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Two Different Methods of Determining B-Type Natriuretic Peptide, Either from Fingertip's Capillary Blood or Antecubital Vein; A Comparison Regarding Diagnostic Accuracy in Patients with Chronic Heart Failure in NYHA Class I-III

Renato De Vecchis,[1,*] and Carmelina Ariano[2]

[1]Cardiology Unit, Presidio Sanitario Intermedio "Elena d'Aosta", ASL Napoli 1 Centro, Napoli, Italy

[2]Neurorehabilitation Unit, Clinica "S.Maria del Pozzo", Somma Vesuviana (NA), Italy

[*]*Corresponding author*: Renato De Vecchis, Cardiology Unit, Presidio Sanitario Intermedio "Elena d'Aosta", ASL Napoli 1 Centro, Napoli, Italy. Tel: +39-3483313530, E-mail: devecchis.erre@virgilio.it, r.de.vecchis@alice.it

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Abstract

Background: In recent years, several systems have been implemented to achieve quick and non-invasive measurements of B-type natriuretic peptide (BNP). Among them, Alere™ heart check (AHC) BNP test represents the most recent advancement. It is a rapid point of care immunoassay (POC), projected for measuring BNP directly from a capillary whole blood sample.

Objectives: This study aimed to compare analytical and clinical performances of this new POC to our reference method (Abbott architect system).

Patients and Methods: 111 patients with stable chronic heart failure (CHF) referred to two cardiac rehabilitation centers were enrolled from December 2013 to January 2015. These patients were subjected to a simultaneous capillary (AHC) and plasma (Abbott) BNP measurements. Clinical and analytical performance of AHC were assessed and compared to the reference method.

Results: Capillary BNP showed a good correlation with the reference method ($r = 0.94$, $P < 0.0001$), although the values diverged when BNP was higher than 1500 pg /mL. Indeed, the AHC had a relatively poor precision and the coefficient of variability was 10.1% and 18% for low and high controls, respectively. However, both methods showed similar diagnostic performances in discriminating patients with heart failure in NYHA class I from those belonging to NYHA classes II-III, with values of area under the curve (AUC) of 0.983 and 0.984, respectively, and equivalent sensitivity, specificity and positive and negative likelihood ratios.

Conclusions: The AHC BNP test is a good POC able to provide reliable information about hemodynamic status of CHF patients, especially of those belonging to NYHA classes I-III.

Keywords: B-Type Natriuretic Peptide, Capillary Measurement, Plasma Measurement, Heart Failure

1. Background

The B-type natriuretic peptide (BNP) and amino-terminal fragment of pro-B-type natriuretic peptide, known as NT-proBNP, are currently seen as gold standard biomarkers for diagnosis and prognostic stratification of heart failure, according to international guidelines (1, 2). These biomarkers are able to discriminate the origin of dyspnea (cardiac versus noncardiac dyspnea, e.g. bronchial asthma) in the setting of emergency medicine (1, 2) and also useful for better managing patients with stable chronic heart failure. Indeed, in the last decade there have been developments in the concept of BNP-guided therapy, in which serial measurements of BNP over time are used to assess the effectiveness of drug treatment for heart failure and guide possible adjustments and modifications to dosage. However, the current methods for the measurement of BNP are invasive, requiring a venous blood sample (e.g. from the antecubital vein of the arm), with specific pre-analytical requirements to make the interpretation of results feasible. In this context, relative discomfort resulting from venipuncture and relatively long period needed to get the test results could be some obstacles to a strict careful monitoring of patient with repeated tests for BNP from venous blood. These constraints prevent monitoring of BNP levels at home or at the office of general practitioner and require patients referring to centers for laboratory investigations. To overcome these limitations, some systems suitable for allowing rapid and noninvasive measurement of multiple biological parameters, including BNP (3), have been developed. Ideally, such a so-called "point of care" (POC) should measure BNP from capillary blood, similarly to blood sugar measurement from the fingertip in patients with diabetes. These POCs have many advantages, e.g. greater convenience and speed of detection of results than the reference methods.

