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Full Length Article

Alterations in the parameters of classic, global, and innovative assays of hemostasis caused by sample transportation via pneumatic tube system

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ABSTRACT

Background: Pneumatic tube system (PTS) is an integral part of large medical facilities providing rapid interconnection between units within the hospital and often used to transport blood samples. The aim of our study was to compare a wide variety of hemostasis assays to identify assays sensitive to this transport method and diagnostic relevance of the alterations.

Methods: Routine coagulation and platelet tests (APTT, PT, TT, fibrinogen, light transmission aggregometry (LTA) with ADP, collagen, ristomycin and epinephrine), whole blood flow cytometry platelet function test (levels of CD42b, CD61, CD62P, PAC1, annexin V binding and mepacrine release) and global coagulation tests (thromboelastography (TEG), thrombin generation (TGT), thrombodynamics (TD), thrombodynamics-4D (TD-4D)) were determined in PTS- and manually transported samples of 10 healthy volunteers.

Results: There were no significant differences between the values of APTT, PT, TT or fibrinogen between the samples transported by PTS or manually. The results for LTA demonstrated increase in the collagen-induced aggregation (84 ± 7% versus 73 ± 5%), while the response to epinephrine was decreased (58 ± 20% versus 72 ± 7.4%). Flow cytometry-based platelet function test showed a pre-activation of platelets by PTS-transportation while all integral assays of coagulation tested in the present study (TEG, TGT, TD, TD-4D) demonstrated a hypercoagulation shift.

Conclusions: Transportation by PTS caused significant shifts in parameters of functional and integral assays that exceeded parameter variation values and sometimes even were comparable to normal ranges. The results obtained in this study indicate that using of PTS for such assays may cause sufficient alterations of results and can lead to patient's mistreatment.

1. Introduction

Pneumatic tube system (PTS) is an integral part of large medical facilities providing rapid interconnection between units within the hospital. Along with the documentation and drugs, PTS is often used to transport blood and other tissue samples to the clinical laboratory. Using of PTS significantly reduces costs and laboratory turnaround time [1]. However, during transportation, various physical factors such as air pressure, accelerations, decelerations, radial gravity forces, and vibrations can cause damage [2,3] and activation of cells [4], which leads to distortion of the analysis results.

Laboratory hemostasis assays are one of the most demanding to comply with the rules of preanalytical phase and each test has different sensitivity. The PTS influence on hemostasis tests has been studied on various systems and gave quite conflicting results [3,5–13]. For example, a number of studies allow the use of PTS for platelet activity tests [3,5], while some declare significant alterations in test parameters and recommend only manual transportation of the tubes for these assays [4,10,11]. Routine coagulation assays, such as activated partial thromboplastin time (APTT) and prothrombin time (PT) in most cases are not affected by PTS transportation [6]: some studies do report their significant shortening [3], but taking into account that these assays

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were designed to be sensitive mostly to hypo-, not hypercoagulation, this shortening cannot be considered clinically relevant.

The need for more sensitive tests led to the introduction of so-called global hemostasis assays into clinical practice. High sensitivity of these tests could not but lead to their increased sensitivity to the methods of sampling and transportation of blood. It is known that for the thrombin generation test, the use of PTS causes significant shifts in results compared to manual transportation [3]. For thromboelastography, as well as for platelet tests, the opinions differ [7,14], presumably, due to differences in PTS models used in conducted studies (mostly variations in travelling speed and distance), and to the variations in thromboelastography settings that were used in each specific study. There are also new promising assays, such as platelet functional activity estimation by flow cytometry [15,16], thrombodynamics and thrombodynamics-4D [17–19], for which no studies on PTS transportation influence have been performed.

Studies on systematic comparison of different categories of hemostasis tests (platelet and plasma, standard and global) on one PTS are absent. Moreover, most of the studies concentrate on the significance of difference between transported manually or via PTS samples, while the probability of test results leave the normal range and indicate false pathology (and vice versa) is much more clinically relevant. To obtain this information it is essential to perform the PTS effect analysis in comparison with each test's normal variability, which is not known for many assays. Thus, the aim of our study was to compare a wide variety of standard, global and innovative hemostasis assays using one PTS to figure out, which assays are most sensitive to this transport method and if the resulting parameters' alterations can be considered clinically relevant.

2. Materials and methods

2.1. Study population, blood collection and sample transport

Blood samples of 10 healthy volunteers (3 males and 7 females) aged 24–37 years (mean ± SD was 27.6 ± 4.2) was used in the main study; additional volunteers were recruited for intrinsic assay variability determination. Written informed consent was given by all participants. The study has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration and has been approved by ethical committee of Center for Theoretical Problems of Physicochemical Pharmacology (Protocol #1). Each volunteer in the main study donated six 3 ml sodium citrate tubes (0.106 M/3.2% S-monovette, Sarstedt, Nümbrecht, Germany). Three tubes were transported to the lab manually, while other three were sent by PTS using a vessel with sample holding insert. The PTS used in the present study had diameter of 110 mm and was produced by Sumetzberger GMBH (Vienna, Austria). The following parameters were used for the transport of the samples: speed 7 m/s, distance 125 m, the route had 8 turns of 90°. The system has a standard bypass brake system near receiving station.

