Evaluation of Analytical Performances and Comparison of 3 NT-proBNP Assays for Diagnosing Heart Failure

Jooyoung Cho, MD, PhD; Jong-Han Lee, MD, PhD; Sang-Guk Lee, MD, PhD

• ***Context.***—**The N-terminal prohormone of the brain natriuretic peptide (NT-proBNP) is a major diagnostic biomarker for heart failure.**

Objective.—**To compare the analytical and clinical performance of 3 NT-proBNP immunoassays: the Atellica IM NT-proBNP assay (Siemens Healthcare Diagnostics), the Alere NT-proBNP assay (Abbott Laboratories), and the Elecsys proBNP II assay (Roche Diagnostics).**

Design.—**For the Atellica IM NT-proBNP assay, analytical performance, including precision, linearity, and carryover, was fully evaluated. Method comparisons among the 3 assays were performed using the Passing-Bablok regression and the κ agreement test. To evaluate the clinical performance of the assays, 160 patient samples were used from patients with (n = 81) or without (n = 79) heart failure.**

Results.—**The analytical performance of the Atellica IM NT-proBNP assay was acceptable according to the manufacturer's claims. The Atellica IM NT-proBNP assay showed a positive bias compared with the Elecsys proBNP II assay. The Cohen κ values among the 3 assays were satisfactory (>0.80) and comparable. There were no significant differences in areas under the curve. However, for the diagnosis of heart failure, the Elecsys proBNP II showed a higher specificity and positive likelihood ratio than the other assays.**

Conclusions.—**All 3 NT-proBNP assays showed acceptable concordance, and their clinical performance was comparable. However, the Elecsys proBNP II might be a more discriminating NT-proBNP assay to diagnose heart failure.**

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Heart failure (HF) remains a major cause of morbidity and mortality despite improvements in treatment.[1–7] The term *HF* refers to a clinical syndrome caused by structural or functional abnormalities. HF results in impairment of left ventricular filling and reduced cardiac output. However, symptoms of HF, such as dyspnea and fatigue, are nonspecific. Thus, it is often difficult to distinguish HF from other diseases. It is confirmed only by the patient's history and physical examination.[3,8] As initial screening tests, serum or plasma natriuretic peptide levels, chest radiography, and electrocardiography (ECG) are recommended for patients with suspected HF. When an abnormality is suspected, echocardiography is recommended.[1]

Brain natriuretic peptide (BNP) and the N-terminal prohormone of BNP (NT-proBNP) are widely used biomarkers for HF diagnosis.[1,3,9,10] Their secretion is stimulated by myocardial wall stress. These specific peptides have been proven to be elevated in HF compared with other natriuretic peptides.[10] In the European Society of Cardiology and American College of Cardiology Foundation/American Heart Association/Heart Failure Society of America guideline, BNP and NT-proBNP are considered to be first-line biomarkers for patients with suspected HF.[2,3]

ProBNP is the 108-amino-acid prohormone, which is cleaved into a biologically active 32-amino-acid hormone, BNP, and a biologically inactive 76-amino-acid N-terminal fragment, NT-proBNP.[9–12] BNP is cleared by binding to a specific natriuretic peptide receptor and proteolyzed by neutral endopeptidase. NT-proBNP is cleared mainly by renal excretion.[13] For these reasons, the half-life of NT-proBNP (approximately 22 minutes) is shorter than that of BNP (approximately 90–120 minutes).[9,11,14] Previous studies have reported that commercially available BNP and NT-proBNP assays are concordant and showed acceptable and comparable performance for diagnosing HF.[14–16] In some studies, NT-proBNP is reported to have more diagnostic benefits than BNP as it has an enhanced sensitivity.[11,17] Currently, there are several NT-proBNP assays available

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From the Department of Laboratory Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea (Cho, J.-H. Lee); and the Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, Korea (Cho, S.-G. Lee).

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Corresponding author: Sang-Guk Lee, MD, PhD, Department of Laboratory Medicine, Severance Hospital, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea (email: comforter6@yuhs.ac).

from numerous manufacturers. However, they can be different because of the variations in monoclonal antibodies used.[10] Therefore, this study aimed to evaluate the analytical and clinical performances of recent NT-proBNP immunoassays for the diagnosis of HF.

MATERIALS AND METHODS

NT-proBNP Assays

Three NT-proBNP assays were evaluated for comparison: the Atellica IM NT-proBNP assay (Siemens Healthcare Diagnostics, Tarrytown, New York), the Alere NT-proBNP assay (Abbott Laboratories, Chicago, Illinois), and the Elecsys proBNP II assay (Roche Diagnostics, Basel, Switzerland). The Elecsys proBNP II assay was used in our laboratory at the time of this study, and the Atellica IM NT-proBNP and Alere NT-proBNP assays were used for comparison.

