Medical Research Center “Immunculus”

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New Approaches

to early detection of pathology changes in the human organism using marker autoantibodies

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Introduction

In recent years, more and more healthy individuals have started to see doctors for non-immediate medical concerns. These people plan to live long healthy lives and confront age as somatically safe and active individuals who consult their doctors for professional advice and help.

Health is difficult to define. Some believe health is the absence of disease and somatic defects. For someone else, health is a state of full physical, mental, and social happiness. Generally speaking though, it is supposed that health is the main condition of individual well-being.

Health is a complex paradigm. Why do some people develop diseases at young ages or later in life? How do some people stay healthy without medical care into old age? How does one know about the risks or early stages of a disease?

A lot of factors influence the state of health in humans. Extensive population studies indicate that the following factors are the biggest determinants of health:

- Mode of living (up to 50%);
- State of the local environment (20-30%);
- Genetic inheritance (from 5% to 10%);
- Quality of the medical service (8-12%).

This data indicates that the primary factor is individual lifestyle, which includes:

- *Dietary habit;*
- *Regular physical activity (or its absence);*
- *Pernicious habits (e.g. smoking or drinking);*
- *The psychological climate;*
- *Personal responsibility for own health state.*

When a doctor regularly observes a patient, he/she is responsible for the following tasks:

- To evaluate an individual's risk of principal diseases;

- To reveal a molecular marker of changes that preceded the disease;
- Select and use adequate individual measures of correction.

One of the most important conditions for health is an opportunity to discover a disease in its early pre-clinical stages. It is during the early stages that pathology may be easily reversed when relatively simple and cheap non-pharmacological and non-surgical measures can be sufficient to arrest and counter the disease.

Most universal and early markers of chronic human pathology can become such specific products of the individual immune system as natural autoantibodies (auto-Abs). Academic understanding of the biological meaning of the immune system has changed significantly during the past 20 years. To a great extent, these changes rethink the essence of natural (physiologic) autoimmunity, auto-Abs, and self-reactive lymphocytes.

The immune system and microbes

All known eukariotic organisms from the most primitive forms to complex mammals coexist with a multitude of viruses and bacteria. The innate immune system (InIS), which appeared in early evolutionary stages and is still present in superior vertebrates, is more likely to be the primary and most effective antimicrobial defender.

The main participants of the adaptive immune system (AdIS) - T- and B-lymphocytes - appeared only at the later stages of evolution from cartilaginous fishes forward (i.e. as soon as a general number and diversity of cells in specialized organs of vertebrata approached a certain threshold of complication and necessity, an additional mechanism of co-tuning appears).

The key missions of the "young" AdIS are self-identification and mutually coordinated adjustment of multiple cellular, subcellular, and epicellular components, in addition to continuous molecular screening and participating in molecular and cellular homeostasis throughout an individual's life. While the AdIS certainly can participate in extermination of potentially dangerous microorganisms, this type of activity seems to be more of an accessory than a priority.

Selectivity is one important feature of the interaction between the AdIS and microbes that was recently revealed. As a result of this phenomenon, homeostatically neutral microorganisms (the main component of the microbiota in host-organisms) are ignored by the immune system, potentially useful ones are even guarded, and only those deemed hazardous become the subjects of attack. This distinction by the immune system is not determined by the usual suspect - "foreignness" of microbes - but instead depends on the extent of the potential danger to homeostasis in general. Mechanisms of such selectivity remain to be elucidated. Regardless, noted phenomena seemingly indicate that antimicrobial activity of the immune system is not solely reliant on the "non-selfness" of microbes since they are all aliens. Rather, it depends upon the ratio of potential "hazard" to "utility" — a reasonable criterion to use when determining an organism's needs.

A short history of the modern immunological ideology

The clonal selection theory proposed by Frank Macfarlane Burnet was an enormous contribution to immunology because it shed light upon the terra incognita that was immunology nearly half a century ago. At the same time, clonal selection theory became a serious obstacle to other prevailing immunological ideologies. From Burnet's theoretical point of view, the immune system was termed a "system" only nominally because it was in fact only considered a conglomeration of nearly independent lymphocyte clones. Each clone was intended to react exclusively with "aliens" and was designed to carry out two main effector functions: 1) to recognize a complementary antigen, and 2) to eliminate any recognized substance by a simple reflex-like principle, stimulus-reaction. As long as the existence of the auto-reactive lymphocytes ("forbidden" clones) that recognize self-antigens was regarded by the clonal selection theory as random abnormal deviation, the idea of physiological or natural autoimmunity simply could not burgeon. There was no place for physiological autoimmunity and all forms thereof were considered pathological by definition. Therefore, the vector of the immune function was directed exclusively outwards (an instrument of defense from environmental hazards) but never inwards. As a result, previously revealed evidence that demonstrated the presence of auto-Abs

in healthy individuals (e.g. against red blood cells) was considered contradictory and practically ignored.

The concepts proposed by Cohn and Langman, as well as some others, are not considered in depth here because, in spite of certain novelty, they were essentially based upon the same grounds as and were equivalent to Burnet's theory. The "danger hypothesis" by Polly Matzinger was based upon different philosophy and better explains the experimental data, originating from ideas about regulatory and homeostatic functions of the immune system. According to Matzinger, the AdIS itself cannot "decide" where, when, and in which way to act, but it is activated instead upon the request of a defined compartment of the organism (by affected tissue, i.e., the microenvironment). Any local organ or tissue can generate signals activating the AdIS whenever in need of additional immunological support (e.g. for intensifying the elimination of damaged cells, for activating tissue regeneration, etc.). In other words, the immune system remains in "standby mode" in the absence of molecular signal-requests from certain tissues or organs. Hence, it is not the presence of some foreign molecular stimulus that is important for inducing the AdIS response but rather it is the endogenous signals that are increasingly released into intercellular spaces of the local environment as a result of definite tissue damage that indicate a potential threat to general homeostasis. Conversely, if the immune system begins to act autonomously, beyond tissue "requests" (e.g. under the influence of lymphotropic viruses or other toxic factors), this may cause serious disturbances that lead to the development of autoimmune disorders or other immunopathological situations.

According to Matzinger, the significant characteristic is not the "foreigness" of microbes or viruses per se but the potential hazard an antigen represents to an organism. Hazardous agents induce cell and tissue injuries and, as a result, lead to production of danger signals — that is, intracellular molecules that begin to excessively appear in extracellular space. Matzinger does not deviate her focus from natural autoimmunity and even considers this phenomenon to be a universal indication of tissue damage, but she unfortunately makes no attempt to explain its biological meaning or relevance.

According to the "immune network theory" by Niels Erne, a healthy individual initially has a well-balanced self-reactive and self-reflective (self-recognizing) idiotype/anti-idiotype network made up of molecular (antibodies) and cellular (lymphocytes) components. Excessive antigenic stimuli may then initially induce perturbations to this precisely balanced system of interconnected elements. It matters not whether the

antigenic stimuli are endogenous or exogenous in origin; it is only important that they are complementary to the components of the global network. Network imbalance is equivalent to system activation. This activated state is unstable and, over a period of time, either reverts to the initial stable state or transitions to a new stable one. There are fundamental differences between the immune network principle and all other immunological concepts: the network theory does not operate within the boundaries of "self – non-self" or "hazard – non-hazard" dichotomies. Network activity is instead based upon collective states (disturbance – resting). It should be noted that the complementary-network principle represents the immune system as a full-fledged system and provides nuance to a previously limited scenario — a literally unlimited capacity to "embrace the unembraceable" variability of antigens.

It especially bears mentioning that the immune network, in spite of its non-topological organization (the immune system is an example of a dissipative system), is robust enough and its main features are defined during the intrauterine period (under the influence of the maternal immune imprinting). If the immune system does operate as a totally integrated network, it is important to bear in mind that we may be wrong to try to comprehend its basic principles through the study of the activities of individual lymphocyte clones because analysis of the features and activities of isolated components of a complicated system has inherently limited potential for understanding the whole.

Natural autoantibodies and the state of health

Within the last twenty 20 years, the field of clinical immunology has been subject to some paradoxes that contradict the positions adopted by most physicians. The puzzle that is natural auto-Abs is a suitable example. In the past, these molecules have been associated exclusively with the state of pathology (autoimmune diseases). However, it is now widely accepted that auto-Abs are permanently and obligatorily produced in all healthy individuals. No longer is the presence of auto-Abs automatically associated with pathological processes; we now know that stable anomalies in their production and serum concentration occur not in relation to failures in immunoregulatory mechanisms.

Today, it has become commonplace to regard increased serum levels of auto-Abs not only as indicators of autoimmune diseases but also as hallmarks of many non-autoimmune illnesses and conditions, including strokes [14], cancer [15], myocardial infarction [9], and complications of pregnancy [6].

It has been proven experimentally that molecular "danger signals" from injured tissue induce transitory autoimmune reactions that are accompanied by increased production of tissue-specific auto-Abs [9]. As noted by Tveita, a temporary rise in autoreactivity per se is a temporal physiological (normal) aspect of all tissue inflammation, and this phenomenon is principally distinct from pathological autoimmunity [16]. Unfortunately, upon analyzing this phenomenon, neither Matzinger nor Tveita went "out of the box" when discussing the reasons why transitory physiological autoimmune reactions following tissue damage do not transition into permanent changes nor lead to the development of autoimmune diseases. However, there are compelling reasons to reconsider the biological meaning of universal injury-associated autoimmune reactions from a physiological point of view. This phenomenon can be interesting not only in abstracto but also in the context of everyday medical practice.

