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Estimation of the imprecision on clinical chemistry testing due to fist clenching and maintenance during venipuncture

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ABSTRACT

Objectives: An experimental study was planned to assess the influence on routine clinical chemistry parameters of fist making prior to, and maintenance during, venipuncture.

Design and methods: Blood was collected from 16 healthy volunteers with two separate sequential procedures, entailing standard venipuncture with hand opened throughout blood collection, or clenching the fist 6 times before venipuncture and maintaining the fist until completion of blood collection. After separation of lithium-heparin plasma at vacuum tubes with gel separator, 28 routine clinical chemistry parameters and serum indices were measured on Roche Cobas 6000 〈c501〉 module.

Results: Fist clenching and maintaining were associated with significant variations of 8/26 (31%) analytes tested. Specifically, aspartate aminotransferase (+2.3%), calcium (+2.2%), chloride (+1.0%), creatine kinase (+2.0%), magnesium (+2.3%), potassium (+13.4%), and sodium (+0.7%) increased, whereas phosphate (−5.0%) decreased. All variations except aspartate aminotransferase and creatine kinase exceeded the quality specifications for desirable imprecision. A remarkable increase of free hemoglobin in plasma (i.e., +28.2%) was also observed. The ratio of plasma potassium was significantly associated with that of plasma CK ($r = 0.55$; $p = 0.029$), but not with variations of other analytes. No significant correlation was observed between the ratio of free hemoglobin and those of other analytes.

Conclusions: The results of our investigation demonstrate that repeated clenching and maintenance of fist during venipuncture may trigger acute variations of several routine clinical chemistry parameters, which may be attributable to muscle contraction, hemolysis or both. Accordingly, venipuncture should be performed avoiding fist clenching and maintenance.

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1. Introduction

Hemolysis is conventionally defined as a process in which red blood cells (RBCs) are severely injured, up to complete breakdown, and which can be attributed to a kaleidoscope of physiological, pathological and spurious conditions. The life span of the erythrocytes is usually comprised between 90 and 120 days [1]. The gradual ageing of these non-nuclear elements increases their physical vulnerability, thus accelerating the clearance from the circulation by uptake by the reticulo-endothelial system of the spleen, liver and bone marrow [1]. A physiological concentration of cell free hemoglobin is hence always present in the blood of healthy subjects, ranging between 0.10 and 0.13 g/L in plasma and between 0.22 and 0.25 g/L in serum, respectively [2]. The pathological causes of erythrocyte injury in vivo, in some cases leading to hemolytic anemia, include congenital hemoglobin disorders (i.e., thalassemias, sickle cell disease and unstable hemoglobin variants), congenital disorders of RBC membrane (i.e., hereditary spherocytosis, elliptocytosis or stomatocytoses), inherited intracellular enzyme disorders (glucose-6-phosphate dehydrogenase deficiency), as well as such as acquired conditions such as autoimmune hemolytic anemia (AIHA), infections, hypersplenism, disseminated intravascular coagulation (DIC), hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura [1]. Beside in vivo hemolysis, many other circumstances may promote a spurious erythrocyte injury. More specifically, inappropriate or mishandled procedures used for collection, handling and storage of diagnostic blood samples are well established causes of RBC breakdown. The most important consequence of in vitro hemolysis is that the values of all those analytes that are more concentrated within the cell than in the plasma spuriously increase, thus generating a meaningful imprecision in the diagnostic reasoning and clinical decision making [3]. This is especially true for certain analytes such as potassium, lactate dehydrogenase (LDH) and aspartate aminotransferase (AST), the concentration of which is considerably high within RBC. Therefore, an accurate and well standardized procedure for collecting diagnostic blood specimens is essential in order to produce reliable and safe laboratory data [4].