The Atellica IM NT-proBNP assay is a 2-site sandwich immunoassay that uses direct chemiluminescence technology. A biotinylated sheep monoclonal anti-human antibody that is directed to N-terminal amino acids 14 to 21 serves as the primary antibody. This is followed by a sheep monoclonal anti–NT-proBNP antibody that is directed to N-terminal amino acids 27 to 32 as the secondary antibody.[18] According to the Urgent Field Safety Notice CC 20-01.A-2.OUS issued in December 2019, Siemens Healthcare Diagnostics[19] corrected the positive bias up to 13% compared with the existing NT-proBNP assays and realigned the lots from those ending at 042 and above. This new lot was used in this study.

The Alere NT-proBNP assay is a 2-step immunoassay that uses chemiluminescent microparticle immunoassay technology. A biotinylated anti–NT-proBNP-coated paramagnetic microparticle directed against N-terminal amino acids 4 to 13 was used in the first step. This was followed by an anti–NT-proBNP acridinium-labeled conjugate directed against N-terminal amino acids 26 to 32 in the second step.[18]

The Elecsys proBNP II assay is a 2-step sandwich immunoassay that uses electrochemiluminescence immunoassay technology. A biotinylated mouse monoclonal anti-NT-proBNP antibody directed against N-terminal amino acids 1 to 21 is the primary antibody. This is followed by an acridinium-ester–labeled sheep monoclonal anti–NT-proBNP antibody that is directed against N-terminal amino acids 39 to 50 as the secondary antibody.[17]

In this study, the Atellica IM NT-proBNP assay using the Atellica IM analyzer (Siemens Healthcare Diagnostics) and the Elecsys proBNP II assay using the Cobas e 801 analyzer (Roche Diagnostics) were performed at the clinical laboratory of our hospital. The Alere NT-proBNP assay using the Allinity I analyzer (Abbott Laboratories) was performed at the clinical laboratory of another hospital.

Evaluation of Analytical Performance

Precision, linearity, and carryover were evaluated for all 3 NT-proBNP assays. For the Atellica IM NT-proBNP assay, 3 levels of quality control materials provided by the manufacturer were measured in duplicate per run, with 2 separate runs per day for 20 days (80 measures per level), according to Clinical and Laboratory Standards Institute (CLSI) guideline EP05-A3.[20] Two (Elecsys proBNP II) and 3 (Alere NT-proBNP) levels of quality control materials were measured in 5 replicates per run, with a single run per day for 5 days (25 measures per level), according to CLSI guideline EP15-A3.[21] The results of the imprecision study were expressed as mean ± SD and coefficient of variation (CV [%]).

The linearity test was performed according to CLSI EP06-A,[22] using linearity validation materials provided by the manufacturer. Seven levels of validation materials were measured in triplicate. The decision for acceptance was determined when the percentage residual, that is, the distance from each data point to the calculated linear fit target for each level, was less than 10%, except for the lowest level.

The carryover study was performed according to CLSI EP10-A3.[23] High-level (H1, H2, H3, and H4) and low-level (L1, L2, L3, and L4) pooled serum samples were analyzed 4 times consecutively. The decision for acceptance was determined when the carryover was less than 1%, according to the following formula: % carryover = $[L1 - (L3 + L4)/2]/[(H2 + H3)/2 - (L3 + L4)/2] \times 100$.

For comparison of the methods, the Passing-Bablok regression with a difference plot and Spearman correlation assay were performed. In addition, the NT-proBNP levels were divided into 3 subgroups: less than 125 pg/mL, 125 to 300 pg/mL, and 300 pg/mL or more. To compare the concordance rate for clinical significance, a weighted κ agreement test was performed.[1,16,24]

Specimens and Data Collection

From July to November 2020, 160 serum specimens were selected from inpatient and outpatient samples submitted for physician-ordered NT-proBNP testing in the clinical laboratory of our hospital. This institution is a tertiary-level, university-affiliated hospital in South Korea. We collected the remaining samples after routine measurements covering various concentration ranges according to CLSI guideline[25] EP09-A3. Each sample was aliquoted into 3 Axygen 1.7-mL MaxyClear Snaplock Microcentrifuge Tubes (Axygen Scientific Inc, Union City, California). At least 0.5 mL of serum was collected in each microtube. Samples were stored at −70°C until the target number of samples was collected and used for the test assays. Each of the 3 aliquots was frozen and transported to the hospital (Seoul, Korea). On the same day, all specimens were thawed at room temperature, and each aliquoted specimen was analyzed using a different assay.

