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ORIGINAL ARTICLE

The Effects of Different Blood Sample and Anticoagulant Types on Quantitative Tests of B-Type Natriuretic Peptide

Liangping Yin [1], Qian Wang [2], Zhikui Jiang [1], Xiufang Meng [1], Yuying Wang [2]

[1] *Emergency Laboratory, Dahua Hospital, Xuhui District, Shanghai, China*
[2] *Research and Development Department, Shanghai i-Reader Biological Technology Co., LTD., Shanghai, China*

SUMMARY

Background: **BNP is a sensitive and widely used biomarker for an early diagnosis of heart failure. Currently, most commercial BNP detection products use EDTA plasma samples. The aim of this study was to evaluate the clinical performance of the BNP test by using whole blood samples compared to plasma samples, and to evaluate the effect of the anticoagulant type on the BNP test result.**
Methods: **In total, 106 patients with different BNP levels from the Dahua Hospital volunteered for this study. Clinically homogenous samples, including EDTA anticoagulant plasma, EDTA whole blood, and heparin anticoagulant plasma, were collected and analyzed by using i-Reader S automatic immuno-analyzer and its supporting reagent kits. Pearson's correlation and weighted least squares linear regression analysis, Bland-Altman plotting, and Kappa test were used for statistical analysis.**
Results: **Correlation analysis showed that BNP concentrations, measured from EDTA anticoagulated plasma samples, had a good linear regression relationship with BNP from whole blood samples, with a slope of 0.9477, $r = 0.9978$, $p < 0.05$. A similar correlation was observed between EDTA anticoagulated plasma samples and heparin anticoagulant plasma, with a slope of 0.8413, $r = 0.9793$, $p < 0.05$. The BNP concentration measured from the heparin plasma samples were lower than of the EDTA plasma samples. Bland-Altman analysis for assessing BNP concentration agreement showed there was no outlier ratio between EDTA whole blood and EDTA plasma within the range of the detection system, as well as no outlier between EDTA anticoagulated and heparin anticoagulant plasma. Kappa coefficient of BNP concentration between homologous EDTA anticoagulated and heparin anticoagulant plasma was 0.8553 ($p < 0.001$), and for EDTA anticoagulated plasma and homologous whole blood it was 0.8941 ($p < 0.001$).**
Conclusions: **The diagnostic performance of EDTA anticoagulated whole blood samples did not differ significantly from EDTA anticoagulated plasma samples for the BNP test. This study showed no big significant difference between EDTA anticoagulated and heparin anticoagulated plasma measurements within 2 hours. The type of anticoagulant should be carefully chosen when performing the BNP test if BNP samples were *in vitro* for a long time.**
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Correspondence:
Liangping Yin
Emergency Laboratory
Dahua Hospital
Xuhui District, Shanghai 200237
China
Email: 18616307081@163.com

KEYWORDS

type natriuretic peptide, blood sample types, EDTA anticoagulated whole blood, EDTA anticoagulated plasma, heparin anticoagulated plasma

INTRODUCTION

B-type natriuretic peptide (BNP) is a highly specific quantitative marker of heart failure (HF) [1]. Changes in

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its concentration in the body reflect changes in ventricular function, such as myocardial ischemia, necrosis, or injury. Quantification of BNP concentration in blood provides important reference values for the diagnosis and severity assessment of HF, as well as for risk stratification and treatment guidance for patients with HF and acute coronary syndrome (CHF) [2].

In clinical testing, blood samples need to be appropriately pretreated, such as by adding coagulant to whole blood and centrifugate to obtain serum for biochemical testing, or by using anticoagulant blood collection tubes to anticoagulate the sample, obtaining plasma after centrifugation [3]. Benefiting from the widespread use of products from manufacturers such as Beckman, Siemens, and Abbott, most emergency departments or clinical laboratories in China can conduct quantitative tests of BNP concentration from plasma samples [4,5]. The commonly used clinical blood anticoagulants include heparin salt, EDTA salt, citrate, etc. However, few studies on the clinical relevance of different anticoagulant homologous plasma have been reported, and often they present contradictory results. Analyzing the different types of blood samples from homologous plasma not only improves the reliability of the quantitative test, but also facilitates more comprehensive mining of patient sample information.

