Evaluation of a New, Rapid Bedside Test for Quantitative Determination of B-Type Natriuretic Peptide, Yuriko Fischer,∗ Karsten Filizmaier, Hugo Stiegler, Jürgen Graf, Simone Fuchs, Andreas Franke, Ulve Janssens, and Axel M. Gressner

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Congestive heart failure (CHF) is among the most frequently encountered cardiac diagnoses, with an estimated prevalence of 1% (1). Plasma concentrations of B-type natriuretic peptide (BNP) and its precursor, N-terminal pro-BNP (NT pro-BNP), are increased in patients with CHF and have been shown to accurately predict clinical severity and left ventricular ejection fraction (LVEF) as well as morbidity and mortality in those patients (2, 3). A major limitation in the routine determination of natriuretic peptides is the time-consuming nature of analytical techniques, e.g., extraction procedures, long incubation times, or radioactive labeling (4–8). A rapid bedside test for determination of BNP was introduced recently (Triage BNP; Biosite Diagnostics, San Diego, CA). We evaluated the assay and compared it, in samples from patients with suspected CHF, with other assay systems for determination of either BNP (Shionoria BNP; CIS Diagnostics) or NT pro-BNP (Roche Diagnostics, Tutzging, Germany).

The Triage BNP test is an immunofluorometric assay for quantitative determination of BNP in EDTA-anticoagulated whole blood or plasma (9, 10). A murine recombinant polyclonal antibody is bound to the fluorescent label, and a murine monoclonal antibody against the disulfide bond-mediated ring structure of BNP 32 is bound to the solid phase.

Briefly, after the addition of 250 μL of EDTA-anticoagulated whole blood, the plasma is separated and allowed to react with fluorescent antibody conjugates within a reaction chamber. After an incubation period, complexes of the analyte and fluorescent antibody conjugates are captured on a detection lane. The concentration of BNP in the specimen is proportional to the fluorescence bound to the detection lane, which is determined quantitatively by a handheld fluorescence instrument (Triage Meter) as described in detail elsewhere (11). The time from application of the sample until the result is reported is ∼15 min.

The Shionoria BNP test is a one-step immunoradiometric assay that uses two different monoclonal antibodies that recognize the C-terminal structure and the disulfide bond-mediated ring structure of BNP 32, respectively. This test requires ∼20 h. NT pro-BNP(1–76) was determined by a recently developed research enzyme immunoassay that uses a polyclonal antibody specific for the N-terminal sequence (amino acids 1–21) of NT pro-BNP as well as a polyclonal antibody specific for the middle sequence (amino acids 30–38) of NT pro-BNP. The assay time is ∼2 h, and no extraction is required. Both assays have been described in detail elsewhere (6, 12–14).

The within-run (n = 11) and between-day (n = 11 days) imprecision of the Triage BNP assay were determined at three different concentrations that were prepared by mixing patient samples with high and low BNP concentrations. Within-run and between-day studies used EDTA-anticoagulated whole blood and EDTA plasma, respectively, each with a single assay lot and unchanged calibration.

For determination of the detection limit, defined as the lowest concentration that could be differentiated from zero (mean ± 3 SD), a zero calibrator was measured 20 times with four lots of reagents on 5 consecutive days.

Plasma samples from 50 apparently healthy individuals (30 males, 20 females; age range, 20–60 years) were measured, and the median and 97.5th percentile concentrations were calculated.

The study population consisted of 100 patients with underlying cardiac disease and suspected CHF. Clinical severity was characterized according to the functional classification system of the New York Heart Association (NYHA) in stages I to IV (15). The LVEF was evaluated times for determination of BNP was introduced recently (Triage BNP; Biosite Diagnostics, San Diego, CA). We evaluated the assay and compared it, in samples from patients with suspected CHF, with other assay systems for determination of either BNP (Shionoria BNP; CIS Diagnostics) or NT pro-BNP (Roche Diagnostics, Tutzging, Germany).

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with biplane transthoracic echocardiography by the modified Simpson rule using second harmonic imaging (16). Five patients were excluded because of insufficient sample material or missing clinical data. A total of 95 patients remained in the study group (Table 1). All patients gave informed consent. The study was approved by the local ethics committee.