Clinical data were retrospectively collected by reviewing patients' electronic medical records. The following demographic and clinical data were collected: age, sex, clinical diagnosis, body mass index, smoking status, and comorbid diseases. Radiologic (chest x-ray), ECG, and echocardiographic data were also collected. The following laboratory data were collected: estimated glomerular filtration rate (eGFR), C-reactive protein, lactate dehydrogenase, BNP, creatine kinase MB, and troponin I levels. The eGFR value was calculated using the Modification of Diet in Renal Disease 4-variable (isotope dilution mass spectrometry traceable) formula. The body surface area was calculated using the Dubois formula. All laboratory data collected in this study were determined using Atellica CH and IM analyzers (Siemens Healthcare Diagnostics). This study was approved by the institutional review board, which waived the requirement for informed consent.

Comparison of Diagnostic Performance

HF diagnostic clinical performance was evaluated using receiver operating characteristic (ROC) curve analysis, and the area under the curve (AUC) values were compared. The clinical diagnosis of HF was determined when a diagnosis of HF was specified in the diagnosis list on the patient's chart. The diagnostic agreement for a reduced left ventricular ejection fraction (LVEF) of less than 40%[11,12] and renal impairment[24] with eGFR less than 60 mL/min/1.73 m^2 were also compared.

Statistical Analysis

Statistical analyses were performed using SPSS (version 25.0; IBM Corp, Armonk, New York) and Microsoft Excel 2019 (Microsoft Corp, Redmond, Washington) with Analyse-it version 5.81 (Analyse-it Software, Ltd, Leeds, United Kingdom). The study participants were divided into HF (n = 81) and non-HF (n = 79) groups, and their clinical data were evaluated. For the numerical data, the Kolmogorov-Smirnov test was used to confirm the normal distribution, in which all numerical data were proven to be nonparametric. Therefore, results were presented as medians and interquartile ranges and were compared using the Kruskal-Wallis test followed by a post hoc analysis using the Bonferroni method. For categorical data, data distributions were presented as frequencies and percentages and compared using a χ^2 test. To compare clinical performance and ROC curve analysis, the sensitivity,

Table 1. Baseline Characteristics of the Study Participants			
	HF Group (n = 81)	Non-HF Group (n = 79)	*P* Value
Age, median (IQR), y	66 (57–75)	63 (54–70)	.19
Sex, No. (%)			.87
Male	41 (50.6)	39 (49.4)	
Female	40 (49.4)	40 (50.6)	
Smoking, No. (%)			.64
Current smoker	13 (16.0)	15 (19.0)	
Ex-smoker	3 (3.7)	5 (6.3)	
Never smoker	65 (80.2)	59 (74.7)	
Body mass index, No. (%), kg/m^2			.59
<18.5	6 (7.4)	8 (10.1)	
18.5–24.9	33 (40.7)	29 (36.7)	
25.0–29.9	33 (40.7)	34 (43.0)	
≥30.0	9 (11.1)	8 (10.1)	
Underlying disease, No. (%)			
Hypertension	57 (70.4)	44 (55.7)	.04[a]
Diabetes mellitus	23 (28.4)	22 (27.8)	.94
Hyperlipidemia	8 (10.4)	9 (11.8)	.78
Pulmonary disease	17 (21.0)	19 (24.1)	.64
Cerebrovascular accident	6 (7.4)	5 (6.3)	.79
Renal disease	12 (14.8)	5 (6.3)	.08
Malignancy	15 (18.5)	7 (8.9)	.11
Other cardiac disease, No. (%)			
Coronary artery disease	36 (45.6)	26 (32.1)	.08
Cardiac myopathy	9 (11.1)	4 (5.1)	.13
Atrial fibrillation	21 (25.9)	4 (5.1)	<.001[a]
Radiologic findings, No. (%)			
Cardiomegaly	40 (49.4)	15 (19.0)	<.001[a]
Pleural effusion	10 (12.3)	9 (11.4)	.72
Other abnormal findings	8 (9.9)	9 (11.4)	.76
Abnormal ECG, No. (%)	62 (76.5)	37 (46.8)	<.001[a]
Laboratory findings, median (IQR)			
eGFR, mL/min/1.73 m^2	76.0 (59.7–90.8)	90.9 (75.7–106.5)	<.001[a]
CRP, mg/L	30.0 (13.0–62.3)	40.0 (14.5–94.5)	.50
LDH, U/L	238.0 (208.3–272.9)	199.5 (186.0–236.0)	.13
BNP, pg/mL	386.7 (111.5–647.1)	28.8 (20.2–75.4)	<.001[a]
CK-MB, μg/L	2.4 (1.9–3.0)	1.5 (1.2–2.0)	.09
Troponin I, pg/mL	33.4 (11.2–55.6)	34.0 (17.2–50.7)	.97
NT-proBNP level, median (IQR), pg/mL			
Elecsys proBNP II	563 (177–1118)	37 (58–108)	<.001[a]
Atellica IM NT-proBNP	728 (252–1412)	79 (53–139)	<.001[a]
Alere NT-proBNP	582 (161–1137)	63 (33–120)	<.001[a]

