Matsumori A, Matoba Y, Sasayama S. Dilated cardiomyopathy associated with hepatitis C virus infection. *Circulation* 1995; 92:2519-25.
2. Matsumori A, Sasayama S. Newer aspects of pathogenesis of heart failure: hepatitis C virus infection in myocarditis and cardiomyopathy. *J Card Fail* 1996; 2(S4):S187-94.
3. Okabe M, Fukuda K, Arakawa K, Kikuchi M. Chronic variant of myocarditis associated with hepatitis C virus infection. *Circulation* 1997; 96:22-4.
4. Matsumori A, Shimada T, Chapman NM, Tracy SM, Mason JW. Myocarditis and heart failure associated with hepatitis C virus infection. *J Card Fail* 2006; 12:293-8.
5. Frustaci A, Calabrese F, Chimenti C, Pieroni M, Thiene G, Maseri A. Lone hepatitis C virus myocarditis responsive to immunosuppressive therapy. *Chest* 2002; 122:1348-56.
6. Frustaci A, Chimenti C, Calabrese F, Pieroni M, Thiene G, Maseri A. Immunosuppressive therapy for active lymphocytic myocarditis: virological and immunologic profile of responders versus nonresponders. *Circulation* 2003; 107:857-63.
7. Kavsak PA, Ko DT, Newman AM, Palomaki GE, Lustig V, MacRae AR, Jaffe AS. Risk stratification for heart failure and death in an acute coronary syndrome population using inflammatory cytokines and N-terminal pro-brain natriuretic peptide. *Clin Chem* 2007; 53:2112-8.
8. Miettinen KH, Lassus J, Harjola VP, Siirilä-Waris K, Melin J, Punnonen KR, Nieminen MS, Laakso M,

- Peuhkurinen KJ. Prognostic role of pro- and anti-inflammatory cytokines and their polymorphisms in acute decompensated heart failure. *Eur J Heart Fail* 2008; 10:396-403.
9. Falasca K, Ucciferri C, Dalessandro M, Zingariello P, Mancino P, Petrarca C, Pizzigallo E, Conti P, Vecchiet J. Cytokine patterns correlate with liver damage in patients with chronic hepatitis B and C. *Ann Clin Lab Sci* 2006; 36:144-50.
 10. Feldmann G, Nischalke HD, Nattermann J, et al. Induction of interleukin-6 by hepatitis C virus core protein in hepatitis C-associated mixed cryoglobulinemia and B-cell non-Hodgkin's lymphoma. *Clin Cancer Res* 2006; 12:4491-8.
 11. Antonelli A, Ferri C, Pampana A, Fallahi P, Nesti C, Pasquini M, Marchi S, Ferrannini E. Thyroid disorders in chronic hepatitis C. *Am J Med* 2004; 117:10-3.
 12. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; 22:696-9.
 13. Januzzi JL Jr, Camargo CA, Anwaruddin S, et al. The N-terminal Pro-BNP investigation of dyspnea in the emergency department (PRIDE) study. *Am J Cardiol* 2005; 95:948-54.
 14. Reis FJ, Viana M, Oliveira M, Sousa TA, Paraná R. Prevalence of hepatitis C and B virus infection in patients with idiopathic dilated cardiomyopathy in Brazil: a pilot study. *Braz J Infect Dis* 2007; 11:318-21.
 15. Prati D, Poli F, Farma E, et al. Multicenter study on hepatitis C virus infection in patients with dilated cardiomyopathy. North Italy Transplant Program (NITP). *J Med Virol* 1999; 58:116-20.
 16. Bojunga J, Sarrazin C, Hess G, Zeuzem S. Elevated plasma levels of N-terminal pro-brain natriuretic peptide in patients with chronic hepatitis C during interferon-based antiviral therapy. *World J Gastroenterol* 2006; 12:5875-7.
 17. Antonelli A, Ferri C, Ferrari SM, Marchi S, De Bortoli N, Sansonno D, Chiavacci C, Ferrannini E, Fallahi P. N-terminal pro-brain natriuretic peptide and tumor necrosis factor-alpha both are increased in patients with Hepatitis C. *J Interferon Cytokine Res* 2010; 30:359-63.
 18. Antonelli A, Ferri C, Ferrari SM, Ghiri E, Galetta F, Franzoni F, Santoro G, Fallahi P. High circulating levels of N-terminal pro-brain natriuretic peptide and interleukin 6 in patients with mixed cryoglobulinemia. *J Med Virol* 2010; 82:297-303.
 19. Cabanes L, Richaud-Thiriez B, Fulla Y, Heloire F, Vuilleumard C, Weber S, Dusser D. Brain natriuretic peptide blood levels in the differential diagnosis of dyspnea. *Chest* 2001; 120:2047-50.
 20. Morrison LK, Harrison A, Krishnaswamy P, Kazanegra R, Clopton P, Maisel A. Utility of a rapid B-natriuretic peptide assay in differentiating congestive heart failure from lung disease in patients presenting with dyspnea. *J Am Coll Cardiol* 2002; 39:202-9.
 21. Motwani JG, McAlpine H, Kennedy N, Struthers AD. Plasma brain natriuretic peptide as an indicator for angiotensin-converting-enzyme inhibition after myocardial infarction. *Lancet* 1993; 341:1109-13.
 22. Latini R, Masson S, Anand I, et al. Effects of valsartan on circulating brain natriuretic peptide and norepinephrine in symptomatic chronic heart failure: the Valsartan Heart Failure Trial (Val-HeFT). *Circulation* 2002; 106:2454-8.
 23. Johnson W, Omland T, Hall C, et al. Neurohormonal activation rapidly decreases after intravenous therapy with diuretics and vasodilators for class IV heart failure. *J Am Coll Cardiol* 2002; 39:1623-9.
 24. Cotler SJ, Reddy KR, McCone J, et al. An analysis of acute changes in interleukin-6 levels after treatment of hepatitis C with consensus interferon. *J Interferon Cytokine Res* 2001; 21:1011-9.
 25. Antonelli A, Ferri C, Ferrari SM, Colaci M, Sansonno D, Fallahi P. Endocrine manifestations of hepatitis C virus infection. *Nat Clin Pract Endocrinol Metab* 2009; 5:26-34.