As regards the activities directly related to blood drawing, the H3-A6 standard (formally known as GP 41-A6 standard) from Clinical

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Laboratory Standards Institute [CLSI] states that "the veins become more prominent and easier to enter when the patient forms a fist". However, it is also stated that "there must not be vigorous hand exercise", since this "may cause changes in the concentration of certain analytes in blood" [5]. An apparently controversial indication is provided by the guidelines of the World Health Organization (WHO) on drawing blood, in that it is stated to "ask the patient to form a fist so the veins are more prominent" and, even more importantly, "ask the donor to open and close the fist slowly every 10–12 seconds during collection" [6]. According to the available indications issued by the CLSI and WHO, it remains thereby uncertain as to whether fist clenching (or pumping) may be allowed or not during the venipuncture procedure, since this would probably cause a damage to RBCs (more or less like "foot-strike hemolysis") or modify the concentration of some analytes in blood due to muscle contraction and hand pressure. It is also noteworthy that fist clenching has recently been discouraged during routine blood pressure measurement, since this practice introduces a significant imprecision in blood pressure readings [7]. Therefore, this study was aimed to assess the influence of repeated clenching and maintenance of the fist during venipuncture on clinical biochemistry testing.

2. Materials and methods

The study population consisted in 16 ostensibly healthy volunteers (12 females and 4 males; mean age 49 years, range 36–64 years), enrolled among the laboratory personnel. Before blood sampling, all subjects observed an overnight fast (12 h) and remained seated for 15 min to eliminate interferences due to both lipaemia [8,9], and posture [10]. Blood was then collected with two separate and sequential procedures. The "standard" procedure, as in use in the local facility, entailed locating a radial vein on the forearm, and performing a standard venipuncture without tourniquet and maintaining the hand opened throughout blood collection. The second procedure entailed again locating a radial vein on the forearm and performing a standard venipuncture without tourniquet, but asking the subject to clench the fist for 6 consecutive times before venipuncture and maintaining the fist until blood collection had been completed. When clenching and maintaining the fist were requested, no specific instructions were given, so that the volunteers exerted their usual force of clenching. Venipuncture on the right arm according to the standard procedure and venipuncture on the left arm according to the second procedure were performed from volunteer 1 to 8, whereas this sequence was inverted from volunteer 9 to 16 (i.e., venipuncture on the left arm according to the standard procedure and venipuncture on the right arm according to the second procedure). Blood samples were always obtained at <1-min interval from both arms. For both procedures, 5 mL of blood was collected using a 20 gauge straight needle (BD Europe, Plymouth, United Kingdom) into a first evacuated blood tube without additives (2 mL, Vacuette®; Greiner Bio-One GmbH, Kremsmünster, Austria) which was then discarded, and was then followed by a second 3.0 mL evacuated blood tube containing lithium-heparin and gel separator (LH PST II REF 367374, Becton Dickinson Europe, Plymouth, United Kingdom, lot 5034469). The use of the discard tube was meant to eliminate potential interferences from endothelial cell injury upon phlebotomy. The routine clinical biochemistry tests were performed immediately after centrifugation of lithium-heparin specimens at 10 min × 2000g on modular pre-analyticals EVO-MPA system (Roche Diagnostics GmbH, Mannheim, Germany) [11] connected to Cobas® 6000 ⟨c501⟩ module (Roche Diagnostics GmbH, Penzberg, Germany), according to manufacturer specifications and using proprietary reagents. The panel of tests included the following parameters: albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), pancreatic amylase, AST, bilirubin (total and conjugated), C reactive protein (CRP), calcium, cholesterol, chloride, creatine kinase (CK), creatinine, gamma-glutamyl transferase (GGT), glucose, iron, LDH, lipase, magnesium, phosphate, potassium, protein (total), sodium,

Table 1
Variation of routine clinical chemistry analytes in plasma collected with (fist clenching) and without (no fist clenching) 6-time fist clenching before, and maintenance during, venipuncture in 16 ostensibly healthy subjects.

