Reference Values and Release Kinetics of B-Type Natriuretic Peptide Signal Peptide in Patients with Acute Myocardial Infarction

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BACKGROUND: The signal peptide for human B-type natriuretic peptide preprohormone (BNPsp), which is released from cardiomyocytes, is increased in plasma of patients with acute myocardial infarction (AMI); however, its exact release kinetics have not been defined.

METHODS: We measured BNPsp and high-sensitivity cardiac troponin T (hs-cTnT) in a reference group of individuals without structural heart disease (n = 285) and determined the release kinetics of these biomarkers in patients (n = 29) with hypertrophic obstructive cardiomyopathy undergoing transcatheter ablation of septal hypertrophy (TASH), a procedure allowing exact timing of onset of iatrogenic AMI. Blood samples were collected before TASH and at numerous preselected time points after TASH.

RESULTS: The reference median BNPsp concentration was 53.4 pmol/L [interquartile range (IQR) 47.0–61.0; 95th percentile 85.9 pmol/L; 99th percentile 116.3 pmol/L]. Baseline concentrations in patients undergoing TASH were higher than in the reference group [91.9 pmol/L (IQR 62.9–116.4); P < 0.0001]. BNPsp increased significantly, peaking at 15 min after induction of AMI [149.6 pmol/L (109.5–204.9) vs baseline; P = 0.004] and declining slowly thereafter, falling below the preprocedural value after 8 h (P = 0.014). hs-cTnT increased significantly 15 min after induction of AMI [26 ng/L (19–39) vs 18 ng/L (11–29); P = 0.001] and remained high at all later time points.

CONCLUSIONS: BNPsp concentrations increased immediately after AMI induction, providing early evidence of myocardial injury. The release kinetics of BNPsp differed from those of hs-cTnT. These findings provide information that should help in establishing the diagnostic value of BNPsp in the setting of early AMI.

Acute chest pain is one of the most common causes of admission to an emergency department (1). The biochemical risk stratification of these patients is mainly driven by determination of cardiac troponin I (cTnI)6, cardiac troponin T (cTnT), B-type cardiac natriuretic peptides (BNP), and N-terminal pro-BNP (NT-proBNP). In particular, the introduction of newer, more sensitive troponin assays has proven to facilitate the early diagnosis of an acute myocardial infarction (AMI) (2–4). This improved discrimination relies mainly on the superior sensitivity of these assays; however, additional diagnostic validation is required with routine clinical chemistry testing after the initial presentation because of the reduced specificity of these tests (approximately 60% of presentations with increased hsTnT results do not have AMI) and a diagnostic gap during the first few hours (5).

Addressing the limitation imposed by the loss of specificity, several studies showed that a multimarker strategy improved diagnostic accuracy in terms of diagnosing or ruling out AMI (6–9).

In this context, a previous study demonstrated increases in the signal peptide for human BNP preprohormone [BNPsp(17–26), here abbreviated BNPsp] in the acute phase of myocardial infarction preceding standard biomarkers of myocardial injury (cardiac troponin) (10). The signal peptide sequence of BNP is present not only...
in cytosolic extracts of tissue from explanted human hearts but also in the circulation of healthy human individuals (10, 11). Furthermore, as BNPsp concentrations increase earlier than standardized biomarkers in AMI patients, the establishment of reference values at early time points is of great clinical interest (10, 12).

We recently reported the early release kinetics of several biomarkers, including cTnT and NT-proBNP, in patients undergoing transcoronary ablation of septal hypertrophy (TASH) as a model for patients with AMI (13–17). There are currently no data, however, regarding the exact release kinetics of BNPsp in patients with AMI. Because of the inaccurate definition of the exact time point of the beginning of myocardial ischemia, and because of patient-related delay before presentation to the hospital, the early release kinetics of BNPsp in human AMI are not well described. Therefore, the objective of the present study was to establish reference values for BNPsp in patients without structural heart disease and, further, to characterize the time course of changes in plasma BNPsp in patients undergoing TASH as a model of patients with AMI.