Abbreviations: BNP, brain natriuretic peptide; CK-MB, creatine kinase–muscle brain; CRP, C-reactive protein; ECG, echocardiography; eGFR, estimated glomerular filtration rate; HF, heart failure; IQR, interquartile range; LDH, lactate dehydrogenase; non-HF, non–heart failure; NT-proBNP, N-terminal prohormone of brain natriuretic peptide.

[a] *P* value < .05 represents statistical significance.

specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR–) were also calculated. The 95% CIs for each item were also calculated, and statistical significance was set at $P < .05$.

RESULTS

Patient Characteristics

The baseline characteristics of the 160 patients are presented in Table 1. Eighty men and 80 women were included in the study. The total number of patients diagnosed with HF was 81. All of these patients had a chronic status at the time of this study. In terms of medical history, hypertension and atrial fibrillation were more frequent in the HF group ($P = .04$ and $P < .001$, respectively). Cardiomegaly and abnormal ECG findings were more frequent in the HF group than in the non-HF group ($P < .001$ for both). Regarding laboratory findings, NT-proBNP and BNP levels were determined and tested using the 3 assays. They were much higher in the HF group

Table 2. Results of the Passing-Bablok Regression (X-Axis Versus Y-Axis)						
	Slope (95% CI)	Intercept (95% CI)	Expected Value at 125 pg/mL (95% CI)	% Bias at 125 pg/mL	Expected Value at 300 pg/mL (95% CI)	% Bias at 300 pg/mL
Elecsys proBNP II versus Atellica IM NT-proBNP	1.14 (1.09 to 1.18)	−3.69 (−5.80 to −1.78)	138.81 (134.99 to 141.56)	11.05	339.17 (326.61 to 347.50)	13.05
Elecsys proBNP II versus Alere NT-proBNP	1.05 (1.00 to 1.11)	−5.03 (−7.77 to −2.18)	126.22 (122.34 to 131.44)	0.98	305.88 (293.03 to 319.13)	1.96
Alere NT-proBNP versus Atellica IM NT-proBNP	1.12 (1.03 to 1.20)	−1.37 (−4.51 to 2.23)	138.63 (129.85 to 146.18)	10.90	336.76 (311.21 to 354.91)	12.25

($P < .001$ for all), whereas the eGFR was significantly lower in the HF group ($P < .001$).

Analytical Performance

The precision results were acceptable, according to the manufacturer's claims (Supplementary Table 1; see the supplemental digital content containing 1 table and 1 figure at https://meridian.allenpress.com/aplm in the August 2023 table of contents). The within-run imprecision (repeatability) of all 3 NT-proBNP assays ranged from 1.5% to 3.0%. The within-laboratory (total) imprecision ranged from 1.6% to 4.5%, which was acceptable according to both the manufacturers' claims and the Westgard database of biologic variation (an imprecision of 5%).[26] In the linearity test, all 3 NT-proBNP assays demonstrated approximate linearity across the claimed analytical measuring range with a percentage residual of less than 10%, except for the lowest level, in which the best-fit regression was a linear equation (data not shown). The carryover test showed an acceptable result of less than 1%.

Method Comparison

The method comparison results using the Passing-Bablok regression are summarized in Table 2 and Figure 1, A through F. The Atellica IM NT-proBNP assay, compared with the Elecsys proBNP II assay, showed 11.1% and 13.1% positive bias at 125 and 300 pg/mL, respectively. Similarly, the Atellica IM NT-proBNP assay compared with the Alere NT-proBNP assay showed 10.9% and 12.3% positive bias at 125 and 300 pg/mL, respectively. In contrast, the Alere NT-proBNP assay showed a relatively low bias of 1% to 2%. However, the Atellica IM NT-proBNP assay showed good concordance in patient classification according to the NT-proBNP value with the other tested assays. The Cohen κ values were 0.85 (95% CI, 0.78–0.93) compared with the Elecsys proBNP II assay and 0.82 (95% CI, 0.74–0.90) compared with the Alere NT-proBNP assay. Moreover, the Cohen κ value between the Alere NT-proBNP and Elecsys proBNP II assays was 0.89 (95% CI, 0.82–0.95) (Table 3). To show the discordant results directly, the overall NT-proBNP concentration values obtained using the 3 different instruments were plotted across the clinical decision points in ascending order in a single chart (Figure 2).

