BRIEF REPORT

The impact of fist clenching and its maintenance during venipuncture on routine hematology testing

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Background: Prevention of a disturbance of the blood vessel allows phlebotomists to collect a blood specimen by venipuncture that will truly mirror the patient condition. This study was aimed to evaluate the impact of repeated fist clenching and maintenance of the fist during blood collection by venipuncture for routine hematology testing.

Methods: Blood were collected from 16 healthy volunteers with two separate sequential procedures, entailing standard venipuncture with hand opened throughout blood collection, or clenching the fist six times before venipuncture and maintaining the fist until completion of blood collection. The parameters tested included red blood cell (RBC) count, hemoglobin, hematocrit, mean corpuscular volume (MCV), RBC distribution width, white blood cell count and differential, including neutrophils, lymphocytes, monocytes, eosinophils, basophils, large unstained cells, platelet count, mean platelet volume, and reticulocytes. The results were reported as median and interquartile range. The comparison of data obtained with the two different venipuncture procedures (i.e., with or without fist clenching and closed hand) was performed with Wilcoxon-Mann-Whitney ranked-pairs test. The degree of statistical significance was set at $P < .01$.

Results and Conclusion: Fist clenching and maintenance during blood collection for routine hematology testing was effective to increase the MCV by 1.2% ($P < .001$). All others hematological parameters were not significantly biased by fist clenching, though hematocrit, neutrophils, eosinophils, and reticulocytes displayed mindful of trends. We hence advise patients against clenching their fist before blood collection for hematology testing.

KEYWORDS

blood specimen collection, clinical laboratory techniques, laboratory variability, medical errors, patient safety, pre-analytical phase

Errors in the pre-analytical phase, especially regarding blood collection represent an important issue on laboratory analyses.¹ Observing phlebotomists while they perform a venipuncture to identify nonconformities² may improve the quality of diagnostic blood specimens, and reduce laboratory variability on pre-analytical phase.²⁻⁴ Moreover, complications associated with blood collection processes are preventable and are directly linked to the technique performed.⁵ Furthermore, opportunities for errors are created when those health professionals

who perform blood collection lack knowledge about laboratory practice or do not have the appropriate skill to perform the necessary procedures.⁶

Prevention of disturbances of blood vessels allow phlebotomist to collect a blood specimen by venipuncture that will truly mirror the patient situation.⁷ Venous stasis by tourniquet application (longer than 60 seconds) as source of laboratory variability has been extensively investigated in the past decade.^{8,9} In fact, it promotes the outflow of

water, diffusible ions and low molecular weight substances from the blood vessels, thus increasing the concentration of biomarkers at the venipuncture site. Fist clenching, is another source of blood vessels disturbance. Presently, the evaluations of fist clenching impact on laboratory analysis are disputed: major variability was observed for clinical chemistry,¹⁰ whereas routine coagulation was not biased.¹¹ Therefore, this study was aimed to evaluate the impact of repeated fist clenching and maintenance of the fist during blood collection by venipuncture for routine hematology testing.

To assess the impact of fist clenching and maintenance during blood collection by venipuncture on routine hematology testing, we followed the protocol previously published.^{10,11} Briefly, 16 healthy volunteers (12 females and four males; mean age 49 years, range 36–64 years), were enrolled among the laboratory personnel for this study. Before blood collection, all volunteers observed an overnight fast (12 hours) and remained seated for 15 minutes to eliminate interferences due to both lipaemia,^{12,13} and posture.¹⁴ 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 venipuncture¹⁵ 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 venipuncture¹⁵ without tourniquet, however, asking the volunteer to clench the fist for six consecutive times before venipuncture and maintaining the fist until blood collection had been completed. When clenching and maintaining the fist was 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 was 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 fewer than 1-minute interval from both arms. For both procedures, 5 mL of blood were collected using a 20 gauge straight needle (BD Europe, Plymouth, UK) 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 3.4 mg of spray-coated of K₂EDTA (BD Vacutainer®, Becton Dickinson Europe, Plymouth, UK). The use of the discard tube was meant to eliminate potential interferences from endothelial cell injury upon phlebotomy. All samples were processed for routine hematology testing immediately after collection (<15 minutes) on the same Advia 2120 automated hematology analyzer (Siemens Healthcare Diagnostics, Newbury, Berkshire, UK). The parameters tested included red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), RBC distribution width (RDW), white blood cell (WBC) count and differential, including neutrophils (NEU), lymphocytes (LYMPHO), monocytes (MONO), eosinophils (EOS), basophils (BASO), large unstained cells (LUC), platelet count (PLT), mean platelet volume (MPV), and reticulocyte count (RETIC). The instrument was calibrated against appropriate proprietary reference standard

material, and verified by third-party control materials (i.e., independent from calibrator materials).¹⁶ The results were reported as median and interquartile range. The comparison of data obtained with the two different venipuncture procedures (i.e., with or without fist clenching and closed hand) was performed with Wilcoxon-Mann-Whitney ranked-pairs test, using a licensed statistical software (GraphPad Prism® version 5.01; La Jolla, CA, USA). The degree of statistical significance was set at $P < .01$. The mean % difference of each analyte was calculated as the ratio of values obtained with the two different procedures (i.e., {[hematological-parameter value in venipuncture with fist clenching and maintenance – hematological-parameter value in venipuncture with hand opened]/hematological-parameter value in venipuncture with fist clenching and maintenance} × 100). All changes were also compared with the desirable quality specifications for imprecision, as provided by Ricos et al.¹⁷ Each patient provided a written consent for being enrolled in the study. The investigation was carried out in accord with the ethical standards established by the institution in which the experiment was performed and the Helsinki Declaration of 1975.

