Clinic substantiation of perspectives of using GDV-technique for etiological diagnostics of allergies

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Nowadays a steady rise of a number of allergy diseases typical of a forth of population is recorded, which causes natural anxiety of medical community and dictates a necessity of research of a more perfect method of diagnostics and treatment of this disease. A priority importance is attached to diagnostics, at that, as the sooner the cause of the allergy state is found, the more effective its complex therapy may be. Laboratory methods of detection of sensitizing agents dominate in this process, as even a thoroughly gathered medical history enables to hypothetically determine the interested (sensitizing) factor [1].

Four types of allergy reactions are distinguished now. In pathogenesis of the three of them, united in a group of humoral reactions (types I-III), a leading role belongs to antibodies, and in that of one of them (type IV) – to sensibilized immunocompetent cells. This division is very relative, as different types of reactions are often developed simultaneously: mechanisms of I and III types take place under anaphylaxis, III and IV types – under autoimmune diseases, and all four types – under drug allergy [1, 2].

Despite of numerous investigations, connected with perfection of the methods of identification of the type of allergy and, what is especially important, the concrete nature of its causally-significant factor, the diagnostics of these states does not fully meet the requirements. The methods, which reveal allergopathologies only in particular parts of immune system, are being used in practice until now; however, they don't allow assessing the whole range of occurring events, which certainly reduces their effectiveness and informativeness. They often give false-positive or negative results even at the presence of quite convincing signs of disease. Therefore, not by chance and in order to raise the reliability, a complex diagnostics is performed by way of applying several complementary methods, allowing to assess the allergy considering disorders in various parts of immune system.

As concerns invasive tests (skin, provocative), despite of their quite high informativeness, they are characterized by high reactogenicity and shall be used very carefully, as they can cause complications of local and system character and provoke undesirable exacerbation of the process [1].

Thus, it is supposed that the methods enabling to determine the aetiology of allergy by way of integral estimation of reaction of immune system of a concrete individual to a certain allergen are required. In this connection, we applied to gas discharge visualization (GDV) technique, which records any shifts of physico-chemical characteristics of liquids, including biological, representing objective information in the form of curves in computer screen [3].

The aim of research consisted in clinico-experimental substantiation of applicability of the GDV technique for etiological diagnostics of allergy. The following tasks were set for its realization:

- To study the capability of the GDV technique to record the presence of mediators of interaction of immunocompetent cells and allergen in biological liquid, as well as the reaction of antigen/antibody, i.e. agglutination reaction;

- To assess the GDV in experiments on animals as a method of determination of allergy status;
- To check the data of experiment (on receipt of positive results) on people suffering from allergy.

Materials and methods

Liophilly dried histamine, serotonin and adrenalin were used as mediators. Vaccine culture of tularemic microbe, B. fragilis and the antibodies complimentary to them were applied for the agglutination reaction.

The allergy was modulated on guinea-pigs according to the well-known technique: 0.1 ml of horse serum (HS) was single-injected intraperitoneally, which stimulated the formation of heightened sensibility to proteins of HS [4].

The following scheme of analysis was used for the detection of allergy. Heparinized blood of each of the investigated animals was poured out in six centrifuge tubes per 0.5 ml. Then 0.2 ml of HS was introduced into the three of them (experimental) and 0.2 ml of bovine serum (BS) – into the other three (control). Heightened sensibility of guinea-pigs to BS was absent a priori. Blood plasma of two samples (experimental and control) obtained by way of centrifugation (1500 rpm during 3 min) was investigated right after mixing the mentioned components, two – after their incubation during 1.5 h under 37 ^{0}C (in thermostat), two – in a day (1.5 h – in thermostat, and then under room temperature). We supposed that the emergence of immune complexes and mediators in the animal blood as a result of repeated interaction of allergen and specific antibodies and cells sensibilized to it in a tube would cause the change of emission properties of blood plasma exposed for 1.5 and 24 h, as compared to the initial (first) sample, which can be registered by means of GDV-graphy.

Moreover, 5 persons with food allergy being under examination of specialists-allergologists of department of military-field therapy of Military Medical Academy were examined. In contrast to the experimental part of work, not only blood serum of patients, but the blood itself was investigated with the help of GDV technique.

Results of research

As it is well known, the participants of allergy reactions developing in the organism are mediators. In this connection, we considered obligatory first, before the experiments on animals, to find out if they could be disclosed by means of the GDV technique in such a complex and multicomponent biological environment as blood. With this purpose we investigated the HS samples, introducing either histamine or serotonin, or adrenalin in concentration 200 mkg/ml. It was found that the serum without mediators (control) was reliably different from experimental samples according to GDV-grams. That was shown, particularly, by such indices as area and average intensity of glow.