All original (primordial) lymphocyte clones are initially moderately autoreactive due to combined negative and positive selection processes undergone during maturation. Thus, the continuous production of a certain amount of auto-Abs with various antigenic specificities is an intrinsic feature of the immune system. Empirical observation proves that the damage (of mechanical, chemical, infectious or any other nature) of specialized cells, expressing particular antigenic profiles, acts as a trigger for increasing the production of auto-Abs to corresponding antigens [6, 9]. Therefore, the question remains: should inborn AdIS autoreactivity be considered a potentially hazardous evolutionary fortuity? Could an increase in the production of auto-Abs of certain specificities, induced in response to tissue damage, be functionally senseless? This eventuality appears more than doubtful. From an evolutionary point of view, there is no place for senseless fortuitousness.

Multitudes of natural auto-Abs of IgG and IgM classes have been permanently synthesized, secreted, and presented in the blood serum of all healthy persons. These data were clearly and repeatedly demonstrated in several laboratories. Even previously accepted as "exclusively pathological" auto-Abs, such as those against myeloperoxidase, proteinase 3, and the glomerular basement membrane, does present in the serum of normal individuals as well as antibodies reactive to autologous ABO blood group antigens. Serum concentrations of auto-Abs may vary dramatically in healthy adults. For example, the average serum content of auto-Abs against Fc-fragments of immunoglobulin is nearly twenty times greater than that of auto-Abs against myelin basic protein. On one hand, the serum concentrations of auto-Abs with the same specificity in healthy individuals (having no tissue or organ damage to express corresponding antigens) is roughly equal; on other hand, several diseases are accompanied by notable deviations in serum content of particular auto-Abs (against specific antigens of certain cells). This secondary rise in ("danger signal"-induced) production and secretion of auto-Abs against antigens of damaged cells should not be considered a side effect but rather a reflection of one of the major roles of the AdIS, namely its ability to execute the function of autoclearance. Large portions of natural auto-Abs specifically bind to oxidation-associated neo-antigens that become exposed on dying cells as well as senescent-associated determinants or molecular "eat me" signals. Any inflammation is accompanied by a rise in respective auto-Ab production, which then reacts with their antigens to form immune complexes and facilitate Fc-mediated clearance by antigen presenting cells (APC). Every day, in various compartments of an organism, clearance of hundreds of billions of apoptotically dying cells (efferocytosis) is required for normal tissue homeostasis and prevention of inflammation. Obviously, the homeostatic importance of local activation of autoclearance mechanisms increases dramatically in the case of tissue damage (of any etiology). That primary tissue damage then induces an evolutionarily-fixed phenomenon of a secondary increase in the production of auto-Abs against tissue-specific antigens (and may be reflected by "mirror" of auto-Abs).

Quantitative changes in the production of autoantibodies: a physiological phenomenon

In accordance with the basic statements of the immunochemical homeostasis concept by Igor Kovaliov, rates of natural auto-Ab production are regulated by quantity/availability of respective antigens via a feedback principle. The rates of production, secretion and/or release of any cytoplasmic, membranous, or nuclear antigen into the intercellular space are nearly equivalent in all healthy individuals (or differ insufficiently); therefore, serum

levels of respective auto-Abs should also demonstrate only slight individual variability. This picture changes dramatically in pathology. Many disorders, especially those of a chronic nature, are directly associated with either abnormally elevated apoptosis or cell necrosis in the involved organ, or deviations in the abnormal production and/or excretion of certain antigens. In turn, a stable increase in the extracellular concentration of any endogenous antigen is inevitably accompanied by deviations in the concentrations of cognate auto-Abs according to Kovaliov's rule. For example, during the preparation phase before in vitro fertilization (IVF), most women receive drugs containing pharmaceutical (that is, excessively large) doses of human choriogonadotropin (HGT). As a result, up to 60% of these women exhibit an increased production of autoAbs against HGT within six months and beyond.

It is typical for some isoforms of insulin receptors to be elevated in skeletal muscle fibers at the pre-disease and early stages of diabetes mellitus (probably as compensation for deteriorating receptor functionality). Accordingly, many patients with pre-clinical diabetes mellitus demonstrate abnormally increased serum levels of auto-Abs against insulin receptors. In most cases, this increase did not relate directly to the pathogenesis of diabetes but, instead, reflects abnormally increased expression of receptors in accordance with Kovaliov's rule: elevated quantity of antigen leading to rise of production of corresponding auto-Ab.

Malignancy-associated increases in auto-Abs against the phosphoprotein p53, a regulator of apoptosis, may be attributable to the same principle. It is known that p53 alterations (missense mutations) appear to be present in 40 to 45% patients with different forms of malignant diseases. Frequently, these alterations are accompanied by compensatory elevations in p53 expression and, secondarily, by a rise in corresponding auto-Abs (directed to non-mutated epitopes of protein!). Lubin and co-authors especially noted that extensive accumulation of p53 is the cause of "self-immunization", i.e., appearance of excess anti-p53 Abs in the patient's serum.

In accordance with the general logic of living systems, quantitative changes in physiologic parameters are usually aimed at correcting or compensating for an abnormal situation in the body. For example, tremendous physical effort is accompanied by elevation of blood pressure, tachycardia, rise of blood glucose level, and other abnormalities that are all untypical of the resting state. Such reactions provide additional resources for "fight or flight" and are physiological and evolutionarily justified. Principally the same physiological (sanogenic) autoimmune reactions may be observed, for example, in patients suffering from ischemic stroke. It was shown that prominent temporary elevation of "neurotropic" IgG auto-Abs in the serum, if observed soon after stroke and for a few weeks thereafter, is a favorable prognostic sign. Conversely, the lack of a notable stroke-induced secondary autoimmune reaction — that is, preservation of normal or low levels of "neurotropic" auto-Abs during the few days following a stroke — is a bad prognostic sign that is typical of non-survivors and of survivors suffering prominent motor and/or cognitive deterioration. As one may suppose, stroke-induced sharp and relatively prolonged (up to 1-2 months) elevation of auto-Abs against proteins of the injured brain cells (GFAP, S100, MBP, and others) is a deeply rational autoimmune sanogenic phenomenon aimed at increasing clearance of damaged neural structures and functional restoration.

Similarly, increased production and serum concentrations of "pulmotropic" auto-Abs are typical of chronic pneumonia, and normalization of serum levels may indicate the resolution of the disease (for example, in response to effective treatment). These examples underscore and further justify the concept of immunochemical homeostasis based on two points: 1) the amount of auto-Abs produced is a function of antigen availability; 2) the main purpose of natural auto-Abs is the clearance of excessive antigens that are formed during normal or deviated vital activity. Some auto-Abs does possess an enzyme-like activity (abzymes), and may inactivate the target antigen directly. However, in the majority of cases, auto-Abs achieve clearance not by means of intrinsic activity but as opsonins, which bind to and mark endogenous molecules, microparticles, or dying cells assigned for consumption and digestion by macrophages.

In addition, IgG auto-Abs that specifically bind to DNA fragments generated during apoptosis can be absorbed by B-cells bearing respective receptors, causing these B cells to proliferate. Likely, equivalent or similar events (pinocytosis of antigen-antibody complexes mediated by membrane Fc-receptors) occur in the case of dendritic cells and macrophages. Coincidentally, it is interesting to note that a substantial number of autoantigens preferentially migrate to the blebs on the surface of apoptotic cells.

Inflammation is considered to be a crucial mechanism of repair and restoration of a damaged organ. When one considers the inflammation-related induction of auto-Ab production (as well as the inflammation-related proliferation of macrophages and elevated production of proinflammatory cytokines), the general picture of sanogenic activity of the immune system appears outlined in general, though lacking certain details.

From a practical point of view, it is crucial to distinguish primary (pathogenic) and secondary (physiological) autoimmune processes. Primary autoimmune reactions (for example, virus-induced) may be observed relatively rare and are usually pathogenic in essence; these events may even cause systemic or organ-specific autoimmune diseases. In contrast, secondary and transitory (physiological) activation of natural autoimmunity, following the primary (injury-associated) events in organs, often seems to be positive in essence by aiming for increased clearance of antigens in the involved organ and recovery of the disturbed physiological functions. Michael Schwartz has presented obvious demonstration of the reparative function of natural autoimmunity in vivo; in experiments involving rats with mechanically damaged nerve fibers, administration of autoreactive (against components of myelin sheets) T-lymphocytes lead to facilitation of functional restoration of the injured nerves. This phenomenon seems to belong to the same cohort as mentioned above: transitory elevation of neurotropic auto-Abs after ischemic stroke to restore disturbed nervous function.

Quantitative changes in autoantibodies as marker of disease

If the immune system is essentially a self-reactive and self-reflective entity, intended for holistic perception and reflection of the antigenic image of the body in both the normal and disease states, the exploitation of these abilities for the needs of medical practice seems very attractive — namely for precision diagnostics and prognostic tools for early detection of pathological situations.

In most cases, an obligatory prerequisite for future development of chronic disease is noted by points (a) and/or (b):

a) The primary biochemical (antigenic) deviation in certain specialized populations of cells is correlated with abnormalities in production, secretion, and presentation of certain antigens. These events are sometimes accompanied by notable elevation of cell destruction and death and may appear months or years before clinical manifestation of the disease.

b) Abnormal increases in the rate of specialized cell death by apoptosis, necroptosis, or necrosis. These events may be accompanied by prominent changes in production and/or secretion of certain antigens; regardless, the immune system experiences significantly higher rates of exposure to intracellular components.

