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The experience of using GDV-graphy technique for the determination of rhesus-factor and human blood groups according to ABO system

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Introduction

The questions of hemotransfusion side reactions and complications were very topical in the works published by the end of XX century.

The analysis of 766 of cases of hemotransfusion complications, which had arisen for patients at various care institutions before 1986, demonstrated that 81.6% of them had been connected with the transfusion of incompatible blood groups (35.8% according to the groups of ABO system, 42.8% - according to rhesus-factor, 3% - according to the antigens of other systems) (1).

The results of research at hematologic scientific center of the Russian Academy of Medical Science in 2000 were even more dramatic: 90.7% of complications were connected with incompatibility (61.7% according to AB0 system, 19.6% - according to rhesus-factor, 2% according to the antigens of other systems), the rest 7.3% of complications were of non-immune nature. The analysis of these complications often demonstrated that the doctors had no knowledge in the sphere of serology (2).

Apart from the fact that the blood antigenic content plays the leading role in the questions of transfusiology, it has great importance in other spheres of medicine and vital activity of human beings as biological species. Thus, A(II) blood group is more frequently met for the patients with pneumonia, sepsis, grippe and breast cancer. These patients more often show a low level of interferon, providing antivirus and antitumoral protection. The frequency of persons of B(III) group is higher among the patients with gastrointestinal tract pathologies. For patients suffering from stomach and duodenum ulcer. The frequency of persons of 0(I) group is 10-12% higher. Rhesus negative people are more inclined to the humoral, rhesus positive – to the cell type of immune response. (3). Inclination of the studied persons to alcohol depending on their group of blood is also worth mentioning: 57% for 0(I) and 100% - AB(IV) group (4).

It is clear why the accuracy of determination of a blood group is so important. However, the performance of pretransfusion immuno-hematologic tests by modern highly sensitive and rapid methods doesn't exclude errors and incorrect results. There are several reasons for that: marginal agglutination, cold and bacterial agglutination; panagglutination, under the presence of pathological processes or absence of agglutination in view of different reasons (irregular reagent ratio, non-compliance of conditions of reaction, usage of old reagents, etc.) (2).

Therefore, the development of new complimentary highly sensitive methods, confirming or refuting serologically obtained results is of great importance.

We have found earlier that the agglutination reaction have been registered with the help of GDV technique (5).

Aim of research

To substantiate the possibility of application of GDV-graphy as a complimentary method in serologic practice for the determination of blood groups in AB0 system and rhesus-factor.

Materials and methods

With the purpose of determination of person's blood groups according to AB0 system, diagnostic liquid tsoliklon anti-A, anti-B and anti-AB (monoclonal antibodies anti-A, anti-B, anti-AB) were used.

Erythrotest-tsoliklon anti-D super was applied for the detection of D antigen of rhesus system in human erythrocytes. On the grounds of the fact that IgM antibodies didn't cause agglutination of some samples of erythrocytes with ill-defined D antigen, the samples of donor blood, determined as D negative from the investigation with tsoliklon anti-D super, were additionally tested at Research Institute of Cardiology by means of anti-D reagents, containing IgG antibodies (polyclonal serum or monoclonal anti-D IgG reagent).

Test-tubes with anticoagulant EDTA -"Microvet" were used in the experiments for blood sampling. Fine-dyspersated anticoagulant powder EDTA at the inner surface of the test-tube quickly dissolved in blood and safely blocked the processes of blood coagulation (6).

Blood groups and rhesus factor (D antigen) were determined from the blood taken from a finger of patients. On the whole, 28 patients-volunteers from 20 to 60 years of both sexes took part in the experiments.

Tsoliklons anti-A, anti-B, anti-AB and tsoliklon anti-D super were applied in big drops (0.1 ml) to a plate with the corresponding labels. Blood samples were applied in small drops (0.01-0.03 ml) near those drops and mixed with reagents. The agglutination of erythrocytes was observed while slightly shaking the plate for 3 minutes.

Having identified a tested volunteer in accordance with the blood group, we passed to the investigation of blood by the GDV-graphy technique. Blood in the amount of 20 mcl was applied to a special glass syringe adapter, filled with a physiological solution (0.5 ml) with the purpose of grounding (7). Each donor blood sample was measured with the device 10 times. All works were performed by means of the same GDV Camera device in "GDV Capture" program. The obtained data was statistically processed with the help of Video Analyzer, Statistica and Microsoft Excel programs.

