The biomarker N-terminal pro-brain natriuretic peptide and liver diseases

Abstract

Purpose: NT-proBNP has emerged as a powerful diagnostic and prognostic biomarker in heart disease. Studies showed that NT-proBNP is a sensitive biomarker for identifying patients with heart failure caused by hepatitis C virus (HCV) related myocarditis. The purpose of this study was to evaluate the correlation between the serum concentration of NT-proBNP and hepatitis virus infection/liver disease.

Methods: 223 serum samples from blood donors (aged 19~50 years old) were collected as a control group, and 644 samples were obtained from patients infected by hepatitis viruses including 493 HBV: 364 chronic hepatitis (CH), 86 hepatocellular carcinoma (HCC) and 43 liver cirrhosis (LC) and 151 HCV (85 CH, 14 HCC, 52 LC). All samples were assayed with an Elecsys immunoassay analyzer for NT-proBNP concentration.

Results: The mean concentration of NT-proBNP in the control group was 21.77 pg/ml and showed no significant variation with either age or gender. Both the mean value and the rate of abnormality of NT-proBNP were significantly higher for the HBV- and HCV-infected groups in comparison with the control group. The mean NT-proBNP value (380.24 pg/ml) and abnormality rate (38.41%) in the HCV group were higher than that of the HBV group. For samples from patients with HBV/HCV-related hepatic disease/pathology, the mean NT-proBNP value (517.19 pg/ml/597.18 pg/ml) were the highest in the liver cirrhosis group.

Conclusions: Hepatic pathologic lesions, particularly cirrhosis, may contribute to the elevation of NT-proBNP in subjects with HBV/HCV infection.
Natriuretic peptides are a group of structurally related but genetically distinct peptides. A total of four types of natriuretic peptides have been found. They are synthesized as high molecular weight precursors that undergo processing into their active forms. Brain natriuretic peptide (BNP) is one of these natriuretic peptides. Although it was first found in the porcine brain [1], it is produced primarily within the heart, and is released into the circulation in response to increased cardiac wall tension [1-3]. Some studies have shown that BNP is secreted not only from the atria, but also from the ventricles, especially in patients with heart failure [4, 5]. ProBNP is the precursor of BNP, and NT-proBNP results from cleavage of ProBNP by protease. NT-proBNP, a 76 amino acid peptide, is the second polypeptide that results from cleavage of the original 108 amino acid preprobrain natriuretic peptide, and it is rapidly released in response to pressure overload, intravascular volume expansion and myocardial ischemia, mainly from myocytes of the cardiac ventricles [6]. ProBNP is stored in secretory granules in myocytes. After synthesis in the ventricle, proBNP is cleaved by a protease into NT-proBNP, the biologically active form, and BNP, the biologically inactive portion of proBNP. NT-proBNP has a longer half-life than BNP, as the latter is degraded by neutral endopeptidase [7]. As a quantitative laboratory test, this molecule may be a useful direct biomarker for macrovascular complications as it has such advantages as greater stability and reliability and has recently shown promise as a more discriminating marker of early cardiac dysfunction than BNP [8]. NT-proBNP has emerged as powerful diagnostic and prognostic biomarker in heart disease. Clinical applications that are currently under investigation include its use as a diagnostic test for the presence of heart failure in newly symptomatic patients, use for risk stratification for prognosis in both recent cardiac decompensation and in chronic heart failure, use for prognostic evaluation after acute coronary events, for monitoring and adjustment of therapy in heart failure, and for screening of asymptomatic at risk populations for significant cardiac impairment and risk stratification [9-15].

In addition, an emerging body of literature suggests that plasma NT-proBNP measurements may have prognostic use in conditions other than heart failure and acute coronary syndromes. Some studies report that increases in NT-proBNP are of prognostic significance in pulmonary embolism [16-18]. Other studies show that NT-proBNP is a prognostic marker of cardiovascular morbidity in hypertension [19]. Moreover, recent data suggest that NT-proBNP may also identify subjects who are at increased risk for cardiovascular morbidity and mortality but who have no evident cardiac abnormalities [20]. Studies have also shown that NT-proBNP concentration are significantly higher in all patients with heart failure caused by hepatitis C virus (HCV) related myocarditis in comparison with patients without HCV infection [21]. These reports stimulated our interest in study of the relationship among the biomarker NT-proBNP and HCV infection, hepatitis caused by viral infections, and liver disease.

The aim of this study was, therefore, to evaluate the correlation between the concentration of NT-proBNP and hepatitis virus infection. This study included three parts. First, serum samples from blood donors were used to establish reference values for NT-proBNP in the general Chinese population. Second, the concentration of NT-proBNP was compared for healthy individuals and patients infected by hepatitis viruses. Finally, the correlation between NT-proBNP levels and various kinds of hepatic pathology/diseases was analyzed.

