

Global Tests Evaluating the Functional Condition of Hemostasis

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Control of effectiveness of global tests evaluation of the functional state regulation of blood aggregation provided an opportunity to obtain timely information about the I-II and III phases of hemostasis while using native substrate - whole blood. *Ex vivo*, registered during hemocoagulation of changing the viscous characteristics of whole blood, allowed to formulate the concept of fibrinogenesis permanence as in vivo existing process, defining proper hemostatic potential – integrative characteristic of the entire cycle of hemocoagulation, ensuring necessary blood fluidity and restricting extravasation of its components in case of defecting its permeability or damaging its vascular wall.

Keywords: Regulation system of aggregate blood condition; Hemocoagulation; Hemostatic potential; Whole blood; Global methods; Low-frequency thromboelastography

Introduction

In recent years, the increase of mortality rates in the world is the result of a variety of diseases associated with thrombosis or hemorrhage. Also, new data on the role of endothelial function in ensuring system RBA has been gained as well as its impact on thromboresistance, vascular tone and permeability, adhesion of blood cells and angiogenesis. This allowed considering endothelial dysfunction (ED) as a key link in the pathogenesis of atherosclerosis, coronary heart disease, hypertension, cancer, diabetes, preeclampsia and many other diseases. For the analysis of system RBA, clotting, amidolytic and immunofluorescence methods are used [1-3]. Detailed evaluation of conducted studies reveals a number of disadvantages, such as lack of standardization, continuous sample preparation, low sensitivity, and most importantly, all the methods are made on the model of citrate plasma [4-7]. Thus, constantly developing new technologies, aiming to identify the genetic thrombophilia, markers of endothelial dysfunction, antiphospholipid antibodies and etc., cannot solve the problem of timely diagnosis and prevention of thrombosis and thrombohemorrhagic complications [7-10]. The global trend - the personification of research in the mode "point-of-care test", using "global" test evaluation system RBA, becomes popular. The main methods of this direction are the thrombin generation test (TGT), thromboelastography (TEG), turbidimetric fibrin formation (TFF), fourier-converting mechanical spectroscopy (FCMS) and low-frequency piezoelectric thromboelastography (LFPTEG) [8,11-14]. This article uses comparative analysis in order to determine their effectiveness in the evaluation of system RBA.

Method

The study was conducted on the basis of clinics of Research Institute of Pharmacology and Regenerative Medicine name of E.D. Goldberg involving 150 conditionally healthy volunteers (mean age 22 ± 6,5 years), after signing a free and informed consent.

As a model of the medium TFF, TGT was used citrate plasma, FCMS, TEG, LFPTEG the original substance - whole blood [8,9,14,15]. The standardization of sample preparation stage of LFPTEG has the following conditions:

- 1 ml of blood from the cubital vein without applying tourniquet;
- 3-component siliconized syringe, V = 2,5 ml;
- single cuvette from medical grade plastic, V = 0,45 ml;

- immediately (10-12 seconds) start of research.

We analyzed the results of "global" test evaluation system RBA such as TEG, TFF, LFPTEG and thrombin generation test (TGT). These FCMS were used as a historical control, since Russia does not have a device for carrying out this research [8]. To register the blood coagulation process using technology LFPTEG, a new technological apparatus of low-frequency piezoelectric thromboelastograph was used (hardware-software complex ARP-01M "Mednord", Russia, registration certificate Rosreestr №2010/09767) [6,16,17]. The analysis of coagulation graph is represented on (Figure 1).

These quantitative data were processed using the programs Microsoft Excel and SPSS 13.0. The first stage has been tested the normality of distribution of quantitative indicators using the Kolmogorov - Smirnov. Then there was a statistical description of the various groups involved in the study, using nonparametric methods. Evaluated quantitative data presented in the form of Me [LQ; UQ] (where Me - median, LQ - the bottom quartile, UQ - top quartile). To test the statistical hypotheses about the difference between the study groups used the nonparametric Mann-Whitney test (where p - the current level of significance) [18].

Results

During the assessment of the hemostatic potential, it is necessary to include all elements of the system RBA in the study, because one cannot exclude the effect of inescapable stressor - a standardized contact activation of coagulation process (const. V seu S cuvette) that is achieved by mode «point - of - care test», or studies *ex vivo*. Evidences show that, in order to achieve maximum reliability of obtaining the results, all studies should be performed using whole blood. Indeed, the whole blood sample, containing aliquots of endothelial producers (some of which trigger effects in a limited time interval from $1 \cdot 10^{-3}$ to 30 sec), formed elements of blood and plasma factors hemostasis, used for operative analysis, provides an opportunity of obtaining objective

