Short- and Long-Term Biologic Variability of Galectin-3 and Other Cardiac Biomarkers in Patients with Stable Heart Failure and Healthy Adults

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BACKGROUND: Galectin-3 (Gal-3) has been suggested as a prognostic biomarker in heart failure (HF) patients that may better reflect disease progression than traditional markers, including B-type natriuretic peptide (BNP) and cardiac troponins. To fully establish the utility of any biomarker in HF, its biologic variability must be characterized.

METHODS: To assess biologic variability, 59 patients were prospectively recruited, including 23 male and 16 female patients with stable HF and 10 male and 10 female healthy individuals. Gal-3, BNP, and high-sensitivity cardiac troponin I (hs-cTnI) were assayed at 5 time points within a 3-week period to assess short-term biologic variability. Long-term (3-month) biologic variability was assessed with samples collected at enrollment and after 4, 8, and 12 weeks.

RESULTS: Among healthy individuals, mean short-term biologic variability, expressed as intraindividual CV (CVI), was 4.5% for Gal-3, 29.0% for BNP, and 14.5% for hs-cTnI; long-term biologic variability was 5.5% for Gal-3, 34.7% for BNP, and 14.7% for hs-cTnI. In stable HF patients, mean short-term biologic variability was 7.1% for Gal-3, 22.5% for BNP, and 8.5% for hs-cTnI, and mean long-term biologic variability was 7.7% for Gal-3, 27.6% for BNP, and 9.6% for hs-cTnI.

CONCLUSIONS: The finding that Gal-3 has minimal intraindividual biological variability adds to its potential as a useful biomarker in HF patients.

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Galectin-3 (Gal-3),3 a soluble B-galactosidase–binding lectin, is produced by activated macrophages and induces cardiac fibroblasts to deposit type 1 collagen. Gal-3 production is increased before and after the onset of heart failure (HF) and is believed to play a role in cardiac fibrosis (1). Studies have shown that increased Gal-3 serum concentrations are associated with increased 2-year risk of HF after acute coronary syndrome (2), increased cumulative 10-year incidence of HF in the Framingham Offspring Cohort (3), and decreased 18-month survival among HF patients (4). More recent studies have shown that Gal-3 is an independent predictor of cardiovascular mortality in patients with and without prior cardiovascular disease (5, 6). Furthermore, a metaanalysis of 3 separate studies suggested that Gal-3 discharge values in the upper tertile (>18 μg/L) were predictive of 30-day readmission in HF patients (7). To fully understand the utility of Gal-3 compared with that of other cardiac biomarkers in managing patients with HF, it will be important to determine its intraindividual biologic variability and compare it to the biologic variability of other cardiac markers, such as B-type natriuretic peptide (BNP) and high-sensitivity cardiac troponin I (hs-cTnI).

To determine what constitutes a true physiologically significant change in the concentration of a biomarker being used to monitor a patient, it is necessary to determine the 2 largest contributors to variability in a biomarker test. These are analytic variability, which is the inherent imprecision of the test method, and biologic variability, defined as the variability one can expect in the blood concentration of a biomarker in a stable patient over a designated period of time, expressed as the intraindividual CV (CVI) (8, 9). To determine whether a change in the blood concentration of a biomarker in con-
Materials and Methods

The definitions of short- and long-term biologic variability are somewhat arbitrary but should take into account the context in which the biomarker is used. For instance, short-term biologic variability for cardiac troponin must be considered as hours, since the diagnosis of myocardial infarction requires serial samples for troponin to be drawn 4–6 h apart. Thus, in 1 study, short-term CV1 for cardiac troponin was considered to be a period of 24 h (12). Long-term biologic variability for cardiac troponin would be important when it is used to assess risk of future cardiac disease by looking for significant changes in healthy or stable patients. In this use, long-term biologic variability of hs-cTnI is considered to be a period of 2 months (12). Because biomarkers that may be of use in monitoring HF patients might be expected to be ordered on a monthly or quarterly basis, we considered short-term biologic variability to be a period of 3 weeks and long-term biologic variability to be 3 months. A recent study examined hourly and 2-month Gal-3 biologic variability among 17 healthy individuals and found RCVs of 39% and 61%, respectively (13). Here, we determined the 3-week (short-term) and 3-month (long-term) biologic variability of Gal-3 in patients with stable HF and healthy individuals and compared it to the biologic variability for BNP and hs-cTnI in the same individuals.