Diagnostic Performance

The ROC curve analyses of the 3 NT-proBNP assays for diagnostic agreement of HF, reduced LVEF (less than 40%), and renal impairment (eGFR <60 mL/min/1.73 m^2) were compared (see Supplementary Figure 1). The AUC values for the diagnosis of HF were as follows: 0.89 (95% CI, 0.84–0.95) for Elecsys proBNP II, 0.90 (95% CI, 0.85–0.96) for Atellica IM NT-proBNP, and 0.87 (95% CI, 0.81–0.93) for Alere NT-proBNP. The AUC values for reduced LVEF were as follows: 0.72 (95% CI, 0.58–0.87) for Elecsys proBNP II, 0.76 (95% CI, 0.64–0.88) for Atellica IM NT-proBNP, and 0.71 (95% CI, 0.57–0.85) for Alere NT-proBNP. The AUC

Table 3. Concordance Analysis of the 3 Assays[a]					
	Atellica IM NT-proBNP				
	<125 pg/mL	125–300 pg/mL	>300 pg/mL	Agreement, % (95% CI)	κ (95% CI)
Elecsys proBNP II					
<125	72	9	0	88.9 (80.2–94.0)	0.85 (0.78–0.93)
125–300	1	16	4	76.2 (54.9–89.4)	
>300	0	0	56	100.0 (93.6–100.0)	
	Alere NT-proBNP				
Elecsys proBNP II					
<125	75	6	0	92.6 (84.8–96.6)	0.89 (0.82–0.95)
125–300	1	18	2	85.7 (65.4–95.0)	
>300	0	2	54	96.4 (87.9–99.0)	
Atellica IM NT-proBNP					
<125	69	4	0	94.5 (86.7–97.8)	0.82 (0.74–0.90)
125–300	7	17	2	65.4 (46.2–80.6)	
>300	0	5	56	91.8 (82.2–96.4)	

[a] N-terminal prohormone of brain natriuretic peptide (NT-proBNP) levels are divided into 3 subgroups: <125 pg/mL, 125–300 pg/mL, and ≥300 pg/mL.