The main results of this study are shown in Table 1. Fist clenching and maintenance during blood collection by venipuncture for routine hematology testing was effective to increase the mean corpuscular volume of erythrocytes by 1.2% ($P < .001$). However, when mean % differences were compared with desirable imprecision derived from biological variation some hematological parameters displayed mindful trends—HCT, NEU, EOS, and RETIC—had shown variability that may lead physicians to misinterpret laboratory data.

Our results show that fist clenching before venipuncture is not associated with statistically significant variations of 14 of the 15 (93.3%) hematological parameters evaluated (Table 1). However, our previous study revealed variations of several routine clinical chemistry parameters, principally electrolytes (i.e., calcium, chloride, magnesium, phosphate, potassium, and sodium), which may be attributable to muscle contraction “clenching”, hemolysis, or both.¹⁰ A theoretical explanation for the significant increase on MCV could be the pH alteration inside vein near the muscle stressed by clenching. It has been established that modification of pH can exert a direct influence on dissociation of ions and consequently impact on passive transport (i.e., osmosis) on erythrocyte membrane.¹⁸ Briefly, the erythrocyte membrane is a plastic molecular mosaic, consisting of proteins, lipoproteins, glycoproteins, and presumably purely lipid zones. Biochemical and biophysical studies showed that cell membranes are porous structures. A common erythrocyte with a membrane area of $176 \times 106 \text{ nm}^2$ contains about 1900 water-permeable pores of $0.007\text{--}0.2 \text{ nm}^2$ in size. Water and certain solutions (electrolytes and nonelectrolytes) penetrate through the membrane via different pores. Some channels are formed by transmembrane proteins (aquaporins) and antibiotics; ionic channels are selectively permeable for ions. Moreover, the ionic channels have hydrophilic walls, and are filled with water due to which water and small soluble molecules penetrate through the membrane, impacting on mean corpuscular volume.^{18,19}

The standard H03-A6 from Clinical Laboratory Standards Institute (CLSI) contains the indication that “the veins become more prominent and easier to enter when the patient forms a fist”. However, it is also

TABLE 1 Impact of fist clenching and maintenance during blood collection by venipuncture for routine hematology testing

Parameter	Unit	Fist clenching	No fist clenching	P	Mean % difference	Desirable imprecision (%)	CVa %
RBC	10 ¹² /L	4.53 [4.22-4.84]	4.46 [4.15-4.90]	.422	1.5	1.6	1.2
HGB	g/L	135 [126-139]	134 [124-137]	.151	0.7	1.4	1.6
HCT	%	40.3 [38.8-41.7]	39.4 [37.6-41.6]	.132	2.2	1.4	1
MCV	fL	89.8 [85.8-92.0]	88.7 [85.5-91.7]	<.001	1.2	0.7	0.5
RDW	%	13.9 [13.4-14.4]	13.9 [13.3-14.4]	.041	0	1.8	1
WBC	10 ⁹ /L	5.36 [4.69-7.34]	5.52 [4.84-7.48]	.352	-3	5.7	1.9
NEU	10 ⁹ /L	2.55 [2.30-4.33]	2.78 [2.58-4.29]	.535	-9	8.6	1.7
LYMPHO	10 ⁹ /L	2.01 [1.83-2.51]	2.08 [1.74-2.72]	.389	-3.5	5.1	2.3
MONO	10 ⁹ /L	0.30 [0.25-0.44]	0.31 [0.27-0.42]	.183	-3.3	8.9	6.5
EOS	10 ⁹ /L	0.15 [0.09-0.22]	0.17 [0.08-0.24]	.363	-13.3	10.5	7.8
BASO	10 ⁹ /L	0.03 [0.01-0.04]	0.03 [0.02-0.04]	.192	0	14	13
LUC	10 ⁹ /L	0.10 [0.07-0.12]	0.10 [0.08-0.14]	.977	0	NA	10.5
PLT	10 ⁹ /L	226 [166-278]	234 [169-286]	.979	-3.5	4.6	2.4
MPV	fL	9.30 [8.92-9.50]	9.25 [8.90-9.80]	.343	0.5	2.2	1.3
RETIC	10 ⁹ /L	60.6 [46.4-78.3]	55.6 [48.4-71.4]	.066	8.2	5.5	3.2

RBC, red blood cell count; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; RDW, RBC distribution width; WBC, white blood cell count; NEU, neutrophils; LYMPHO, lymphocytes; MONO, monocytes; EOS, eosinophils; BASO, basophils; LUC, large unstained cells; PLT, platelet count; MPV, mean platelet volume; RETIC, reticulocyte; NA, not available.

Results are presented as median [interquartile range]. P-values represent the significance by Wilcoxon ranked-pairs test. The P-values in bold are statistically significant ($P < .01$), and bold mean % differences represent potential impact clinically significant, when compared with desirable imprecision derived from biological variation.

stated that “there must not be vigorous hand exercise”, since this “may cause changes at the concentration of certain analytes in blood”.²⁰ An apparently controversial indication is provided by the guidelines of the World Health Organization (WHO) on drawing blood, affirming to “ask the patient to form a fist so the veins are more prominent”.²¹ 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 for routine hematology testing. According to our data, we discourage phlebotomists to allow patient clenching the fist before collecting diagnostic blood specimens for hematology testing. However, a general step could be included on phlebotomy procedure stating: “request the patients to gently close him/her hand (but never ask the patient to “pump”)”.

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