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Influence of Sample Matrix and Storage on BNP Measurement on the Bayer Advia Centaur

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The assessment and management of congestive heart failure relies increasingly on the measurement of B-type natriuretic peptide (BNP). However, the effective contribution of this biochemical test in the clinical decision making is influenced by reliability of the measure, which also depends on several preanalytical issues. Since there is controversy on the influence of the matrix and the storage conditions on BNP measurement, we compared results of BNP in serum, K2 ethylene diamine tetra-acetic acid (EDTA) plasma and lithium heparin plasma fresh samples and in matching samples stored at −20 and −80°C for 1 week. BNP measured on the Bayer Advia Centaur was systematically underestimated in heparin plasma (−47%) and serum (−62%) when compared to K2 EDTA plasma. According to the established 100 ng/L cutoff value, 25% and 37% of the fresh samples collected in heparin plasma or serum were misclassified from the reference K2 EDTA fresh specimen, respectively. When compared to the fresh specimens, the mean and interindividual bias observed for samples stored at either −20°C or −80°C was, overall, modest for K2 EDTA plasma (−2%) and heparin plasma (+6% and −4%, respectively), though it appeared clinically meaningful in serum (+47% and +28%, respectively). Although we can not rule out that other BNP assays using different antibodies may be not affected from degradation during storage to the same extent, results of our investigation demonstrate that K2 EDTA plasma is the most suitable specimens for BNP testing on fresh and frozen samples stored at either −20°C or −80°C for up to 1 week. J. Clin. Lab. Anal. 21:293–297, 2007. © 2007 Wiley-Liss, Inc.

Key words: B-type natriuretic peptide; natriuretic peptides; preanalytical variability; storage

INTRODUCTION

B-type natriuretic peptide (BNP) belongs to a class of structurally similar proteins, the natriuretic peptides (NP), which also include atrial NP (ANP), c-type NP (CNP), dendroaspis NP (DNP), and urodilantin (1). BNP is derived from the precursor peptide proBNP, which is cleaved into the inactive amino-terminal fragment (NT-proBNP) comprising the first 76 amino acids of proBNP and the biologically active BNP hormone (BNP 1-32) (2). BNP is constantly elevated in states of increased ventricular wall stress and the measurement of circulating BNP and N-terminal proBNP (NT-proBNP) help to identify and guide the therapy of patients with both acute and chronic heart failure (HF) (3,4) and might also have a significant role in differentiating origin of dyspnea either cardiac or pulmonary in the emergency room (5). Additionally, in patients with chronic HF and acute and chronic coronary syndrome, both elevated BNP and NTpro-BNP are markers of unfavorable prognosis, being associated with increased mortality (6). Finally, BNP

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is elevated in other disease states such as atrial fibrillation, amyloidosis, restrictive cardiomyopathy, valvular heart disease, pulmonary hypertension, chronic obstructive pulmonary disease, pulmonary embolism, renal failure, and sepsis (7). Since variations of the BNP concentration across different clinical settings may have significant implications in the assessment of either clinical status or patient response to treatment, standardization of both the analytical and preanalytical phases of BNP testing is essential to maximize its clinical usefulness.

NT-proBNP is biologically inactive and relatively stable both in circulation and in serum in vitro (8); on the other hand BNP is characterized by a relatively short circulating half-life of nearly 20 min (2) due to proteolytic degradation in vivo, which produces several breakdown products, including small amounts of des-SerPro-BNP (9,10). Plasma collected in glass tubes containing ethylene diamine tetraacetic acid (EDTA) as anticoagulant shows proteolytic degradation of BNP with loss of amino acids from the C-terminus due to active kallikrein (9). Moreover prolonged incubation of EDTA-anticoagulated plasma collected in plastic tubes results in des-SerPro-BNP production due to proteolytic degradation of the N-terminus by an aminopeptidase (10). In any case, the immediate cold storage of EDTA plasma collected in plastic (polypropylene) tubes is conventionally recommended for quantification of both ANP and BNP (11,12). In the laboratory setting, EDTA plasma is mostly an unsuitable sample for the measurement of several clinical chemistry analytes (13), hindering the parallel determination of the above peptides along with other biomarkers on a single specimen. Therefore, the identification of an alternative, more conventional and suitable sample matrix, such as serum or heparin plasma, for collection and storage of samples would facilitate blood collection for BNP testing, especially in the emergency units. Some studies have been published so far on the effect of the matrix and the storage conditions on BNP measurement, though most of them are fragmentary and even controversial.