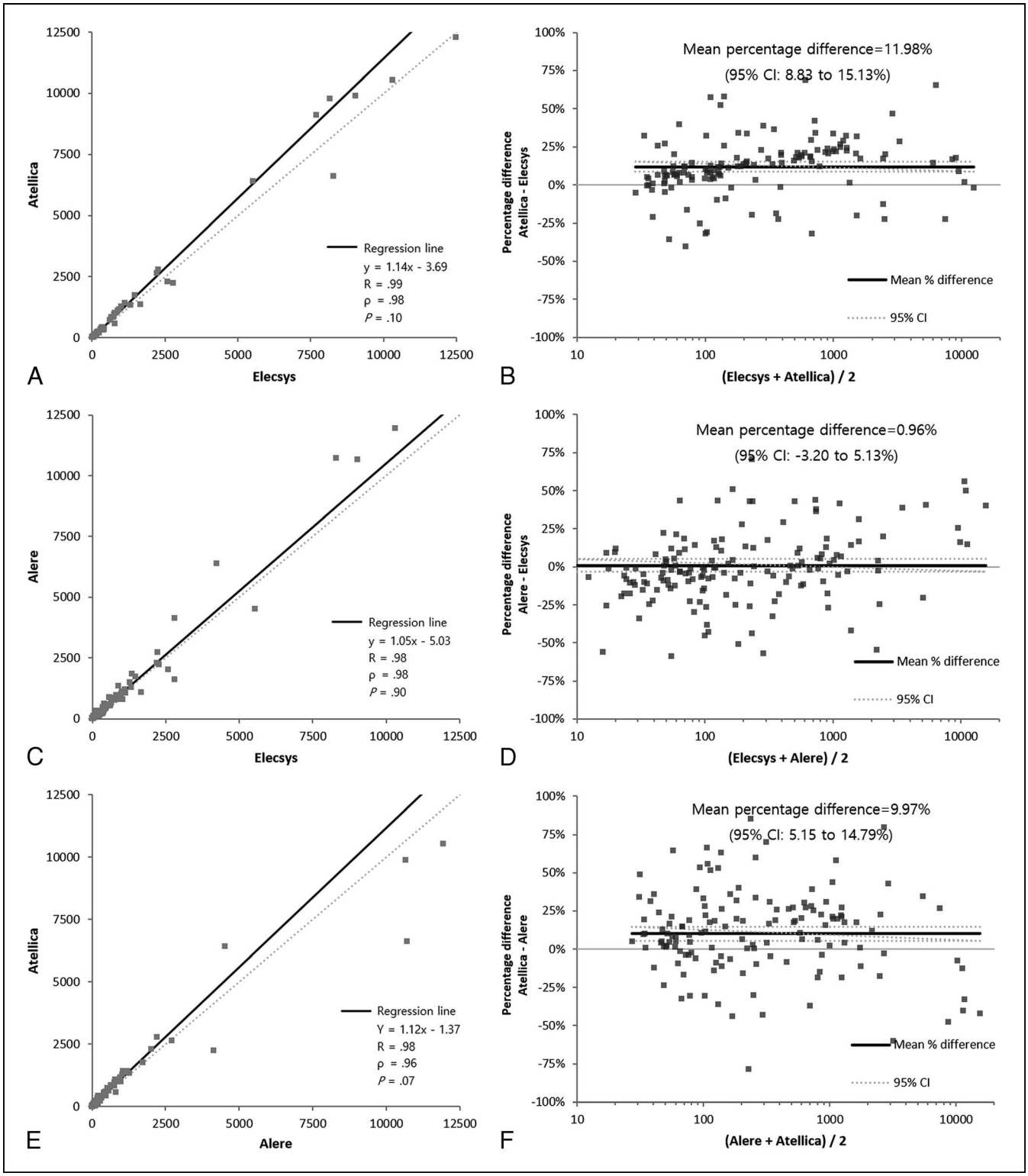

Figure 1. *Passing-Bablok regression and difference plots for the Atellica IM NT-proBNP (y-axis) versus the Elecsys proBNP II (x-axis), the Alere NT-proBNP (y-axis) versus the Elecsys proBNP II (x-axis), and the Atellica IM NT-proBNP (y-axis) versus the Alere NT-proBNP (x-axis): (A) and (B), (C) and (D), and (E) and (F), respectively. For (A), (C), and (E),* P *values > .05 for the CUSUM test for linearity indicate that the relationship between 2 assays is linear and therefore the Passing-Bablok regression is applicable.*