On the day of echocardiography, peripheral venous blood was collected into sampling tubes containing EDTA as the anticoagulant. Within 20 min after venipuncture, BNP concentrations were determined by the Triage system. The remaining sample material was centrifuged, and EDTA plasma was frozen at −80 °C until further determination.

Correlation of BNP Triage with Shionoria BNP and NT pro-BNP was evaluated by the statistical procedure by Passing and Bablok (17). For calculation of assay comparison, results of the 95 study patients and the 50 healthy subjects were included (n = 145). Results of ROC analysis are expressed as areas under the individual ROC curves, including the 95% confidence interval. The diagnostic sensitivity of each assay was calculated as the number of test results with a BNP or NT pro-BNP value equal to or higher than a specific cutoff value among all patients with an LVEF ≤50%, whereas diagnostic specificity was defined as the number of test results below the cutoff value among all patients with an LVEF >50%.

For imprecision, the within-run CVs of the Triage BNP assay were 9.4%, 13%, and 15% at 40, 450, and 800 ng/L, respectively. At the same mean concentrations, the between-day CVs were 11%, 13%, and 16%, respectively. The detection limit was 6 ng/L. The 97.5th percentile of the healthy subjects was 82 ng/L for the Triage BNP assay, 64 ng/L for the Shionoria BNP, and 57 pmol/L for the NT pro-BNP ELISA.

Comparison of results by regression analysis (Fig. 1) revealed the following: Triage BNP = 1.579(Shionoria BNP) − 2.947 (r = 0.963); Triage BNP = 1.198(NT pro-BNP) + 1.419 (r = 0.947); NT pro-BNP = 1.309(Shionoria BNP) − 3.780 (r = 0.948). The 95% confidence intervals for slope and intercept were as follows: Triage BNP vs Shionoria BNP, 1.490–1.659 and −4.909 to −1.884; Triage BNP vs NT pro-BNP, 1.136–1.270 and −0.664 to 3.500; NT pro-BNP vs Shionoria BNP, 1.251–1.388 and −6.493 to −1.004. Comparison of BNP and NT pro-BNP concentrations with NYHA classes revealed only moderate correlations for all assays: rs = 0.689 (Triage BNP), 0.667 (Shionoria BNP) and 0.718 (NT pro-BNP); P < 0.001; n = 95. Nevertheless, BNP and NT pro-BNP values showed a strong inverse correlation with LVEF: rs = −0.816 (Triage BNP), −0.762 (Shionoria BNP), and −0.718 (NT pro-BNP); P < 0.001; n = 95.

ROC analysis regarding impaired ventricular function, defined as LVEF ≤50%, revealed areas under the ROC curve (95% confidence interval) of 0.91 (0.83–0.98) for Triage BNP, 0.88 (0.80–0.95) for Shionogi BNP, and 0.86 (0.77–0.94) for NT pro-BNP, respectively. At a cutoff value of 130 ng/L, the Triage assay had a sensitivity of

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**Table 1. Patient characteristics according to LVEF (n = 95).**