A system for detecting analytes at the bedside (POC) would enable easy and quick BNP monitoring even in a remote way and would be useful for routine checks at the offices of primary care physician or community cardiologist. In addition, POC may also be used in hospitals for rapid monitoring of BNP-guided treatment of heart failure during an outpatient visit in department of cardiology and for a rapid triage of patients with acute dyspnea in emergency department. However, although promising, preparation and evaluation of a POC as a diagnostic routine tool requires assessing adequately its reliability in comparison with the reference methods, i.e. those used in laboratories for blood analyses. Until a short time ago, the two POCs available for the measurement of BNP were Alere™ Triage (4) and Abbott i-STAT (5, 6), the latter using venous or arterial whole blood, while the former uses plasma treated with ethylenediaminetetraacetic acid (EDTA) as a chelating agent (see also Table 1) (7). In contrast, the Alere™ heart check BNP test is the first POC designed as a type of immunological assay for the measurement of BNP from whole untreated capillary blood. Two preview studies demonstrated the safety and feasibility of this test at home for patients (8) and healthcare providers (9). However, no peer-reviewed studies have compared the Alere™ heart check BNP test with the reference methods used in the medical laboratory, except for data provided by the Alere™ heart check BNP test compared with the Beckman coulter access II BNP assay (10).

2. Objectives

The aim of this study was to evaluate clinical performance of this new POC to measure BNP by comparing it with our consolidated reference method (Abbott architect system), as an analytical prerequisite for a large deployment of this system in hospital.

3. Patients and Methods

3.1.Patients

We enrolled 111 patients with stable chronic heart failure (CHF) followed at two cardiac rehabilitation units who participated in our search (E.d'A. and S.M. d. P.) from February 2013 to January 2015.

3.2. BNP Measurements

These patients were subjected to simultaneous capillary (Alere™ heart check system) and plasma (Abbott architect System) BNP measurement. Plasma was obtained from EDTA blood samples centrifuged at 3500 g for 15 min at 4°C. Plasma BNP was subsequently measured on an Abbott Architect i2000. The Alere™ heart check BNP test uses capillary whole blood to measure BNP. This method is a one-step immunoassay that uses biotinylated anti-BNP monoclonal antibody and streptavidin-coated magnetic solid-phase particles used to attract the immune binding of latex particles coupled with horseradish peroxidase and a monoclonal antibody fragment. This generates an electrochemical detection signal proportional to the level of BNP in the patient sample. The limits of detection are 10 pg/mL to 4955 pg/mL. The Alere™ heart check is ready to use on a static or mobile device; capillary blood obtained by pricking is deposited on a single-use test strip and can be performed by patients themselves. There is no pre-analytical requisite for this method. Results are provided within 15 minutes. The Abbott architect i system is the lab-based system used in our hospitals for routine measurement of plasma BNP; this method is valid and considered as a reference method. It is based on a two-step sandwich immunoassay using monoclonal antibodies specific for human BNP; detection is performed by chemiluminescence. The limits of detection are 10 pg/mL to 5000 pg/mL. Unlike the Alere™ heart check, the Abbott architect i system requires plasma to be obtained from EDTA whole blood by centrifugation at 3500 g for 15 min at 4°C, within 4 hours after collection. Results are provided within 15 minutes.

3.3. Analytical Performance. Precision

Repeatability was assessed by performing six repeated measures of low and high BNP controls on the same day by the same operator using strips and controls from the same batch. Reproducibility was assessed by performing six measures of low and high BNP controls per day, over three consecutive days using three different strip batches.

3.4. Statistical Analysis

Values were expressed as median [interquartile range], mean $\pm$ SD or number (%) as appropriate. The difference between capillary and plasma BNP was assessed by Mann-Whitney test (independent samples). Receiver operator characteristic curves were generated for evaluating diagnostic accuracy of the two tests (Alere™ heart check and Abbott architect). All statistical analyses were performed using Stata version 10 statistical software (StataCorp LP, College Station, TX, USA). A P value < 0.05 was considered significant.

Table 1. Comparison of Analytical Performance of POC and Lab-Based BNP Assays[a]

System	Sample Type	CV Low Control	CV High Control	Reference
Abbott Architect**	EDTA plasma	5.5%	3.2%	(6)
Abbott i-STAT*	EDTA whole blood	14%	9.8%	(6)
Alere Heart check*	Capillary whole blood	10.1%	18%	This study
		8.4%	14.1%	(9)
Alere Triage*	EDTA plasma	9.2%	11.4%	(10)

Abbreviation: CV, coefficient of variation; EDTA, ethylenediaminetetraacetic acid.
[a] *POC and **Lab-Based BNP Assays.