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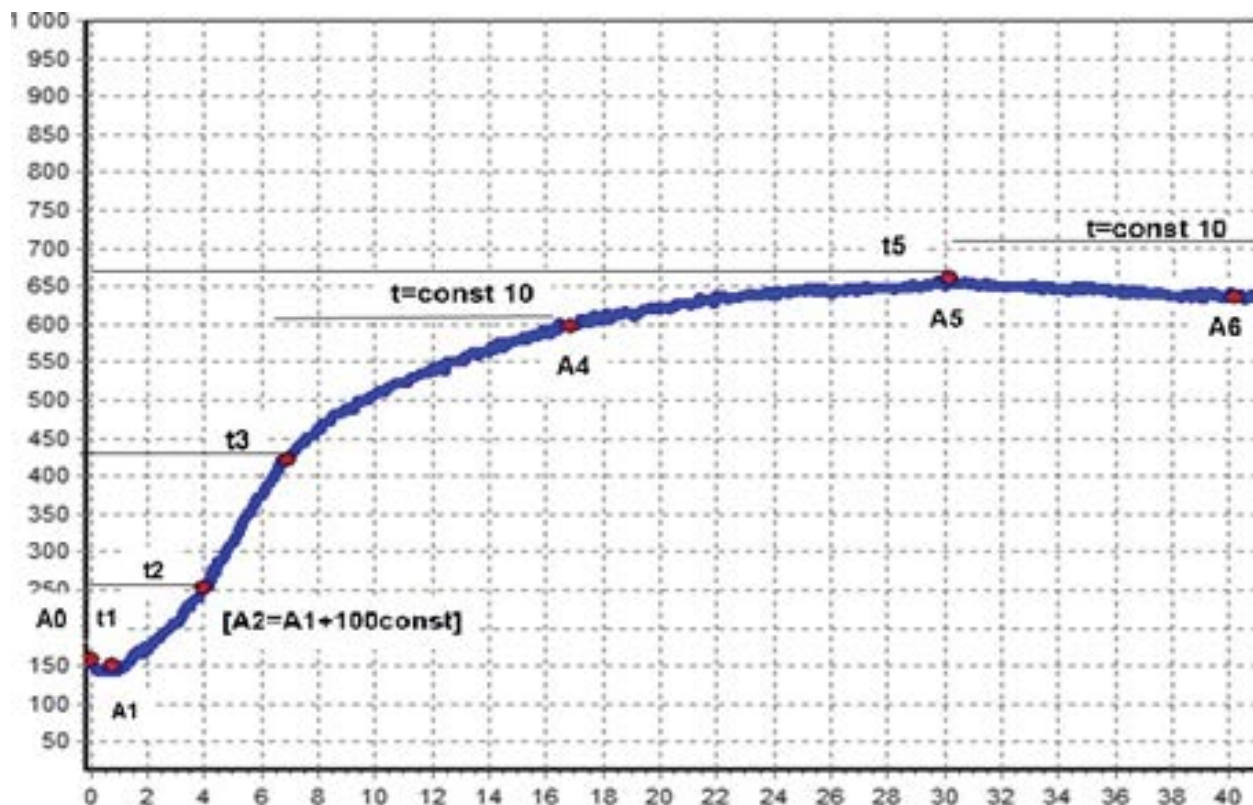


Figure 1: A graph of changes condition of blood aggregation (LFPTEG) healthy volunteer, which is estimated along the ordinate axis the amplitude research process (A1) in relative units (r.u.) and the abscissa axis – time or research (T1) in minutes
 A0 – initial value of the amplitude at the moment of time t0, in r.u.;
 t1 – period of reaction (time from the beginning of research to maximum decrease of amplitude LFPTEG – A1, in minute;
 A1 – maximum decrease in amplitude of the time «t1» (period of reaction), in r.u.;
 t2 – time to achieve the amplitude A2 LFPTEG, in minute;
 A2 – increase of the amplitude LFPTEG on 100 r.u. (A2-A1), in r.u.;
 t3 – blood coagulation time (point of gelling) is determined automatically when you change tg angle of the curve 50%, in minute;
 A3 – amplitude at the point of gelling LFPTEG, in r.u.;
 A4 – amplitude LFPTEG after 10 minutes reaching attaining point of gelling, in r.u.;
 t5 – time of achievement maximum amplitude LFPTEG (A5) (time of formation a fibrin-platelet clot structure), in minute;
 A6 – amplitude LFPTEG after 10 minutes reaching the maximum amplitude, in r.u.

information about the “time slice” state of hemostatic potential (HP) and viability function of the endothelium [7,13].

