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The Effects of Sampling Procedures and Storage Conditions on Short-term Stability of Blood-Based Biochemical Markers of Bone Metabolism

To the Editor:

The prevalence and incidence of metabolic bone disease are rising as the population ages (1). In conjunction with imaging and clinical history, biochemical bone markers have become increasingly important in diagnosis and for monitoring bone disease (2, 3).

Little evidence is available regarding preanalytical influences on short-term stability of bone markers. We have evaluated the effects of sample type, processing, and storage procedures on the stability of biochemical markers of bone turnover [C-terminal telopeptide of type 1 collagen (β -CTX), N-terminal propeptide of procollagen type 1 (P1NP), osteocalcin (OC), bone-specific alkaline phosphatase (bone ALP), and parathyroid hormone (PTH)].

After obtaining informed consent, we collected venous blood samples from 18 patients with metabolic bone disease into lithium heparin-containing, EDTA-containing, and serum Sarstedt blood-collection systems.

¹ Nonstandard abbreviations: β -CTX, C-terminal telopeptide of type 1 collagen; P1NP, N-terminal propeptide of procollagen type 1; OC, osteocalcin; bone ALP, bone-specific alkaline phosphatase; PTH, parathyroid hormone.

We investigated the use of 3 sample-processing protocols (6 patients each): (a) delayed separation from cells (samples stored at room temperature for 1, 2, 4, 8, 24, or 48 h after venesection before separation); (b) immediate separation and storage at room temperature (plasma and serum aliquots left at room temperature for 2 h, 4 h, 8 h, 24 h, 48 h, or 7 days); and (c) immediate separation and storage at 2 °C–8 °C (plasma and serum aliquots stored at 2 °C–8 °C for 24 h, 48 h, 7 days, 14 days, or 28 days).

All samples were then stored at –70 °C until analysis of each batch. Baseline samples for each sample type (produced by immediate separation and storage of plasma or serum aliquots at –70 °C) were analyzed for all patients. We measured β -CTX, P1NP, OC, and PTH on the Modular Analytics E 170/Elecsys® platform (Roche). In addition, we used the Metra Biosystems enzymatic immunoassay to measure bone ALP (in duplicate, serum samples only) in 3 patients from each sample-processing protocol. The assays were assessed for intraassay imprecision by duplicate analysis of each sample for each container type, and interassay imprecision was assessed with QC material. For all assays, mean CVs were <10%.

After analysis, we used 1-way ANOVA with Bonferroni-corrected post hoc *t*-tests to compare analyte concentrations at each time point with the baseline concentration (Table 1). Mean values were considered significantly different at *P* values <0.05.

The stability of each analyte was evaluated according to the criteria of the 2002 WHO guidelines (4, 5) and was defined as the amount of time the change in concentration remained less than half of the total error of the sum of the biological and analytical variation. The calculated value was <5.4%

Table 1. Analyte concentrations compared with baseline values for samples stored unseparated for up to 48 h at room temperature, stored separated for up to 7 days at room temperature, and stored separated for up to 28 days at 2 °C–8 °C.^a