The *in vitro* stability of BNP is poor [6], and the conventional preparation of serum or plasma blood samples is time-consuming and hinders the use of BNP as a rapid diagnostic indicator in emergency clinics. Using whole blood samples can greatly reduce the turn-around time (TAT) and operation complexity of BNP testing in clinical use, achieving immediate sampling and testing, and improving the detection efficiency of BNP in blood samples.

In this study, EDTA- and heparin-anticoagulated plasma or whole blood samples from the same patient were analyzed by using the same protocol to investigate the effects of different sample types on BNP quantification. Statistical analysis found that both the EDTA-anticoagulated whole blood and the heparin-anticoagulated plasma samples showed a good clinical correlation and agreement with the corresponding EDTA- anticoagulated plasma samples. These results revealed the possibility of using whole blood instead of plasma samples for BNP tests in clinical diagnostics, paving the road for a broader clinical application of BNP tests. However, the same type of anticoagulation tube should be used for BNP tests to avoid the inaccurate results caused by matrix effects.

MATERIALS AND METHODS

Collection of blood specimens

The collection of blood specimens was performed by an expert phlebotomist, following the international standard of the Clinical Laboratory Standard Institute (CLSI) [7]. The blood from a single patient was collected in both an EDTA-K2- and a heparin-anticoagulant tube, and the standard anticoagulants were used for a routine blood analysis [8]. The whole blood samples in each tube were divided evenly into two parts. One part was centrifuged at 3,500 rpm/minute for 8 minutes for plasma sample collection, and the other part was directly applied as whole blood sample. Samples with obvious hemolysis, lipemia, and jaundice were eliminated to minimize the interference on the test results. The BNP tests were applied within 2 hours after blood specimen collection.

The samples were collected from 126 volunteers that underwent BNP testing in the Dahua Hospital, Xuhui District, Shanghai, from October 2020 to April 2021. Among them, 106 volunteers that were included in the final statistical analysis, after eliminating the cases that were exceeding the instrument detection range. These volunteers consisted of 54 males and 52 females, with an average age of 79 years. The BNP concentrations of the selected volunteers ranged from 0.1 pg/mL to 5,000 pg/mL and were evenly distributed in low, medium, and high levels.

The protocol was approved by the ethics committee of the Shanghai Dahua Hospital (Shanghai Dahua Hospital DHYY-20191230-08), and informed consent was signed by all patients or their close relatives.

Instruments and reagents

All the tests were operated on the i-Reader S automatic immune-analyzer (Shanghai i-Reader Biotechnology Co., Ltd., Shanghai, China). The instrument was calibrated by using appropriate reference standard material. BNP assay kits and supporting sample dilution buffer were provided by Shanghai i-Reader Biotechnology Co., Ltd., (Shanghai, China). Single-use EDTA-K_2 anticoagulant and heparin anticoagulant vacuum blood collection tubes were purchased from Henan Zhiyuan Medical Technology Co., Ltd., (Henan, China) and BD Medical (the United States).

Statistical analysis

Minitab 21 and Microsoft Excel 2019 were used for data processing and statistical analysis. Pearson's correlation analysis and M + 2r were used for correlation analysis ($p < 0.05$ was considered statistically significant). Bland-Altman plotting and Kappa test were used for consistency analysis and the determination of consistency intensity, respectively [9].

RESULTS

Correlation analysis of different blood sample types and different anticoagulants

Correlation analysis of different blood sample types and different anticoagulants was performed to investigate the effects of the different blood sample types (plasma and whole blood) and the different anticoagulants (EDTA and heparin) on the BNP quantification test.