2.2. Laboratory assays

2.2.1. Routine clotting assays

Assays were performed using an ACL TOP 700 coagulometer (Instrumentation Laboratory, Bedford, MA, USA) in platelet-poor plasma (PPP), obtained by centrifugation at 1750*g* for 15 min. The following assays were performed: APTT (SynthASil, Instrumentation Laboratory, Bedford, MA, USA), PT (RecombiPlasTin 2G, Instrumentation Laboratory, Bedford, MA, USA), thrombin time (Thrombin Time, Instrumentation Laboratory, Bedford, MA, USA), fibrinogen (QFA Thrombin, Instrumentation Laboratory, Bedford, MA, USA).

2.2.2. Light transmission aggregometry

LTA was measured using ALAT-2 aggregometer (Biola, Moscow, Russia) in platelet-rich plasma (PRP) obtained by centrifugation at 200*g* for 10 min. The platelet count in PRP was measured on the hematology analyzer Sysmex KX-21N and was 341 ± 81 (mean ± SD). The following agonists were used: ADP, 5 μM (adenosine diphosphate, Sigma Aldrich, Munich, Germany); collagen, 2 mg/ml (NPO Renam, Moscow, Russia); ristomycin 15 mg/ml (NPO Renam, Moscow, Russia); adrenaline, 5 μM (epinephrine, Moscow Endocrine Factory, Moscow, Russia).

2.2.3. Flow cytometry platelet function analysis

Platelet function was analyzed as described in [15,16] with minor modifications. Whole blood samples were diluted with buffer A (150 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 0.4 mM NaH_2PO_4, 20 mM HEPES, 5 mM glucose, 0.5% bovine serum albumin, pH 7.4). Platelets were either left intact or loaded with mepacrine (1 mM) for 30 min at 37 °C. Subsequently, platelets were either left unstimulated or stimulated with a mixture of CRP at 1.5 μg/μl, SFLLRN at 20 μM, AYPGKF at 80 μM, and ADP at 100 μM for 10 min in the presence of 2.5 mM calcium chloride. Both resting and activated samples were incubated with antibodies against CD61, CD42b, CD62P, as well as PAC1 and annexin V for 10 min. Subsequently, they were diluted 10-fold with buffer A containing 2.5 mM calcium, and analyzed using Novocyte (Acea Bioscience, San Diego, CA, USA) flow cytometer. Annexin V-Alexa647 and antibodies against P-selectin (CD62P-Alexa647), glycoprotein Ib (CD42b-PE), glycoprotein IIb/IIIa (CD61-PE) and its activation marker (PAC1-FITC) were from Sony Biotechnology (San Jose, CA, USA). Cysteine-containing version of collagen-related peptide (CRP) was custom-synthesized and purified by VCPBIO (Shenzhen, China) and then crosslinked in-house. All other reagents were from Sigma-Aldrich (St Louis, MO, USA).

2.2.4. Thromboelastography

Thromboelastography was performed on a TEG 5000 thromboelastograph (Haemoscope Corp, Niles, IL, USA) using 340 μl whole citrated blood recalcified with 20 μl of 0.2 M $CaCl_2$. The following parameters were used for analysis: R – the time until the fibrin formation start; K – the time needed to reach a certain level of clot strength; alpha (α) angle - the rapidity of fibrin build-up and cross-linking (clot strengthening); MA - maximum amplitude, a direct function of the maximum dynamic properties of the fibrin-platelet aggregate.

2.2.5. Thrombin and fibrin generation test

Thrombin generation test was performed with the Thrombodynamics Analyzer System T-2T and a thrombodynamics-4D PLS kit (HemaCore Labs LLC, Moscow, Russia) in PFP, obtained by serial centrifugation at 1600*g* for 15 min and at 10,000*g* for 5 min. PFP was pre-incubated with a fluorogenic substrate for thrombin Z-Gly-Gly-Arg-AMC·HCl (Bachem, Bubendorf, Switzerland) (400 μM) and artificial phospholipid microvesicles (HemaCore Labs LLC, Moscow, Russia) (4 μM), as it was done in thrombodynamics-4D assay. Clotting was activated by 5 μl of recombinant tissue factor (TF, Instrumentation Laboratory, Bedford, MA, USA) diluted in distilled water after being supplemented with dried calcium acetate (final concentration 20 mM). Thrombin activity was measured by the rate of fluorogenic substrate cleavage. Fibrin concentration depends linearly on the light scattering [20], so we presented these data as light scattering in arbitrary units. For a detailed protocol and typical plots please see Supplementary Appendix S1 and Supplementary Fig. S1. The following parameters were used for analysis: T ½ - the time to reach half-maximal light scattering; Tmax – the time to reach thrombin peak; Amax – the maximum concentration of thrombin; ETP – the area under the curve of thrombin generation.

2.2.6. Thrombodynamics

Thrombodynamics assay was performed with a thrombodynamics