In both cases the immune system meets with quantitative abnormalities in the molecular composition of bodily fluids and reacts by secondary changes in production and serum content of auto-Abs with respective specificity. Accordingly, long-term abnormalities in the level of particular auto-Abs could in most cases identify the population of cells/which organ is suffering the molecular disturbance. However, before we can learn to effectively speak this molecular language, we should first comprehend the peculiar code (i.e., antibodies/symbols) by which the “magic mirror” of the immune system reveals secrets of the bodily state. Practical realization of these ideas may help us transition to a new medical paradigm based upon early detection of the “disease-before-disease” and stop or slow development of pathology by implementing very early preventive measures.

Holographic immunculus

In spite of continuing pressure from habitual ideas about the immanently aggressive nature of all autoimmunity (rising from Ehrlich’s “horror autotoxicus”), it is high time to admit: increased intensity of secondary (that is, induced by tissue damage or by abnormal expression of certain molecules) autoimmune reactions does not always relate to nor always reflect the potentially self-destructive activity of the immune system.

The clearance function of the immune system, mediated by natural auto-Abs (together with macrophages), was first proposed more than half a century ago by Pierre Grabar. Earlier, in the thirties, the idea of regulatory auto-Abs was also mentioned by Karl Landsteiner. However, the real “prophet” of the new attitude concerning immune functions became Elia Metchnikoff. He claimed that it would be wrong to consider the immune system mainly as a gendarme of the organism; its participation in a constant struggle between host and parasite is only a particular fragment of a much more broad biological destiny —namely dynamic participation in self-maintenance, self-reparation,

self-optimization, and maintenance of a harmonious state under the constant pressure of the environment. Metchnikoff's concept was far too ahead of his time and only now may we admit that he was correct and wonder at his staggering intuition. Today, owing to the pioneers E.Metchnikoff, P.Matzinger, O.Parnes, and others we must recognize that the global function of the adaptive immune system is the maintenance and regulation of an optimal molecular homeostasis of the entire body (including struggles against hazardous microbes when necessary).

One of the most important instruments used by the AdIS in this venture is natural auto-Abs of the IgG, as well as IgM class. The most peculiar feature of auto-Abs is their permanent presence in nearly every compartment of the body (in the blood stream, interstitial fluid, lymphatics). According to our experience, the auto-Abs present is nearly the same regardless of whether one examines capillary, venous, or arterial samples of blood. Therefore, quantitative evaluation of auto-Abs directed against specific sets of antigens of heart, liver, lungs, or other specialized organs ("cardiotropic", "hepatotropic", "pulmotropic", etc., auto-Abs) in a blood sample, regardless of origin, may provide equally valuable information about the state of specialized cells, tissue injuries, abnormalities in hormonal state, or steady changes in expression/production of defined receptors, etc., allowing us to monitor the state of any organ as well as general health. In other words, the immune "image model" presents a multi-dimensional molecular "picture" of the body, seemingly almost similar to the holographic principle. The main features of a holographic image are virtual three-dimensionality and intrinsic indivisibility (each small portion of a hologram does necessarily reflect the entire picture; they must be viewed as a whole). The same seems to be true of the network of auto-Abs "mirroring" the body's state (the immunculus). Any small piece of holographic film contains information about a whole image; the image will get hazier as the portion of the hologram being viewed gets smaller, but there will be no "gaps". Similarly the "mirroring" properties of the immunculus are non-localized, distributed throughout the whole system (in contrast to the topologically organized neurological homunculus), and removal of a piece of the immunculus "puzzle" (for example, after massive bleeding) would not detract from the reflection of any part of the organism nor create any "gaps" in the immunological image of the body. Accordingly it have been rather strange if permanent changes in auto-Abs content will be marker signs only for autoimmune diseases (as it was proposed initially by Ernst Witebski and Noel Rose) but not any of chronic disorders in the human body.

Changes in serum autoantibodies as clinical laboratory data

The methods of the ELI-Test Group)[1] (see below) for diagnostic purposes have been used in medical practice in Russia since 1996. These methods are based upon the simultaneous detection of many specific marker autoantibodies of the IgG class (auto-Abs) in the serum blood of an investigated person and the consequent analysis of profiles of multiple auto-Abs directed to tissue-specific antigens. Detection of abnormal changes in profiles of auto-Abs reflects abnormal molecular changes in respective organs and systems of the body. This information is used to analyze the pathology of molecular changes in a patient's body (including pre-disease ones) and could be valuable for precise diagnostics as well as for the most adequate individual therapy prescription. Moreover, this information also evaluates the effectiveness and sufficiency of said therapy.

Why is multiparametric analysis of auto-Abs profiles used?

Evaluation of human autoantibodies (auto-Ab) with different antigen specificity is carried out around the world in a large number of clinical laboratories. Dozens of companies provided the specialized kits to assess the blood serum content of the auto-Ab against DNA, cardiolipin, beta2-glycoprotein I, collagen, thyroglobulin, etc. All commercially offered kits intended to detect separate auto-Abs in serum samples, considering they are markers of certain autoimmune diseases. However, our practice and observations of colleagues indicates that this approach may be grounds for incorrect clinical conclusions. If the content of serum auto-Abs is investigated in patients with immunodeficiency or polyclonal immune activation states, it may lead to frequent errors. In contrast, analysis of changes in a relative ratio of different auto-Abs (i.e. profiles of serum immune reactivity of investigating person) can provide an objective assessment of the clinical picture regardless of whether there is an observed patient with immunodeficiency, immune activation, or a normal immune reactivity. This approach uses the methods of the ELI-Test group that were elaborated soon after the Chernobyl Disaster (1986) to investigate the population's state of health in radioactive polluted areas.

1 ELI-Test – abbr. from Enzyme-Linked-Immuno-Test

Now, ELI-Tests have been successfully used in Russia for routine clinical investigations of auto-Abs in patients' blood serums for diagnostic and prognostic needs. These methods provide the possibility to analyze individual profiles of dozens of markers auto-Abs of the IgG class present in serum samples.

What should one know about the results obtained by the ELI-Tests?

1) It is necessary to note that the assessment of absolute contents (concentration) of auto-Abs in the investigated serum sample was not provided by the ELI-Test group method.

2) The results obtained do not characterize concentrations (serum level) of analyzed auto-Ab but rather characterize the deviations in auto-Ab partial ratios in the sample (distortion in physiological "immunoreactivity profiles").

3) Therefore, general activity of the immune system of the observed person had no influence on the results obtained; it does not matter whether there is an immunodeficiency (immune depressive state), immune activation, or a normal immune reactivity. Also, it is unimportant whether the investigated person is an adult, elderly, or an infant in the first months of life (at which time activity of auto-Abs is 2-3 times lower than adults).

4) Irrespective of the general level of activity of the immune system, partial ratios between the serum contents and different auto-Ab (with different antigen specificity) are very stable and dispersion (if investigated individuals are healthy) does not exceed 10-20%. Moreover, the results do not depend on gender or age of the person, provided there are no any chronic diseases.

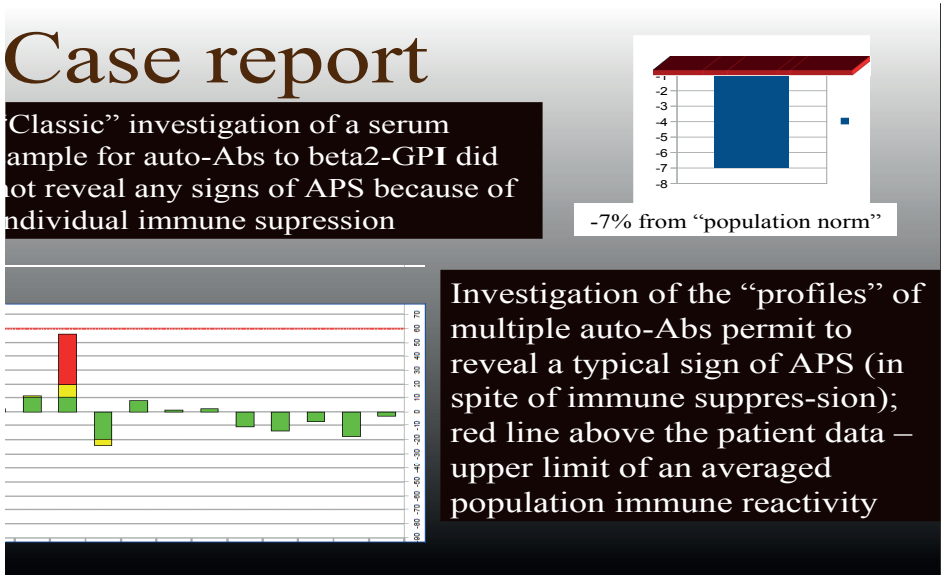
5) Therefore, even if the observed person is characterized by deep general immune suppression with a prominently decreased serum level of immunoglobulins (e.g. it is typical for professional athletes), the situation will not be an obstacle to receiving correct data using ELI-Test methods. Undistorted results would also be obtained in the situation of polyclonal activation (e.g. induced by acute EBV infection). In both, the results obtained will reveal abnormality (or lack of abnormality) in the normalized profiles of different auto-Abs in investigated persons.

NB: Nonetheless, we cannot recommend using any of the ELI-Test investigations at the period of active infection because of possible distortion of auto-Ab profiles by means of non-specific and uneven activation of the different lymphocyte clones. The same can be noted in regards to the situation of using immune suppressor medicines, most of all corticosteroids.

Once again we emphasize: Changes of profiles directly depend on SELECTIVE (partial) changes in serum contents of defined auto-Abs but not with changes of the general level of serum immunoglobulins. Therefore, "immunoreactivity profiles" of any healthy persons are exceedingly similar. Nevertheless, in the case of any chronic disease, the general picture changes considerably. The serum content of some auto-Ab significantly increases, decreases in other, or does not change. As a result, characteristic (physiological) profiles of serum immune reactivity will change significantly and form peculiar patterns, typical for any definite form of pathology.

