Influence of method of venous blood collection on the blood cell count determination result

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Abstract

Introduction: The preanalytical phase of laboratory testing, including collecting of the material, is one of the most important factors affecting the quality of the results of analyzes.
Aim of the research: To evaluate the influence of the method of collecting venous blood on the result of blood cell count determination.
Material and methods: Venous blood of 20 healthy people was collected into tubes with K_2EDTA using two types of blood collection system: an open blood collection system (consisting of a syringe and needle) and a vacuum blood collection system (consisting of a vacuum tube for blood collection and matching needle).
Results: Statistical analysis of the results showed that the method of blood venous collection influenced the result of some parameters of blood cell count determination, such as red blood cell count, white blood cell count, hemoglobin, hematocrit and platelet count, but did not influence mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. These differences are clinically relevant because they do not exceed 5% of the value.
Conclusions: Results of blood morphology appears to depend on the method of sample collection. The observed differences are not clinically significant, because they do not exceed 5%.

Introduction

Appropriate blood collection and preparation is one of the most important factors affecting the quality of laboratory results [1]. Median antecubital veins are the most popular site for venipuncture [2]. Recent standards of collecting blood for laboratory tests suggest the use of closed systems for blood. Only in exceptional cases is the use of open systems for blood sampling recommended.

In an open system, blood collection is performed using a needle or needles with drain connected to a dry, plastic syringe. The collected blood is then carefully injected into the tube without any additives, in order to obtain the serum, or to a tube containing an anticoagulant, if it is intended for liquid whole blood or plasma. The vacuum or aspiration-vacuum blood collection system consists of a needle, adapter and vacuum tubes with anticoagulant or without any additives. The enclosed system provides clean, safe blood sampling, thus protecting medical personnel from contact with potentially infectious material [3, 4]. At the same time it provides immediate mixing of the blood removed from the possible additives contained in the test tube already during aspiration of the sample. It also eliminates the need for re-transfusion of blood from a syringe into the blood collection service. The aim is to obtain material with the highest possible quality [3, 4].

Selection of blood collection technique is one of the pre-analytical factors that may significantly affect the test results.

Aim of the research

The aim of this study was to assess the impact of venous blood collection method on the result of complete blood count.

Material and methods

The material was venous whole blood containing K_2EDTA. Material was obtained from 20 healthy individuals (men and women) aged 23 to 34 years. Blood was collected twice a day within a few minutes from the veins of both upper limb elbows. Material was collected in accordance with the standard conditions, between 7.00 am and 9.00 am, the patients remaining on fasting after an overnight rest [5].

Two methods of blood sampling were used. Venous blood was collected from the median cubital vein using both an open system with a needle, dry, plastic syringe and pre-open tube with the K_2EDTA Vacutainer system, Becton Dickinson, and a closed vacuum system (Vacutainer, Becton Dickinson), using a needle system and a vacuum tube containing K_2EDTA. In

both cases the same tubes were used. The blood was always collected by the same qualified person. The material was obtained from all individuals in one day.

Complete blood count (hemoglobin (HGB), hematocrit (HCT), red blood cell count (RBC), white blood cell count (WBC), platelet count (PLT) and red blood indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)) were analyzed within 2 h of collection of the material, using the Sysmex hematology analyzer XE-2100 (SYSMEX Corporation, Japan).

The Sysmex XE-2100 is a hematology automated analyzer, used to quickly perform full blood counts and reticulocyte counts. Blood, collected with anticoagulant, is sampled and diluted (1 : 500), and moves through a tube thin enough that cells pass by one at a time. Characteristics of the cell are measured using lasers (fluorescence flow cytometry) or electrical impedance. Red blood cells and platelets are counted in a dedicated channel using the direct current detection method with hydrodynamic focusing technology. Automatic discriminators separate the two cell populations. The intensity of the electronic pulse from each analyzed RBC is proportional to the cell volume. The hematocrit is directly determined based on the count and volume detection of each individual RBC. The sodium lauryl sulfate (SLS) method is used for hemoglobin concentration analysis. White blood cells are counted by fluorescent flow cytometry method. From the results of erythrocytes, hemoglobin and hematocrit values red blood indices are automatically calculated.

Statistical analysis

Analysis of the normal distribution for the studied haematological parameters was performed using the Shapiro-Wilk test. For comparison of RBC and HGB in blood samples collected by different techniques Student's *t*-test for dependent samples was used. For comparison of WBC, HCT, PLT and MCV, MCH and MCHC in blood samples collected by the vacuum system and the open system Wilcoxon test was used. The value of $p \leq 0.05$ was considered statistically significant.