Picture 2 shows a graph of blood coagulation, obtained by means of TFF, TEG, LFPTEG, TGT, FCMS. From the represented data, of the process of lateral assembly and the formation of cross-linked fibrin takes $\approx 30 \pm 5$ minutes and is determined by almost all methods. “Point of gelling” (blood coagulation time) is the key indicator of the process start of clot formation - transition in the coagulation of blood from a liquid (sol) into a solid (gel). This is estimated only in two ways: FCMS and LFPTEG. Fibrinolytic activity was evaluated by TEG, FCMS and LFPTEG. As for the initial phase of coagulation, its analytical estimation is possible only with the use of LFPTEG. For all other methods, the determined step is regarded as a «lag time» (TGT, TFF), reaction time (TEG), the phase of pre-emerging clot (FCMS) [2].

The graphs (Figure 2) representing the registered blood clotting process to TEG «lag time» until the lateral assembly; for FCMS «lag time» takes place until “gelation point”, and the TGT, describing the kinetics of thrombin activity, also has a «lag time» in the time interval 2-3 minutes when setting and within ≈ 30 minutes due to sample

preparation (taking into account the fact that the coagulation process takes place even in the stabilized blood.

Thus, among the “global” tests to evaluate the HP in whole blood, the closest to the observance of the fundamental requirements for the research methods of hemostasis (efficiency, availability, informativity, reliability) is LFPTEG. The results are graphically overlay registration process of whole blood clotting obtained by LFPTEG on a constructed hypothetical model of coagulation. This overlap, reflecting all the stages, was the basis for a new view on system RBA as resulting participation geographically and functionally separates its units (vascular-platelet, coagulation, anticoagulant and fibrinolytic) to ensure proper HP where, in vivo, the process fibrinogenesis in terms of activation, management and restrictions is present permanently (Figure 3).

From positions of system analysis, the resulting functions of the system RBA in vivo is HP of whole blood, and ensuring its consistency over time is determined by nonlinear on time process fibrinogenesis with known and already axiomatic stages of initiation / amplification, propagation, lateral assembly, formation of cross-linked fibrin, until retraction and lysing of the clot. Temporary determinant nonlinearity

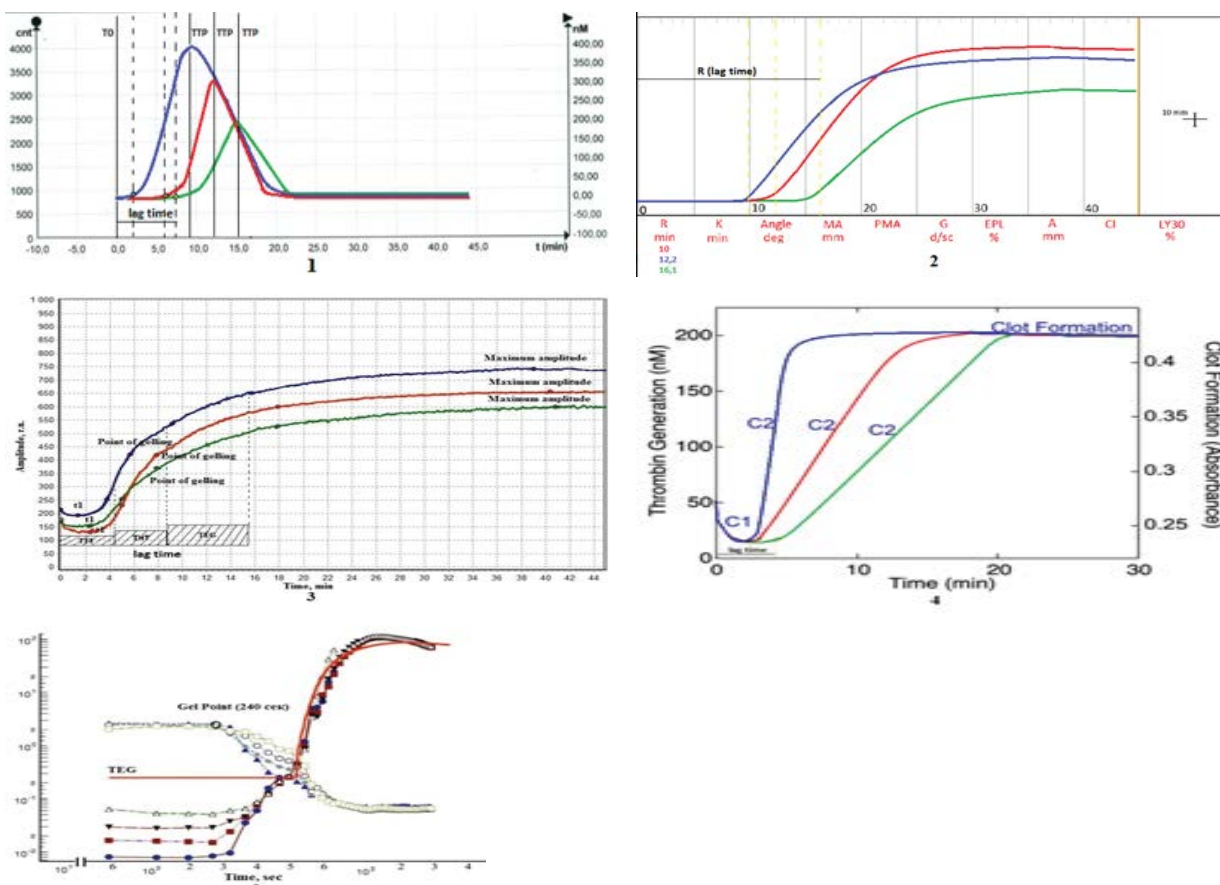


Figure 2: Graphs of blood coagulation, obtained by the methods: 1- THT; 2-TEG; 3- LFNTEG, 4- TFF, 5-FCMS.