MATERIALS AND METHODS

Three blood samples were simultaneously collected without venous stasis into evacuated tubes containing no additives (Terumo Europe, Haasrode, Belgium), K2 EDTA (Terumo Europe), or Lithium Heparin+gel (Terumo Europe) from 32 consecutive outpatients afferent to our phlebotomy service for routine laboratory testing. All subjects recruited to the study gave informed consent for being tested. For the optimum sample type studies, the specimens were centrifuged within 2 hr of collection and immediately processed or stored at either $-20^{\circ}C$ or $-80^{\circ}C$ for 1 week and assayed after thawing. BNP was measured on the ADVIA Centaur® System (Bayer Healthcare LLC, Diagnostics Division, Tarrytown, NY) according to manufacturer specifications, employing proprietary reagents, the same calibration curve, and an identical lot of reagents. The Bayer ADVIA Centaur BNP assay is a two-site dual-monoclonal immunochemiluminescent assay. Both antibodies are supplied by Shionogi & Co., Ltd. (Osaka, Japan) and have been widely used in the ShionoRIA BNP assay to demonstrate the clinical efficacy of BNP measurements. The capture biotinylated antibody is directed to the C-terminal part of the peptide (amino acids 27–32) and is coupled to streptavidin paramagnetic particles. The acridinium ester-labeled F(ab′)2 antibody is directed against the ring portion of BNP (amino acids 14–21). The analytical signal, in relative light units, is detected by release of chemiluminescent light. The assay has a sample volume of 100 μL and the time to first result is 18 min with the next result available in 15 sec. Samples up to 20,000 ng/L give linear results, whereas samples between 40,000 and 100,000 ng/L exhibit a plateau, although no high-dose hook effect is reported up to this limit. Bayer ADVIA Centaur BNP assay recommends the use of a single worldwide BNP cutoff of 100 ng/L for both genders (14). The total imprecision of the assay has been reported to be consistently $<3.5\%$ (14). Significance of differences between samples was assessed by Wilcoxon's paired test and the level of statistical significance was set at $P<0.05$. Bland-Altman plots were used to estimate the biases from the reference specimens and differences were reported as mean and 95% confidence interval (CI) percentage bias. The chi-squared test analysis was used to compare the percentage of specimens with BNP values exceeding the recommended <100 ng/L diagnostic threshold (14).

RESULTS

The limited number of specimens notwithstanding, results were homogeneously distributed within a broad measuring range, including either normal and pathological values. Spearman correlations (r) for BNP measured on fresh serum and lithium heparin specimens vs. K2 EDTA plasma were $r = 0.978$ ($P<0.001$) and $r = 0.987$ ($P<0.001$), respectively. A significant underestimation of values was observed with Passing and Bablok regression in specimens collected in either serum or heparin plasma (serum = 0.397 [EDTA] – 0.354 ng/L; heparin = 0.563 [EDTA] + 0.08 ng/L) (Table 1). According to the established 100 ng/L cutoff value, 25% and 37% of the fresh samples collected in heparin plasma or serum, respectively, were misclassified in respect of the reference K2 EDTA fresh specimen. The

TABLE 1. Influence of the matrix and the storage condition on results of BNP testing on the Bayer Advia Centaur*

	K2 EDTA plasma	Lithium heparin plasma	Serum
Fresh samples			
Values (ng/L)	131 (87–175)	71 (46–95)[a]	50 (35–65)[a]
Difference from K2 EDTA samples		−49% (−26 to −62%)	−62% (−41 to −83%)
−20°C samples			
Values (ng/L)	127 (84–170)[b]	72 (47–98)	64 (45–83)[b]
Difference from fresh samples	−2% (−10 to +6%)	+6% (−16 to +28%)	+47% (−93 to +186%)
−80°C samples			
Values (ng/L)	127 (84–169)[b]	66 (42–90)[b]	61 (43–79)[b]
Difference from fresh samples	−2% (−12 to +8%)	−4% (−33 to +25%)	+28% (−55 to +110%)

*BNP values are given as mean and 95% CI of the mean. Recovery of BNP expressed in percent of the initial value for the given reference specimen is shown as mean and 95% CI.
[a]$P < 0.01$ versus K2 EDTA plasma specimens under the identical storage conditions.
[b]$P < 0.01$ versus room temperature specimens collected in the identical matrix.

freezing of the specimens introduced a statistically significant mean bias in BNP values, though this variation was modest and clinically negligible for samples collected in K2 EDTA (−2%; 95%CI = −10 to +6% at −20°C, and −2%; 95%CI = −12 to +8 at −80°C) and lithium heparin (+6%; 95%CI = −16 to +28% at −20°C, and −4%; 95%CI = −33 to +25% at −80°C) when compared with the established day-to-day mean reference change value (RCV) for this marker (38%) (15). Conversely, the mean increase and unpredictable interindividual variation of recovery observed in serum specimens stored at either −20°C (+47%; 95%CI = −93 to +186%) or −80°C (+28%; 95%CI = −55 to +100%) largely exceeded the RCV.