**Methods**

**REFERENCE GROUP**

From July 2009 to January 2014, 5329 patients were referred to the Kerckhoff Heart and Thorax Center for elective coronary angiography and participation in blood-based biomarker studies. From this population, we selected 285 individuals who in several imaging modalities showed no evidence of structural heart disease and were therefore included in the study as a reference group to establish reference values for BNPsp. Coronary angiography, echocardiography, and measurement of high-sensitivity (hs)-cTnT and NT-proBNP were performed for all individuals. Patients in the reference group were subdivided into patients with hs-cTnT values above and below the assay limit of blank (LOB) for further analysis (Fig. 1). Basic biochemistry and hematologic testing indicated that all individuals were free of renal or liver dysfunction and had healthy blood values. All individuals from the reference group provided written informed consent for their participation in the study, and approval of the institutional ethics board (FF 43/2010) was obtained. The investigation conforms to the principles outlined in the Declaration of Helsinki.

**PATIENTS WITH HYPERTROPHIC OBSTRUCTIVE CARDIOMYOPATHY UNDERGOING TASH**

Another group of 29 patients with hypertrophic obstructive cardiomyopathy (HOCM) who were scheduled for TASH from January 2010 to January 2014 were included in the study. Pre- and postprocedural management as well as the proof of concept of the TASH method as an equivalent for AMI have been recently published (13–17). In brief, a clinical history and the results of a physical examination, 12-lead electrocardiography, laboratory tests, echocardiography, and coronary angiography were assessed for all patients. The final diagnosis of HOCM was made according to the current guidelines on the basis of severe symptoms during physical activity, asymmetrical septal hypertrophy >15 mm, systolic movement of the anterior mitral valve leaflet, and an intraventricular pressure gradient of ≥30 mmHg at rest and/or >50 mmHg after provocation by the Valsalva maneuver (18). All patients received analgesic and anxiolytic pretreatment. Patients were free of renal or liver dysfunction and had healthy blood values. TASH was performed according to standard clinical practice with temporary septal branch occlusion for selective therapeutic injection of 96% ethanol. All TASH procedures were
performed in a single session with a single septal branch occlusion. During the procedure, the mean (SD) volume of ethanol administered was 1.67 (0.52) mL. The median occlusion time was 15.0 min [interquartile range (IQR) 12.0–26.0 min]. Postprocedural management included monitoring in the intensive care unit for 48 h. The pre- and postprocedural data documented the success of the procedure as a reduction in the mean intraventricular pressure gradient.

**BLOOD SAMPLE COLLECTION AND PROCESSING**

We collected venous blood samples for determination of BNPsp and hs-cTnT in gel-filled tubes without additives and in EDTA-filled tubes before the procedure; at 15, 30, 45, 60, 75, 90, and 105 min; and at 2, 4, 8, and 24 h after induction of myocardial infarction. In the control group, we collected venous blood samples for determination of hs-cTnT and BNPsp in gel-filled tubes without additives and in EDTA-filled tubes before coronary angiography. Serum or plasma samples were processed immediately and frozen at −80 °C until assay.

**BNPsp MEASUREMENTS**

BNPsp was measured at the Christchurch Heart Institute, New Zealand, via specific immunoassay as previously described (10) with a methodological change in sample processing before assay. The samples were air-shipped on dry ice within 36 h without thawing. To maximize recovery and reduce nonspecific interactions, plasma samples were mixed with an equal volume of 0.1 mL HCl and centrifuged to remove high molecular weight protein debris before extraction on Sep-Pak C18 cartridges. This method modification improved recovery of synthetic and endogenous BNPsp from 74% to 90% and stabilized resulting assay samples through reduced protein interference. Because of the improved recoveries, a new reference group was required to establish appropriate healthy range and 99th percentile values, as reported here.

**hs-cTnT MEASUREMENTS**

We measured cTnT in serum with a high-sensitivity electrochemiluminescence immunoassay (Elecsys Analyzer 2010, Roche Diagnostics). For the hs-cTnT assay, LOB was 3.0 ng/L, limit of detection was 5.0 ng/L, and limit of quantification was 13.0 ng/L. The lowest concentration measurable with a CV <10% for this assay is 13.5 ng/L (13). The recommended clinical decision limit to rule out AMI with this assay is 14.0 ng/L.

**NT-proBNP MEASUREMENTS**

We measured NT-proBNP in serum with an electrochemiluminescence immunoassay that uses monoclonal antibodies (Elecsys Analyzer 2010, Roche Diagnostics). The lower detection limit for the NT-proBNP assay is 5.0 ng/L, and concentrations above the upper limit of the analytical measurement range are reported as >35 000 ng/L. The lowest concentration measurable with a CV of 20% for this assay is 50.0 ng/L. At the cutoff value of 150 ng/L, the CV is <3%. The upper reference limit is 300.0 ng/L.

