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Preliminary Evaluation of the AxSYM B-Type Natriuretic Peptide (BNP) Assay and Comparison with the ADVIA Centaur BNP Assay

To the Editor:

Blood measurements of B-type natriuretic peptide (BNP), a cardiac neurohormone, are proposed as a diagnostic and prognostic aid in congestive heart failure (CHF) and as a prognostic marker in acute coronary syndromes (ACS) *(1)*. In addition, there are preliminary data indicating a possible role of BNP as an aid in guiding medical therapy in patients with CHF *(2, 3)*. The growing interest in clinical determination of BNP has led to the development of immunoassays for the determination of this analyte that are suitable for fully automated, high-throughput clinical instruments with random access, e.g., the ADVIA Centaur BNP (Bayer Diagnostics) and AxSYM BNP (Abbott Laboratories). We aimed to perform a preliminary analytical evaluation of the recently developed AxSYM BNP assay and to compare its clinical performance with that of the ADVIA Centaur BNP assay. The properties of the ADVIA Centaur BNP assay have been reported previously *(4)*.

As indicated by the manufacturer, the AxSYM BNP assay, produced by Axis-Shield plc for Abbott Laboratories, is a fully automated microparticle enzyme immunoassay that uses two monoclonal mouse antibodies in a two-step sandwich format. The analyte is captured on anti-BNP-coated latex microparticles with detection via alkaline phosphatase and measurement of fluorescence produced from the breakdown of 4-methylumbelliferyl phosphate substrate. The solid-phase antibody is directed at the NH_2 terminus of the BNP peptide, whereas the conjugate antibody is directed at the COOH terminus. Six calibrators are used for the AxSYM BNP assay. Calibrator A is an acetate buffer with protein stabilizers. Calibrators B–F contain increasing concentrations of synthetic BNP (100, 400, 1000, 2000, and 4000 ng/L) in acetate buffer with protein stabilizers. These calibrators are traceable to a reference standard that was prepared gravimetrically with synthetic BNP; the reference standard underwent a value assignment to align with the Biosite Triage BNP assay with a decision threshold of 100 ng/L. The AxSYM BNP assay, thus being harmonized to the Biosite assay, has a dynamic range of 0–4000 ng/L. The required specimen type is EDTA plasma, and samples should be collected in plastic collection tubes.

For the present evaluation, commercially available reagents for BNP measurements were used for the ADVIA Centaur method, whereas BNP measurements in the AxSYM method were done with investigational reagents not approved for routine clinical application. However, all investigational reagents were the same as for the recently Food and Drug Administration-cleared AxSYM test. The two assays were performed according to the manufacturers' recommendations. Blood for BNP measurements was collected by venipuncture into Vacuette polyethylene terephthalate glycol–EDTA tubes (Greiner Bio-One). Blood samples were centrifuged at 3500*g* for 10 min at 4 °C immediately after collection, and the BNP in each sample was measured within 4 h after blood collection on the two analyzers unless otherwise stated.

We evaluated the linearity of the AxSYM method according to NCCLS guideline EP6-A *(5)*, using 11 different peptide concentrations. Fresh plasma samples were used to prepare high- and low-concentration pools covering the whole measurement range of the assay. We then constructed a direct dilution series with the low- and high-concentration patient sample pools in the following volume ratios (low-concentration pool:high-concentration pool): low only; 0.9:0.1; 0.8:0.2; 0.7:0.3; 0.6:0.4; 0.5:0.5; 0.4:0.6; 0.3:0.7; 0.2:0.8; 0.1:0.9; and high only. Two measurements were done on each concentration, with the default criterion set at 7.5% for repeatability (also see results of precision testing). The standard errors of regression ($S_{y|x}$) and *t*-tests for the regression analyses showed that the first-order model fitted better than the second- and third-order models: first-order model b_1, $S_{y|x} = 51.2$; $t = 107.2$ ($P < 0.001$); second-order model b_2, $S_{y|x} = 51.6$; $t = -0.9$ ($P = 0.406$); third-order model b_3, $S_{y|x} = 49.9$; $t = -1.5$ ($P = 0.148$). In addition, the repeatability estimate was <7.5% (pooled difference, 3.55%); therefore, the method is linear within its measurement range (3627 ng/L was the mean of the high-concentration pool). The detection limit for the AxSYM BNP assay was determined by assaying the zero calibrator (calibrator A) in replicates of 20 and was calculated as 3 SD added to the mean response of the zero calibrator. On the basis of this procedure, the detection limit was 11.9 ng/L for the AxSYM BNP assay.