DISCUSSION

The management of congestive HF relies heavily on the judgment and skills of the physician (1,2). Indeed, accurate diagnosis and successful treatment depend on appropriate interpretation of the history, physical examination, and production of reliable laboratory information, which is currently based on BNP or NT-proBNP testing. Although BNP testing is most commonly and usefully done immediately after collection of the specimens for immediate clinical management (16,17), there may be valid reasons for long-term storage of the samples, including instrument malfunctioning, retesting, and clinical retrospective studies. Moreover, the storage of the specimens may be indicated for serial measurements used to interpret results of therapeutic studies whereby serial changes in BNP concentrations would predict the therapeutic success of short-term (inpatient) and long-term (outpatient) management (15). Some previous reports have been published on the stability of BNP, but these involved different methods or protocols. Plasma EDTA and centrifugation of the tubes at 4°C are widely recommended to warrant reliable results of BNP testing (11). Although the presence of aprotinin may be effective to prevent the degradation of BNP in whole blood specimens stored at room temperature up to 12–24 hr (18), standard blood tubes containing EDTA as anticoagulant could be used when the sample is immediately centrifuged and frozen at either −20°C or −80°C, since the addition of aprotinin does not improve significantly the long-term stability of the molecule as compared with EDTA alone (18,19). However, since the measurement of several clinical chemistry analytes is not feasible on EDTA plasma (13), identification that a more conventional sample matrix such as heparin plasma or serum might be suitable for testing and storage of BNP would facilitate blood collection and handling of the specimens. Evidence has also been provided that heparin plasma may be an attractive alternative to EDTA, since many laboratories use heparin plasma for many or most of their clinical assays and, in particular, for cardiac markers testing (13). Dupuy et al. (20) observed that with freshly collected EDTA plasma as the reference sample, 90% vs. 89% of subjects were classified as "concordant" with heparin fresh vs. heparin frozen plasma samples by Biosite BNP testing (Biosite, San Diego, CA) on the Beckman Coulter Access Immunoassay System (Beckman Coulter, Brea, CA). Moreover, after storage at −20°C, 86% of the values of frozen EDTA plasma were concordant with values of fresh EDTA plasma, though no subject was misclassified according to the classification of the New York Heart Association (NYHA). At variance with these data, Wu et al. (14) demonstrated that BNP concentrations on the Bayer ADVIA Centaur are 60%, 39%, 70%, and 48% lower in blood collected into tubes containing citrate, heparin, fluoride, and no anticoagulants, respectively, compared with values for samples collected into EDTA-containing

tubes. Accordingly, Mueller et al. (21) demonstrated that the determination of endogenous BNP with the AxSYM assay using frozen EDTA plasma might not be valid since the mean recovery of BNP was less than 70% after 1 day of storage at −20°C and decreased to less than 50% after 2 to 4 months of storage. It was also concluded that the stability of BNP in samples stored at less than −70°C remains to be established. No statistical difference can be observed in BNP measurements when EDTA-anticoagulated blood is collected and stored in glass or plastic tubes for 24 hr at room temperature or at 4°C. However, unlike NT-proBNP, BNP is unstable in EDTA plasma for 3 days at room temperature at 4°C (22). Results of our investigation confirm that the sample matrix strongly influences the immunorecovery of BNP assayed on the Bayer ADVIA Centaur. The mean and interindividual variations in BNP immunoreactivity in EDTA plasma stored at either −20°C or −80°C is clinically negligible (15) and even lower than the estimated imprecision for this assay (3.5%) (14), making this matrix the most suited for collection and storage of the specimens. Samples collected in heparin plasma showed a constant decrease of immunoreactivity, regardless of the storage conditions. The mean bias observed in frozen serum specimens over the fresh ones is still limited, though the wider CI limits indicate a great inter-individual variation of recovery. Thus, the individual recovery of BNP values after a distinct duration of sample storage would be unpredictable. The large decay observed in serum specimens from the reference ones collected in K2 EDTA and the highly unpredictable individual response to the storage conditions both make this matrix unsuitable. This is consistent with the evidence that contact activation of blood coagulation system and blood coagulation factors, especially kallikrein, play an important role in digestion of BNP (9). Although we can not rule out that other BNP assays using different antibodies may be not affected from degradation during storage to the same extent, we conclude that K2 EDTA plasma is the most suitable specimens for BNP testing on fresh and frozen samples stored at either −20°C or −80°C for up to 1 month. We also point out that the minor bias observed in NT-proBNP measurement regardless of the preanalytical handling (23) would make it more suitable than BNP for routine assessment and management of cardiac dysfunction, allowing the simultaneous determination of this promising peptide along with other biomarkers on a single serum or heparin plasma specimen.

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