**STATISTICAL ANALYSIS**

All data for continuous variables are expressed as mean (SD) or median (IQR), as appropriate. Categorical variables are reported as n (%). Continuous variables were compared with the Wilcoxon signed-rank test. Within-individual comparisons were made across repeated observations without correction for multiple comparisons. The TASH cohort data including BNPsp and hs-cTnT were tested for conformity with the normal distribution by applying the Kolmogorov–Smirnov test. All statistical tests were performed with SPSS software, version 22.0. A 2-tailed P value <0.05 was considered to be statistically significant.

**Results**

Table 1 shows the clinical characteristics of the reference group, consisting of 285 individuals [117 men, 168 women; age 58.9 (11.1) years] with no coronary artery disease, hs-cTnT concentrations <99th percentile, and NT-proBNP concentrations below the upper reference limit. Individuals in the subgroup with hs-cTnT concentrations below the LOB (n = 220; <3 ng/L) were younger (P < 0.0001), had a lower heart rate (P = 0.004), and had lower systolic blood pressure at rest (P < 0.0001). Furthermore, the prevalence of arterial hypertension (P = 0.014) was lower, and they had better renal function (P < 0.0001).

The reference group had a median plasma BNPsp concentration of 53.4 pmol/L (IQR 47.0—61.0) with a 95th percentile of 85.9 pmol/L and a 99th percentile of 116.3 pmol/L. Individuals with hs-cTnT concentrations below the limit of detection had median BNPsp 52.0 pmol/L (IQR 46.6—59.5) with a 99th percentile of 92.1 pmol/L. The distribution pattern of BNPsp in these individuals is depicted in Fig. 2. Plasma concentrations of BNPsp correlated positively with hs-cTnT concentrations (r = 0.135; P = 0.023) and age (r = 0.119; P = 0.045). There was no difference in BNPsp between sexes (P = 0.29). Healthy volunteers ≥65 years old (n = 92) had higher BNPsp values [55.8 pmol/L (IQR 48.7–67.9)] than those <65 years old (n = 193) [52.3 pmol/L (46.7–59.5); P = 0.03].

Dividing the reference group into those with hs-cTnT below the LOB and those with hs-cTnT above the LOB, BNPsp concentrations were slightly but significantly lower in the group with hs-cTnT below the LOB.
[52.0 pmol/L (IQR 46.6–59.5) vs 56.4 pmol/L (49.6–69.8); \(P = 0.014\)] (Fig. 1).

Table 2 shows the clinical characteristics of all 29 TASH patients [13 men, 16 women; age 60.4 (13.4) years]. As expected, creatine kinase (CK) serum concentrations significantly increased 1 day after TASH compared with baseline values [maximal postprocedural CK 672.5 U/L (IQR 453.0–846.3) vs baseline CK 93.0 U/L (IQR 71.5–135.5); \(P = 0.014\)]. Pre- and postprocedural echocardiographic findings are given in Supplemental Table 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol61/issue12.

hs-cTnT was increased in TASH patients at baseline [18 ng/L (IQR 11–31)]. Measurement of serum cTnT concentrations by the high-sensitivity assay revealed a significant increase at 15 min compared with baseline concentrations [27 ng/L (IQR 20–42) vs 18 ng/L (IQR 11–31); \(P = 0.001\)] after induction of myocardial infarction, with a continuous rise at all prespecified time points (Fig. 3). In all patients, there was a significant increase in the hs-cTnT concentration of \(\geq 50\%\) compared with the baseline value after 30 min [range of percent increase (minimum–maximum) 57.3%–1331.7%; range of absolute increase (minimum–maximum) 9–137 ng/L]. The hs-cTnT concentrations measured 30 min postpro-
were >99th percentile in all patients. Twenty (68.9%) patients had hs-cTnT concentrations >99th percentile at baseline; however, the interaction term for time point of blood draw and increased baseline cTnT was not significant (P for interaction = 0.31). Therefore, the change in hs-cTnT in patients with increased baseline hs-cTnT was not significantly larger than in patients without increased baseline cTnT (<14 ng/L).

Preprocedural BNPsp concentrations were significantly higher in patients with HOCM undergoing TASH [91.9 pmol/L (IQR 62.9–116.4)] compared with the reference group (P < 0.0001). Fifteen (51.7%) patients had BNPsp concentrations >99th percentile at baseline. Plasma BNPsp concentrations were significantly increased 15 min after induction of AMI compared with baseline values by 8 h after initiation of myocardial infarction (P = 0.014) (Table 3). Twenty-one of 29 patients showed a significant increase in BNPsp of >50% compared with the baseline value after 15 min [range of percent increase (minimum–maximum) 54.4%–412.8%; range of absolute increase (minimum–maximum) 63.3–309.6 pmol/L]. At 24 h, all TASH patients had BNPsp concentrations comparable to those of the reference group [48.6 (42.3–56.1); P = 0.62].