Analyte	Fist clenching	No fist clenching	p	Mean % difference	Desirable imprecision
Albumin, g/L	42.6 [41.4–43.6]	42.2 [40.7–43.5]	0.208	0.9	±1.6
ALP, U/L	56.0 [52.2–67.5]	55.0 [50.8–67.2]	0.507	1.8	±3.2
ALT, U/L	20.5 [15.0–35.5]	21.0 [15.0–35.8]	0.484	−2.4	±9.7
Amylase pancreatic, U/L	26.0 [18.0–29.8]	26.0 [17.8–29.0]	0.571	0.0	±5.9
AST, U/L	22.1 [20.0–25.5]	21.6 [18.5–24.0]	**0.023**	2.3	±6.2
Bilirubin total, μmol/L	7.15 [6.50–11.3]	7.20 [6.12–11.0]	0.141	−0.7	±10.9
Bilirubin conjugated, μmol/L	2.57 [1.96–3.68]	2.65 [2.17–3.68]	0.737	−3.1	±18.4
C reactive protein, mg/L	0.95 [0.40–1.78]	1.00 [0.40–1.70]	0.892	−5.3	±21.1
Calcium, mmol/L	2.33 [2.28–2.36]	2.28 [2.23–2.33]	<**0.001**	**2.2**	±1.0
Cholesterol, mmol/L	4.80 [4.15–5.57]	4.78 [4.16–5.52]	0.056	0.4	±3.0
Chloride, mmol/L	103 [102–105]	102 [101–104]	**0.010**	**1.0**	±0.6
Creatine kinase, U/L	99.0 [87.5–116]	97 [86.2–116]	**0.003**	2.0	±11.4
Creatinine, μmol/L	71.0 [64.0–80.8]	73.0 [63.5–81.5]	0.193	−2.8	±3.0
GGT, U/L	23.5 [13.0–38.0]	23.5 [14.0–38.8]	0.821	0.0	±6.7
Glucose, mmol/L	4.40 [4.20–4.88]	4.40 [4.02–5.00]	0.916	0.0	±2.3
Iron, μmol/L	16.3 [12.9–19.6]	16.4 [12.8–19.3]	0.114	−0.6	±13.3
LDH, U/L	312 [265–325]	315 [276–329]	0.518	−1.0	±4.3
Lipase, U/L	32.0 [25.5–35.8]	31.0 [25.2–36.0]	0.386	3.1	±16.1
Magnesium, mmol/L	0.86 [0.82–0.90]	0.84 [0.80–0.88]	<**0.001**	**2.3**	±1.8
Phosphate, mmol/L	1.00 [0.90–1.09]	1.05 [0.96–1.11]	**0.014**	**−5.0**	±4.1
Potassium, mmol/L	4.34 [4.03–4.71]	3.76 [3.47–3.88]	<**0.001**	**13.4**	±2.3
Protein total, g/L	71.6 [70.6–75.6]	71.4 [69.0–76.2]	0.133	0.3	±1.4
Sodium, mmol/L	141 [140–142]	140 [139–142]	**0.001**	**0.7**	±0.3
Triglycerides, mmol/L	1.07 [0.87–1.80]	1.06 [0.91–1.80]	0.798	0.9	±10.0
Urea nitrogen, mmol/L	4.87 [4.30–6.29]	4.91[4.40–6.44]	0.932	−0.8	±6.0
Uric acid, μmol/L	260 [190–292]	264 [192–291]	0.469	−1.5	±4.3
Hemolysis index	5.75 [3.25–7.75]	4.13 [2.25–6.00]	**0.008**	28.2	NA
Icteric index	1.00 [1.00–1.00]	1.00 [1.00–1.00]	0.999	0.0	NA
Lipaemic index	12.0 [9.00–14.0]	12.0 [10.2–16.2]	0.138	0.0	NA

Legend: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; NA, not available. Values are shown as median [interquartile range]. The bold p values are statistically significant ($p < 0.05$), according Wilcoxon-Mann-Whitney ranked-pairs test. Mean % difference in bold was higher than desirable imprecision.

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