Patients and controls

This study has been approved by, and carried out according to the instructions of, the institutional Human Investigations/ Ethics Committee of Peking University Health Science Center, China.

To investigate the mean value of NT-proBNP in the general Chinese population, 223 serum samples were collected from blood donors of the Beijing Red Cross Blood Center as a normal control group including 84 women and 139 men, all of whom were between 19 and 50 years of age. The samples were divided into three groups by age (19-29, 30-50, 19-50 years), and these groups were further subdivided according to gender.

To compare the concentration of NT-proBNP among the groups of different hepatitis virus infections, 644 samples were selected from 1056 patients infected by hepatitis viruses, including 493 HBV (364 chronic hepatitis-CH, 86 hepatocellular carcinoma-HCC, and 43 liver cirrhosis-UC), 151 HCV (85 CH, 14 HCC, 52 LC). There were total 95 samples from LC patients: most were in the stage of Child-Pugh A, with less than 10% in the stage of Child-Pugh B. For this study, 644 samples were selected from patients under 50 years old without evident cardiac abnormalities (based on ECG examination and chest X-ray).

All patients were diagnosed according to criteria set forth in “The guideline on the prevention and treatment of hepatitis C” and “The guideline on the prevention and treatment of chronic hepatitis B” [22, 23]. The diagnoses of liver cirrhosis were made by histological examinations, imaging procedures, and several liver function tests. Patients with liver cirrhosis were staged according to the Child-Pugh classification. Patients with HCC were diagnosed histologically by biopsy and surgical specimens.
and clinically by ultrasonography and/or computed tomography scanning in a regular examination and this was combined with the measurement of AFP.

All the samples collected in this study were evaluated for markers of hepatitis virus, including HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb, by means of a chemiluminescent micro-particle immunoassay (CMIA, Roche Diagnostics, Switzerland), and an enzyme-linked immunosorbent assay for evaluation of anti-HCV (ELISA, Abbott Laboratories, USA). The samples were selected for normal control if the results for the above tests were normal.

**Methods**

Blood samples for measurement of NT-proBNP were centrifuged at 3,000g, and the supernatants were stored at -80°C until use for pooled analysis. NT-proBNP was measured by a double antibody sandwich technique using electrochemiluminescence as the signal (Elecsys 2010, proBNP, Roche Diagnostics) according to the manufacturer’s recommendations. This test uses two polyclonal antibodies that bind to the NT-proBNP peptide and form a stable sandwich complex. The sensitivity of the test is 5pg/ml, and the intra-assay and inter-assay coefficients of variance at 175 pg/ml are listed by the manufacturer as 2.7% and 3.2%, respectively. This test, moreover, shows no cross reactivity with other hormones.

A serum concentration of NT-proBNP greater than or equal to 53 pg/ml was defined as abnormal in this study. Continuous data are presented as the median and interquartile range (range from the 25th to the 75th percentile). The Student’s t-test was used for comparison of quantitative variables. The chi-square test was applied to examine qualitative differences and abnormal distribution data were analysed by a non-parametric test. P values <0.05 were taken to be statistically significant, and all values are expressed as mean (SD) or median (interquartile range). Statistical analyses were performed with SPSS 15.0 for Windows software (SPSS, USA).

### TABLE 1. NT-proBNP serum levels in general population stratified by age and sex

<table>
<thead>
<tr>
<th>NT-proBNP Concentration</th>
<th>Age 19-29 yr</th>
<th>Age 30-50 yr</th>
<th>Age 19-50 yr</th>
<th>Age 19-50 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (IQR) (pg/ml)</td>
<td>15.40 (11.61-24.81)</td>
<td>15.80 (12.62-31.89)</td>
<td>16.26 (12.54-24.70)</td>
<td>16.46 (12.0-25.9)</td>
</tr>
<tr>
<td>Mean (SD) (pg/ml)</td>
<td>20.17 (13.63)</td>
<td>25.70 (26.39)</td>
<td>19.91 (15.16)</td>
<td>24.30 (15.68)</td>
</tr>
<tr>
<td>MAX/MIN (pg/ml)</td>
<td>95.81/5.00</td>
<td>126.79/7.66</td>
<td>77.55/7.05</td>
<td>77.55/7.05</td>
</tr>
<tr>
<td>Median value &gt;53 pg/ml (n)</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>95th percentile (pg/ml)</td>
<td>47.14</td>
<td>58.3</td>
<td>39.67</td>
<td>53.9</td>
</tr>
</tbody>
</table>