4. Results

4.1. Clinical Performance and Characteristics of the Study Population

From December 2013 to January 2015, we enrolled 111 patients with stable chronic heart failure, mostly men (65%), whose characteristics are presented in Table 2. Fifty four patients (48.6%) had a heart failure with reduced ($\leq$ 50%) left ventricular ejection fraction (HFREF) mainly due to ischemic cardiac disease, whereas 57 of them (51.3%) had heart failure with preserved ejection fraction (HFpEF).

Forty nine (44%) patients had symptomatic heart failure (NYHA functional class II-III). The patients underwent two types of blood sampling, one venipuncture and one fingertip capillary blood sample.

4.2. Comparing Alere™ Heart Check BNP Test and the Reference Method

Overall, BNP values obtained ranged between 95 and 2178 pg/mL using the Alere™ heart check, and 89 to 1435 pg/mL using the architect system. There was a strong positive correlation between the two measurements (r = 0.947, P < 0.0001), although the values diverged more when BNP was higher than 1500 pg/mL (Figure 1). Bland-Altman analysis confirmed this difference (bias of 46.9 pg/mL), some values above 1500 pg/mL being outside the 95% limit of agreement (Figure 2). We subsequently compared both methods for their diagnostic accuracy in distinguishing asymptomatic CHF patients, i.e. belonging to NYHA functional class I, from those with symptoms, i.e. belonging to NYHA functional classes II-IV. For this purpose, we generated specific receiver operating characteristic curves. The methods (Alere™ heart check vs. Abbott architect) showed similar diagnostic performances (Figures 3 and 4) with values of area under the curve (AUC) of 0.983 and 0.984, respectively, and equivalent sensitivity, specificity and positive and negative likelihood ratios.

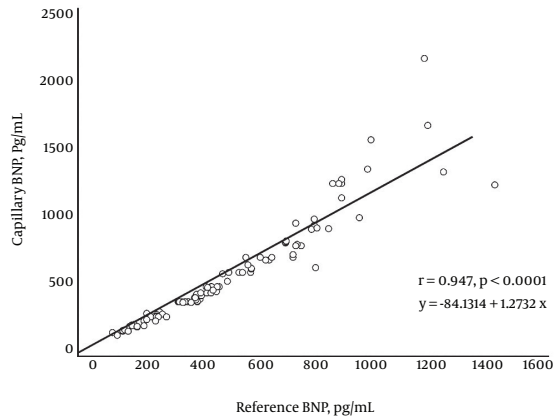

Figure 1. Distribution of Capillary BNP Measured on the Alere Heart Check System According to Plasma BNP Measured on the Abbot Architct System Among the 111 Patients with CHF, Examined in the Study

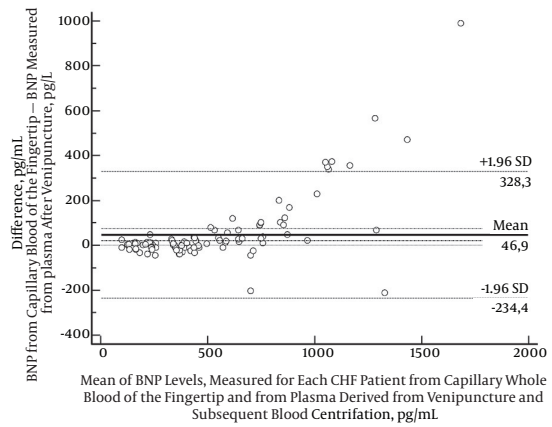

Figure 2. Bland Altman Comparison of Capillary and Plasma BNP

5. Discussion

In this study, we evaluated clinical performance of a new method for hematochemical diagnosis of cardiac de-

Table 2. Characteristics of the Study Population (n = 111)[a]

