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ORIGINAL ARTICLE

Inorganic Phosphorus and Potassium Are Putative Indicators of Delayed Separation of Whole Blood

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Abstract
Objectives: The delayed separation of whole blood can influence the concentrations of circulating blood components, including metabolites and cytokines. The aim of this study was to determine whether clinical-biochemistry analytes can be used to assess the delayed separation of whole blood.
Methods: We investigated the plasma and serum concentrations of five clinical-biochemistry analytes and free hemoglobin when the centrifugation of whole blood stored at 4°C or room temperature was delayed for 4 hours, 6 hours, 24 hours, or 48 hours, and compared the values with those of matched samples that had been centrifuged within 2 hours after whole-blood collection.
Results: The inorganic phosphorus (IP) levels in the plasma and serum samples were elevated $\geq$ 1.5-fold when whole-blood centrifugation was delayed at room temperature for 48 hours. Furthermore, the IP levels in the plasma samples showed excellent assessment accuracy [area under the receiver-operating-characteristic curve (AUC) $>$ 0.9] after a 48-hour delay in whole-blood separation, and high sensitivity (100%) and specificity (95%) at an optimal cutoff point. The IP levels in the serum samples also exhibited good assessment accuracy (AUC $>$ 0.8), and high sensitivity (81%) and specificity (100%). The potassium (K^+) levels were elevated 1.4-fold in the serum samples following a 48-hour delay in whole-blood separation. The K^+ levels showed excellent assessment accuracy (AUC $>$ 0.9) following a 48-hour delay in whole-blood separation, and high sensitivity (95%) and specificity (91%) at an optimal cutoff point.
Conclusion: Our study showed that the IP and K^+ levels in the plasma or serum samples could be considered as putative indicators to determine whether whole-blood separation had been delayed for extended periods.

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1. Introduction

Plasma and serum samples have been used to diagnose a variety of diseases, predict prognoses, or identify disease-related biomarkers [1−4]. These samples are obtained by centrifugation after anticoagulation (using an anticoagulant, such as EDTA or heparin) and coagulation of whole blood, respectively [4]. Currently, the delay between collection and whole-blood centrifugation can influence the concentrations of circulating blood components, including metabolites and cytokines, due to prolonged contact with cells [5−10]. Hemolysis, which results in the release of hemoglobin and other intracellular components from red blood cells, might also occur when whole-blood processing is delayed [11]. Therefore, plasma and serum samples should be prepared as soon as possible after whole-blood collection by venipuncture [6]. However, whole-blood separation can be delayed for various reasons.

In laboratory tests (such as clinical biochemistry and hematology) routinely performed for diagnosis or prediction of prognosis in a clinical laboratory, the main causes of laboratory errors are preanalytical variables involved with biospecimen collection, processing, and storage conditions [12−14]. Several studies demonstrated that delayed whole-blood separation affects the plasma and serum concentrations of biochemical analytes [e.g., alanine aminotransferase (ALT), aspartate aminotransferase (AST), inorganic phosphorus (IP), potassium (K^+), and lactate dehydrogenase (LDH)] [6−9]. In this study, we investigated whether clinical-biochemistry analytes can be used to assess the delayed whole-blood separation. Moreover, to determine whether hemolysis influences variations in the tested analytes, we also investigated the variation of free plasma and serum hemoglobin concentrations induced by delayed whole-blood separation.

2. Materials and methods

2.1. Sample preparation

Whole-blood samples were collected from 135 participants into Vacutainer serum-separator tubes (Becton Dickinson, East Rutherford, NJ, USA) and plasma-separator tubes (Becton Dickinson). Each sample was divided into several 1.5-mL tubes (300 μL × 5 tubes), and stored at 4°C or room temperature until centrifugation for 15 minutes at 1,600*g*. Among the samples, one aliquot was centrifuged within 2 hours after whole-blood collection, and other aliquots were centrifuged at 4 hours, 6 hours, 24 hours, or 48 hours after the first centrifugation. (These represent "delay times" in the separation of plasma and serum.) The plasma and serum samples were separated, and then stored at −70°C before being used for clinical-biochemistry or enzyme-linked immunosorbent assay experiments.

2.2. Sample analysis

Concentrations of ALT (U/L), AST (U/L), IP (mg/dL), K^+ (mmol/L), and LDH (U/L) in the plasma and serum samples were determined using an automated chemistry analyzer (Roche Modular P800 autoanalyzer; Roche Diagnostics GmbH, Mannheim, Germany) with the appropriate reagents (Roche Diagnostics) according to the recommended protocol. Free-hemoglobin concentrations (mg/dL) in the plasma and serum samples were measured using the QuantiChrom Hemoglobin Assay Kit (Bioassay Systems, Haywood, CA, USA) according to the manufacturer's recommendations. All samples were measured in duplicate.

2.3. Statistical analysis

The analyte concentrations were expressed as means ± standard deviation. To identify analytes whose concentrations increased or decreased with statistical significance in plasma and serum samples following delayed whole-blood separation, we performed a paired two-tailed *t* test and a repeated-measures analysis of variance using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

To determine whether the plasma or serum concentrations of clinical-biochemistry analytes can be used for assessing delayed whole-blood separation, we carried out receiver-operating-characteristic (ROC) curve analysis using MedCalc software for Windows (MedCalc Software, Ostend, Belgium). The area under the ROC curve (AUC) values < 0.7, between 0.7 and 0.8, between 0.8 and 0.9, and > 0.9 were considered as poor, fair, good, and excellent, respectively. Statistical significance was determined as $p < 0.05$.

3. Results

3.1. Effect of delayed whole-blood separation on the stability of clinical-biochemistry analytes

We evaluated the concentration changes of ALT, AST, IP, K^+, and LDH in the plasma and serum samples when the centrifugation of whole blood stored at 4°C or room temperature was delayed for 4 hours, 6 hours, or 24 hours as compared to the separation being performed within 2 hours after whole-blood collection (baseline; Tables 1 and 2). The concentrations of AST, K^+, and LDH increased slightly (3.7−21.2%), but significantly ($p < 0.05$), in the plasma samples when whole-blood centrifugation was delayed at 4°C for 24 hours. Additionally, the AST and LDH concentrations changed more markedly at room temperature as compared to those at 4°C. The IP and LDH levels in the plasma were elevated $> 20\%$ relative to baseline when separation was delayed for 24 hours at room temperature. In the serum samples, the K^+ and LDH concentrations increased when whole-blood centrifugation was delayed