Table 1. BNP concentration of different blood sample types.

	Number of samples	BNP concentration (pg/mL) median (quartiles)	Positive rate
EDTA plasma	**106**	**194.01 (66.64, 741.00)**	**66.04%**
EDTA whole blood	**106**	**169.84 (66.65, 768.71)**	**66.98%**
Heparin plasma	**88**	**148.12 (48.43, 589.08)**	**59.09%**

Table 2. Interpretation of the Kappa coefficient.

Kappa	Agreement
< 0	**less than chance agreement**
0.01 - 0.20	**slight agreement**
0.21 - 0.40	**fair agreement**
0.41 - 0.60	**moderate agreement**
0.61 - 0.80	**substantial agreement**
0.81 - 0.99	**almost perfect agreement**

Table 3. Kappa analysis of different sample types.

Blood samples	Negative or positive judgment	EDTA plasma			Kappa
		Positive	Negative	Total	
EDTA whole blood	**positive**	**68**	**3**	**71**	**0.8941**
	negative	**2**	**33**	**35**	
	total	**70**	**36**	**106**	
Heparin plasma	**positive**	**52**	**0**	**52**	**0.8553**
	negative	**6**	**30**	**36**	
	total	**58**	**30**	**88**	

The quartiles of BNP concentration and the positive rates of each group are shown in Table 1 and Figure 1. A sample is judged positive if the BNP concentration is greater than the cutoff value, 100 pg/mL.

To evaluate the clinical correlation between the BNP concentrations measured from different blood sample types, the correlation and linear regression analysis (weighted least squares (WLS) model) was used. The results are indicated in Figure 2. The results showed that BNP concentrations, measured from EDTA anticoagulated plasma samples, had a good linear regression relationship with BNP from EDTA whole blood samples, with a slope of 0.9477, $r = 0.9978$, $p < 0.05$. A similar correlation was observed between EDTA anticoagulated plasma samples and heparin anticoagulant plasma, with a slope of 0.8413, $r = 0.9793$, $p < 0.05$. The BNP concentration measured from heparin plasma samples were lower than that of the EDTA plasma samples.

Bland-Altman analysis of different sample types

To assess the variance of the BNP concentration across the different blood sample types, the Bland-Altman plot was applied. The results are shown in Figure 3.

Notably, Bland-Altman analysis for assessing the BNP concentration agreement between EDTA whole blood and plasma revealed, that among these 106 volunteers, 3 data points (plasma BNP concentration: 7.36, 22.7, and 22.2 pg/mL) did not conform to statistical analysis, which is possibly caused by the fact that the linearity of the BNP assay kits provided by Shanghai i-Reader Biotechnology Co., Ltd., (Shanghai, China), is within a range from 25 to 5,000 pg/mL. Similarly, 3 data points (BNP concentration 7.36 and 25.26 pg/mL in EDTA plasma, and 11.25 pg/mL in heparin plasma) did not

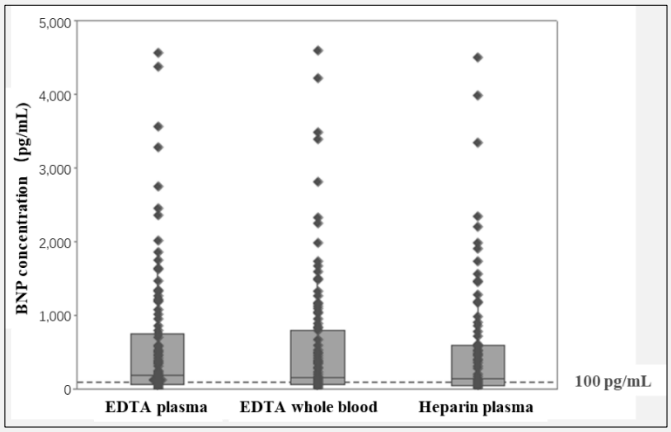

Figure 1. Boxplot of the BNP concentration measured from different types of blood samples.