Characteristics	
Age	58 (47 - 65)
BMI	24.7 (21 - 29.2)
Men	72 (65%)
NYHA I	62 (55.8%)
NYHA II	30 (27%)
NYHA III	19 (17.1%)
LVEF, $\leq$ 50%	54 (48.6%)
LVEF, > 50%	57 (51.3%)
Patient history	
AF	34 (31%)
Moderate-to severe CKD, eGFR < 60mL/min/m^2	47 (42%)
Hypertension	53 (48%)
Diabetes	24 (22%)
ICD/PM	17 (15%)
Etiology of heart failure	
Ischemic	49 (44.1%)
Valvular	17 (15.3%)
Hypertensive	22 (19.9%)
Idiopathic	20 (18%)
Others	3 (2.7%)
Biology	
eGFR, mL/min/m^2	67 (54 - 82)
Hematocrit (%)	42 (39 - 45)
Capillary BNP, pg/mL	400 (245 - 678.75)
Plasma BNP, pg/mL	399 (246.5 - 699)
Bilirubin, μmol/L	13 (9 - 17)
Therapy	
Beta-blocker	100 (90%)
Diuretics	82 (74%)
ACE-i/ARB	94 (85%)

Abbreviations: BMI, body mass index; NYHA, New York heart association; LVEF, left ventricular ejection fraction; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ICD, implantable cardioverter defibrillator; PM, pace-maker; BNP, B-type natriuretic peptide; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.
[a]The numbers are absolute numbers followed by corresponding percentages or median values plus interquartile range.

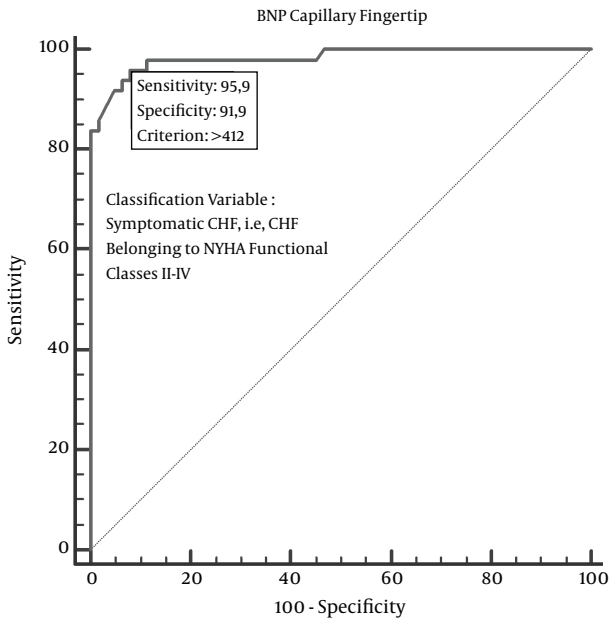

Figure 3. In this ROC plot there is the representation of the very good diagnostic performance (AUC = 0.983) of the Alere check system as a tool for predicting a clinical picture of CHF belonging to NYHA class II - IV. By adopting this method, the best diagnostic accuracy for identifying a condition of NHYA class II or higher has been attributed to the BNP threshold-value of 412 pg/mL. This means that this value, when derived from a measurement made by the Alere Check system on capillary blood, is associated to present of heart failure symptoms with a sensitivity of 95.9% and a specificity of 91.9% (see the note on the top of the graph). CHF, chronic heart falure; NYHA, New York heart association; ROC, receiver operating characteristic; AUC, area under the curve; BNP, B-type natriuretic peptide; pg, pictograms.

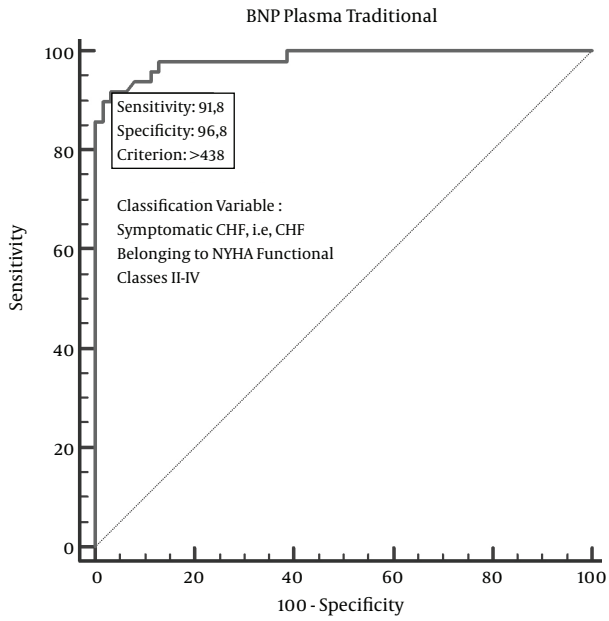

Figure 4. In this ROC plot there is the representation of the very good diagnostic performance (AUC = 0984) of the Abbott architect system as a tool for predicting a clinical picture of CHF belonging to NYHA classes II-IV. The criterion value of 438pg/mL has been identified as the BNP level that exhibites the best diagnostic accuracy for done from plasma using the Abbott architect system, is associated with heart failure symptoms with the best combination of sensitivity and specificity (91.8% and 96.8%, respectively.) CHF, chronic heart failure; NYHA, New York heart association; ROC, receiver operating characteristics; AUC, area under the curve; BNP, B-type natriuretic peptide; pg, pictograms.