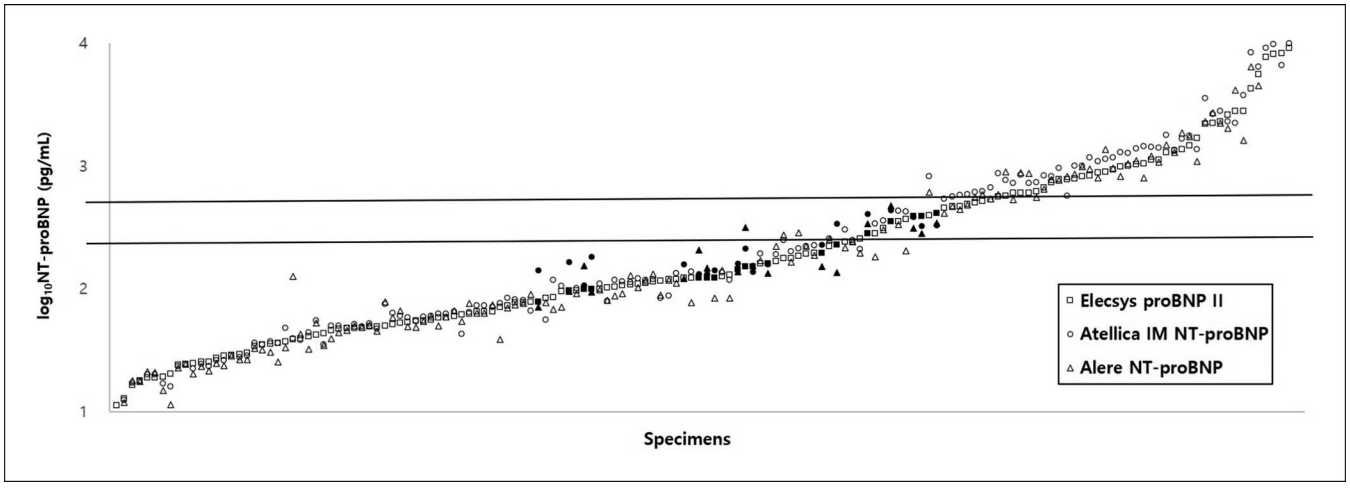

Figure 2. *Scatterplot showing log-transformed N-terminal prohormone of the brain natriuretic peptide (NT-proBNP) concentrations determined using the 3 NT-proBNP assays. Samples are ordered using the median NT-proBNP concentration. Horizontal lines at the proposed medical decision points (2.09 and 2.48, corresponding to the log-transformed values of 125 and 300 pg/mL, respectively) are included. The discordance between the interpretative categories (log-transformed values of 2.09 and 2.48) is indicated by solid markers; all other results are indicated by hollow markers.*

values for renal impairment were as follows: 0.77 (95% CI, 0.67–0.88) for Elecsys proBNP II, 0.77 (95% CI, 0.66–0.88) for Atellica IM NT-proBNP, and 0.79 (95% CI, 0.68–0.89) for Alere NT-proBNP. There were no statistical differences between the AUC values (*P* values of AUCs for reduced LVEF, Atellica versus Alere .30, Atellica versus Elecsys .43, and Elecsys versus Alere .59; and *P* values of AUCs for renal impairment, Atellica versus Alere .62, Atellica versus Elecsys .28, and Elecsys versus Alere .92).

Table 4 shows a comparison of the clinical performance for diagnostic agreement, presented as sensitivity, specificity, PPV, NPV, LR+, and LR-. The cutoff value of the 3 NT-proBNP assays was 125 pg/mL.[1,3,8,14,24] For the diagnosis of HF, Elecsys proBNP II showed lower sensitivity (70.4%) but higher specificity (91.1%) and LR+ (7.94) than the other assays. The diagnostic performances of the 3 assays were similar with respect to the estimation of reduced LVEF and renal impairment.

DISCUSSION

Serum NT-proBNP concentration is useful for diagnosing, monitoring, and determining the prognosis of patients with HF.[1–3,9,27–30] In this study, the analytical performances of 3 NT-proBNP assays, including precision, linearity, and carryover, were acceptable according to the manufacturer's claims. The comparison results obtained from the Passing-Bablok regression showed more than 10% positive bias at the medical decision level compared with the Elecsys proBNP II and Alere NT-proBNP assays. These relative biases among assays may be related to the calibrators and antibodies used by each manufacturer. However, the concordance rates using weighted κ agreement analysis were like those of the Elecsys proBNP II assay. Biases determined using the Passing-Bablok equation varied among the 3 assays. However, the concordance rates were consistent with those from a previous study, in which the Roche assay was evaluated with other assays.[14] The ROC curve analysis of the 3 NT-proBNP assays showed no significant differences in the AUC values for diagnosing HF,

Table 4. Comparison of the Diagnostic Performance of the 3 Assays for the Diagnosis of Heart Failure, Reduced Left Ventricular Ejection Fraction (LVEF; <40%), and Renal Impairment (Estimated Glomerular Filtration Rate [eGFR] <60 mL/min/1.73 m^2), Mean (95% CI), With Cutoff of 125 pg/mL

	Sensitivity, %	Specificity, %	PPV, %	NPV, %	LR+, %	LR−, %
Heart failure						
Elecsys proBNP II	70.4 (59.7–79.2)	91.1 (82.8–95.6)	89.1 (79.8–94.4)	75.0 (68.0–80.9)	7.94 (4.03–16.34)	0.33 (0.23–0.45)
Atellica IM NT-proBNP	74.1 (63.6–82.4)	82.3 (72.4–89.1)	81.1 (72.4–87.5)	75.6 (67.9–81.9)	4.18 (2.63–6.92)	0.32 (0.21–0.45)
Alere NT-proBNP	72.8 (62.3–81.3)	83.5 (73.9–90.1)	81.9 (73.1–88.4)	75.0 (67.5–81.3)	4.43 (2.72–7.49)	0.33 (0.22–0.46)
LVEF <40%						
Elecsys proBNP II	75.0 (50.5–89.8)	58.6 (48.1–68.4)	25.0 (18.6–32.7)	92.7 (84.3–96.8)	1.81 (1.15–2.56)	0.43 (0.17–0.87)
Atellica IM NT-proBNP	81.3 (57.0–93.4)	55.2 (44.7–65.2)	25.0 (19.3–31.7)	94.1 (85.0–97.8)	1.81 (1.21–2.46)	0.34 (0.12–0.80)
Alere NT-proBNP	81.3 (57.0–93.4)	55.2 (44.7–65.2)	25.0 (19.3–31.7)	94.1 (85.0–97.8)	1.81 (1.21–2.46)	0.34 (0.12–0.80)
eGFR <60 mL/min/1.73 m^2						
Elecsys proBNP II	66.7 (48.8–80.8)	68.0 (59.3–75.6)	33.9 (26.3–42.4)	89.2 (83.1–93.3)	2.09 (1.41–2.95)	0.49 (0.28–0.77)
Atellica IM NT-proBNP	73.3 (55.6–85.8)	62.3 (53.4–70.4)	32.4 (25.9–39.6)	90.5 (83.8–94.6)	1.95 (1.38–2.63)	0.43 (0.23–0.73)
Alere NT-proBNP	73.3 (55.6–85.8)	63.9 (55.1–71.9)	33.3 (26.6–40.8)	90.7 (84.1–94.7)	2.03 (1.43–2.77)	0.42 (0.22–0.71)