The dotted line indicates the cutoff point, 100 pg/mL, of the BNP concentration.

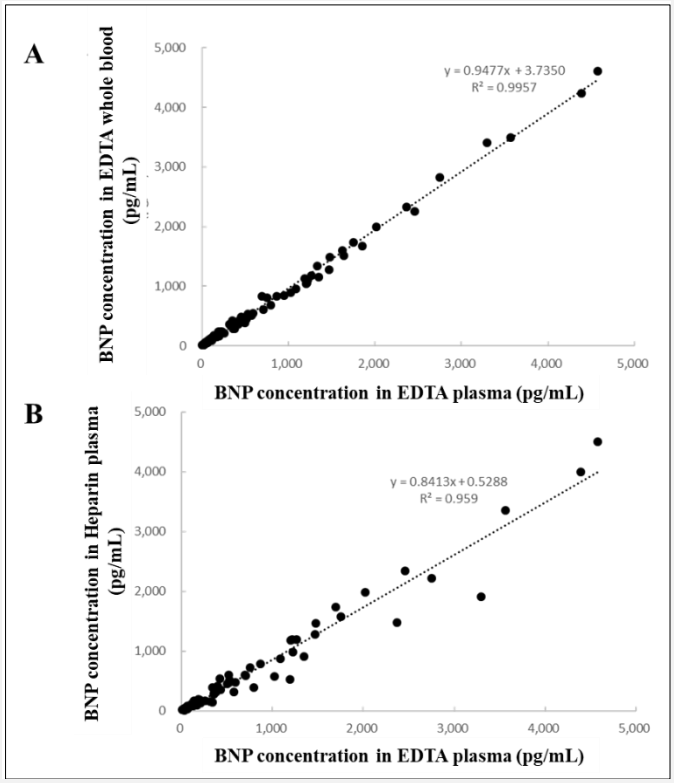

Figure 2. The correlation and linear regression analysis (WLS model) of different blood sample types for BNP concentration.

A - correlation between EDTA plasma and whole blood samples, correlation coefficient r = 0.9978, p < 0.05; and B - correlation between EDTA plasma and Heparin plasma, correlation coefficient r = 0.9793, p < 0.05.