compensation at patient's bedside, the Alere™ heart check system, by comparing it with our standard laboratory method (Abbott architect), which measures the plasma BNP. Overall, the Alere™ heart check system showed good performance as evidenced by a good correlation with the results of Abbott architect found in the entire investigated cohort. Due to technical limitations, the point-of-care testing methods are usually less accurate than the automata-based methods for quantifying biomarkers. The Alere™ heart check system is no exception and its accuracy falls within the range observed for other POCs measuring BNP at low concentrations of BNP, Table 1 (5). For higher concentrations of BNP, the Alere™ heart check system exhibited a relatively high coefficient of variation (18%) compared to other POCs, which is in line with the correlation rather low between the values of capillary (Alere™ heart check) and plasma (Abbott architect) BNP at higher concentrations (> 1500 pg / mL), as seen in NYHA class III (see also the box-and-whisker plots displayed in Figures 5 - 7). A similar variation, related to the concentration, was also observed in lab-based essays such as i-STAT Abbott (Figure 1). These results suggest that the Alere™ heart check system is an optimal tool for accurate identification of BNP levels, when they are located within its low-to-mid range. Overall, these data strongly suggest that Alere™ heart check system is reliable for measuring BNP in patients with chronic heart failure, particularly those within NYHA classes I - III, although important changes to the BNP should be confirmed by lab-based tests. From a practical standpoint, this system is the only test for BNP from capillary blood; the Abbott i-STAT and the Alere™ triage systems used in emergency departments measure BNP using either venipuncture EDTA whole blood (Abbott i-STAT) or EDTA plasma (Alere™ triage). Therefore, the Alere™ heart check is the only noninvasive system that uses capillary blood from the fingertip and could easily implemented as a rapid method for evaluating BNP by general practitioner, community cardiologist or for home monitoring, as evidenced by the recent HABIT trial (8). The differences observed between the two methods (Alere™ heart check and Abbott architect) are quite small, which suggests that both could be used, in alternation, to follow the efficacy of treatment in patients with chronic heart failure. Moreover, some ergonomic improvements could be made to facilitate deposition of blood droplet on the strip, which would result in fewer

errors. In addition, the machine lacks traceability of the results since patients' ID cannot be entered the machine. Importantly, the data we obtained showed that the Alere™ heart check BNP assay performed as well (AUC = 0.983) as the Abbott architect assay (AUC = 0.984) in distinguishing patients with asymptomatic (NYHA I) and symptomatic (NYHA II - IV) chronic heart failure. These results suggest that the Alere™ heart check BNP assay could be used in an emergency to triage dyspneic patients. However, our data suggested that more studies are needed to validate this method in emergency conditions. Indeed, capillary (Alere™ heart check) BNP was biased by 46.9 pg/mL (see Bland-Altman plot in Figure 2) compared to plasma (Abbott Architect) BNP. Currently, the algorithm available for the diagnosis of acute decompensated heart failure (ADHF) in international guidelines refers to a BNP cutoff of 100 pg/mL, i.e. ADHF can be excluded in acutely dyspneic patients whose BNP levels fall below this threshold value (1, 2). Given the bias observed in our Bland-Altman comparison, the use of Alere™ heart check system in emergency conditions would most likely overestimate patients who would fall into the gray zone of BNP (100 - 400 pg/mL), among patients with acute dyspnea, unless a new threshold is determined for this system.

