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Preanalytical phase: Effects of water ingestion during fasting on routine hematological parameters in a small cohort of young women

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ABSTRACT

Background: We analyze the effects of water ingestion before blood extraction on routine hematological parameters.

Methods: Twenty female volunteers –mean 24 y– were included. Blood was collected after a 12 h fast period (T_0) and 1 h after the ingestion of 300 ml water (T_1). These parameters were analyzed: white blood cell (WBC) count; WBC differential count including lymphocytes (LYM), monocytes (MONO), neutrophils, eosinophils (EOS), and basophils; red blood cell (RBC) count; hematocrit (HCT); hemoglobin (HGB); mean cell volume; mean cell hemoglobin; RBC distribution width; and platelet count (PLT). Statistical significance: $P < 0.05$. Mean difference % (MD%) was calculated for each parameter and was compared with reference change value (RCV). A change was considered clinically significant when MD% exceeded the RCV.

Results: Significant differences were observed in (medians, T_0 vs T_1, P): WBC $\times 10^9$/l (6.51 vs 6.12, 0.002); LYM $\times 10^9$/l (2.90 vs 2.19, 0.000); MONO $\times 10^9$/l (0.50 vs 0.48, 0.031); EOS $\times 10^9$/l (0.17 vs 0.16, 0.003); RBC $\times 10^{12}$/l (4.46 vs 4.40, 0.024); HCT l/l (0.38 vs 0.37, 0.036); HGB g/l (129 vs 129, 0.009). All MDs% were lower than their respective RCV.

Conclusion: Ingestion of 300 ml water 1 h before blood extraction does not alter the hematological parameters studied.

1. Introduction

Quality policies are necessary to guarantee reliability in laboratory results along the biochemical process, including the preanalytical phase, because a large number of medical decisions and procedures depend on such results [1]. Although error probability has been observed to be highest in the preanalytical phase, the latter has often been underestimated and its significance in the results collected could therefore be either minimized or unidentified by laboratory professionals [2]. In addition, a crucial issue that has been reported to be an important source of error but is key to secure result reliability ensuring safety is patient preparation [3]. The absence of standardization and harmonization among laboratory professionals at the time of providing patient preparation instructions before blood sample extraction tests is consequently a matter of concern [3]. In 2014, the following specific patient preparation recommendations –before routine phlebotomy– were issued by the Working Group on Preanalytical Phase (WG-PRE) of the European Federation of Clinical Chemistry and Laboratory Medicine [4]: to collect blood samples in the morning in the basal state between 7 and 9 a.m., during which water ingestion is permitted, not to smoke, not to drink alcohol 24 h before blood extraction, and not to ingest caffeine-containing drinks during the morning of blood extraction. These recommendations are a relevant step forward towards the standardization of patient preparation for phlebotomy. Nonetheless, although these recommendations clearly allow water ingestion during the fast period, what precise water volume can be permitted and whether or not the permitted water volume is a source of preanalytical variability are questions whose answers are yet not known.

2. Materials and methods

2.1. Study design

This study is a continuation of our research on the analysis of routine laboratory parameters and therefore uses the same materials and methods as those of a previous study from our laboratory [5]. It was conducted in a laboratory without ISO 15189 accreditation in the Hospital Municipal de Agudos "Dr. Leónidas Lucero" in Bahía Blanca

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city, province of Buenos Aires, Republic of Argentina, in August 2017, and was approved by the Bioethics Committee of this hospital. The methodology used was that followed in previous studies on pre-analytical variability [6–8].

2.2. Subjects

The studied population included 20 women, aged 22–50 y (mean 24 y), from the Universidad Nacional del Sur, Bahía Blanca, Argentina, who voluntarily consented in writing to participate in this study. All volunteers, who had been previously informed of the aims of the present study, declared neither to have health problems nor to be taking medications.

Inclusion criteria: not to be pregnant and not to be suffering from any chronic or acute disease.

Exclusion criteria: to be under medical treatment or to have done physical activity 24 h before blood extraction.

2.3. Blood sampling

Blood samples were collected in the basal state on the same day, after a 12 h nocturnal fasting period without water ingestion. Blood extraction was performed by an experienced phlebotomist following the standards set by the Clinical and Laboratory Standards Institute (CLSI) [9] with adaptations by Lima-Oliveira et al. [10]. Once in the clinical blood laboratory, volunteers were asked to remain sitting for 15 min after which the first blood collection was performed at 9 a.m. After the first phlebotomy, they drank 300 ml water and continued sitting in the blood collection room for an additional hour after which the second phlebotomy was performed. Two blood samples −5 ml each− were thus collected per volunteer, namely a basal one (T_0) and a second one 1 h after water ingestion (T_1).

Blood samples were obtained through antecubital vein puncture using 21Gx1 needles (Neojet, Zhejiang Ougian Medical Apparatus Co.) and 10 ml syringes (Hongda, Jiangxi Hongda Medical Equipment Group Ltd). After blood extraction, samples were distributed in 1.3 ml plastic tubes with EDTA K_3 additive (Tecnon, Laboratorios Argentinos, Berisso, Argentina) for further analysis.

