Clinica Chimica Acta 415 (2013) 196–201

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Random variation and systematic error caused by various preanalytical variables, estimated by linear mixed-effects models

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ARTICLE INFO

Article history:
Received 28 September 2012
Received in revised form 16 October 2012
Accepted 17 October 2012
Available online 29 October 2012

Keywords:
Preanalytical uncertainty
Preanalytical variables
Random variation
Sample handling
Pneumatic tube transport
Linear mixed-effects models

ABSTRACT

Background: We wanted to determine whether specific, preanalytical sample handling increases preanalytical variation and bias test results compared with optimal handling.
Methods: Blood was collected into 4 serum-separation tubes from each arm of 60 outpatients. In 30 of the patients, half of the tubes were transported in the pneumatic tube system, while the other half were manually delivered. In the remaining patients, the blood samples were collected using 21-gauge straight needles (green needles) and 23-gauge butterfly needles. Half of the tubes were mixed by inverting 5–6 times, and the other half by one inversion. Linear mixed-effects models were used as statistical method.
Results: Transporting samples in the pneumatic tube system caused a significant bias to the results for LD (4.5 U/L, $p<0.001$) and magnesium (0.0021 mmol/L, $p=0.003$). For CK and glucose, the preanalytical variation was significantly higher for samples transported in the pneumatic tube system vs manual delivery. Using butterfly needles resulted in lower values ($p<0.05$) for calcium (−0.0072 mmol/L), CK (−0.75 U/L) and LD (−1.6 U/L) compared with 21-gauge needles. The preanalytical variation for ALP was significantly higher with butterfly needles.
Conclusions: The specific sample handling had significant but small random and systematic effects on results for some analytes.

1. Introduction

Preanalytical variables such as transportation of blood samples in pneumatic tube systems, type of needles used when collecting blood, and suboptimal mixing of the samples may influence test results. With pneumatic tube systems, which have become common in many hospitals, shaking and gravity forces may influence the specimens, e.g. by hemolysis, and thereby change the concentration of some analytes [1–6]. Hemolysis may also be a problem when venipuncturing using thin needles [7–10]. The 23-gauge butterfly needle has a smaller inside diameter than the 21-gauge straight needle, and is often used when venipuncturing children and patients with fragile blood vessels. The manufacturers of blood tubes emphasize that the gel tubes have to be mixed by 5–6 inversions after the phlebotomy to disperse the clot activator. The activation occurs when blood makes contact with the micronized silica particles on the tube wall.

In some preanalytical studies, the deviations were estimated between a handling practice and different alternative practices [6,11,12]. However, some researchers have estimated the preanalytical variation for a specific practice without distinguishing between random and systematic effects [13–15]. In a previous study, we estimated the biases between specific preanalytical practices and optimal practices based on the distribution of the alternative sample handling [16]. We found linear mixed-effects models [17] useful in estimating different sources of variation and fixed effects in a data set of patients' results from optimally handled blood samples [18].

The aim of current study was to expand the number of preanalytical variables, and apply linear mixed-effects models, to examine whether specific, preanalytical treatments within current practice increase the preanalytical variation and bias test results compared with optimal treatment for 21 common clinical chemistry analytes. The effects of a pneumatic tube system, using different needles and suboptimal mixing of specimens were tested out. We hypothesized that transporting samples in a pneumatic tube system may increase the preanalytical variation and bias test results compared with manual gentle delivery. Further, we hypothesized that choice of needle for blood collection may influence the test results, and that collecting blood using

Abbreviations: GUM, Guide to Expression of Uncertainty in Measurement; SST, Serum separation tube; ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; CK, Creatine kinase; GGT, γ-Glutamyltransferase; LD, Lactate dehydrogenase; H-index, Hemoglobin index; TOOS, *N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3-methylaniline.

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http://dx.doi.org/10.1016/j.cca.2012.10.045

23-gauge butterfly needles causes a higher preanalytical variation and a bias in the test results compared with using the 21-gauge straight needles. Finally, we hypothesized that mixing blood samples by only one inversion instead of optimal mixing would affect the results similarly.

2. Materials and methods

2.1. Sample handling

The study was approved by the Regional Committee for Medical and Health Research Ethics, Western Norway, and adhered to the tenets of the Declaration of Helsinki. The patients signed an informed consent. We performed phlebotomies on a total of 60 non-fasting, consecutively recruited patients at our outpatient clinic. To limit the amount of blood collected, patients with only a few routinely ordered tests were randomly chosen. The study was in two parts.

In experiment 1, blood samples were obtained from 30 patients, 16 men and 14 women, aged 27–86 years. Experiment 2 included another 30 patients, 16 men and 14 women, aged 22–82 years. The phlebotomies and the sample handling were performed optimally according to current guidelines [19,20]. In experiment 1, the phlebotomy was performed using 21-gauge straight needles (0.8×32 mm, Becton Dickinson, USA) (green needles) in both arms. In experiment 2, blood was collected using a 23-gauge winged blood collection set with flexible tube (0.6×19 mm×178 mm, Becton Dickinson) (butterfly needle) in one arm, and the straight green needle in the other arm. The average duration of the blood collection was 3.5 min (range 2.5–6 min) for both arms.