Analyte and sample type	Unseparated and stored at room temperature					Separated and stored at room temperature					Separated and stored at 2 °C–8 °C				
	Immediate	8 h	24 h	48 h	Immediate	Immediate	24 h	48 h	7 days	7 days	48 h	7 days	14 days	28 days	
β -CTX, $\mu\text{g/L}$															
Lithium heparin	0.56 (0.04)	0.51 (0.04)	0.42 (0.02)	0.29 (0.02)	0.30 (0.08)	0.24 (0.07)	0.19 (0.05)	0.09 (0.03)	0.45 (0.09)	0.36 (0.08)	0.31 (0.07)	0.18 (0.04)	0.17 (0.04)		
EDTA	0.56 (0.05)	0.53 (0.04)	0.55 (0.05)	0.51 (0.05)	0.29 (0.08)	0.31 (0.08)	0.28 (0.08)	0.26 (0.08)	0.45 (0.10)	0.39 (0.09)	0.38 (0.09)	0.41 (0.09)	0.35 (0.08)		
Serum	0.57 (0.05)	0.49 (0.03)	0.43 (0.03)	0.29 (0.01)	0.30 (0.08)	0.30 (0.07)	0.24 (0.06)	0.14 (0.04)	0.47 (0.10)	0.45 (0.09)	0.40 (0.08)	0.34 (0.07)	0.24 (0.06)		
OC, $\mu\text{g/L}$															
Lithium heparin	30.9 (5.2)	27.2 (4.6)	20.3 (2.1)	23.6 (5.4)	19.5 (6.1)	15.8 (5.3)	17.1 (5.2)	13.9 (4.6)	27.3 (9.0)	26.0 (8.6)	25.2 (8.3)	23.9 (8.1)	22.7 (7.9)		
EDTA	30.6 (5.6)	32.1 (5.1)	27.5 (4.7)	25.4 (4.9)	19.6 (6.1)	20.8 (5.8)	18.0 (5.6)	17.1 (5.5)	28.8 (10.4)	28.6 (10.0)	27.6 (9.5)	26.5 (9.5)	25.4 (9.3)		
Serum	30.6 (4.7)	25.7 (5.0)	23.6 (4.9)	20.6 (4.6)	18.7 (6.0)	17.2 (5.6)	14.4 (4.9)	13.1 (4.5)	28.4 (10.1)	26.8 (9.5)	25.2 (9.2)	23.1 (9.0)	21.6 (8.4)		
PTH, pmol/L															
Lithium heparin	9.1 (2.0)	8.4 (1.9)	7.8 (2.0)	7.9 (1.5)	5.7 (2.0)	5.4 (1.8)	5.4 (1.8)	5.0 (1.8)	4.2 (0.3)	4.2 (0.2)	4.0 (0.3)	4.1 (0.2)	3.7 (0.3)		
EDTA	8.4 (1.7)	8.4 (1.8)	8.5 (1.5)	7.4 (1.1)	5.8 (1.9)	6.0 (1.9)	5.5 (1.8)	5.1 (1.7)	4.4 (0.4)	4.4 (0.5)	4.5 (0.4)	4.2 (0.4)	4.2 (0.3)		
Serum	9.6 (1.9)	8.5 (1.9)	7.6 (1.8)	6.5 (1.6)	5.6 (1.9)	5.3 (2.0)	4.2 (1.7)	2.9 (1.3)	4.1 (0.3)	4.0 (0.3)	3.6 (0.3)	2.7 (0.4)	2.2 (0.5)		

^a Data are presented as the mean (SE). Values in boldface indicate a significant difference from the baseline (immediate) value ($P < 0.05$). Samples were also separated 1, 2, and 4 h after collection at room temperature and stored at room temperature for 2, 4, and 8 h after separation and at 2 °C–8 °C for 24 h after separation. No significant differences between these samples and baseline values were found for any of the analytes.

for β -CTX, <3.7% for P1NP, <4.2% for OC, and <4.3% for bone ALP. Because PTH is a regulatory hormone and because quantifying its biological variation is difficult, PTH stability was defined by the amount of time a change of <10% occurred.

We found P1NP and bone ALP to be stable and identified no significant changes for any sample types over the time periods investigated. By the WHO criteria, β -CTX was stable in EDTA for 48 h at room temperature and for 7 days at 2 °C–8 °C. In lithium heparin, β -CTX was stable for 4 h at room temperature and for <24 h at 2 °C–8 °C. β -CTX in serum was stable for 4 h when stored unseparated at room temperature, for 8 h when stored separated at room temperature, and for 48 h when stored separated at 2 °C–8 °C.

OC in EDTA was stable for 8 h when stored unseparated or separated at room temperature and was stable for 7 days when stored separated at 2 °C–8 °C. Lithium heparin and serum samples were less stable, however, with unseparated samples being stable for 2 h and 1 h, respectively. Separated samples, however, were stable for 2 h at room temperature and for 24 h at 2 °C–8 °C. PTH was stable for 8 h when stored unseparated in lithium heparin and was stable for 48 h when stored unseparated in EDTA. PTH was stable for 48 h and 28 days when stored separated at room temperature and at 2 °C–8 °C, respectively. The stability of PTH in serum was decreased to 4 h when stored unseparated, to 8 h when stored separated at room temperature, and to 48 h when stored separated at 2 °C–8 °C.

Baseline analyte concentrations were highly correlated, with similar values measured in lithium heparin (n = 18), EDTA (n = 18), and serum (n = 18). Although a

difference between EDTA and serum was observed for β -CTX and P1NP (3.4% and 3.7% lower, respectively, in EDTA), this difference was deemed statistically insignificant and consistent with inter-assay imprecision.

For the measurement of β -CTX and OC, we recommend that samples be collected into EDTA-containing tubes and undergo separation procedures as soon as possible. If samples are not analyzed immediately, we recommend they be stored frozen. This information is vital to ensure the validity of results for any laboratories referring or accepting samples. Serum bone ALP and plasma or serum P1NP are stable and suitable for routine processing. For PTH, collection into EDTA- or lithium heparin-containing tubes is recommended. We recommend that serum samples be processed immediately or be separated and stored frozen until analysis. These results have important implications for laboratories that accept PTH samples from primary care clinics or that use PTH as an “add-on” test for investigation of an abnormal calcium result.

Our data have produced stability guidelines that will allow laboratories to follow optimal sample-collection and -storage procedures for each analyte investigated, thereby improving their analysis and consequently their clinical utility.

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