Analysis of the discussed data

The results of serological reactions for the identification of blood groups according to AB0 system and rhesus factor, for tested volunteers were compared with the results of GDV-graphy.

The experimental data were evaluated with parameters of dynamic GDV-grams – entropy, fractality, mean square deviation of fractality, power of time series, as well as exponential and polynomial coefficients of approximation of time series of background area and form coefficient. No significant results were found for such parameters as intensity, length of isoline, etc.

For the convenience of data interpretation, the following denotations were taken: the tested volunteers were divided by sex - M (male), F (female) and numbers were assigned to each person in every group particularly (fig. 1 and 2).

The cluster analysis was carried out in multiparameter space of the given parameters. The following results were obtained on the basis of it. Four areas were registered in the space of parameters of time series of form coefficient (fig. 1). The first of them was represented by individuals with II blood group (F2, F7, F9, F14, M1, M2, M7, M8, M9), as well

as one person with I blood group (M12). On the whole, this area included 9 of 10 persons with II blood group.

The second area was made up by the individuals with I blood group (6 of 9). And namely, F5, F6, M4, M5, M6, M8.

Four of six tested volunteers with IV blood group made up the third area (F3, F10, F11, M11).

The forth area included persons with different blood groups, who didn't fall into the previous three areas (F1, F4, F13, F15, M3, M10, M12, M13).

Three areas were revealed in the space of parameters of time series of background area (fig 2.): two of them with rhesus positive individuals and one – rhesus negative. The group of rhesus negative individuals consisted of F2, M3, M4, M9, the others made up "positive" group.

Basing on the results obtained, we can conclude that the tendency to the division of the tested volunteers into blood groups took place in multiparameter space, formed by the parameters of time series of form coefficient. As to the rhesus factor, the results of processing of time series of background area demonstrated a distinct division of people into rhesus positive and rhesus negative.

It is worth mentioning that no differences by sex or age were found in the given sampling.

Thus, the application of GDV-graphy in serological practice as a complimentary method has been proved to be effective under further perfection of measurement technique and laboratory practice.

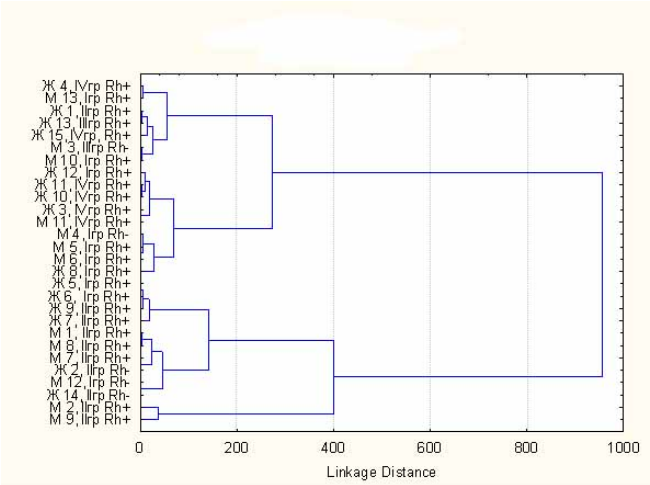

Fig.1.Cluster analysis by the Form Coefficient

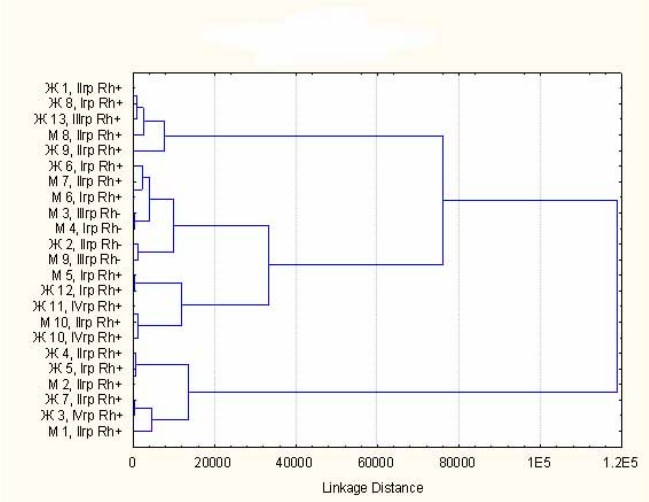

Fig.2. Cluster analysis by the Area

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