To compare the precision of the AxSYM and ADVIA Centaur BNP assays, we performed a replication study according to NCCLS guideline EP5-A *(6)*. Three pooled patient plasma samples were aliquoted into forty 1.5-mL plastic tubes for each

concentration and frozen at −70 °C. We analyzed these samples in duplicate in two runs per day for 20 days on the two analyzers with a single calibration for each assay. Within-run and total imprecision (CV) was calculated with the NCCLS double-run precision evaluation test *(6)*. Precision data for the two methods were as follows: The AxSYM BNP assay had a within-run CV of 6.0% and a total CV of 8.1% at a mean concentration of 108 ng/L (pool 1), a within-run CV of 4.3% and a total CV of 7.5% at a mean concentration of 524 ng/L (pool 2), and a within-run CV of 5.1% and a total CV of 10% at a mean concentration of 2117 ng/L (pool 3). The ADVIA Centaur BNP assay had a within-run CV of 1.6% and a total CV of 4.8% at a mean concentration of 82 ng/L (pool 1), a within-run CV of 1.8% and a total CV of 4.7% at a mean concentration of 379 ng/L (pool 2), and a within-run CV of 1.5% and a total CV of 4.4% at a mean concentration of 1525 ng/L (pool 3).

Passing and Bablok regression analysis *(7)* and Bland–Altman plots *(8)* were used for method comparison of the AxSYM BNP assay with the ADVIA Centaur BNP assay as comparative method. To avoid any dilution for the method comparison, we limited the range for this experiment to the reportable ranges of the two BNP methods. For the comparison study, we used samples from 177 patients scheduled for BNP determinations for diagnostic and prognostic purposes or for monitoring of medical treatment (in patients with CHF). When we compared the BNP values obtained with the AxSYM and the ADVIA Centaur analyzers, Passing and Bablok regression analysis revealed an intercept of −10.4 ng/L [95% confidence interval (CI), −14.0 to −8.4 ng/L] and a slope of 1.55 (95% CI, 1.51–1.58), suggesting, in addition to a small but significant constant bias, a considerable proportional difference between the two methods. The Cusum test showed no significant deviation from linearity ($P > 0.10$). Nonparametric correlation analysis revealed a correlation coefficient (r_s) of 0.992 (95% CI, 0.989–0.994; $P < 0.001$). Bland–Altman difference plots are shown in Fig. 1, A and B.

From October 1, 2003, to November 25, 2003, all patients of the St. John of God Hospital (Linz, Austria) presenting with dyspnea at the emergency department were eligible for the present prospective clinical study. Inclusion criterion was shortness of breath as a chief compliant at the initial patient examination in our emergency department. Patients with ST-elevation myocardial infarction, non-ST-elevation myocardial infarction, or cardiac troponin-positive ACS were excluded, as were trauma victims. During the initial patient examination in our emergency department, a blood sample for the determination of BNP was collected and analyzed within the next 4 h. The complete clinical evaluation, including echocardiography, and the classification of patients had to be completed within 3 days after the initial patient examination. In total, 104 patients were included in the present study. However, in one patient BNP values (index tests) were not determined as requested in the study protocol, and in three patients complete clinical evaluation (reference standard) as described below was not available. Thus, 100 patients were enrolled in the present clinical study. The study protocol was approved by the local ethics committee in accordance with the Helsinki Declaration, and all study participants gave informed consent.

For each patient enrolled in the clinical part of the present study, a thorough survey of the patient's history, physical examination, 12-lead electrocardiogram, chest x-ray, and sonography of the liver were performed. All study participants underwent subsequent echocardiography within 3 days after their first examination at the emergency department. Patients were defined as having systolic dysfunction if the left ventricular ejection fraction was ≤50%. Patients with a left ventricular ejection fraction >50% were examined for diastolic dysfunction according to standard procedures. One cardiologist reviewed all medical records pertaining to the patient after completion of the clinical evaluation. Thereafter, the final classification was done by means of the Framingham Criteria for the Clinical Diagnosis of Congestive Heart Failure *(9)*. For the diagnosis of CHF, both of the following had to be present: (*a*) Framingham CHF score with two major or one major and two minor criteria; and (*b*) evidence of systolic or dia-