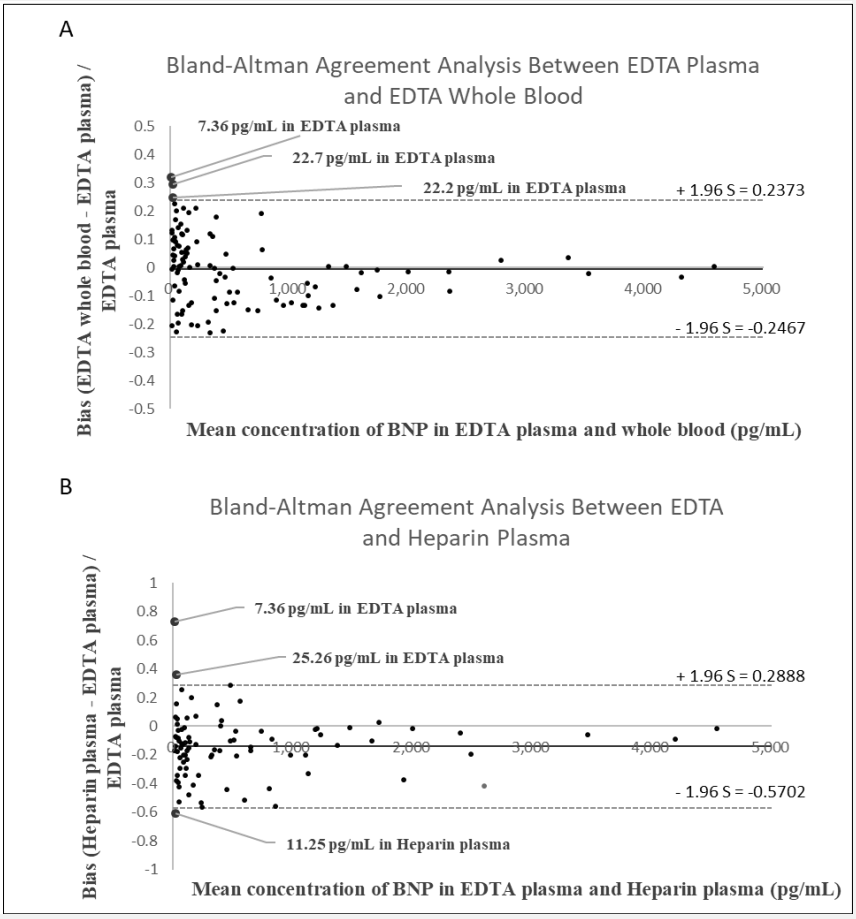

Figure 3. Bland-Altman plots for assessing the BNP concentration agreement between A) EDTA plasma and whole blood samples and B) different anticoagulants in plasma samples.

Solid lines are the mean differences; dotted lines represent the limits of agreement (from -1.96 S to 1.96 S), large solid dots indicate the data points did not conform to statistical analysis. The x-axis indicates the average BNP concentration between EDTA plasma and whole blood (3A) or EDTA plasma and heparin plasma (3B), and the y-axis indicates the relative bias of the BNP concentration. The dotted blue lines represent the limits of agreement (from -1.96 S to +1.96 S).

conform to statistical analysis in the heparin vs. EDTA plasma analysis, and were also nearly outside of linearity. In conclusion, the different sample types for BNP detection had statistically significant consistency.

Kappa analysis of different sample types

To evaluate BNP detection between different blood sample types, the kappa analysis was applied. Table 2 lists the interpretation of the kappa coefficient [10].

In this study, the kappa coefficient was used to evaluate the consistency of the BNP concentration measured from different types of blood samples. The results are shown in Table 3.

The Kappa coefficient between EDTA plasma and whole blood sample was 0.8941, and the kappa coefficient between EDTA plasma and heparin plasma was 0.8553. These results indicate that BNP detection of different types of blood samples reached an almost perfect agreement.

DISCUSSION

In this study, we found that EDTA whole blood samples showed a statistically significant linear correlation and consistency with EDTA plasma samples. Currently, plasma samples are commonly used in BNP testing, which requires a long preparation time. Given the poor

in vitro stability of BNP [11], using whole blood samples in BNP testing can significantly reduce TAT and establish a faster and simpler BNP test.

We also found that the type of anticoagulant has no significant effect on the BNP measurement within 2 hours. However, the BNP concentration measured from heparin plasma is slightly lower than that of EDTA plasma. Both EDTA and heparin are commonly used anticoagulants in clinical blood tests. EDTA has little effect on the shape and volume of blood cells and is often used in blood cell analysis. Heparin has less interference with blood components and is often used for blood chemistry tests. There is considerable controversy over the results of BNP testing in EDTA and heparin plasma. A short communication published in 2010 found that BNP concentration in heparin plasma is higher than that in EDTA plasma if the test is performed within one hour after blood collection, where a fast degradation of BNP would lead to an underestimate of the BNP concentration [12]. Therefore, it is important to carefully choose the anticoagulant when performing a BNP test if the blood sample is *in vitro* for a long time; EDTA plasma seems more suitable than heparin plasma due to its higher stability.

Ethics Approval Statement:

Our research includes “Human studies” due to the need of human experiments. We obtained approval from the Human Experiments Ethical Review Application (Shanghai Dahua Hospital, DHYY-20191230-08). Before we conducted our study, we had inspected the feasibility of the experiment scheme from numerous studies. During the experiment, volunteers were ethically treated.

Declaration of Interest:

The authors declare that they have no competing interests.

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