In the basis of humoral mechanisms of development of allergy reactions lies the interaction of allergen with antibodies complementary to it. Therefore, the capability to fix the agglutination reaction of the studied method was assessed in special experiments. It turned out that the GDV allowed revealing those immune complexes, forming as a result of specific immune reactions between the antigen and the antibody corresponding to it (in our case – reaction between the vaccine culture of tularemic microbe, B. fragilis and the antibodies complimentary to them).

The obtained results enabled to pass to the experiments on guinea-pigs, modeling their allergy to foreign protein – normal horse serum.

In accordance with the scheme of reaction chosen by us, the first two samples investigated right after the mixing of reactive components, were intended for the assessment of the initial state of blood plasma, and the next – for the disclosure of changes which could take place as a result of the subsequent contact of blood with foreign serum (HS or BS).

We supposed that having such a scheme of analysis the blood of allergen-challenged animals would react in a different way to the antigen which caused the allergization, i.e. allergen (HS), and heterological (control) antigen (BS). Under the contact with allergen within 1.5 h color-sensitive, sensibilized cells would actively synthesize and secrete biologically active factors of interaction, and, when decomposed, the cytoplasm could pass into the liquid part of the blood. The reaction of blood cells of the same animals to control antigen (BS) could probably be less active as compared to its intensity to antigen.

In 24 hours the agglutination reaction of allergen with antibodies specific to it should take place, too, while in control sample it should be absent (blood + BS).

We intended to disclose the above mentioned differences in the reaction of blood of experienced animals to allergen and heterological antigen with the help of GDV technique.

As a result of the investigation, it turned out that the blood of most (88 %) of the sensibilized animals reacted quite actively to the cause-significant allergen: GDV-grams of plasma, obtained from blood, which contacted with HS in the tube within 1.5 h or 24 h, reliably differed from the initial lines, characterizing emission properties of plasma, obtained right after mixing the blood and allergen, i.e. before their interaction. The GDV-grams of one of the guinea-pigs can serve an example, where the animal's allergization is demonstrated by such parameters as the area of glow of plasma, average radius of isoline, normalized mean square deviation of the radius of isoline, and form coefficient. In contrast to it, the reaction of blood of the same guinea-pig to the heterologous antigen (BS) was significantly less pronounced.

We observed such differences for 8 of 9 investigated animals. The result was considered to be positive when:

- the reaction to HS was present and demonstrated by one or several parameters and the reaction to BS was absent at all (animals NN 1, 2, 4, 8, 9);
- the reaction to HS was disclosed judging by a bigger number of criteria than that to BS (animals NN 3, 5, 7).

In case of negative approach (absence of allergy to HS) we evaluated the result of examination of guinea-pig N 6: reliable differences for HS were found only for one parameter – form coefficient, and for SC – for two – average radius of isolines and average intensity.

It is worth mentioning that we received positive reply in 70 % of cases investigating the blood after its exposure in thermostat during 1.5 h. This indicates that the GDV technique is an express method, which shows its big advantage among other methods used nowadays.

In the closing stage of work the studied method was tested on people, whose personal allergic history showed that they were allergic to the albumin (hen's egg). Not only was the blood serum investigated, but also the blood itself. Samples of the investigated material with diluting liquid and causally insignificant allergen – house dust and meat of duck, were used as a control.

The result of research was considered positive in case if:

- the GDV-gram of the blood sample with the suspected allergen (according to the data of anamnesis) exposed during 90 min. was reliably different from the analogous curve obtained from the analysis of samples with diluting liquid and control (heterologous) allergens (criterion A);
- The GDV-gram of the initial blood sample with the suspected allergen was reliably different from the analogous curve, obtained from the investigation of exposed sample, in the absence of such time dynamics in the samples with diluting liquid and control antigens (criterion B).

With the purpose of confirmation of informativeness and reliability of the studied method, the data of GDV-grams was compared with the results obtained by means of such a well-known test as leukocyte migration inhibition test (LMIT) in every particular case.

As a result, the three of five patients examined with the help of both methods were found to have allergy, conditioned by albumen, and the two – to have no allergy.

An example can be shown by the GDV-grams of patient A, whose allergic history gave grounds to suppose that his allergy was conditioned by albumen. Therefore, in the investigation of blood, the preparation of this protein was "experimental" and the diluting liquid and house dust

served as controls. As a result, the diverse intensity of blood reaction to etiologically significant allergen, heterogeneous antigen and diluting liquid was graphically determined.

Thus, estimating by criterion A (fig.1), it was found that the reaction of blood to house dust in 90 min. of exposure was the same as to the diluting liquid, which indicated that the patient had no sensitization to this substance. At the same time, the curve reflecting physico-chemical properties of blood after the interaction of its components with albumin, had reliably different spatial position as compared to the control lines, indirectly indicating heightened sensitivity (allergy) of the patient to this foreign protein.