We observed a highly significant difference in the kinetics of the 2 biomarkers (P < 0.0001, Friedman test for multiple comparisons). In addition, we performed a correlation analysis between the maximum hs-cTnT concentration, representing the extent of myocardial injury, and BNPsp concentrations for the first 240 min after induction of AMI. BNPsp and hs-cTnT concentrations were not significantly correlated (r = 0.304, P = 0.22).

Pre- and postprocedural echocardiographic data are presented in the online Supplemental Table 1. BNPsp at baseline correlated with pressure gradient during Valsalva (r = 0.440; P = 0.06) and myocardial mass (r = 0.431; P = 0.07), but without reaching significance. BNPsp was not correlated with NT-proBNP at baseline (r = 0.285; P = 0.13) or pressure gradient at rest (r = 0.291; P = 0.19). BNPsp 24 h after the TASH procedure did not correlate with NT-proBNP at 24 h (r = 0.273; P = 0.31). Similar results were found for BNPsp 24 h after TASH and left ventricular outflow tract pressure gradient, both at rest (r = −0.213; P = 0.29) and during Valsalva (r = −0.195; P = 0.47), and myocardial mass (r = 0.067; P = 0.74).

Discussion

Our study reports reference values of BNPsp established in a group without any structural heart disease to enable...
a comparison with patients with heart disease such as HOCM or AMI. We describe the early release kinetics of BNPsp after induction of myocardial infarction in a cohort of patients undergoing TASH, a clinical model of AMI. This is the first study to document the precise early time course for this potentially valuable biomarker of AMI.

There is an ongoing discussion about how to define the health or “normality” of the reference population that is used to establish accurate reference values for cardiac biomarkers (19–21). First, the reference population should be free of structural heart disease. To investigate this characteristic, we used several surrogate markers to screen for a reference population without structural heart disease, including biomarkers and imaging modalities. The individuals were characterized with echocardiography and coronary angiography to exclude cardiac abnormalities. For more extensive screening, we measured cTnT by a high-sensitivity assay and divided the population into those with hs-cTnT concentrations below and above the LOB (3 ng/L). Because various circulating biomarkers (especially cTnT and NT-proBNP) are sensitive to changes in glomerular filtration rate (GFR) (22, 23), we also assessed renal function. The median and 99th percentile concentration of BNPsp was greater in individuals with hs-cTnT concentrations ≥3 ng/L than in those with hs-cTnT concentrations <3 ng/L. Sex and age have been identified as important factors in the selection of reference individuals (24, 25). We observed a significant correlation between BNPsp concentration and age but not sex. In comparing the median and 99th percentile concentrations among different age groups, we found that these values were higher in individuals ≥65 years old than in younger individuals.

Patients undergoing TASH had significantly higher preprocedural values of BNPsp compared with the reference group, which may reflect myocardial wall stress from the high left ventricular outflow tract gradient or increased overall myocardial mass. This is consistent with the previously described higher NT-proBNP values in this study group (15). BNPsp at baseline correlated with pressure gradient during Valsalva and myocardial mass,