The water ingested by the volunteers was bottled table water from a local company (Aristu Hnos) which had been subjected to reverse osmosis, filtration, ionization, and ozonization to ensure physical, chemical and biological quality for human consumption.

2.4. Hematological parameters

Hematological determinations were performed using an automated hematological counter Sysmex XN 1000 (Sysmex) with reagents, calibrators, and controls from this manufacturer. The following hematological parameters were analyzed: white blood cell (WBC) count; WBC differential count including lymphocytes (LYM), monocytes (MOO), neutrophils, eosinophils (EOS), and basophils; red blood cell (RBC) count; hematocrit (HCT); hemoglobin (HGB); mean cell volume; mean cell hemoglobin; RBC distribution width; and platelet count. Table 1 shows the hematological parameters analyzed in the present study and the analytical CV_A obtained from the internal laboratory quality control for each hematological parameter. The hematological determinations were performed using the same lot of reagents within the hour in which blood samples had been collected.

2.5. Statistical analysis

Nonparametric statistics [11] was applied for data processing on account of the fact that the number of volunteers studied was lower than 30. Medians between T_0 and T_1 were compared using the non-parametric Wilcoxon Signed Rank Test for paired samples. Statistical significance was fixed at $P < 0.05$. Data analysis was performed using the Statistical Package for Social Science for Windows (SPSS) software (ver 15.).

The reference change value (RCV) was calculated for each hematological parameter using standard formula [8,12,13]. CV_I is the within-subject biological variation obtained from Westgard database [14], and CV_A is the analytical CV from the internal laboratory quality control. All these data are included in Table 1.

Mean difference % (MD%) was calculated for each hematological parameter using the following formula [15–19]:

$$MD\% = \frac{100}{N} \sum_{i=1}^{i=20} \frac{T_{1(i)} - T_{0(i)}}{T_{0(i)}}$$

where N = 20 (total number of volunteers that participated in the study), $T_{1(i)}$ is the value of the hematological parameter in the blood sample of each individual 1 h after the ingestion of 300 ml water (T_1), $T_{0(i)}$ is the value of the hematological parameter in the blood sample of each individual at basal time (T_0), and i means individual. All these data are included in Table 1. Every change detected in an hematological parameter was considered to be clinically significant if MD% exceeded the respective RCV.

3. Results

The results collected from the present work are expressed as the median and interquartile range (Percentile 25 - Percentile 75) and are all detailed in Table 1. The samples collected after the ingestion of 300 ml water 1 h before blood extraction showed statistically significant decreases in (medians, T_0 vs T_1, P): WBC $\times 10^9$/l (6.51 vs 6.12, 0.002); LYM $\times 10^9$/l (2.90 vs 2.19, 0.000); MONO $\times 10^9$/l (0.50 vs 0.48, 0.031); EOS 10^9/l (0.17 vs 0.16, 0.003); RBC $\times 10^{12}$/l (4.46 vs 4.40, 0.024); HCT l/l (0.38 vs 0.37, 0.036); and HBG g/l (129 vs 129, 0.009). Table 1 also shows that none of the parameters analyzed showed a clinically significant change, (MD% vs RCV): WBC (−9.3 vs 31.9); LYM (−24.0 vs 28.9); MONO (−6.2 vs 50.5); EOS (−12.1 vs 61.3); RBC (−1.3 vs 8.9); HCT (−1.4 vs 7.5); HGB (−1.1 vs 8.1). The rest of the parameters studied (NEU, BASO, MCV, MCH, RDW, and PLT) showed no clinically significant changes as well. Interestingly, the decrease observed in LYM was statistically significant and it was the one which was closest to RCV. Fig. 1 shows this change [20].

4. Discussion

The results collected show that the ingestion of 300 ml water 1 h before blood extraction yields no clinically significant changes in the routine hematological parameters evaluated. Nonetheless, it is important to identify the possible cause of the statistically significant decrease in LYM because it was the only hematological parameter whose MD% was close to its respective RCV.

Bishop et al. [21] observed a statistically significant decrease in LYM 1 h after the ingestion of artificially sweetened and lemon-flavored water. The water volume used in the protocol they followed depended on the body weight of each individual included in the study. Therefore, individuals drank −on average− 230 ml. They concluded that the cause that led to LYM decrease is not clear. Still, they discarded the effect of hemodilution produced by the intake of liquids as a possible reason for this decrease because they adjusted cell count according to the estimation of the change they detected in plasma volume [21]. As this estimation [22] presents controversies [23], it was not applied to our study. Interestingly, previous research reported that the count of certain LYM subpopulations evidenced a circadian decrease dependent on morning cortisol increase [24–29]. The decrease in LYM observed after water ingestion could thus be a consequence of this circadian rhythm decrease rather than of water ingestion per se.

The evaluation of the clinical significance of a statistically significant difference by means of an objective tool, such as RCV, is of