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Postural change during venous blood collection is a major source of bias in clinical chemistry testing

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ABSTRACT

Background: To investigate the influence of different phlebotomy postures on clinical chemistry testing.
Materials and methods: Nineteen volunteers were recruited from the laboratory staff. A first set of samples was drawn after 25 min of resting in supine position, a second after 20 min in sitting position, and a third after 20 min in upright position. Clinical chemistry testing was performed on Roche Cobas C501.
Results: The plasma volume change (PVC) was −3.4% from supine to sitting, −14.1% from supine to standing and −9.7% from sitting to standing. Compared to quality specifications for bias, hemoglobin, hematocrit, albumin and total proteins exhibited meaningful increases from supine to sitting, whereas meaningful increases were observed for hemoglobin, hematocrit, albumin, alkaline phosphatase (ALP), amylase, aspartate aminotransferase (AST), total bilirubin, calcium, total and high-density lipoprotein (HDL) cholesterol, gamma-glutamyl transferase (GGT), glucose, lactate dehydrogenase (LDH), magnesium, total protein and triglycerides from sitting to standing. The parameters with meaningful bias from sitting to upright were hemoglobin, hematocrit, albumin, ALP, total bilirubin, calcium, total and HDL cholesterol, glucose, LDH and total protein.
Conclusions: These results provide further support to the need of standardizing patient's posture during phlebotomy.

1. Introduction

According to the historical definition coined with felicitous intuition by George D. Lundberg in the early 1980s [1], the total testing process can be divided into three discrete parts, that are the preanalytical, analytical and postanalytical phases. This thoughtful picture of laboratory diagnostics can then be further clustered in more specific activities, which include test ordering, patient preparation and identification, sample collection, transportation, preparation and analysis, test reporting and interpretation [2]. Several lines of evidence now attest that most errors in laboratory diagnostics emerge from inappropriate, incorrect or mishandled procedures during collection of diagnostic samples [3]. More specifically, problems in the preanalytical phase account for as many as 70% of all errors throughout the total testing process [4,5].

Major focus has been placed in harmonizing sample collection over the past decades, by development and implementation of national and international guidelines [6]. Reference documents such as the Clinical and Laboratory Standards Institute (CLSI) H3-A6 standard [7], or the World Health Organization (WHO) guidelines [8], contain a large number of recommendations, which are aimed to standardize the procedure of collecting venous blood, from patient preparation to sample treatment prior to analysis. Specific indications are also provided regarding patient posture during venipuncture. In the CLSI H3-A6 standard, the item "patient position" indicates that "specimens should be drawn with the patient seated comfortably in an appropriate chair or lying down" [7]. The WHO guidelines on drawing blood contain a different indication, that is "make the patient comfortable in a supine position (if possible)" [8]. As such, two major drawbacks seemingly emerge from these indications. First, no clear distinction is made between supine or sitting position during venipuncture, thus assuming that the two postural positions may be virtually interchangeable and the shift from one posture to the other may not generate meaningful bias of laboratory testing. This may be a major issue for hospitalized patients, especially when blood is drawn at different times of the day (e.g., in sitting position during the day or in supine position during the night) [9]. Then, it is not clearly stated for how long the patient should maintain a stable position (either supine or sitting) before venipuncture. This is noteworthy, because blood may be occasionally drawn from subjects (especially outpatients) who have walked or remained in upright position for long and had seated for a very short time before phlebotomy. Since it is now clearly acknowledged that either hemoconcentration and hemodilution

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may be associated with different postural positions (i.e., supine, sitting or standing) [10], this study was aimed to establish to what extent the concentration of a large number of clinical chemistry analytes may be influenced by phlebotomy posture.

2. Materials and methods

The study population consisted in 19 ostensibly healthy volunteers (mean age 44 ± 11 years; 7 males, 12 females; mean height 1.68 ± 0.11 m; mean weight 68 ± 12 kg), recruited from the staff of the laboratory of the University Hospital of Verona (Italy). Venous blood sampling was performed after an overnight fast, between 9 and 12 AM, by the same experienced phlebotomist and without venous stasis. Three separate sets of samples were collected from each subject. The first was drawn after the volunteer had rested for 25 min in supine position, the second after 20 min in comfortable sitting position, and the last after 20 min of permanence in upright position. At each time point one serum vacuum tube with clot activator and gel separator (Venosafe, Terumo Europe N.V., Leuven, Belgium) and another vacuum tube containing 5.9 mg K_2EDTA (Venosafe, Terumo Europe N.V.) were collected from a forearm vein and immediately transported to the laboratory. The serum samples were separated with standard centrifugation, according to manufacturer's instructions (i.e., 1300 × *g* for 15 min, at room temperature). No samples ought to be discarded for unsuccessful venipunctures or spurious hemolysis. The following analytes were measured on a Cobas c501 (Roche Diagnostics GmbH, Mannheim, Germany), using proprietary reagents: albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), alpha-amylase, aspartate aminotransferase (AST), bilirubin total and conjugated, calcium, cholesterol total and high-density lipoprotein (HDL), chloride (mmol/L), creatine kinase (CK), creatinine, C reactive protein (CRP), gamma-glutamyl transferase (GGT), glucose, iron, lactate dehydrogenase (LDH), lipase, magnesium, phosphate, potassium, total protein, sodium, triglycerides, urea and uric acid. Hematocrit and hemoglobin were also assessed on K_2EDTA blood, using an Advia 2120 (Siemens Healthcare Diagnostics, Deerfield, IL). The plasma volume change (PVC) was then calculated with the reference formula of Dill and Costill [11]. Results of measurements were finally reported as median and interquartile range (IQR). The significance of differences was assessed with Wilcoxon's signed rank test, and potential associations between variables were explored by univariate and multivariate analysis, using Analyse-it (Analyse-it Software Ltd, Leeds, UK). The degree of statistical significance was set at $p < 0.05$. The percentage variation calculated from the different postural positions was also compared with the desirable quality specifications for bias, as provided by Ricos et al. [12]. Each patient provided a written consent for being enrolled in the study, which was performed in accord with the ethical standards established by the institution in which the experiments were performed and the Helsinki Declaration of 1975.