<table>
<thead>
<tr>
<th>LVEF</th>
<th>&gt;50%</th>
<th>40–50%</th>
<th>30–39%</th>
<th>&lt;30%</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>53</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Age, mean (range), years</td>
<td>60 (19–85)</td>
<td>65 (52–86)</td>
<td>67 (58–76)</td>
<td>61 (41–75)</td>
</tr>
<tr>
<td>M/F</td>
<td>37/16</td>
<td>11/3</td>
<td>8/6</td>
<td>8/6</td>
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<tr>
<td>NYHA classification</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>I</td>
<td>38 (72%)</td>
<td>2 (14%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>8 (15%)</td>
<td>8 (57%)</td>
<td>7 (50%)</td>
<td>5 (36%)</td>
</tr>
<tr>
<td>III</td>
<td>7 (13%)</td>
<td>5 (36%)</td>
<td>4 (29%)</td>
<td>5 (36%)</td>
</tr>
<tr>
<td>IV</td>
<td>1 (7%)</td>
<td>1 (7%)</td>
<td>1 (7%)</td>
<td>4 (28%)</td>
</tr>
<tr>
<td>Underlying cardiac disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CADb</td>
<td>29 (55%)</td>
<td>11 (79%)</td>
<td>10 (71%)</td>
<td>2 (21%)</td>
</tr>
<tr>
<td>HTN</td>
<td>5 (9%)</td>
<td>3 (21%)</td>
<td>3 (21%)</td>
<td>8 (57%)</td>
</tr>
<tr>
<td>DCM</td>
<td>1 (2%)</td>
<td>1 (7%)</td>
<td>3 (21%)</td>
<td>3 (21%)</td>
</tr>
<tr>
<td>ARRH</td>
<td>11 (21%)</td>
<td>2 (14%)</td>
<td>3 (21%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>VHD</td>
<td>3 (6%)</td>
<td>2 (14%)</td>
<td>2 (14%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Otherd</td>
<td>16 (30%)</td>
<td>1 (7%)</td>
<td>1 (7%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>BNP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triage, ng/L</td>
<td>60 (28–109)</td>
<td>333 (217–616)</td>
<td>482 (229–1087)</td>
<td>811 (500–1420)</td>
</tr>
<tr>
<td>Shionoria, ng/L</td>
<td>45 (18–83)</td>
<td>186 (92–415)</td>
<td>242 (121–541)</td>
<td>375 (261–892)</td>
</tr>
<tr>
<td>NT pro-BNP, pmol/L</td>
<td>64 (31–104)</td>
<td>277 (106–650)</td>
<td>321 (195–798)</td>
<td>538 (312–973)</td>
</tr>
</tbody>
</table>

*a Absolute values and percentages given.
*b Suspected or diagnosed underlying cardiac disease in patient population. Absolute values and percentages given, because of comorbidity numbers, may exceed total number of patients.
*c CAD, coronary artery disease; HTN, hypertension; DCM, dilated cardiomyopathy; ARRH, arrhythmias; VHD, valvular heart disease.
*d Myocarditis, pericarditis, pulmonary embolism, pneumonia, chronic obstructive pulmonary disease.
*e Values for BNP and NT pro-BNP are given as median and 25th and 75th percentiles.
93% and a specificity of 79% to detect patients with impaired LVEF. Similar results were obtained for the other assays: for the Shionogi BNP, at a cutoff of 100 ng/L, the sensitivity was 80% and the specificity was 79%; for the NT pro-BNP assay, at a cutoff of 100 pmol/L, the sensitivity was 90% and the specificity was 66%.

The Triage BNP assay seems to be a sensitive screening method to decide which patient with suspected CHF warrants further investigation, particularly when assessment by echocardiography is not readily available. The major advantage of the Triage test system compared with the other investigated assays is its rapid and accurate measurement of BNP from whole blood with 24-h availability in a routine laboratory or at the point of care. Additional time-consuming preparation, centrifugation, extraction, and incubation steps can be omitted. Rapid measurement of either BNP or NT pro-BNP seems useful, especially in the triage of patients with suspected CHF presenting to the emergency room, where these results add important information for faster diagnostic evaluation (8, 18). It might also be useful in patients admitted with decompensated CHF as a previous study has shown that changes in BNP during treatment are a strong predictor for mortality and early readmission (19). Furthermore, the recently introduced BNP-guided therapy for chronic CHF will require faster determination of the analyte (20). Nevertheless, additional clinical trials need to be designed to clarify the detailed clinical benefit in patients diagnosis, management, and therapy as well as the cost-effectiveness of this marker (8).

We gratefully acknowledge VIVA Diagnostika Germany, Biosite Diagnostics Europe, and Roche Diagnostics Germany for providing reagents free of cost.

Fig. 1. Method comparison using the Passing and Bablok (17) regression model.
(A), Triage BNP (ng/L) vs Shionoria BNP (ng/L): Triage BNP = 1.579(Shionoria BNP) − 2.947; r = 0.963; n = 145. (B), Triage BNP (ng/L) vs NT pro-BNP (pmol/L): Triage BNP = 1.198(NT pro-BNP) + 1.419; r = 0.947; n = 145. The solid line represents the regression line; the dotted line denotes the identity y = x. Note that for better visualization, concentrations >1000 ng/L (pmol/L) are not shown.