As an illustration, let us consider two clinical cases observed in our laboratory.

Fig. 1.



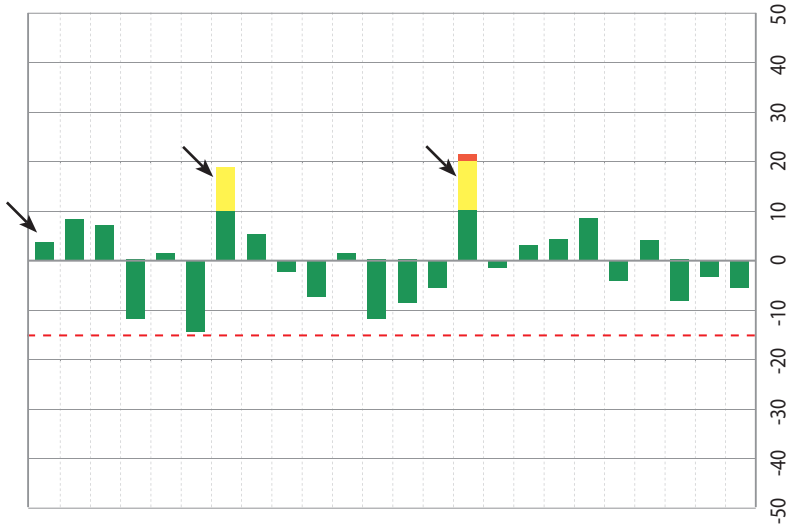
Case 1. Fig. 1. (female; 29 y.o.); clinically healthy; third pregnancy; 11 weeks of pregnancy; clinical signs for possible miscarriage appeared at 4-5 weeks of gestation; threat of miscarriage. During additional investigation, abnormally increasing coagulation in her

blood (hypercoagulation) was found. Deterioration of placental blood flow was also noted. The blood check: all parameters in a normal range except leucopenia (4000) and ESR 21. In spite of used treatment, the spontaneous interruption of her pregnancy took place at 12-13 weeks of pregnancy. Clinical and laboratory data were typical for antiphospholipid syndrome (APS) but only low serum levels of auto-Abs against cardiolipin and against beta2-Glycoprotein-I were found during repeated investigations of the blood serum at 6 and 10 weeks of pregnancy by using of a standard ELISA Kits ("Orgentec," Germany). In contrast, abnormal increases of auto-Abs against beta2-Glycoprotein (a marker of APS), reveals changes in the relative content (profiles) of multiple auto-Abs analyzed using ELI-Tests technology instead of a separate evaluation of auto-Abs against cardiolipin and beta2-Glycoprotein-I in the serum sample. In this case, the relative elevation of auto-Abs against beta2-Glycoprotein-I happened in a woman with prominent immune suppression. APS could not be revealed in this situation by using a standard kit.

Clinical conclusion. The pregnant woman was characterized by symptoms typical of antiphospholipid syndrome. This diagnosis used data to confirm the relative increased level of auto-Abs against beta2-Glycoprotein-I in her blood serum sample when compared to many other natural auto-Abs. Common immunochemical methods of analysis for serum auto-Abs to cardiolipin and beta2-Glycoprotein-I were not informative (i.e. marker signs of APS have not been found) because of prominent immune suppression observed in the woman.

Case 2. Fig. 2. (male 54 y.o); the man complains during a visit to a physician about "heavy head," headache, and muscular pain during the last 2 or 3 days; body's temperature is in normal range. The blood check: all parameters in a normal range except ESR 16. A day before, the man requested a clinical laboratory to investigate his serum level of antibodies against DNA because he was "afraid of the inheritable risk of SLE in his case." An official laboratory conclusion was presented. In accordance with the conclusion, the serum level of the IgG auto-Abs elevated significantly (> 30 U/ml) against dsDNA. His serum blood sample was checked by the ELI-Viscero-Test Kit and specialized PC software that analyzes profiles of 24 IgG auto-Abs with different antigen specificity ("Immunculus," Russia). Elevated immune reactivity of auto-Abs against dsDNA had not been noted in profiles. Relative weak elevation of immune reactivity of ANCA (a marker of small vessel vasculitides) and auto-Abs against liver mitochondrial antigen were noted in profiles. An important feature was general immune activation, which was reflected by the concordant increase of immune reactivity of many auto-Abs with different specificities. An acute viral infection (infectious mononucleosis) clinically manifested in the patient a few days later. It may be supposed that anti-dsDNA antibodies and profiles of 24 IgG auto-Abs (including auto-Abs to DNA) measured by ELISA in patient serum samples were obtained during the prodromal period of the acute viral infection.

Fig. 2 The results of the same patient investigation by the ELI-Viscero-Test method (profiles of 24 auto-Abs in the blood serum) confirm an absence of specifically elevated anti-DNA auto-Abs.



Notes:

Left arrow – auto-Abs against DNA (an elevation did not reveal)

Medium arrow – auto-Abs ANCA (marker sign of vasculitis)

Right arrow – auto-Abs against mitochondrial liver antigen (marker sign of changes in the liver)

Red line below the patient data – an average population immune reactivity

Clinical conclusion. Clinical and laboratory data indicates generalized immune activation in the patient's body related to the acute viral infection. The infection was accompanied by polyclonal activation of lymphocytes and elevated production of

numerous auto-Abs with different antigen specificity, including auto-Abs against DNA, which did not strictly relate to the infection.

Both examples clearly illustrate the restrictions related to evaluating the serum content of auto-Abs with any sort of separate antigen specificity. Measuring separate auto-Ab content may provide clinically reliable information only if the individual activity of the immune system is normal. Conversely, it is applicable to situations of immune suppression, as well as to the situation of polyclonal immune activation, because alternative separate evaluations of any auto-Ab in serum may lead to diagnostically incorrect conclusions about the patient's health.

These examples may be considered additional evidence for P-L. Meroni's argument (2007), "The initial paradigm 'one autoantibody for one disease' does not appear to be useful any longer. An autoantibody profile does seem to offer more diagnostic and prognostic power than the determination of single autoantibody specificity." C. Backes et al. recently (2011) wrote about the same saying, "Instead of allocating single antigens to a specific group of diseases and even to a specific disease, it appears more appropriate to allocate seroreactivity patterns." The idea of identification of autoantibody reactivity patterns has also been addressed as autoantibody signatures that are highly specific for various diseases, as others and we demonstrate.

The general activity of the immune system of the person observed has no influence on the results obtained by the ELI-Test methods. It does not matter whether there is an immunodeficiency (immune depressive state), immune activation, or a normal immune reactivity. Also, it is unimportant whether the investigated person is an adult, elderly, or a baby in the first months of life (at this stage, activity of auto-Abs is 2-3 times lower than that in the adults). Irrespective of the general level of activity of the immune system, partial ratios between the serum contents of different auto-Abs (with different antigen specificity) are quite stable and dispersion (if investigated individuals are healthy) does not exceed 10-20%. Moreover, the results do not depend on gender and age of the person, if there are no chronic organ diseases. Therefore, even if the observed person is characterized by deep general immune suppression with prominently decreased serum levels of immunoglobulins (e.g. it is typical for professional athletes), this situation will not be an obstacle to receiving correct data using the ELI-Test methods. Undistorted results would also be obtained in the case of polyclonal activation (e.g. induced by acute EBV infection). In both cases, the results obtained will reveal abnormality (or lack of abnormality) in the normalized profiles of different auto-Abs in the investigated person.

Let us additionally stress that we should orient to the common normative index for small children, adults, and old people (for each of the analyzing auto-Abs). If there are no abnormally elevated death rates of cells and/or abnormally elevated expression of macromolecules, then the number of relative changes in any auto-Abs in the investigated organism must be in the range -20…+10% from the Mean Individual Immune Reactivity (MIR) of the investigated person. The personal MIR may be below an average MIR, typical for some populations (e.g. in a baby), or above an average MIR (e.g. polyclonal immune activation) but personal MIR normalizations of each investigated healthy person (in men, women, children, and old people) should be nearly the same (Fig. 3).

The differences in the intensities of auto-Ab reactions is clear (see Fig. 3) from the different serum samples, with each antigen nearly the same (separate pikes), and the individual profiles of serum immune reactivity, wherein the used set of antigens are nearly the same in each case (personal distinctions kept at a range 20-30%).

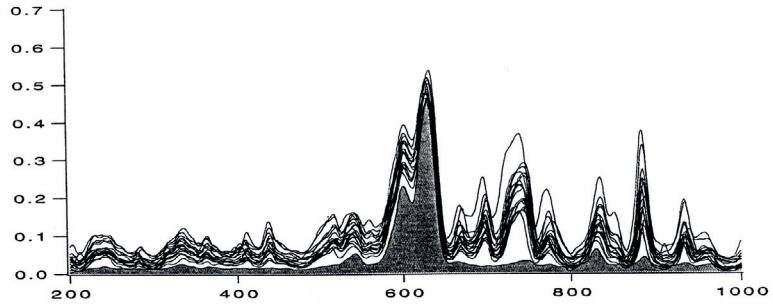
Ours empirical data[2] also demonstrated the same range for normal immune reactivity with each investigated self-antigens (-20...+10% from the individual MIR), although we did not use immunobloting on nitrocellulose sheets but instead used ELISA methods wherein antigens adsorbed in the wells of polystyrene immunoplates.