### TABLE 2. Concentration of NT-proBNP in patients with viral hepatitis infection and HBV/HCV-related liver disease

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Cases</th>
<th>NT-proBNP Abnormality % (n)</th>
<th>MAX/MIN (pg/ml)</th>
<th>NT-proBNP value Mean (SD) (pg/ml)</th>
<th>NT-proBNP value Median (IQR) (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>223</td>
<td>5.59 (8)</td>
<td>133.9/5.06</td>
<td>21.77 (15.56)</td>
<td>16.46 (12.0-25.9)</td>
</tr>
<tr>
<td>HBV</td>
<td>493</td>
<td>32.25 (159)</td>
<td>17321/5</td>
<td>129.16 (889.35)</td>
<td>35.72 (21.2-25.9)</td>
</tr>
<tr>
<td>CH</td>
<td>364</td>
<td>24.73 (90)</td>
<td>9220/6.01</td>
<td>82.17 (409.49)</td>
<td>30.50 (19.41-55.28)</td>
</tr>
<tr>
<td>HCC</td>
<td>86</td>
<td>51.16 (44)</td>
<td>1368/5</td>
<td>134.01 (229.4)</td>
<td>63.52 (29.81-128.9)</td>
</tr>
<tr>
<td>LC</td>
<td>43</td>
<td>58.14 (25)</td>
<td>17321/14.09</td>
<td>517.19 (2626.65)</td>
<td>69.62 (34.23-159.1)</td>
</tr>
<tr>
<td>HCV</td>
<td>151</td>
<td>38.41 (58)</td>
<td>32829/9.01</td>
<td>346.80 (2735.71)</td>
<td>44.76 (21.17-80.17)</td>
</tr>
<tr>
<td>CH</td>
<td>52</td>
<td>3.85 (8)</td>
<td>1429/13.77</td>
<td>66.49 (198.46)</td>
<td>34.58 (20.71-76.63)</td>
</tr>
<tr>
<td>HCC</td>
<td>14</td>
<td>50.0 (7)</td>
<td>1459/41.60</td>
<td>268.59 (394.02)</td>
<td>60.45 (25.73-361.75)</td>
</tr>
<tr>
<td>LC</td>
<td>85</td>
<td>57.65 (49)</td>
<td>32839/9.01</td>
<td>561.65 (3637.46)</td>
<td>64.43 (33.05-110.95)</td>
</tr>
</tbody>
</table>
Results

The NT-proBNP level in the general Chinese population

Serum samples of 223 blood donors, collected as a normal control group, were analyzed for concentration of NT-proBNP. The upper limit for the normals (mean +/- 2 SD: 21.77 +/- 31.36) was assessed as 52.93 pg/ml (53 pg/ml) in this study. 8/223 (96%) of the samples’ NT-proBNP value were under 53 pg/ml in this group (Table 1). The concentration of NT-proBNP showed neither age- nor gender-related differences in the general population under 50 years (age groups 19-29, 30-50 years). In comparing any two groups, the P values were >0.05.

Overall comparison of NT-proBNP values in patients with hepatitis virus infection

To evaluate the relationship between the concentration of NT-proBNP and different types of viral hepatitis infection, serum concentrations of NT-proBNP from two groups of patients with HBV and HCV infection were compared. The results

FIGURE 1. NT-proBNP values in groups of viral hepatitis. The values of NT-proBNP in the HBV- and HCV-infected groups were significantly higher than those of the control group (P<0.05).
showed that the concentrations of NT-proBNP were apparently different in these three groups (Table 2). Based on the results from the first part in this study, only eight of the 223 samples from the general population had NT-proBNP values over 53 pg/ml, giving an NT-proBNP value abnormality rate of 3.59%. The NT-proBNP abnormality rate, however, was higher in the groups of hepatitis virus infection than in the control group, and was 32.25% for HBV group and 38.41% for HCV group. Similarly, the mean values of NT-proBNP in the HBV (129.16 pg/ml) and HCV (346.80 pg/ml) infected groups were significantly higher than in the healthy groups (Fig.1).

**Comparison of NT-proBNP values in HBV/HCV-infected individuals with different hepatic disease/pathology status**

To further assess the association between the NT-proBNP value and liver diseases, samples from patients with different hepatic diseases/pathologic status, including HBV/HCV re-
lated CH (364/52), HCC (86/14) and LC (43/85), were compared. It was clear that both the NT-proBNP values and the rates of abnormality in these disease groups were significantly higher than in the control group (P<0.05). The NT-proBNP values in the LC group were the highest among these groups (Table 2). While comparing the NT-proBNP value of LC group with other two groups (CH and control), the difference was statistically significant (P<0.05) but there was no significant difference between the LC and HCC groups (P>0.05) (Fig 2).

Discussion
Recent studies have suggested that elevated NT-proBNP levels may identify subjects without evident cardiac abnormalities who are at increased risk for cardiovascular morbidity and mortality [20]. In addition, one study showed that NT-proBNP levels were higher than 55 pg/ml in all patients with CHF caused by HCV-related myocarditis [21]. This finding suggested the value of studying the relationship among the biomarker NT-proBNP and HCV infection and liver disease.