Results

Statistical analysis of the research results (Table 1) indicated that the red blood cell count in the blood sample collected by the open system was significantly lower than in the samples collected by the vacuum system. The difference was 1.3%. Also, the hematocrit and concentration of hemoglobin were significantly lower in the blood sample collected using a needle and syringe as compared to the values of these parameters in the samples collected using the vacuum technique. Differences were 1.4% for the hematocrit and 1.3% for hemoglobin. Red blood indices (MCV, MCH and MCHC), calculated for a sample of blood taken by both methods, did not differ significantly. It was also observed that for the blood sample taken by the open system the white blood cell counts was significantly lower than in the blood sample collected using a vacuum set, and the difference was 4.4%. Platelet counts in samples taken by syringe were statistically significantly lower in comparison to the number of platelets in the samples collected by closed system vacuum. The difference was 1.7%.

Discussion

Venous blood is a basic biological material used for laboratory tests. Although it is now recommended to use closed vacuum systems or the aspiration-vacuum

Table 1. Comparison of the parameters identified in edetate blood samples taken by an open system and a closed system – a vacuum

Parameter	Venous blood collection method				Value of *p*
	Open system – syringe		Closed system – vacuum		
	Mean	SD	Mean	SD	
RBC [10^6/μl]	4.56	±0.37	4.62	±0.32	≤ 0.007
HGB [g/dl]	13.33	±1.12	13.51	±1.04	≤ 0.006
HCT [%]	39.41	±2.63	39.96	±2.39	≤ 0.007
MCH [pg]	29.21	±1.47	29.21	±1.38	> 0.05
MCHC [g/dl]	38.80	±0.95	38.81	±0.94	> 0.05
MCV [fl]	86.44	±3.74	86.48	±3.75	> 0.05
WBC [10^3/μl]	5.83	±1.36	6.10	±1.50	≤ 0.003
PLT [10^3/μl]	264.0	±53.50	268.4	±54.72	≤ 0.05

RBC – red blood cells count, WBC – white blood cell count, HGB – hemoglobin, HCT – hematocrit, PLT – platelet count, MCH – mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration, MCV – mean corpuscular volume

method, in special cases it is acceptable to use traditional methods of open system blood collection, using a needle and syringe [5]. The complete blood count evaluates the morphology of individual blood cell count, hemoglobin concentration, hematocrit and red blood indices. Laboratory medicine has many anticoagulants, which in various ways affect the properties of the blood from their participation. The addition of anti-clotting agents has an impact on both the biochemical composition of blood samples and their cellular elements. The International Committee for Standardization in Hematology (ICSH) recommends the use of dipotassium salt of ethylene diamine (K_2EDTA) as the anticoagulant of choice for complete blood counts [6]. Salts of EDTA (EDTA) do not interfere with the absorption of hematology dyes. It has also been shown that blood containing K_2EDTA is characterized by considerable stability. Erythrocytes in the blood edetate remain stable for at least 6 h [7] and according to some sources overnight [8]. Hemoglobin in blood samples collected with the addition of dipotassium edetate retains its properties up to 48 h [8] from the time of collection. The stability of leukocytes in the blood edetate largely depends on their type. It has been shown that the cells under these conditions are much more stable than monocytes or neutrophils [9]. Blood samples for laboratory testing should always be taken while maintaining a constant concentration of anticoagulant which for the salt of EDTA is between 1.2 mg/ml and 2.0 mg/ml of blood [9]. Increasing the concentration of EDTA above indicated values results in the flow of water from the cells into the plasma, which can lead to abnormally low hematocrit value and number of blood cells [8]. A high concentration of EDTA in the blood sample causes the failure of bridges between segments of nuclei in neutrophils and loss of cytoplasmic granules. These morphological changes in the white blood cells can lead to erroneous results in terms of the count and differentiation of leukocytes [9]. Anticoagulant also affects on the size and shape of the cellular components of blood. EDTA salts cause, among others, reduction of the volume of red blood cells, which can be observed as a decrease in hematocrit, when the examination is carried out by conventional centrifugal method [4, 10].