The immunoreactivity level of each investigated sample with each antigen was evaluated in arbitrary units, or the percentage of intensity of the sample reaction to the reaction of the control serum with the same antigen. It was more convenient compared to using O.D. units because arbitrary units permit the possibility to compare the relative changes in profiles of very different auto-Abs while using the same numeric scale. For example, the intensity of the reaction of minor auto-Abs (left arrow) of control serum with some antigens is 100%, and the intensity of the reaction of major auto-Abs (right arrow) of control serum with some other antigens is 100%. Thus, the deviation in reactivity of an investigated sample will be expressed in a percentage to the reactivity of the control serum with the same antigens.

2 Over the last 30 years we studied the profiles of a reaction more than 17000 serum samples obtained from healthy children, men, and women of different ages, as well from patients with respect to over 500 antigens belonging to various organs and tissues.

Lacroix-Desmazes S., et al. (1998):

Serum content and relative rates of different auto-Ab is nearly the same in each healthy adults



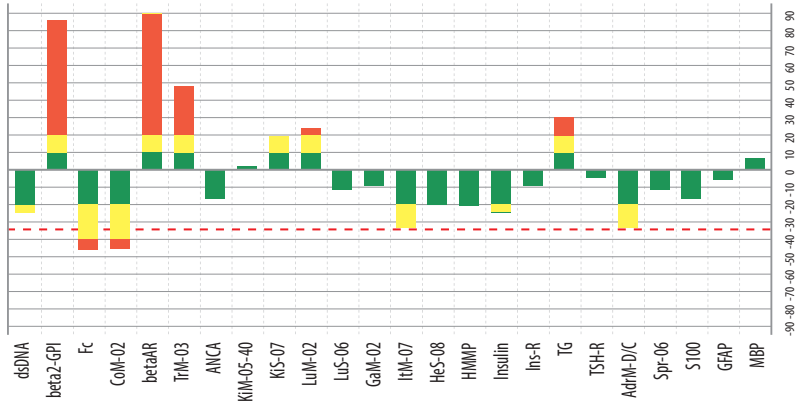
If the profiles of auto-Abs immune reactivity in the blood serum of healthy individuals are very similar, then the picture changes at the disease stage because production and serum content of some auto-Abs increase and others decrease. Character deviations in the profiles strictly depend on the character of pathological molecular changes. Such changes can be transitory and normalize as soon as an organism overcomes the abnormal situation, or they can be conserved for months (Case 4).

Let us also note: the normal (physiological) level of an individual MIR (Median Immune Reactivity) of an investigated sample fits in the range -30%...0% compared to the MIR of the control sample. If individual MIR of an investigated sample is above the upper limit of the MIR of the control sample (red dotted line in the histogram), then it should be considered an indication of general immune activation of the investigated person. If individual MIR of an investigated sample is below -30% of the MIR of the control sample, then it should be considered indicative of the general immune suppression or immune deficiency of the investigated person.

Case 4. Fig. 4. (Female, 67 y.o); investigated by the ELI-Viscero-Test method twice with a 6-month interval. The main clinical features: prominent disturbances of heart rhythm (during the last 6-7 years), trombocytosis, cancer of the thyroid gland was revealed shortly before the first ELI-Test investigation. She declined any proposed treatments at the 6-month mark between investigations.

Fig. 4. Patient (female, 67 y.o.); recently diagnosed thyroid cancer; ELI-Viscero-Test data from June 2012 (a) and from December 2012 (b)

a)



b)

Comments. The amplitude of each bar of the histogram reflects the comparative levels of immune reactivity to respective antigens (relative content of respective auto-Ab) in

relation to MIR. In each case, MIR corresponds to the zero level at X-axis. The red dotted line corresponds to the population's average immune reactivity with respective antigens (indicated at the bottom of the picture).

- Green bars correspond to the normal levels of immune reactivity;
- Yellow portion corresponds to weak deviations from the normal reactivity;
- Red portion corresponds to prominent deviations from the normal reactivity.

The results of Fig. 4 indicate the following:

1) Profiles of auto-Abs can be relatively stable during a half-year period.
2) The peak of auto-Abs to thyroid antigens was stable at the first and second profiles (some elevation is noted).
3) The peak of auto-Abs to platelet antigens was stable at the first and the second profiles (some elevation is noted).
4) The peak of auto-Abs to platelet antigens was stable at the first and the second profiles.
5) The peak of auto-Abs against beta2-glycoprotein I (marker sign of antiphospholipide syndrome; it is typical for many cancer cases) was stable at the first and the second profiles.
6) The elevated level of an individual MIR (Median Immune Reactivity) was conserved during a 6-month period.

Results of the ELI-Test: Artifacts and other difficult questions

1) It is hardly possible to identify laboratory methods sans false and/or erroneous results. Technical bugs, careless mistakes of inexperienced personnel, etc. may occur from time to time during the implementation of different laboratory investigations. Besides, any methods based upon immunochemical interactions, including ELI-Tests, are principally stochastic. Therefore, some scattering (usually very small) will appear at stages of test executions– this phenomenon may illustrate the scattering of results in the doubling implementation. Scattering results of multi-component ELI-Tests may be more comparable to the 'classical' mono-component tests. Therefore, the ELI-Test methods require particularly high-quality professional execution.

2) A universal laboratory diagnostic method that can find the answer to any physician's question is rather impossible. Therefore, it is important to delineate the area of the most advisable uses of ELI-Test methods. It should be noted that these methods are screening methods of the primary level first of all. Or, in other words, the methods of the ELI-Test group should be attributed to pre-disease screening investigations. This technology may be considered a laboratory analogue of the traditional prophylactic medical examination of the whole body, except it is cheaper and quicker. Let us imagine such a situation. A clinically healthy woman has decided to carry out the wide prophylactic medical examination. She may do step-by-step gastroscopy, X-ray examinations of the lungs, mammograms, cardiographs, Holter monitoring, computed tomographic scanning of abdominal organs and kidneys, gynecologic examinations, and electroencephalograms, in addition to executing a lot of biochemical, bacteriological and other investigations. Such complicated systemic examinations will take excessive time, utilize many specialized personnel, and be rather expensive.

On the other hand, there is an alternative decision. The patient could choose a two-step check-up. At the first check-up, there is a wide immunochemical investigation of the main organs and systems of the body using the ELI-Viscero-Test method ("molecular prophylactic examination"), and, if there are alarming "molecular symptoms" in certain organs, there is a more specialized investigation of the at-risk organ (i.e. lungs, stomach, etc.). Such two-level diagnostic and prognostic approaches seem to be optimal because they can avoid complicated, expensive, and even risky procedures that may be unnecessary. It should be specifically noted that ELI-Test methods do not replace other methods of patient investigation, but they permit us to use the latter on reasonable and individual terms.

The other aspect of ELI-Test methods is correcting diagnoses if the clinical case is not quite clear. *Elucidation:* patient complains about pain in the heart; preliminary investigation by electrocardiography and Holter monitoring do not reveal any changes; additional investigation by the ELI-Test method discover changes in the stomach wall; additional gastroduodenoscopy confirms a gastric ulcer; effective treatment of the ulcer did not cease complaints of "pain in the heart".

Moreover, the ELI-Test methods can be effectively used for anticipatory monitoring of changes in a patient's state of health. That is the standard for effectiveness and sufficiency of used therapy.

3) Sometimes, we meet patients who already know about certain pathologies that they have been previously diagnosed with but the pathology is not revealed by ELI-Test data. Usually the patient asks, "Doctor, for some reason the ELI-Tests did not see my duodenal ulcer that was diagnosed a year ago (or long-lasting thrombophlebitis, diabetes, etc.). Why is this so?"

The physician should bear in mind and explain to the patient that changes in ELI-Test data only indicates currently active pathology processes. It usually does not reflect former events that ceased to exist a long time ago. The reason is simple; the formation of any pathological focus in an organ increases the activity of apoptosis (necrosis) and elevated intracellular releases of different antigens of specialized cells. These events have lead to elevated production of auto-Abs of respective specificity (feedback principle) and assist the healing of an injured organ. The methods of the ELI-Test group were elaborated and used to reveal the changes of marker auto-Abs in the blood serum. Therefore, any presently active pathology processes can be easily detected by the ELI-Test methods. But, these methods reveal nothing if abnormal cell destruction and/or abnormal expression of specific molecules were completed post-treatment or spontaneously much later because, in a such situation, the levels of production of marker auto-Abs was normalized long before the ELI-Test group. In other words, ELI-Tests, lacking specific changes in patients with earlier diagnosed ulcer duodeni, can indicate pathological remission.

Another example is that elevated destruction of the Lanhergans islets beta-cells have been accompanied by elevation of the serum's auto-Abs against insulin/proinsulin and other specific antigens of beta-cells at the very beginning of insulin-dependent diabetes mellitus type I, a few years before the disease manifests. The elevation of marker auto-Abs could be seen before disease manifestation and over months or even the early years of diagnosed diabetes. But, later levels of such auto-Abs (when destruction of the beta-cells has been nearly completed) decrease to the normal level or below. That is, the disease (diabetes type I) is identified as a result of the earlier completed pathological process, but typical marker auto-Abs did not reflect it. Elevated production of typical marker auto-Abs reflects active pathology, but does not show the disease as a result of former breakage (completed long before).

4) In any case, no laboratory investigation can replace an experienced physician.