Since there has been no previous report on the serum concentration of NT-proBNP in the general population in China, the NT-proBNP concentration was measured in 223 samples from healthy blood donors at the Beijing Red Cross Blood Center. Our results showed that in the general population, 96% of individuals aged 19-50 years showed NT-proBNP concentrations less than 53 pg/ml. The mean value was 21.77 pg/ml for these normal blood donors, and the NT-proBNP values showed no significant correlation with age or gender. This result was similar to that reported in previous studies but in different populations, [10, 24-26] although some studies have shown that concentrations of NT-proBNP increase with increasing age, notably in women over 45 years old [27-30]. The reasons for this elevation with age and female gender remain unclear. Because the subjects in this study were limited to ages between 19 and 50 years, the results here may provide a practical reference index for NT-proBNP values for clinical diagnosis in the general population under 50 years of age, regardless of gender.

To clarify the relationship between the concentration of NT-proBNP and hepatitis virus infection, NT-proBNP values were measured in patients with HBV and HCV infections. The NT-proBNP values for both HCV and HBV groups were found to be significantly higher than the control group, and the NT-proBNP value from the HCV group was much higher than that from the HBV group. These results suggest that the elevation of NT-proBNP was related to both HCV and HBV infection. Because samples in the HBV and HCV groups were all selected from patients under 50 years of age, without evident cardiac abnormalities, the elevation of NT-proBNP in the group of HBV/HCV infection may be caused by hepatic pathological lesions.

To investigate this possible link between NT-proBNP levels and hepatic lesions, the relationship between the biomarker NT-proBNP and different hepatic diseases was studied. The samples from patients with HBV/HCV infection were subdivided into different hepatic pathologic conditions and disease status, including HBV/HCV-related CH (the samples in this group were from patients positive for serum HBsAg, HBeAg/ anti-HBe, anti-HBc, anti-HCV and with normal levels of ALT and AST without diagnosed cirrhosis), LC and HCC, and compared the results from these three groups with the control group. Our results showed that both the value and the abnormality of NT-proBNP in HBV/HCV-related diseases groups were significantly higher than that in the normal group. The NT-proBNP level in advanced hepatic diseases was significantly higher than that of the control and chronic hepatitis groups. Importantly, both the value and the abnormality of NT-proBNP in LC group were the highest among the diseases groups, the mean value was over 500 pg/ml and the abnormality was up to 60%. There were no significant differences for either the NT-proBNP levels or abnormalities between LC and HCC groups. Since more than 80% of the patients with HCC had progressed from LC; therefore, these results strongly suggest that hepatic pathologic lesions, particularly cirrhosis, contribute to the elevation of NT-proBNP in patients with liver diseases. This hypothesis is in accordance with Galasko’s explanation for increased NT-proBNP levels with age [30] and is also consistent with the results of Bernal’s study where elevated levels of NT-proBNP were found to be independently related to the severity of cirrhosis [31]. Since elevated levels of NT-proBNP have been regarded as a risk factor for death, the extremely high levels of NT-proBNP in patients with LC predict poor outcomes. The possibility that some patients with progressed liver disease in this study might have had altered cardiac output cannot be completely excluded; thus, the exact mechanism of NT-proBNP elevation in patients with liver disease requires further study.

A limitation of this study is that, the relationship between NT-proBNP levels and increasing age could not be analysed due to the relatively limited age distribution of healthy individuals. A second limitation is that the number of subjects in each group of liver disease was small, which may have affected the significance of the results.

In summary, there was no gender-specific differences in concentration of NT-proBNP in the general population under
50 years of age. The mean values of NT-proBNP for the groups with HBV and HCV infection, as well as hepatic pathology groups with LC and HCC, were significantly higher than the control group; this was especially true for the LC group with the highest value of NT-proBNP, which suggests that cirrhosis may contribute to the elevation of NT-proBNP in subjects with HBV/HCV infection. These data are consistent with the point of view expressed by de Lemos [32] that further investigation of non-cardiac sources, which might account for variation in NT-proBNP, is warranted.

Acknowledgments

The authors thank Dr. Michael A. McNutt for critically proof-reading this manuscript. This work was funded by the National Science Foundation of China (Grant No. 2008ZX10002-012 & 2008ZX10002-013).

References

19. Talwar S, Siebenhofer A, Williams B, Ng L. Influence of hypertension, left ventricular hypertrophy, and left ventricular systolic dysfunction on plasma N terminal proBNP. Heart 2000;83:278-282
27. de Lemos JA, Hildebrandt P. Amino-terminal pro-B-type natriuretic peptides: testing in general populations. Am J Cardiol 2008;101:16-20