Materials and Methods

Participants

We enrolled 59 participants: 23 male and 16 female stable HF patients, as well as 10 healthy men and 10 healthy women. Stable HF patients were defined at enrollment by the following: (a) no hospital admissions during the previous 3 months; (b) ≤50% change in dosage of HF medications in the previous 3 months; (c) no overt changes in clinical symptoms of HF; and (d) no more than a 1-category change in New York Heart Association (NYHA) classification of HF in the previous 3 months. Exclusion criteria for the stable HF patients included inability or unwillingness to provide consent, cancer, use of a left ventricular assist device, or continuous infusion of inotropes. Participants were dropped from the study if there was a change in medication, hospitalization, or a change in NYHA classification during the study period. Exclusion criteria for healthy individuals were any self-reported history of cardiovascular disease, including HF, myocardial infarction, and congenital heart disease, hypertension, or any history of taking certain cardiac medications, including beta-blockers, calcium channel blockers, and antihypertensives. Venous blood samples for all participants were collected at scheduled appointments between 0900 and 1500 at the Washington University Center for Clinical Studies Volunteers for Health office, except for the initial sample from the stable HF patients, which was drawn during a routine visit to the heart failure clinic where they provided consent and were enrolled. All samples were drawn in K2EDTA tubes and processed and frozen at –70 °C within 2 h of collection. To determine short-term CV1, each participant provided 5 samples over the first 3 weeks of the study, with ≤2 samples within the same week. For long-term CV1, the participants provided 3 additional samples at 4, 8, and 12 weeks. Long-term CV1 (3 months) was calculated with the initial and 4-, 8-, and 12-week samples. Participants were retained in the study if they missed ≤2 of the short-term variability samples and ≤1 of the 3 long-term variability samples. To minimize run-to-run variability, all samples from any 1 individual were analyzed together as a batch for each assay. This study was approved by the Human Research Protection Office (institutional review board) of Washington University.

Assays

We measured hs-cTnI, BNP, and Gal-3 with Abbott Architect i2000 chemiluminescent microparticle immunoassay methods according to the manufacturer’s instructions. The 99th percentile for the hs-cTnI assay is 16 ng/L for females and 34 ng/L for males (26 ng/L overall) (14). Per the package insert, the limit of detection is 1 ng/L, and the 10% CV is 5 ng/L. The limit of detection for Gal-3 is 1 μg/L, and a cutoff of 18 g/L has been suggested (7). Per the package insert, the BNP assay has a limit of detection of 10 ng/L, and ROC curve analysis shows that the optimal decision limit for BNP is 100 ng/L for diagnosing or ruling out HF.

Statistical Analysis

All statistical analysis was carried out in Microsoft Excel 10 and SPSS 21. We assessed biological variation by the method of Fraser and Harris (9) unless otherwise stated.
Because data from all biomarkers was slightly right-skewed, we performed assessment of outliers and subsequent data analysis on log-transformed data. Analytical CV (CVA) was determined from 32 individuals for whom duplicate measurements were available. We performed linear regression analysis to identify individuals with a consistent increase or decrease in biomarker concentration over time. No significant trends were found (see Supplemental Fig. 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol62/issue2). Outlying values were identified and eliminated at the individual level with the Cochran test and Reed criteria.

Overall, only 1 individual per group was identified as an outlier with log-transformed data (Fig. 1). We calculated variance within and between individuals with ANOVA, with individuals as a random effect. Variances were homogeneously distributed. We calculated within-individual CV (CVW) and between-individual biological variation (CVG) as the square root of the respective log-transformed variance. CVW was partitioned to CVI as follows:

\[
CV_I = \sqrt{CV_W^2 - CV_A^2} 
\] (1)

Index of individuality (II) was calculated as

\[
II = \frac{\sqrt{CV_A^2 + CV_I^2}}{CV_G} 
\] (2)

Homeostatic set point was estimated within 10% with 95% confidence with the equation of Fraser and Harris (9). We evaluated RCVs for log-transformed data by the method of Fokkema et al. (15). For comparison, RCVs were also calculated from untransformed data.