<p>| Table 3. Concentrations of the indicated biomarkers in 29 patients undergoing TASH. |
|---------------------------------|-----------------|------------------|</p>
<table>
<thead>
<tr>
<th>Variable</th>
<th>BNPsp, pmol/L</th>
<th>hs-cTnT, ng/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Median (IQR)</td>
<td>Minimum-maximum</td>
</tr>
<tr>
<td>9.1 (62.9–116.4)</td>
<td>43.3–270.5</td>
<td>18 (11–29)</td>
</tr>
<tr>
<td>149.6 (109.5–204.9)</td>
<td>78.6–384.6</td>
<td>26 (19–39)</td>
</tr>
<tr>
<td>135.4 (105.7–180.7)</td>
<td>62.0–316.9</td>
<td>51 (33–72)</td>
</tr>
<tr>
<td>130.2 (98.6–158.9)</td>
<td>59.3–298.5</td>
<td>83 (68–112)</td>
</tr>
<tr>
<td>131.1 (88.5–150.5)</td>
<td>60.6–380.6</td>
<td>118 (80–174)</td>
</tr>
<tr>
<td>123.5 (85.1–154.3)</td>
<td>63.8–224.6</td>
<td>149 (109–217)</td>
</tr>
<tr>
<td>112.2 (81.8–155.2)</td>
<td>63.3–235.5</td>
<td>197 (126–309)</td>
</tr>
<tr>
<td>112.3 (93.2–149.0)</td>
<td>64.6–263.7</td>
<td>234 (154–324)</td>
</tr>
<tr>
<td>109.1 (79.9–130.9)</td>
<td>67.4–300.2</td>
<td>284 (172–508)</td>
</tr>
<tr>
<td>81.2 (65.3–102.6)</td>
<td>50.8–274.4</td>
<td>553 (360–861)</td>
</tr>
<tr>
<td>60.9 (50.0–86.2)</td>
<td>39.1–176.3</td>
<td>974 (751–1640)</td>
</tr>
<tr>
<td>48.6 (42.3–56.1)</td>
<td>38.1–67.6</td>
<td>2239 (1639–2571)</td>
</tr>
</tbody>
</table>
but because of the low number of patients, this correlation was not significant.

Nevertheless, our data show that there is a rapid and robust release of BNPsp within the first 15 min after induction of myocardial infarction; subsequently, concentrations decrease and return to levels comparable to those of the reference population after 24 h, possibly from a reduction in myocardial wall stress. We observed a correlation between concentrations of hs-cTnT and BNPsp in the reference population; however, there was a significant difference in the release kinetics of the 2 biomarkers after TASH. Whereas hs-cTnT concentrations increased continuously throughout the observation window, BNPsp concentrations peaked within 15 min and then decreased to baseline levels or lower within 8 h after TASH. This rapidly rising and falling (i.e., dynamic) profile is consistent with our previous observations in ST-segment elevation myocardial infarction patients (10) and in those undergoing dobutamine stress echo testing (26). The early rise after induction of AMI is also in accordance with previous data describing the release of NT-proBNP after TASH (15). Nevertheless, BNPsp seems to have a shorter half-life than NT-proBNP and might be more comparable to the release and half-life of BNP. There are no reports of data showing the exact release kinetics of BNP.

Understanding the time course of the release of cTnT and BNPsp and correlating the concentrations with patient symptoms and the results of electrocardiogram and imaging studies is important for early diagnosis, individual risk stratification, and individualized therapy, especially in the hours soon after symptom onset. Measurement of BNPsp could be helpful in diagnosing or excluding AMI in early presenters, as has been shown for other biomarkers (6, 8), and may offer increased specificity for AMI when combined with high-sensitivity troponin assay results. Measurement of both cTnT and BNPsp concentrations might assist in estimating the time of onset of an AMI: high BNPsp and low cTnT concentrations might indicate very recent onset, whereas low BNPsp and high cTnT concentration might reflect an AMI several hours into its evolution. This hypothesis needs to be tested in a patient cohort with suspected AMI.

In summary, our study shows that BNPsp concentrations in a healthy reference group correlate with concentrations of hs-cTnT. BNPsp concentrations increase immediately after induction of myocardial infarction, providing early evidence of myocardial injury. BNPsp shows different release kinetics than those of hs-cTnT. These findings provide information that may be helpful in establishing the diagnostic value of the relatively new biomarker BNPsp in the setting of early AMI.

This is the first study of serial BNPsp measurements in patients with HOCM undergoing TASH. However, the small number of enrolled consecutive patients from a single center is a major limitation of our study that must be considered. Additionally, the kinetics of biomarker release after alcohol ablation might be different from the release from the stuttering thrombotic occlusion of an epicardial coronary artery. The data nevertheless demonstrated a significant increase in BNPsp concentrations at 15 min after TASH in almost three-quarters of the patients, especially in patients with BNPsp baseline values <99th percentile.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:

Employment or Leadership: None declared.

Consultant or Advisory Role: C.W. Hamm, BRAHMS.

Stock Ownership: None declared.

Honoraria: None declared.

Research Funding: The William G. Kerkhoff-Stiftung Foundation, Bad Nauheim, Germany.

Expert Testimony: None declared.


Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

Acknowledgments: We thank Elizabeth Martinson, PhD, of the KHFI Editorial Office for editorial assistance and Anett Kirchhof of the KHFI laboratory for help in biomarker determination.

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Clinical Chemistry 61:12 (2015) 1539