References


Measurement of Low Apolipoprotein Concentrations by Optimized Immunoturbidimetric Applications, Mustafa Porsch-Oezcueruemez, Sabine Westphal, and Claus Luley

Apolipoproteins define the functional properties of lipoprotein particles. In addition to their stabilizing features, several apolipoproteins have ligand functions. Some are also responsible for the modulation of enzymes involved in the homeostasis of lipid metabolism. Thus, apolipoprotein concentrations may provide essential information about lipid metabolism and associated diseases. There is evidence, for example, that apolipoprotein concentrations provide clinically relevant information concerning risk for coronary heart disease (1).

The determination of apolipoproteins in distinct lipoprotein subfractions narrows the conclusions that can be drawn for risk assessment as determined by or the discriminative power of HDL- and LDL-cholesterol (2, 3). In research, the determination of apolipoproteins encompasses their measurement in cell culture supernatants (4), lipoprotein subfractions separated by ultracentrifugation (5), isoschophoresis (6), immunoaffinity chromatography (7), or size-exclusion chromatography (8). The measurement of apolipoproteins in samples derived from such procedures requires appropriate methods reliable at concentrations below the physiological range.

Five analytical techniques are commonly used to quantify apolipoproteins. No delipidation is necessary in any of the advanced assays.

The use of radioactive reagents in RIAs is problematic (9). Radial immunodiffusion (RID) is simple to perform but time-consuming. Difficulties can occur when analyzing lipemic sera if the diffusion of the lipoprotein particles is complicated by particle size (5). Electroimmunodiffusion using the Laurell-Rocket technique (9) requires less time than RID, but large amounts of antibodies are needed. ELISAs provide several advantages (10), including good precision and a sensitivity comparable to RIAs. ELISAs are useful in routine clinical determinations because of the availability of automated methods. Nephelometry and immunoturbidimetry provide additional advantages in apolipoprotein measurement (11, 12). Whereas ELISAs are highly sensitive, nephelometry and immunoturbidimetry are superior with respect to precision, time, and cost. Therefore, nephelometry and immunoturbidimetry seem to be the most suitable methods for routine analysis of apolipoproteins.

To improve the relatively low sensitivity compared with ELISA, we optimized and evaluated immunoturbidimetric applications for low concentrations of the most frequently measured apolipoproteins, i.e., apolipoprotein (Apo)A-I, ApoA-II, ApoB, ApoC-III, and ApoE, attaining 12.5- to 45-fold higher sensitivities for these assays.

Commercially available assays for ApoA-I and ApoB (“ApoA-I, immunologischer Trübungstest” and “ApoB, immunologischer Trübungstest”) were obtained from Rolf Greiner Biochemica and modified to measure physiological concentrations of ApoA-II, ApoC-III, and ApoE. Assays were performed on a Hitachi 911 automated analyzer (Boehringer Mannheim).

The application characteristics of assays for physiological applications and the final low-range conditions are given in Table 1. Initially, samples were incubated for 5 min with buffer 1 (“Immunofluid”; Greiner Biochemica) containing different concentrations of polyethylene glycol (PEG 10000) dissolved and stabilized in 100 mmol/L Tris buffer (pH 7.5) as provided ready to use by the manufacturers. Sample volumes were increased 10- to 12.5-fold in low-range applications. Commercially available human polyclonal goat antibodies specific against ApoA-I, A-II, B, C-III, and E (Rolf Greiner Biochemica) were used without additional dilution in all assays. Antisera were added after the first incubation step. The resulting absorbance was determined after an incubation interval of another 5 min.

Calibrators and control sera were purchased from Behringwerke (“N-Apolipoprotein” and “Apolipoprotein Control Serum CHD (human”)]. Analytical values of ApoA-I and ApoB were based on IFCC reference preparations (11). For ApoE, reference values provided by the manufacturers for nephelometry were used. The ApoC-III concentration of the N-Apolipoprotein standard was repeatedly measured by RID, and the mean value was used for calibration. Calibrators for the low-range applications were within the experimentally determined linearity range (see below) starting with a dilution of 1:16 of the N-Apolipoprotein standard. In all assays, a six-point calibration was performed. Sample concentrations were estimated by the logit-log method.