3. Results

The main findings of this study are reported in Table 1. The change from supine to sitting posture generated a median PVC of −3.4%, whereas the PVC from supine to standing posture was −14.1%, and that from sitting to standing posture was −9.7% (Fig. 1). No significant correlation was found between PVC (at any time point) and weight, height, body mass index, sex and age in both univariate and multivariate analysis (all interactions, $p > 0.05$). Statistically significant differences from supine to sitting posture were found for hemoglobin, hematocrit, albumin, ALP, ALT, amylase, AST, total and HDL cholesterol, CK, GGT, iron, LDH, magnesium, total protein, triglycerides, and urea. When compared to the quality specifications for bias [12], only hemoglobin, hematocrit, albumin and total proteins exhibited meaningful increases (Table 1). Statistically significant differences from supine to standing posture were found for all analytes except chloride, CRP, phosphate, potassium, sodium and uric acid. When compared with the quality specifications for bias [12], meaningful increases were observed for hemoglobin, hematocrit, albumin, ALP, amylase, AST, total bilirubin, calcium, total and HDL cholesterol, GGT, glucose, LDH, magnesium, total protein and triglycerides (Table 1). The statistically significant differences from sitting to standing posture were identical to those observed between supine and standing position, with the exception of magnesium, which remained unchanged (Table 1). When compared with the quality specifications for bias [12], the number of analytes exhibiting meaningful variation included hemoglobin, hematocrit, albumin, ALP, total bilirubin, calcium, total and HDL cholesterol, glucose, LDH, and total protein.

4. Discussion

The plasma volume reacts dynamically to substantial modifications of gravitational force and hydrostatic pressure [13]. It is hence obvious that postural changes may also exert a strong influence on plasma volume distribution, especially in bipedal species such as humans and other primates, that can assume an erect position. This is mainly attributable to the fact that the venous pressure in the lower parts of the body increases after a prolonged standing position, thus generating an enhancement of capillary pressure, which ultimately leads to ultrafiltration of plasma in the interstitial space [14]. In this process of plasma extravasation, larger and nondiffusible plasma components remain entrapped within the blood vessels, whereas smaller and filtrable elements migrate along with water in the interstitial space. This is clearly reflected by the results of our study, wherein the concentration of virtually all analytes except non protein-bound ions were significantly modified by postural changes and the relative gap of gravitational force (Table 1).

Some studies have previously addressed the influence of posture on some laboratory parameters. The first ever human investigation about the effect of postural position on laboratory testing was published by Stoker et al. in 1966 [15], who investigated 13 healthy subjects and 4 patients with hypercholesterolaemia. In brief, mean increases of 12.9% for plasma cholesterol, 8.5% for plasma protein and 8.6% for hematocrit were recorded after 15 min permanence in upright posture compared to a reference supine position.

In 1974, Statland et al. studied 11 healthy men (ages 20–25 years), who had their blood collected in three separate days, after being supine for 30 min, remaining seated for 30 min and standing for 30 min [16]. When the sitting posture was used as a reference, a significant increase was observed after 30 min standing for albumin (+3.0%), ALP (+4.6%), total cholesterol (+2.5%), phosphate (+6.1%) and total protein (+2.5%), whereas the values of AST, ALT, total bilirubin, calcium, chloride, creatinine, iron, potassium, sodium, urea and uric acid remained unchanged. When the sitting posture was instead compared with 30 min in supine position, a significant decrease was observed for albumin (−6.7%), AST (−4.7%), calcium (−3.1%), total protein (−6.1%), potassium (−3.9%), whereas the remaining analytes tested remained unchanged. Interestingly, these findings seem rather different from those reported in other investigations, and also differ from data obtained in our experiments. This is probably attributable to the different study design, since Statland et al. performed sample collection in separate days, so that the final results may have been at least partially biased by a greater degree of inter-day biological and analytical variation.

In 1978, Dixon and Paterson studied 12 healthy students (8 men and 4 women, with age comprised between 20 and 25 years) [14], and reported that the values of albumin (+12%), ALP (+12%), total bilirubin (+17%), calcium (+5%), cholesterol (+18%), and total protein (+11%) significantly increased when the subjects changed position from supine to standing (for at least 20 min). No significance difference was instead observed for creatinine and phosphate. Interestingly, despite our study population was older than that studied by Dixon and Paterson, all changes were reproduced rather similarly in our investigation (Table 1), including the invariability of phosphate. At variance with Dixon and Paterson, we observed a modest increase of creatinine,