Relative decreasing of autoantibody content is a sign of pathology

Most physicians who apply immunochemical methods in their clinical practice fail to take into account two significant peculiarities. First, a free molecule of idiotypic antibody that is not bound by respective anti-idiotypic auto-Ab or antigen can only be measured by routine laboratory methods in a blood serum sample. Second, anti-idiotypic auto-Abs, permanently present in human blood circulation, will prominently influence the apparent reactivity/content of the measuring idiotypic antibodies. No more than one-third of routinely measured auto-Abs is present in serum in free form and two-thirds of them form inbound with mostly appropriate anti-idiotypic antibodies. The bound auto-Abs may be measured after their dissociation only by using some of the chaotropic agents. If the relative production of specific anti-idiotypic antibodies notably exceeds the production of respective idiotypic ones, then laboratory data will indicate the decrease of immune reactivity related to the content of non-bound idiotypic antibodies. Abnormal (non-proportional) rises of anti-idiotypes per se may cause pathological changes because anti-idiotypic antibodies, as well as idiotypic ones, possess their own biological activity. The necessity to take these reasons into account is illustrated in the following example.

Case 3. Pregnant woman (first pregnancy); 28 years old; clinically healthy; routine biochemical investigation revealed no abnormalities. Blood serum profiles related to 12 auto-Abs with different specificity repeatedly analyzed at 8, 20, and 34 weeks of pregnancy using the ELI-P-Complex Kit and specialized PC software ("Immunculus", Russia). Prominent decrease of relative immune reactivity of auto-Abs against platelet membrane antigen TrM-03 was found during each analysis. Profuse pathological hemorrhage (life-threatening) occurred during delivery. Recently, it has been repeatedly confirmed that abnormally low-levels of auto-Abs to platelet membrane antigen TrM-03 in pregnant women is a specific and informative marker for expected pathological hemorrhage at delivery. It has been proposed that lower levels of auto-Abs against platelet antigen TrM-03 may be related to excessive production of respective anti-idiotypic antibodies. The hypothesis has been experimentally checked using quantitative immunoaffinity chromatography. Elevation of serum content of specific anti-idiotypes (bounded to mini-columns with anti-TrM-03 auto-Abs) was experimentally demonstrated. The obtained data provides evidence of biological activity of anti-idiotypic auto-Abs and possible clinical (pathophysiological) consequences related to abnormalities in serum balance between idiotypic and anti-idiotypic auto-Abs.

The aforementioned situation is not unique or singular. Daily laboratory practice indicates that the steady abnormal decrease of serum immune reactivity of many natural idiotypic auto-Abs depends on different somatic, neurologic, and malignant diseases. However, the phenomenon remains practically uninvestigated. It is, however, assumed that possible clinical consequences of abnormal elevation of different anti-idiotypic Abs (reflected by an abnormal decrease of serum immune reactivity in appropriate natural idiotypic auto-Abs) should be elucidated.

The main methods of the ELI-Test group

The following variants of ELI-Test group laboratory methods are currently used in Russian clinical practice.

1. ELI-Viscero-Test Method: ("the prophylactic examination at the molecular level") This is used for complex health evaluations of primary organs and systems of the human body. The method is based on the analyses of serum immune reactivity profiles of 24 marker auto-Abs of IgG class.

2. ELI-P-Complex Method: ("the molecular evaluation of female reproductive functions") This is used for complex evaluations of the reproductive function in women of fertile age. The method is based on the analyses of serum immune reactivity profiles of 12 marker auto-Abs of IgG class.

3. ELI-Andro-Test Method: ("the molecular evaluation of male reproductive functions") This is used for complex evaluations of the reproductive function in men. The method is based on the analyses of serum immune reactivity profiles of 12 marker auto-Abs of IgG class.

4. Specialized methods of the ELI-Test group: There are a few additional methods used for detailed analysis of the cardiovascular system (Method ELI-ANCOR-Test), the nervous system (Method ELI-Neuro-Test), the gastrointestinal system (Method ELI-GST-Test), lungs (Method ELI-Pulmo-Test), and kidneys (Method ELI-Nephro-Test). The ELI-V-Test method evaluates the state of the immune system and should be used in children and adults before preventive vaccinations in order to minimize risks of vaccination-related complications related to abnormal activity of the immune system.

ELI-Viscero-Test Method ("prophylactic examination at the molecular level")

This method is based on analyses of serum immune reactivity profiles of 24 marker auto-Abs of the IgG class directed towards the antigens of different organs and systems of the body. The following antigens are used in the Kit:

1. **Antigen dsDNA** is the main component of cell nucleus. Excess of relevant auto-Abs is most likely the marker sign of the virus-induced activation of apoptosis. It may on occasion indicate systemic autoimmune diseases and malignancy.

2. **Antigen β2-Glycoprotein I** is the main phospholipid-binding protein of the blood. Excess of auto-Abs against β2-Glycoprotein I is a marker of anti-phospholipid syndrome. The latter may be the cause of thrombosis in different organs and can lead to miscarriages, strokes, or infarctions.

3. **Antigen Fc-fragment of IgG.** Excess of auto-Abs against the Fc-fragment of IgG (rheumatoid factor) is an indicator for chronic inflammation of the local area.

4. **Antigens LuM-02** and **LuS-06** are specific antigens of lungs. Excesses of auto-Abs against any one or both of these antigens are marker signs of destructive and/or inflammatory changes in lung tissue.

5. **Antigens KiS-07** and **KiM-05** are specific antigens of kidneys. Excesses of auto-Abs against any one or both of these antigens are marker signs of destructive and/or inflammatory changes in kidney tissue.

6. **Antigen GaM-02** is a specific antigen of the cytoskeleton of stomach wall cells. Excess of auto-Abs against this antigen is a marker sign of destructive and/or inflammatory changes in the stomach wall.

7. **Antigen ItM-07** is a specific antigen of the cytoskeleton of intestinal wall cells. Excess of auto-Abs against this antigen is a marker sign of destructive and/or inflammatory changes in intestinal wall.

8. **Antigens HeS-08** and **HMMP** are specific cytoplasmic (first) and mitochondrial membrane (last) antigens of the liver. Excesses of auto-Abs against any one or both of these antigens are marker signs of destructive and/or inflammatory changes in liver tissue.

9. **Antigen β1-Adrenoreceptor** is a specific antigen of the heart's autonomous nervous system. Excess of auto-Abs against this antigen is a marker sign of pathology changes in the heart's nervous structures and may be often accompanied by arrhythmia. Rarely, it is a marker sign for dilated cardiomyopathy.

10. **Antigen CoM-02** is a specific antigen of the cytoskeleton of myocardial cells. Excess of auto-Abs against this antigen is a marker sign of degenerative changes in the heart muscle.

11. **Antigen TrM-03** is a specific antigen of platelets' membranes. Excess of auto-Abs against this antigen is a marker sign of trombocytopathy of a different kind. It may leads to blood hypercoagulation as well as hypocoagulation.

12. **Antigen ANCA** is a specific antigen of vascular endothelium. Excess of auto-Abs against this antigen is a marker sign of a small vessel's vasculitis.

13. **Antigens Thyroglobulin** and **TSH Receptors** are specific antigens of thyroid glands. Excesses of auto-Abs against any one or both of these antigens are marker signs of thyroid pathology changes.

14. **Antigen Insulin** is a specific antigen of pancreatic islets beta-cells (insulin-secreting cells). Excess of auto-Abs against this antigen is a marker sign of pancreatitis and may be a prognostic sign of future insulin-dependent diabetes mellitus type-1.

15. **Antigen Insulin receptors** are transducers of insulin's physiological action upon target cells. They are complicated molecular structures associated with superficial membranes of myocytes and many other cells. Excess of auto-Abs against this antigen is a marker sign of some variant insulin-independent diabetes mellitus type-2. It may be a prognostic sign for future diabetes mellitus type-2.

16. **Antigen Adr-DE/CM-0** is a specific antigen of cortex and medulla cells of adrenal glands. Excess of auto-Abs against this antigen is a marker sign of transitory changes in adrenal glands, most likely due to prolonged stress. It also may be a sign of Addisson's disease or other adrenal diseases.

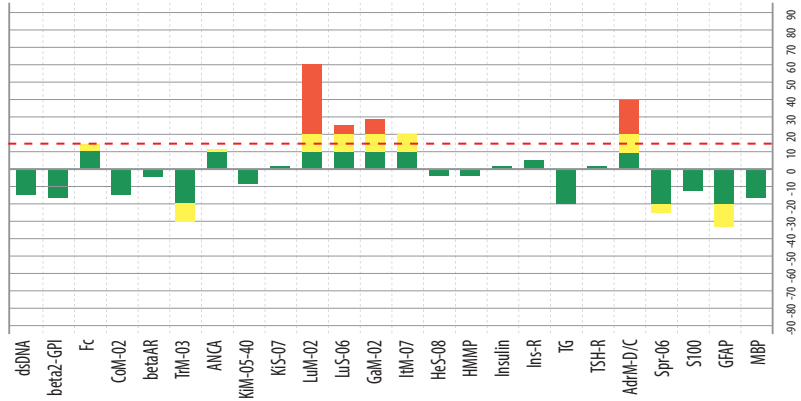
17. **Antigen Spr-06** is a specific membrane antigen of spermatozoids and prostate cells. Excess of auto-Abs against this antigen is a marker sign of pathology changes in the prostate and indicates an abnormal rise of immune reactivity against spermatozoa. In women, abnormal rise of immune reactivity against Spr-06 is a marker sign of inflammatory changes in the endometrium, or in other organs of the pelvis minor.

18. **Antigen S100** is one of the regulators of apoptosis. It is a specific trophic factor for serotoninergic neurons of the brain. One of the most common reasons for excessive production of such auto-Abs is the human papilloma virus infection by molecular mimicry mechanisms. Excess of auto-Abs against this antigen may lead to emotional disturbances (e.g. aggressiveness, phobias, depression) and be related to papillomatosis and some forms of cancer.

