METHODS

A Noninvasive Method of Examination of the Hemostasis System

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We propose a noninvasive method of *in vivo* examination the hemostasis system based on speckle pattern analysis of coherent light scattering from the skin. We compared the results of measuring basic blood coagulation parameters by conventional invasive and noninvasive methods. A strict correlation was found between the results of measurement of soluble fibrin monomer complexes, international normalized ratio (INR), prothrombin index, and protein C content. The noninvasive method of examination of the hemostatic system enable rough evaluation of the intensity of the intravascular coagulation and correction of the dose of indirect anticoagulants maintaining desired values of INR or prothrombin index.

Key Words: *soluble fibrin monomer complexes; non-invasive method; hemostasis*

Non-invasive techniques are now widely used to measure various blood parameters. These technologies are usually based on optical techniques; the most popular of them is so called pulse oximetry [11], *i.e.* evaluation of the percent of oxygen saturation of hemoglobin in arterial blood using an optical sensor fixed on the tip of index finger or earlobe. In the visible and near-infrared light absorption, The spectrum of oxygenated hemoglobin differs from that of reduced hemoglobin. Measuring the signal by pulse-oximetry at several wavelengths and using the photoplethysmographic effect to isolate the signal from arterial blood, one can calculate the ratio of oxyhemoglobin to total hemoglobin. The function of another device, which recently passed clinical trials and is designed to measure hemoglobin, is based on a similar principle. It also determines the effects associated with absorption and scattering of light in blood and tissues [7]. Devices for measuring carboxyhemoglobin and bilirubin in the blood operate on a similar physical principle. The important fact contributing to the introduction of optical methods of analysis is the existence of the so-called "transparent window" of tissue namely in the visible and near infrared region of the spectrum.

All these methods are designed to measure the concentration of certain particles or molecules in the blood. However, non-invasive methods of measuring concentrations are more convenient in clinical practice, but always less accurate than the laboratory standard methods.

The situation is quite different when it comes to the system and rheological parameters of flowing blood. If there is a predisposition to thrombosis or bleeding, a set of parameters are measured in the laboratory: INR (international normalized ratio to determine the level of prothrombin), APTT (activated partial thromboplastin time), fibrinogen concentration, SFMC (soluble fibrin monomer complexes), content of D-dimer, *etc*. All these parameters provide information on the presence of certain factors involved in blood clotting, but they cannot replace the integral clinical

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picture of what is really going on in blood vessels. Thus, there is a need for additional methods, which would give a systematic assessment of tendency for thrombosis. This technique, of course, may only be implemented *in vivo*, otherwise it will not adequately reflect the processes occurring in the bloodstream.

An Israeli company Elfi-Tech Ltd has developed and patented a technique that makes it possible to measure the intensity of blood clotting by an optical technique based on the methods of speckle analysis [8-10].

The measurement is made on one of the patient's finger. A temporary arrest of blood flow of the finger rapidly induces aggregation of red blood cells, because shear stress is zero. The formed aggregates are suspended and move randomly in the plasma. At this moment, dynamic light scattering is measured and the effective viscosity of the plasma is calculated on the basis of fluctuation analysis of the reflected signal. It was assumed that this parameter correlated with the intensity of blood clotting.

The purpose of the work was to compare the reliability of the determination for main indicators of blood clotting by invasive and non-invasive methods.

MATERIALS AND METHODS

We observed 117 patients with various cardiovascular diseases. Coagulograms were examined with STA-Evolution-R (Roche) hemostasis analyzer using Reanal reagents. The following indicators characterizing the general state of blood coagulation system and individual phases of clotting and fibrinolysis were determined: platelet count, APTT, INR, prothrombin index, fibrinogen concentration, content of SFMC, D-dimer, antithrombin III (A-III), plasminogen, and erythrocyte sedimentation rate (ESR).

In all patients, the optical speckle signal of the reflected light was simultaneously measured by ELFI-3 unit. The patient was sitting or lying down. The sensor was attached to the base of the middle or index finger. The operator activated the beginning of the measurement by computer, and pressure 280 mm Hg was applied to the air ring. Within 0.1 seconds, the vessels were pinched at the measuring point, and the optical system began to illuminate the constricted site of the finger. The measured signal was saved in computer memory for further processing. After 25 seconds, the pressure was reset to zero. The measurement was repeated after 15 seconds. A total of 5 consecutive measurements were carried out.

Statistical data processing was performed using fast Fourier transform of the received signal so that the end result of the measurement was converted to vector C (frequency f and time t), describing changes in the spectrum of density as a function of time. Vectors of measurements for all patients were put together into a single matrix. These matrices were compared to laboratory values of SFMC concentration.