Abbreviations: LR−, negative likelihood ratio; LR+, positive likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

estimating reduced LVEF, and determining renal impairment. All 3 NT-proBNP assays showed acceptable diagnostic performance as determined by ROC curve analysis, consistent with a previous study.[31] Moreover, diagnostic agreement, such as sensitivity, specificity, PPV, NPV, LR+, and LR–, was also similar for estimating reduced LVEF and determining renal impairment. The sensitivity and specificity were similar to those in the previous study.[11] However, for the diagnosis of HF, the Elecsys proBNP II showed low sensitivity but high specificity and LR+. This is in line with the positive biases of the Atellica IM NT-proBNP and Alere NT-proBNP compared with those of the Elecsys proBNP II, which were determined using the Passing-Bablok equations. This may be due to the variability and bias among the NT-proBNP assays.[14,17,32]

The high sensitivity for reduced LVEF and relatively low sensitivity for diagnosing HF may be due to the presence of HF with preserved ejection fraction (HFpEF). In acute settings, the diagnostic performances of HF with reduced ejection fraction and HFpEF are reported to be similar.[30] However, a previous meta-analysis study[33] reported that NT-proBNP showed a higher PPV than NPV. In a nonacute setting, distinguishing HFpEF from other diseases is difficult, as the NT-proBNP level in patients with HFpEF can be closer to normal than in acute settings. For estimating renal impairment, NT-proBNP assays showed a relatively good concordance with NPV; however, they lacked specificity. A previous study reported that the NT-proBNP to BNP ratio is higher in patients with an eGFR less than 60 mL/min/1.73 m^2 than in those with an eGFR greater than 60 mL/min/1.73 m^2.[24] It has been reported that NT-proBNP is thought to be mainly cleared by the kidneys. BNP is thought to be cleared primarily by endopeptidases and receptor-mediated clearance.[13] Increased NT-proBNP levels may be associated with renal impairment. However, a systematic review suggested that the cutoff value in patients with renal impairment should be raised.[8] Therefore, the relationship between the NT-proBNP level and renal impairment remains unclear, and further studies with larger populations are recommended.

This study had certain limitations. First, we collected patient samples focused on NT-proBNP concentration distribution rather than the overall incidence rate of HF to cover various concentration ranges for comparison. Therefore, the proportion of HF in our samples was higher than the prevalence rate of HF.[7] Therefore, the statistical results could be overestimated.

Second, this study focused on the comparison among 3 assays. We collected only 160 samples, which met the minimum number of 120 for method comparison according to CLSI guideline[25] EP09-A3. For a comparison of diagnostic and clinical performance and for consideration of the prevalence of HF, further studies with a larger number of samples are required.

Third, we relied solely on the electronic medical record diagnosis to determine patients with HF. We could collect only limited clinical data for method evaluation and comparison because the need for informed consent was waived by the institutional review board. For this reason, we could not directly select patients. None of the patients were diagnosed with acute HF. Furthermore, we could not collect data on New York Heart Association functional classification. It has been reported that serum NT-proBNP concentration is strongly associated with the New York Heart Association classification.[9,11,29] Further studies based on clinical research are warranted to cover disease severity and prognosis.

This study evaluated and compared the analytical and diagnostic performances of recent NT-proBNP immunoassays. There were certain analytical differences among the 3 assays. However, these assays showed acceptable clinical and diagnostic performance and could be used to diagnose HF in clinical settings. In conclusion, this study provides clinical evidence that NT-proBNP assays have beneficial diagnostic performance for routine testing.

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