The same medical technician performed all phlebotomies with the patient remaining in a sitting position for approximately 10 min at ambient temperature between 9 AM–1 PM. The tourniquet was loosely fastened and released after <1 min, immediately when blood appeared. Repeated clenching and unclenching of the fist was not allowed. The arm to be phlebotomized first was randomly chosen in both experiments, but in experiment 2 the green needle was always used first. Blood was collected using four plastic serum-separation Vacutainer SST II Advance gel tubes 3.5 mL (Becton Dickinson), containing silica clot activator, in each arm in both experiments, for a total of 8 tubes from each patient.

In experiment 1, the tubes were completely filled, mixed gently by 5 inversions, and put in a vertical position. One inversion was one complete turn of the wrist, 180°, and back. After 10 min (range 10–11.5 min), two gel tubes randomly chosen from each arm, i.e. a total of 4 tubes from each patient, were transported by the pneumatic tube system, TranspoNet Pneumatic Tube Systems (Swisslog, Switzerland), to the laboratory. The samples were padded in bubble plastic for transportation in the pneumatic tube system. The other 4 tubes were manually delivered in a vertical position. The average duration of both pneumatic and the hand delivery transport was approximately 2.5 min.

In experiment 2, two gel tubes, randomly chosen from each arm, i.e. a total of 4 tubes from each patient, were optimally mixed by gently inverting the tubes 5–6 times immediately after the phlebotomy. The other 4 gel tubes were only mixed by one inversion.

In both experiments clotting time was standardized according to the manufacturer's recommendation to 30 min (range 30–35 min) after the last tube was collected, and centrifuged for 10 min at 1600 *g* in a swing-out centrifuge Kubota 5930 (Kubota Corporation, Japan) at 20 °C. Immediately after centrifugation, the serum from each gel tube was separated into 2 secondary tubes.

2.2. Analytical methods

The samples were analyzed under repeatability (within-run) conditions, on average 2 h (range 1–4 h) after the phlebotomy, on Roche Modular Analytics SWA (serum work area) instruments by photometric methods (Roche Diagnostics, Germany).

On a P800 module, albumin was measured with the bromcresol green method; alkaline phosphatase (ALP) liquid according to IFCC; alanine aminotransferase (ALT) according to IFCC with pyridoxal phosphate activation; bilirubin, total with the Diazo method; calcium with *o*-cresolphthalein; cholesterol with CHOD-PAP (cholesterol oxidase phenol 4-aminophenazone peroxidase); creatine kinase (CK) liquid according to IFCC; creatinine with creatinine plus; γ-glutamyltransferase (GGT) liquid according to IFCC; glucose with Gluco-quant Glucose/HK; HDL-cholesterol (HDL-C) with HDLC3 (HDL-C plus, 3rd generation), no pre-treatment; iron with the FerroZine method without deproteinization; lactate dehydrogenase (LD) liquid according to IFCC; magnesium with xylidyl blue; phosphate with ammonium molybdate to form ammonium phosphomolybdate without reduction; potassium and sodium with ISE (ion-selective electrode) indirect; total protein with the biuret method; triglycerides with GPO-PAP (glycerol phosphate oxidase 4-aminophenazone); and uric acid by peroxide reacts in the presence of peroxidase, TOOS, and 4-aminophenazone to form a quinine-imine dye. Folate was measured with the Elecsys competitive method Folate III on two different E 170 modules (Roche Diagnostics). A photometric method, the hemoglobin index (H-index), was used for measuring hemolysis; 100 H-index units correspond to approximately 0.06 mmol/L (0.1 g/dL) of hemoglobin.

2.3. Statistical analysis

Data were analyzed by use of linear mixed-effects models [17]. Mixed-effects models allow analyses of multi-level data, and thereby allow separate estimates of fixed and random effects.

Random effects are expressed as standard deviations for variation between groups at each level, e.g. between persons, between arms, between tubes from each arm, and duplicates of each tube (measurement uncertainty). The between-arm SD represents the between-venipuncture SD. By drawing blood from both arms, we could estimate the between-venipuncture SDs separately for each experiment. The between-tube SD is defined as the preanalytical SD, and does not include the venipuncture variation.

We used the package Linear and Nonlinear Mixed Effects Models in R (The R Foundation for Statistical Computing) for the mixed effects analyses [21]. The level for statistical significance was set to 0.05. We calculated 95% CIs for both the fixed and random effects. Comparisons of the SDs for random effects were performed by evaluation of the overlap of their CIs, and the SDs were considered significantly different when their confidence intervals did not overlap.

3. Results

Table 1 presents the estimated mean differences (95% CI) between transporting samples in a pneumatic tube system vs manual delivery, use of butterfly vs green needles, and suboptimal vs optimal mixing. Table 2 presents the estimated preanalytical SDs (95% CI) for each of the paired treatments.

3.1. Pneumatic tube system vs manual delivery

Transporting samples by pneumatic tube system added a significant bias to the test results for LD ($p<0.001$) and magnesium ($p=0.003$) (Table 1). For CK and glucose, the preanalytical SD was significantly higher for samples transported in the pneumatic tube system than for those transported manually (Table 2).

3.2. Use of butterfly vs green needles

Using butterfly needles resulted in significantly ($p<0.05$) lower values for calcium, and CK compared with the results when using