19. **Antigen GFAP** is a specific antigen of intermediate filaments of the cytoskeleton of the astrocytes (glial cells). Excess of auto-Abs against this antigen indicates reactive gliosis (abnormal proliferation of astrocytes). Gliosis is usually induced by mechanical trauma of the brain, ischemic or hemorrhagic events, brain tumors, or neuroinfections. Excess of auto-Abs against this antigen may be a prognostic sign for epilepsy risk or other disturbances in the bioelectrogenesis of the brain.

20. **Antigen Myelin Basic Protein (MBP)** is a specific antigen of myelinated nervous fibers. Excess of auto-Abs against this antigen is a marker sign of pathology changes in nervous fibers (i.e. radiculitis, neuritis, plexites, etc.). More rarely, it may be a marker sign of demyelinating disease (i.e. multiple sclerosis).

Fig. 5. An example: Data obtained by the ELI-Viscero-Test method



The data obtained by the ELI-Viscero-Test represents a serum profile of the individual immune reactivity composed by 24 different auto-Abs interacting specifically with the above-noted antigens and is calculated in arbitrary units (AU; or percentages from an

idealized level zero). In a healthy state, amplitude of each auto-Ab should be placed in a range from -20 to +10 AU (physiologic range). If amplitude of some auto-Abs is above +10 AU or below -20 AU, it may indicate pathology changes in certain cell populations in certain organs or systems. In the graphic form (Fig. 5) the data is represented as bars in the histogram:

- Green bars correspond to the norms of immune reactivity of respective auto-Abs;
- Yellow parts of bars correspond to weak deviations from the norms of reactivity of respective auto-Abs;
- Red parts of bars correspond to prominent deviations from the norms of reactivity of respective auto-Abs.

Besides the detailed profile of immune reactivity relating to the changes in production of auto-Abs to the mentioned antigens, the mean individual immune reactivity (MIR) of investigated women is also calculated and represented in graphic form (as X-axis). MIR implies an average deviation of the immune reactivity of investigated persons from the control data and is calculated in percentage from the reactions of the control sample with the same antigens. In a normal state, MIR should be situated in a range from -25% below to +5% above the control data (the idealized norm). The "idealized norm" is sketched out as a red dotted line in the histogram. In cases of polyclonal immune activation (e.g. a systemic autoimmune disease, the onset of the cancer, etc.), the line of "idealized norm" is placed below MIR of the investigated patient (Fig. 5. red dotted line) at more than 5%. Conversely, if a case of generalized immune suppression (immune deficiency) occurs, the "idealized norm" (red dotted line) is placed above the X-axis at more than 25%.

How often should a patient be investigated?

Changes in the intensity of pathology size and activity usually reflects dynamic changes in the relative content of blood serum profiles of respective auto-Abs. Nonetheless, even prominent changes in relative content of some auto-Abs can be transitory and disappear in one to two weeks.

If serious changes were not revealed during a patient's prophylactic investigation using the methods of the ELI-P-Group, there will still be enough auto-Abs to repeat the study once or twice per year. If prominent changes in marker auto-Abs profiles were found, then it is desirable to repeat the study two or three weeks later. If the changes were not revealed during the repeat study (i.e. the situation of short-term disturbances/self-recovery; does not lead to evident pathology), then it means medical intervention is unnecessary. In contrast, if revealing changes earlier saves repeated control studies, then the situation may require certain prophylactic measures aimed at preventing the manifestation of clinical pathology.

Dynamic monitoring of marker auto-Ab profiles can be useful for tracking the changes in patient health during treatment. It is especially important if the patient has suffered from any chronic disease whereby treatment should be individually selected on a trial basis. NB: If adequate and effective treatments lead to normalization of the tissue/organ/organism state, it will be reflected by improved/normalized ELI-Test data a few weeks before evident clinical signs appear. Vice-versa, an absent tendency of normalized ELI-Test data usually indicates the ineffectiveness of therapeutic measures used in the case.

ELI-P-Complex Method (used to evaluate the state of female reproductive functions)

The ELI-P-Complex Kit (from the Possibility of Pathology in Pregnancy) was the first method of the ELI-Test Group. It was developed, successfully tested, and approved for diagnostic purposes in Russia between 1989 and 1996. The ELI-P-Complex Kit is intended to measure specific profiles of the serum immune reactivity (IR), which depends on the serum content of twelve auto-Abs of the IgG class. Changes in such auto-Abs may prominently influence pregnancy and fetus formation if their serum content has deviated from normal parameters. The antigens contained in the Kit are the following:

1. **Antigen Chorionic gonadotropin** is a peptide hormone, which regulates the process of implantation of a zygote, and is important for placenta formation and maturation. Excess of auto-Abs against choriogonadotropin indicates endocrine malfunctions and may cause functional insufficiency of choriogonadotropin. This may be the reason for infertility and placenta malformation and lead to placental insufficiency. Large dosages of this hormone (e.g. Pregnil, Horagon, or similar medicines) taken by

women preparing for IVF are the most common cause of long-lasting abnormally elevated productions of such auto-Abs. Additionally, excess of such auto-Abs is typical for women with premature ovarian failure. More rarely, these auto-Abs may indicate the presence of hormone-producing adenoma (hypophyseal).

2. **Antigen dsDNA** is the main component of cell nucleus. Increase of respective auto-Abs is a marker sign of virus-induced activation of apoptosis in most cases. Rarely, this rise may indicate a systemic autoimmune process or malignant tumor growth.

3. **Antigen β2-Glycoprotein I** is a phospholipid-binding protein in the blood. Excess of auto-Abs against this antigen is a marker sign of anti-phospholipid syndrome, which may be the cause of thrombosis in the placenta and other organs and leads to placental insufficiency. Excess of such auto-Abs may reveal embryotoxic effects.

4. **Antigen Fc-fragment of IgG.** Excess of auto-Abs against Fc-fragment (Rheumatoid factor) indicates chronic inflammatory processes. Excess of such auto-Abs may also reveal embryotoxic effects.

5. **Antigen Collagen II** is the main protein of the intercellular matrix. Excess of auto-Abs against collagen is a marker sign of an active adhesive process of any region. Moreover, it may be a sign of pathological changes in connective tissue. Excess of such auto-Abs may reveal embryotoxic effects.

6. **Antigen Insulin** is a specific antigen of the pancreatic islet cells. Excess of auto-Abs against insulin is a marker sign of pancreatitis and may indicate developing insulin insufficiency due to gestational diabetes or insulin-dependent diabetes type I. Excess of such auto-Abs may be a pathogenic factor for development of diabetic fetopathy.

7. **Antigen Thyroglobulin** is the specific protein of the thyroid. Excess of auto-Abs against thyroglobulin may be a sign of existing or forming thyroid gland malfunction. Excess of such auto-Abs may reveal embryotoxic effects.

8. **Antigen Protein S100.** Proteins of the S100 family take part in regulating a lot of cellular functions, including apoptosis. Excess of auto-Abs against S100 may lead to deviations of the general morphogenesis and tissue differentiation in embryo. Moreover, S100 proteins participate in the differentiation of neuroblasts of the neural tube; thus, excess of auto-Abs against S100 may cause deviation of the nervous system ontogenic formation. One of the most common reasons for excessive production of such auto-Abs is the human papilloma virus infection by molecular mimicry mechanisms. Therefore, the human papilloma virus in pregnant women may lead to frequent miscarriages or malformation of the fetus' nervous system.

9. **Antigen Spr-06** is an important antigen of the membranes of the spermatozoids and some prostatic gland cells. Excess of auto-Abs against Spr-06 may cause decreased fertility in women and men, and it is a specific marker sign of endometritis. Rarely, there is inflammation in other pelvis minor organs.

10. **Antigen TrM-03** is one of the most important antigens of platelet membranes. Excess of auto-Abs against TrM-03 is a sign of trombocytopathy and may lead to the following deviations: 1) excessive lysis of platelets, which leads to thrombocytopenia and abnormal bleeding; 2) excessive aggregation of platelets (without their lysis) and frequent thromboses.

11. **Antigen ANCA** expression increases during the systemic or local small vessel vasculitis (i.e. inflammation of internal blood vessel walls by any cause). Excess of such auto-Abs is a typical sign of different forms of vasculitides.

12. **Antigen KiM-05** – is one of most common antigens of kidney cell membranes. Excess of auto-Abs against KiM-05 should be considered an indication of renal tissue inflammation and degeneration.

Besides the detailed profile of immune reactivity related to the changes in production of auto-Abs to the mentioned antigens, the mean individual immune reactivity (MIR) of investigated women was also calculated and is represented in graphic form (as X-axis). MIR implies an average deviation of the immune reactivity of the investigated person from the control data and calculates a percentage from the reactions of the control sample with the same antigens. In a normal state, MIR should be situated in a range from -25% below to +5% above of the control data (the idealized norm). The "idealized norm" is sketched out as a red dotted line in the histogram. In cases of polyclonal immune activation (e.g. a systemic autoimmune disease, the onset of the cancer, etc.), the line of "idealized norm" is placed below MIR of the investigated patient (Fig. 5. red dotted line) at more than 5%. Conversely, if cases of generalized immune suppression (immune deficiency) occur, the "idealized norm" (red dotted line) is placed above the X-axis more then 25%.

Elevated above 5% from the "idealized norm," the general immune reactivity (general immune activation) in pregnant women may also cause miscarriage, placental insufficiency, fetal retardation, or fetal death.

Decreased below 25% from the "idealized norm," the general immune reactivity (general immune suppression or general immunodeficiency) may also cause miscarriage,

placental insufficiency, fetal retardation, or fetal death. Furthermore, more than 80% of pre-eclampsia cases developed at the state of deep immune suppression (-40% or below).