Results

Analytic Performance

Within-laboratory imprecision for the 3 immunoassays with Abbott QC samples (n = 38–43) in our laboratory was <5% CV at all QC concentrations, and mean values were 3.5%, 3.1%, and 3.6% for hs-cTnI, Gal-3, and BNP, respectively. We determined CV_A per
Fraser and Harris (9) with duplicate measurements from 32 individuals; the mean CV_j values were 2.2%, 4.1%, and 4.8% for Gal-3, BNP, and hs-cTnI.

**DEMOGRAPHICS AND CARDIAC BIOMARKER VALUES**

Table 1 summarizes the demographics and mean and median values at the initial visit for Gal-3, hs-cTnI, and BNP in the healthy and stable HF individuals. In the healthy population, 2 individuals had Gal-3 values at or above the highest tertile cutoff that has been suggested to predict risk (18 μg/L). These values were 18 and 19 μg/L, and subsequent values for both of these individuals remained within 4 μg/L of their initial value (see online Supplemental Fig. 1). One woman had an hs-cTnI value of 18 ng/L, which is above the sex-specific 99th percentile, and all of her subsequent hs-cTnI values were 15–17 ng/L (see online Supplemental Fig. 1). The highest initial BNP value in the healthy individuals was 45 ng/L. Ninety-five percent of values from healthy individuals over all time points were 1102120 μg/L for Gal-3, 30 ng/L for BNP, and 1102115 ng/L for hs-cTnI.

In contrast, the stable HF patients had 23 Gal-3 values >18 μg/L, 9 patients had initial hs-cTnI above the sex-specific 99th percentile values, and 19 patients had initial BNP values >100 ng/L. Among the male stable HF patients, 3 were NYSHA class I, fifteen were class II, and 5 were class III. Among the female stable HF patients, none were class I, 14 were class II, and 2 were class III.

**SHORT- AND LONG-TERM INTRINDIVIDUAL VARIABILITY**

Twenty healthy individuals (10 males and 10 females) provided samples to assess both short- and long-term biologic variability. All were able to provide all 8 samples. Short- and long-term CV_j for the healthy individuals is shown in Table 2. On the basis of these findings, the RCV for Gal-3 in healthy individuals was calculated to be 15%/−13% and 18%/−15% for short- and long-term changes, respectively (Table 2). Long-term variability of Gal-3 for each individual is shown in Fig. 1, and for hs-cTnI and BNP, in online Supplemental Figs. 2 and 3.
Thirty-nine stable HF patients (23 males and 16 females) provided samples to assess both short- and long-term biologic variability. Thirty-one of the HF patients were able to provide all 8 samples; 1 provided 3 short-term samples; and 2 provided 3 of the 4 long-term samples. Additionally, 1 HF patient was excluded from the long-term analysis because of an insufficient number of long-term samples, 1 withdrew from the study after providing the short-term samples, and 3 were excluded from analysis of long-term variability because of changes in health status such that they no longer fit our definition of stable HF (hospitalization in 2 patients, medication dosage change in 1 patient).

Table 3. Short- and long-term biological variation in stable HF.

<table>
<thead>
<tr>
<th>Marker</th>
<th>CV_A</th>
<th>CV_I</th>
<th>CV_G</th>
<th>II</th>
<th>RCV lognormal</th>
<th>Samples required, n^*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Short-term</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gal3</td>
<td>2.2</td>
<td>7.1</td>
<td>40.8</td>
<td>0.2</td>
<td>23</td>
<td>−19</td>
</tr>
<tr>
<td>BNP</td>
<td>4.1</td>
<td>22.5</td>
<td>92.1</td>
<td>0.2</td>
<td>87</td>
<td>−47</td>
</tr>
<tr>
<td>hsTnI</td>
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<td>8.5</td>
<td>99.3</td>
<td>0.1</td>
<td>31</td>
<td>−24</td>
</tr>
<tr>
<td>Long-term</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gal3</td>
<td>2.2</td>
<td>7.7</td>
<td>39.7</td>
<td>0.2</td>
<td>25</td>
<td>−20</td>
</tr>
<tr>
<td>BNP</td>
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<td>27.6</td>
<td>93.6</td>
<td>0.3</td>
<td>114</td>
<td>−53</td>
</tr>
<tr>
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<td>9.6</td>
<td>100.1</td>
<td>0.1</td>
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<td>−26</td>
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^* Required to estimate homeostatic set point within 10% with 95% confidence.