To calculate optical equivalent of desired quantity related to the clotting factor, multiple linear regression model was used, with an assumption that dependent variable (*e.g.*, 1/SFMC) was a linear function of independent variables from measurement vector C. The correlation regression analysis was performed for each selected coagulation factor. The number of acceptable parameters in the regression function was determined by a technique known as jackknifing [6]. The application of this technique prevents redundant degrees of freedom in determining correlation between the

TABLE 1. Comparative Analysis of Laboratory Tests and Noninvasive Measurements

Parameter	Range	Mean	Standard deviation	Correlation (R)	Confidence
Platelets, $\times 10^9$	78-568	270	79	0.22	0.02
APTT, sec	20.6-79.0	35	8.6	0.27	0.07
INR	0.81-5.80	1.3	0.75	0.55	0.00012
Prothrombin index, %	10.4-142	83	32	0.54	0.0012
A-III, %	35-151	97	24	0.11	0.5
Protein C, %	29.4-186.0	101	33	0.35	0.0025
Fibrinogen, g/liter	1.40-7.16	3.7	1.08	0.35	0.001
D-dimer, mg/liter	0.04-4.00	0.78	0.8	0.18	0.028
ESR, mm/h	3-66	20	17.5	0.37	0.002
Plasminogen, %	58-140	103	18	0.20	0.1
1/(SFMC+fibrinigen), g/liter	0.035-0.227	0.11	0.036	0.65	5×10^{-7}

measured optical parameters, expressed in the vector of measurements, and laboratory tests.

RESULTS

The range of changes of some blood parameters associated with thrombosis is presented in Table 1. It was found that significant correlation with 1/SFMC was achieved when the second consecutive measurement is considered, which can be explained as follows.

Blood coagulation differs in different vessels. Thus, venous blood coagulates more quickly than arterial [3,5]. Clotting in small vessels can be faster than in larger ones, depending on the expression of tissue coagulation factor [7]. The first and second clampings of the finger leads to hypoxia promoting blood clotting and prerequisites appear for increasing blood viscosity, while the concentration of SFMC increases. It is quite possible that in this case SFMC levels in blood vessels of the finger and in the cubital vein (usually punctured for blood sampling for invasive study) become equal.

Our experiments revealed the highest correlation of optical equivalent with 1/(SFMC+fibrinogen) and 1/SFMC (r=0.6, $p<10^{-6}$; Fig. 1).

Moreover, our observations showed that optical signal had statistically significant correlations with the following parameters: fibrinogen, INR, prothrombin index, protein C, and ESR.

The fact that the best results were obtained after the second measurement, can be explained as follows. Intravascular blood coagulation constantly going on in the body leads to the formation of SFMC and D-dimers in the plasma of healthy individuals [2,4,5]. At the same time, in different parts of the of the vascular bed, fibrilation activity of the blood is not the same [4,5]. In large vessels (arteries, veins), coagulation is enhanced compared with small ones (arterioles, precapillaries, capillaries) [7]. Hypoxia induced by finger clamping promotes local expression of tissue factor on endothelial cells [4,5], which leads to an increase in permanent blood clotting in the finger vessels, so the content of SFMC in the blood of large (vein) and small finger vessels should be leveled.

Strict correlation between the indices of optical signal and SFMC allows us to conclude that the proposed method provides information on the intensity of intravascular coagulation. Taking into account relatively high correlation between optical equivalent and the level of fibrinogen, the presence of disseminated intravascular coagulation can be assumed with high probability from this parameter and the level of SFMC.

At the same time, high correlations were found between indicators of INR, prothrombin index, and protein C, on the one hand, and measured optical signal, on the other. The facts indicate that therapy with indirect anticoagulants can be monitored and corrected by measuring the optical signal for maintaining the required level of prothrombin and protein C.

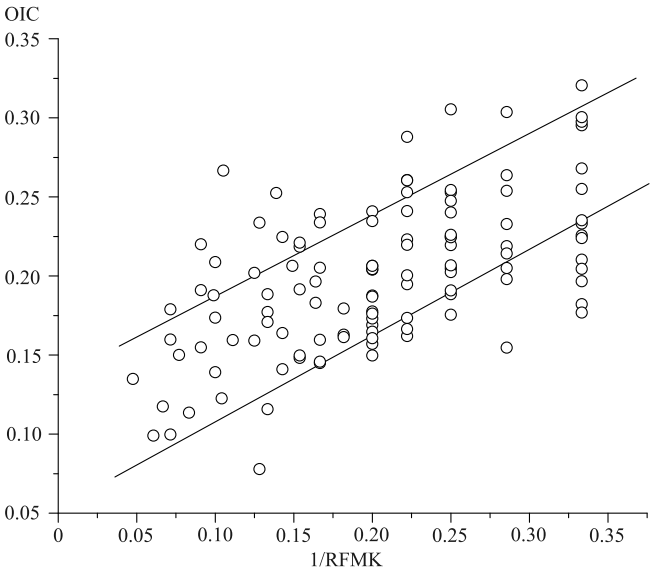

Fig. 1. Optical index of coagulability (OIC) as a function of 1/RFMK.

However, some studied parameters, *e.g.* platelet count, APTT, level of A-III, plasminogen, and others, weakly correlated with optical signal. Apparently, some modifications of the technique, *e.g.* increasing or decreasing the duration of finger compression, and standardization of the studied parameters are required for estimation of these parameters by the proposed method.

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