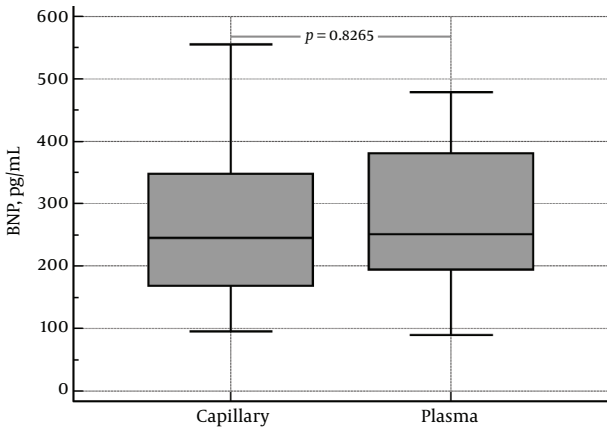

Figure 5. Comparison of Capillary (Alere Heart Check System) and Plasma (Abbott architect System) BNP Levels in Patients with Chronic Heart Failure NYHA Class I (no. 62 pts)

5.1. Study Limitations

Our study had many limitations. First, only two centers participated in the study was a monocentric study, with a small population younger than the current population with CHF and a few women. However, the range of BNP measured was sufficiently broad to test the limits of measurements. Another limitation of the study was that we

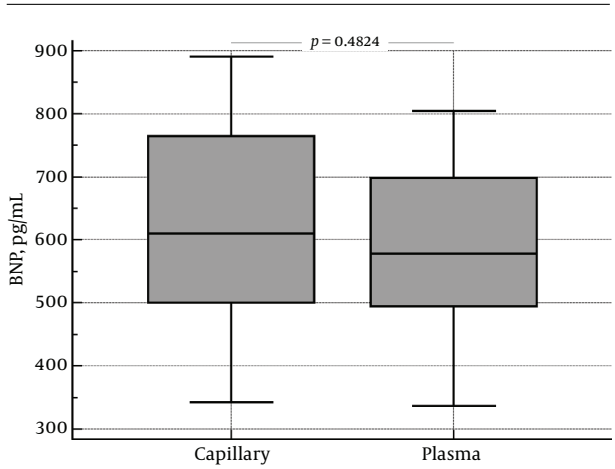

Figure 6. Comparison of Capillary (Alere Heart check System) and Plasma (Abbott Architect System) BNP Levels in Chronic Heart Failure Patients With NYHA Class II (no.3)

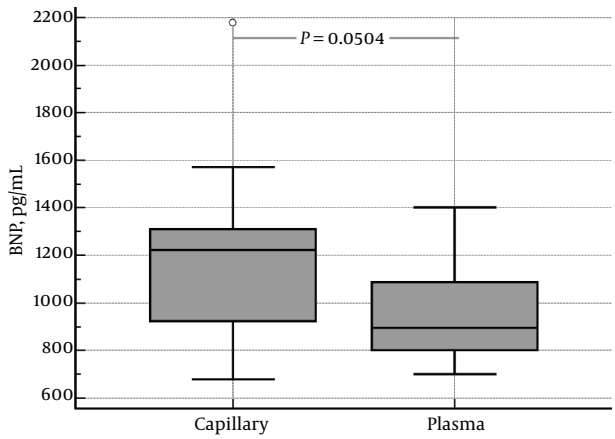

Figure 7. Comparison of Capillary (Alere Heart check System) and Plasma(Abbott architect system) BNP Levels in Chronic Heart Failure Patients With NYHA Class II (no.19)

did not make any measurements concerning serum natriuretic peptide levels grouped by gender. It is plausible that in the preliminary assessment of a new reagent for determination of serum BNP, the choice of executing separate calculations for males and females may be more correct, considering the higher average values of the circulating hormone detectable in females. Moreover, compared to other POCs available for blood glucose, INR or plasma BNP, we postulated a higher cost than laboratory-based methods. However, cost-effectiveness should be evaluated in a larger-scale study, according to the aimed application, e.g. remote vs. in-hospital monitoring.

5.2. Conclusions

The Alere™ heart check BNP test is a good POC for management of heart failure despite a relatively poor precision in higher BNP values. There was a good agreement between the Alere™ heart check system and the lab-based test (Abbott architect system). Thus, further studies are required to evaluate the real cost and real application of this device in emergency departments or by patients at home.

Footnote

Authors' Contribution: Study concept and design: De Vecchis. Analysis and interpretation of data: De Vecchis and Ariano. Drafting of the manuscript: De Vecchis. Critical revision of the manuscript for important intellectual content: De Vecchis and Ariano. Statistical analysis: De Vecchis and Ariano.

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