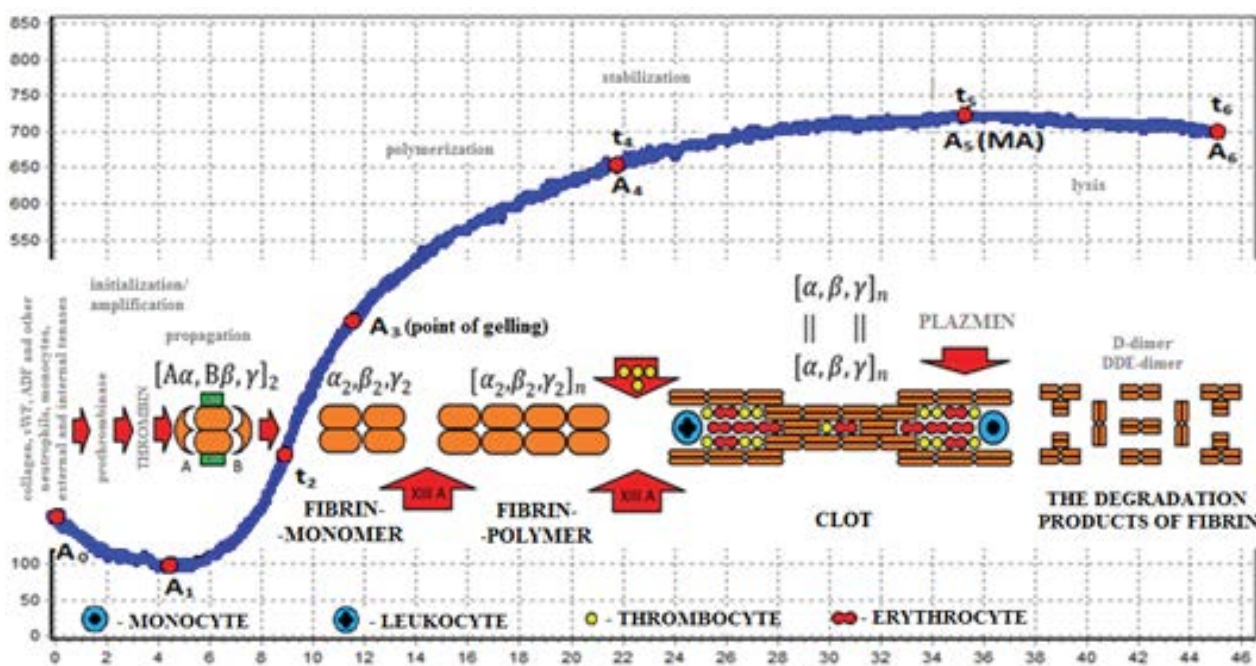


Figure 3: Graph of low-frequency thromboelastography is approximated on a modified version blood coagulation pathway.

fibrinogenesis determined enzymatic potential endothelial-intravascular continuum. While not disputing the main provisions of the title role of thrombin in that process it should be understood that it is its amount (level) [14], and most likely – activity [7] that regulated by anticoagulant potential, provides protein C, and S, thrombomodulin, AT III, α 2-macroglobulin, NO and prostacyclin, determines the completeness or incompleteness fibrinogenesis in terms of deterrence and limit its rate of formation of metabolic products of fibrinogen and producers of the vascular wall. Thus, in vivo, a second and even a millisecond required for thrombin generation are sufficient to initiate fibrinogenesis, but its viability is directly dependent on the intensity formation and size of thrombin pool, together with the characteristics of blood flow in different parts of vascular channel[19].

Conclusion

Thus, in view of the existence of a multitude of methods for studying the hemostatic system, it is necessary to distinguish between methods that evaluate the viscoelastic properties of the blood, provided the length of the control scale and timing of deformation, in contrast to other more simple techniques that give the most complete picture of the hemostatic system. Currently, there are a large number of devices that allow for LFPTEG, TEG, TGT, etc., but we must remember that it is necessary to strive for maximum production of early research results system of RBA, close to the bedside, and receive all the information about the picture forming pre-emerging bunch.

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