The blood is potentially infectious biological material. Therefore, it is preferable to use a closed system for collecting, largely because it restricts the possibility of direct contact with blood. There are, however, cases where the closed system of blood sampling is not possible. Such a situation may occur in the blood collection of premature infants or patients with brittle or poorly filled veins. Then the blood is often sampled by an open system using a needle and syringe, or even the same needle, the free flow out of the blood vessel through an injection needle directly into a substituted open tube.

The blood collection procedure by a vacuum system differs significantly from the blood collection by syringe. If research is needed on venous whole blood, the collected blood must be immediately mixed with an anticoagulant. Immediate mixing of the blood sample with anticoagulant is only possible if the blood is collected from a vein directly into a tube containing suitable additives. In this case, after gentle mixing, the coagulation cascade is not initiated. In an open system, the blood is collected into a dry syringe and it is not possible to immediately connect to the anticoagulant. After collecting the blood into the syringe, the needle is removed and blood is injected into a tube containing an anticoagulant and mixed gently to obtain a blood fluid. It takes a long time for the blood samples to freely flow out of the blood vessel, and in consequence the coagulation process in these samples is already well advanced. This will lead to a delayed stop of the coagulation cascade and may be the cause of the invisible to the naked eye micro-clots that can lead to a slight decrease in the content of blood cells in a blood sample collected via syringe for a blood sample collected via the vacuum system, which also can be confirmed by the results of the discussed research. Transfer of blood from the syringe into the tube further requires re-enforcement of the erythrocytes by squeezing the syringe outlet, which in turn may lead to mechanical damage and low hemolysis of erythrocytes. Then, both the hematocrit and the content of the erythrocytes can be reduced [11]. Also the results of the experiment showed a slight decrease for these parameters in a blood sample obtained by a syringe as compared to those obtained by vacuum system. The vacuum tube closed system is calibrated to a specific volume. After the puncture needle stopper vacuum tube is located in the patient's vein there immediately occurs aspiration into the tube of the volume of blood for which it is calibrated. The defined volume of the vacuum ensures always the same volume of blood collected in relation to the volume of anticoagulant contained in the tube, which in turn ensures the same proportions of blood and an anticoagulant agent [3]. However, in the case of blood collection by syringe it is always possible to pour too small or too large a portion of the blood into a tube containing anticoagulant. The volume of the transferred blood from the syringe into the tube depends largely on the manual dexterity and the ability of visual perception of the person collecting. In such cases, the condition of the sample can affect both its failure and excessive filling. Tube overflow causes a real anticoagulant shortage in relation to the volume of blood and makes it difficult to initiate mixing of blood with an anticoagulant in the tube. Both of these processes can lead to the formation of micro-clots and reduction of the cellular components. On the other hand, if overflow of the syringe occurs, too small amount of blood in relation to the

volume of anticoagulant can cause a real dilution of the blood, reflected in the results of blood counts, as a reduction in blood cell counts, hemoglobin, and hematocrit [12], which is also observed in the results of the experiment.

At present, it is estimated that regarding the lack of agreement between the results of laboratory tests of actual clinical presentation, more than 60% is caused by factors that are not related to the analytical process. Ultimately, this leads to a situation in which the result of properly conducted in the laboratory analysis of biological material delivered does not reflect the current status of the patient, visualized with changes resulting secondarily in the sample collected or transported improperly. This phenomenon is sometimes referred to as the analytical uncertainty [13]. Human intervention in the process of collection and preparation of biological material for research has a significant impact on the quality of the result in the laboratory. Choice of method of blood collection can also be reflected in the quality of the analysis. An open system of blood samples using a syringe by a qualified person associated with aspiration blood from the vein and transfer it to the secondary tube. This delays, inter alia, mixing the blood with anticoagulant located in the tubes and can provide an imbalance in the mutual ratio of the volume of blood and anticoagulant. No effect of manual methods for the blood collection process means that sampling error, in the process of blood aspiration by vacuum tube, is reduced to a minimum, which in turn contributes to improving the quality of laboratory results obtained from this material [14].

Conclusions

Statistical analysis of the results of laboratory tests suggests that the method of collection of venous blood using a syringe or vacuum significantly affects the results of the measured parameters, blood cell count (RBC, WBC, HGB, HCT, PLT) but does not affect the calculated parameters (MCH, (MCH, MCV, MCHC). These differences, however, do not exceed 5%, which makes them insignificant from a clinical point of view. It does not free one from the necessity of using such methods of obtaining material that is compatible with the current state of medical knowledge and current standards with regard to exceptions.

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