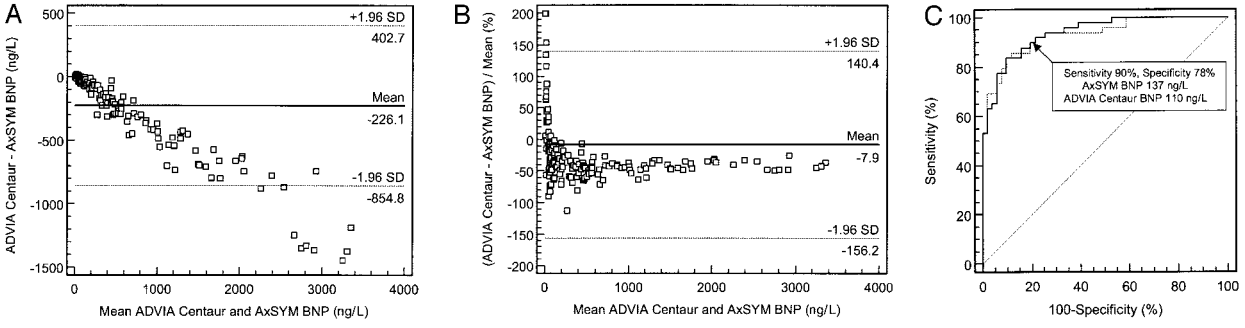

Fig. 1. Bland–Altman analyses (n = 177) and ROC plots for the AxSYM vs the ADVIA Centaur BNP.

(*A*), Bland–Altman difference plot; (*B*), Bland–Altman percent difference plot. (*C*), ROC plots for BNP values in patients with dyspnea attributable to CHF (n = 49) compared with individuals with dyspnea from other causes (n = 51). AUC for AxSYM BNP, 0.938; AUC for ADVIA Centaur BNP, 0.932. *Solid line*, ROC curve for AxSYM BNP; *dashed line*, ROC curve for ADVIA Centaur BNP.

stolic dysfunction as determined by echocardiography. All attending physicians/cardiologists were blinded to the BNP results.

To determine the diagnostic accuracy of the two assays for CHF, we performed ROC plot analysis, and areas under the curve (AUC) were calculated for both BNP assays. Comparisons between AUC were assessed according to the method of Hanley and McNeil *(10)*. Cutoff values for the two methods were determined at the 90% sensitivity criterion derived directly from the ROC curves. Statistical analysis for the clinical evaluation was performed with the MedCalc 7.2.1.0 package (MedCalc Software) and the software N (IDV). All probabilities were two-tailed, and P values <0.05 were regarded as statistically significant.

Of the 100 patients enrolled in the clinical study, 49 were classified as having dyspnea attributable to CHF, and 51 were classified as having dyspnea attributable to other reasons. The reasons for dyspnea in these 51 patients were as follows: chronic obstructive pulmonary disease (n = 21), pneumonia (n = 10), bronchitis/asthma (n = 5), malignancy of the lung (n = 1), musculoskeletal chest pain (n = 4), hypertension (n = 2), cardiac troponin-negative ACS (n = 2), tachycardia/arrhythmia (n = 2), and other causes (n = 4), such as pulmonary embolism, interstitial lung disease, or anemia. In distinguishing between patients with (n = 49) and patients without CHF (n = 51), the AUC (SE) were 0.938 (0.025) for the AxSYM BNP assay (95% CI, 0.872–0.977) and 0.932 (0.027) for the ADVIA Centaur assay (95% CI, 0.863–0.972; Fig. 1C). AUC were not significantly different for the two BNP assays [difference (SE) of AUC, 0.007 (0.006); 95% CI, −0.005 to 0.019; P = 0.265]. A power calculation showed that the power of this analysis was >99%. On the basis of the ROC curves, the cutoff values with a 90% sensitivity for CHF were 137 ng/L for the AxSYM BNP method [95% CI for sensitivity, 78–97%; specificity, 78% (95% CI, 65–89%)], and 110 ng/L for the ADVIA Centaur BNP method [95% CI for sensitivity, 78–97%; specificity, 78% (95% CI, 65–89%)].

In conclusion, we demonstrated a considerable proportional difference between results obtained with the AxSYM and ADVIA Centaur BNP assays. Given the limitations of low enrollments, our preliminary clinical study suggests that both assays provide comparable diagnostic information for the diagnosis of CHF in an emergency setting. Further studies are needed to clarify additional analytical issues concerning the AxSYM BNP assay (including sample stability data and reference intervals by age and gender) and to expand the database for the clinical relevance of this assay with respect to diagnostic and prognostic issues as well as its potential role in guiding medical treatment of CHF patients.

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Bicarbonate Interference with Chloride-Ion-Selective Electrodes

To the Editor:

Plasma chloride is of value in the assessment of acid/base and electrolyte abnormalities. It is a component in calculation of both the anion gap and strong ion differences. The analytical method used extensively today, particularly in high-throughput analyzers, involves an ion-selective electrode incorporating a quaternary nitrogen compound. This has superseded amperometric titration with silver electrodes, which is not amenable to high-throughput or multianalyte instrumentation, and mercuric thiocyanate-based colorimetric methods, which require disposal of a toxic waste product.

In 1986 and 1991, two reports *(1, 2)* were published that documented marked positive interference by bicarbonate on plasma chloride analyses performed by Hitachi 705 and 717 analyzers. No further reports have been published since then. We suspected that similar problems