Discussion

The clinical course of HF varies widely, with some patients demonstrating a rapidly progressive decline while others remain stable over long periods of time. Additionally, individual patients may plateau symptomatically for months or years between acute exacerbations. Traditionally, the decision to escalate monitoring and treatment has been largely based on patient symptoms. Recently, it has been shown that cardiac remodeling ultimately drives disease progression and that such remodeling is not consistently reflected in patient symptoms. The need for an accurate marker of cardiac remodeling to guide risk stratification and optimize patient outcomes is clear. Numerous studies have provided data suggesting that Gal-3 is such a marker (2, 16–18), and more recent data have highlighted the utility of serial monitoring (19).

The prognostic value of serial Gal-3 measurement was assessed in the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA) and the Coordinating Study Evaluating Outcomes of Advising and Counseling Failure (COACH). Gal-3 concentrations were obtained at baseline and 3 months in 1329 acute HF patients in the CORONA study and at baseline, 3 months, and 6 months in 324 chronic HF patients in the COACH study (16). A threshold value of 18 μg/L or 15% change from baseline was used to stratify patients. Van der Velde et al. (19) suggested that patients in both cohorts whose Gal-3 increased by >15% between measurements had a significantly higher relative hazard of all-cause mortality and HF hospitalization within the follow-up period that was independent of age, sex, diabetes, left ventricular ejection fraction, renal function, cardiac medication, and BNP. Although impressive, these data must be interpreted within the context of biologic and analytic variability in both healthy individuals.
and stable HF patients. Recently, Wu et al. (13) showed that the long-term RCV of Gal-3 in 17 healthy individuals was 61%, which would imply that a >15% change in Gal-3 might be needed to be considered physiologically significant.

Our results show that the Gal-3 assay from Abbott has analytical variability similar to that observed for the BNP and hs-cTnI assays used in this study. Consistent with the values reported by Tsai et al. (18), we found the mean initial Gal-3 concentration in healthy individuals to be 13 μg/L. As expected, the mean Gal-3 value among stable HF patients was higher, at 24 μg/L. Among healthy controls, Gal-3 shows minimal biologic variation in both the short and long term (<10% CV) without sex differences. Furthermore, the CVa among healthy individuals was that in stable HF patients was very similar to that found for hs-cTnI (12). The biologic variability in Gal-3 among HF patients was somewhat less than the biologic variability of hs-cTnI and far less than that of BNP. We found lower RCVs for Gal-3 than Wu et al. (13), but the CVa of the automated method used here is much lower than the CVa of the microtiter plate ELISA used in their study. The II of Gal-3 is low relative to BNP but comparable to that of hsTnI. Low II indicates that population-based reference intervals may be less useful for monitoring individual patients than observing a change from previous values that exceeds the RCV.

Strengths of this study include an appropriately selected patient cohort reflective of the population in whom the assay will be used for clinical decision making, and serial laboratory measurements combined with clinical follow-ups. Our data with both healthy individuals and stable HF patients is consistent with previously published long-term RCVs for cardiac troponin and BNP, which range from 40% to 138% for troponins and 66% to 198% for BNP (20–29), suggesting that the included cohorts are representative of the intended groups we wished to study (20–29). There are some notable limitations of this study. The modest sample size prevents some relevant analyses such as an assessment of biologic variability with respect to ethnicity, but the number of individuals studied is larger than or comparable to that of previous studies of biological variation of cardiac biomarkers (13, 20–29). Another limitation is that blood draw times were not at precisely controlled intervals for the short-term (3-week) variability (i.e., not same time of day or day of week) but rather at times that fit the schedules of the participants and the staff at the Volunteers for Health office. Finally, these studies were performed with a single lot of reagent for each test.

In summary, our data show that Gal-3 has relatively low biologic variability in healthy individuals and stable HF patients, which will improve its potential as a marker to serially monitor HF patients. RCVs for Gal-3 found here suggest that an upward change >25%–30% is likely to be clinically important in stable HF patients, a lower value than found for other cardiac biomarkers directly compared in this study or reported previously.

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References