Criterion B is another indication of that fact. The samples of blood containing diluting liquid and house dust had the same physico-chemical characteristics both in the first and second (after exposure) investigation, i.e. the reaction of blood components was absent. The reaction to albumin was different: GDV-grams obtained before the interaction of blood with albumin (0 min. exposure) and after it (90 min. exposure) were reliably different between one another (fig. 2), which was an absolute indication of change of emission properties of blood and, respectively, of sensitization of patient with this very protein.

Certainly, such narrow clinico-laboratory examination of people doesn't allow making some final generalization. The started work of examination of allergic people shall be continued in order to formulate recommendations on the application of the GDV technique in allergology.

Nevertheless, both the experimental data and the results of research on people presented in the given report can be considered as a quite weighty evidence of perspectives of the new application of the GDV technique, concerned with the detection of a concrete cause of allergy of people.

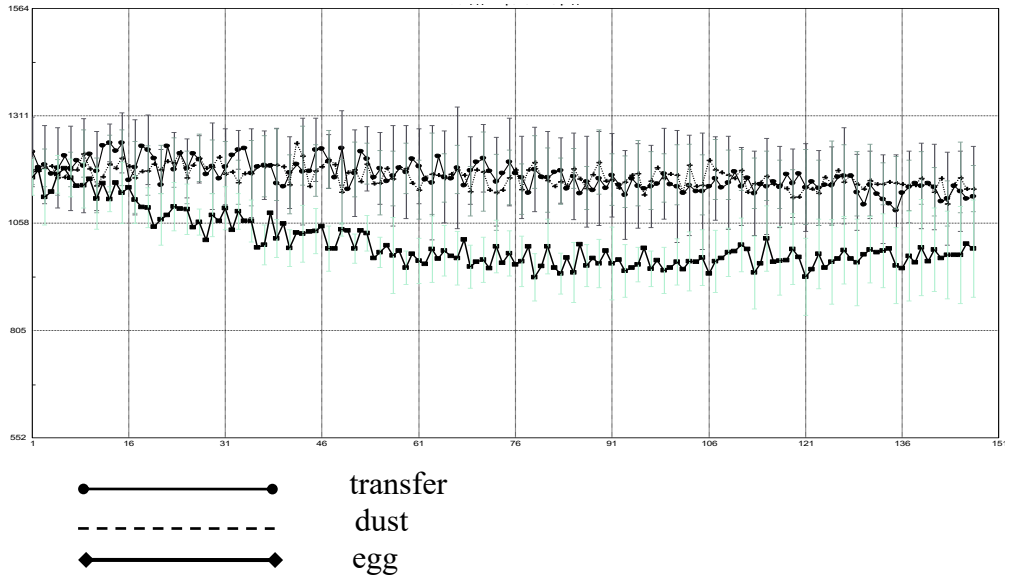

Fig. 1. Area

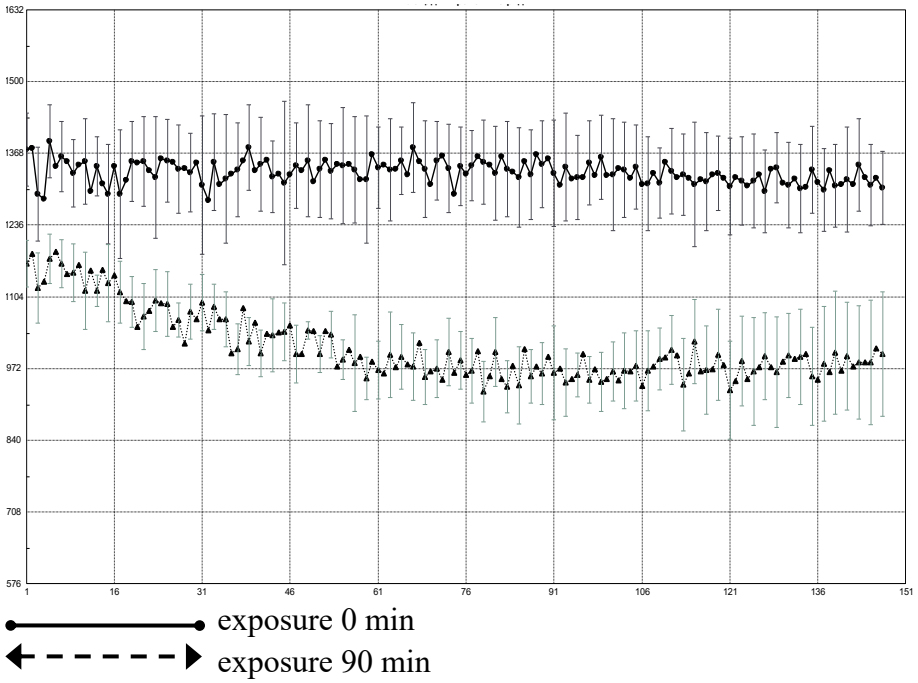

Fig. 2. Area

Literature

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