NB: Short-term (up to 1-2 weeks) deviations in the serum content of any of the auto-Abs, even substantial, do not usually lead to evident damage of the embryo and fetus because of multiple effective mechanisms of correction and compensation. However, long-lasting deviations, even modest, may become fatal.

ELI-Andro-Test Method (used to evaluate the state of male reproductive functions)

The ELI-Andro-Test is intended to measure specific profiles of the serum immune reactivity (IR), which depends on the serum content of twelve auto-Abs of the IgG class. Changes in this auto-Abs may reflect reproductive dysfunction in men. The method can be used to evaluate the effectiveness of individually used treatments. Antigens contained in the Kit are the following:

1. **Antigen Spr-06** is one of the important antigens of the spermatozoids membranes and some prostatic gland cells. Excess of auto-Abs against Spr-06 may decrease fertility in women and men and is also a marker sign for prostatitis.

2. **Antigen α1-Adrenoreceptor.** Excess of auto-Abs against the antigen is a specific marker sign of change in the autonomous nervous system, often accompanied by erectile dysfunction (men) and refractory hypertension (men and women).

3. **Antigen ec-NOS.** Excess of auto-Abs against the antigen is a specific marker sign of endothelial dysfunction, which occurs in hypertension, diabetes, aging, and as a prelude to atherosclerosis (men and women), and is often accompanied by erectile dysfunction (men).

4. **Antigen PAPP-A.** Excess of auto-Abs against the antigen is a specific marker sign of obstructed blood vessels related to changes in myocardium and endocrine dysfunctions. It may be accompanied by erectile dysfunction (men).

5. **Antigen ANCA.** Expression of this antigen increases during systemic or local small vessel vasculitis (inflammation of internal wall of blood vessels by any cause). Excess of such auto-Abs is a typical sign of different forms of vasculitides.

6. **Antigen TrM-03** is one of the antigens of platelet membranes. Excess of auto-Abs against TrM-03 is a sign of trombocytopathy and may lead to the following deviations: 1) excessive lysis of platelets, which leads to thrombocytopenia and abnormal bleeding; 2) excessive aggregation of platelets, without their lysis, and frequent thrombosis events.

7. **Antigen Insulin receptors.** Excess of auto-Abs against this antigen is a marker of insulin-independent diabetes mellitus type-2. It may be used for disease prognosis.

8. **Antigen KiM-05** is one of the most common antigens of kidney cell membranes. Excess of auto-Abs against KiM-05 should be considered an indication of renal tissue inflammation and degeneration.

9. **Proteins of the S100 family** take part in the regulation of many cellular functions, including regulation of apoptosis. Excess of auto-Abs against S100 may relate to the deviations of the general morphogenesis and tissue differentiation and accompany some malignancies. One of the most common reasons for excessive production of such auto-Abs is the human papilloma virus infection by molecular mimicry mechanisms.

10. **Antigen Interferon alpha** is one of the macrophagal secretory antigens. Excess of the auto-Abs against this antigen should be considered an indication of inflammation of any region.

11. **Antigen dsDNA** is the main component of cell nucleus. Excess of respective auto-Abs is a marker sign of the virus-induced activation of apoptosis, mostly. Rarely, it may indicate systemic autoimmune diseases and malignancy.

12. **Antigen β2-Glycoprotein I** is the main phospholipid-binding protein of the blood. Excess of auto-Abs against β2-Glycoprotein I is a marker of anti-phospholipid syndrome. This may cause thrombosis in different organs and lead to miscarriages, strokes, and infarctions.

Additional methods of the ELI-Tests group used to Evaluate the state of specialized organs and systems

ELI-ANCOR-Test Method

The ELI-ANCOR-Test method is intended to measure specific profiles of the serum immune reactivity of 12 auto-Abs of the IgG class, related to or reflecting dysfunctions in the cardiovascular system. The method can also be used to evaluate the effectiveness of individually used treatments. Antigens contained in the Kit are the following:

1. **Antigen dsDNA** is the main component of cell nucleus. Excess of respective auto-Abs is the marker sign of the virus-induced activation of apoptosis, mostly. Rarely, it may indicate systemic autoimmune diseases and malignancy.

2. **Antigen β2-Glycoprotein I** is the main phospholipid-binding protein of the blood. Excess of auto-Abs against β2-Glycoprotein I is a marker of anti-phospholipid syndrome. This may cause thrombosis in different organs and lead to miscarriages, strokes, and infarctions.

3. **Antigen CoM-02** is a specific antigen of the cytoskeleton of myocardial cells. Excess of auto-Abs against this antigen is a marker sign of degenerative changes in heart muscle.

4. **Antigen CoS-05-40** is a specific cytoplasmic antigen of myocardial cells. Excess of respective auto-Abs is a marker sign of inflammation in the heart muscle (myocardites) and, in most cases, is not accompanied by prominent degenerative processes.

5. **Antigen β1-Adrenoreceptor** is a specific antigen of the heart's autonomous nervous system. Excess of auto-Abs against this antigen is a marker sign of pathological changes in nervous structures of the heart and may often be accompanied by arrhythmia. Rarely, it may be a marker sign of dilated cardiomyopathy.

6. **Antigen Cardiac myosin L** is an organ-specific form of myocardial myosin. Increase of respective auto-Abs is a marker sign of degenerative changes in heart muscle.

7. **Antigen TrM-03** is one of the antigens of platelet membranes. Excess of auto-Abs against TrM-03 is sign of trombocytopathy and may lead to the following deviations: 1) excessive lysis of platelets, which leads to thrombocytopenia and abnormal bleeding; 2) excessive aggregation of platelets, without their lysis, and frequent thrombosis events.

8. **Antigen ANCA.** Expression of this antigen increases during systemic or local small vessel vasculitis (inflammation of internal wall of blood vessels by any cause). Excess of such auto-Abs is a typical sign of different forms of vasculitides.

9. **Antigen Nitric oxide synthase (e-NOS).** Excess of auto-Abs against the antigen is a specific marker sign of endothelial dysfunction, which occurs in hypertension, diabetes, aging, and as a prelude to atherosclerosis (men and women).

10. **Antigen Angiostatin** is a participant of fibrinolysis. It takes part in the mechanisms of neoangiogenesis. Excess of auto-Abs against the antigen may lead to changes in blood coagulation and be related to active formations of collateral blood vessels.

11. **Antigen PAPP-A.** Excess of auto-Abs against the antigen is a specific marker sign of obstructed blood vessels related to changes in myocardium and of endocrine dysfunctions. It may be accompanied by erectile dysfunction in men.

12. **Antigen Collagen II** is the main protein of the intercellular matrix. Excess of auto-Abs against collagen is a marker sign of an active adhesive process of any region. Furthermore, it may be a sign of pathological changes in connective tissue.

ELI-GIC-Test Method

The ELI-GIC-Test method is intended to analyze specific profiles of the serum immune reactivity of 12 auto-Abs of the IgG class, related to or reflecting the dysfunctions in the gastro-intestinal system. The method can be used to evaluate the effectiveness of individually used treatment. Antigens contained in the Kit are the following:

1. **Antigen GaM-02** is a specific antigen of the cytoskeleton of stomach wall cells. Excess of auto-Abs against this antigen is a marker sign of destructive changes in the stomach wall.

2. **Antigen GaS-03 02** is a specific cytoplasmic antigen of stomach wall cells. Excess of auto-Abs against this antigen is a marker sign of inflammatory changes in the stomach wall.

3. **Antigen ItM-07** is a specific antigen of the cytoskeleton of intestinal wall cells. Excess of auto-Abs against this antigen is a marker sign of destructive and/or inflammatory changes in the intestinal wall.

4. **Antigen SCM** is a specific antigen of the cytoskeleton of colon wall cells. Excess of auto-Abs against this antigen is a marker sign of destructive and/or inflammatory changes in the colon.

5. **Antigen HMMP** is a specific antigen of the liver's mitochondrial membrane. Excess of auto-Abs against this antigen is a marker sign of destructive changes in liver tissue.

6. **Antigen HeS-08** is a specific cytoplasnic antigen of liver cells. Excess of auto-Abs against this antigen is mostly a marker sign of inflammatory processes in liver tissue.

7. **Antigen Tubulin** is an antigen of the cytoskeleton (microtubuli) of hepatocytes. Excess of auto-Abs against this antigen is a marker sign of destructive changes in liver tissue.

8. **Antigen Actin** is an antigen of the cytoskeleton of hepatocytes. Excess of auto-Abs against this antigen is a marker sign of acute chronic hepatitis and autoimmune hepatitis.

9. **Antigen Insulin** is a specific antigen of pancreatic islet beta-cells (insulin-secreting cells). Excess of auto-Abs against this antigen is a marker sign of pancreatitis and may be a prognosis for future insulin-dependent diabetes mellitus type-1.

10. **Antigen dsDNA** is the main component of cell nucleus. Increase of respective auto-Abs is the marker sign of the virus-induced activation of apoptosis, mostly. Rarely, it may indicate systemic autoimmune diseases and malignancy.

11. **Antigen Collagen II** is the main protein of the intercellular matrix. Excess of auto-Abs against this antigen is a marker sign of an active adhesive process of any region. Furthermore, it may also be a sign of pathological changes in connective tissue.

12. **Antigen Fc-fragment of IgG.** Excess of the auto-Abs against Fc-fragment (Rheumatoid factor) is an indicator of chronic inflammatory processes of any region.

ELI-Neuro-Test Method

The ELI-Neuro-Test method is intended to analyze specific profiles of the serum immune reactivity of 12 auto-Abs of the IgG class, related to or reflecting nervous system dysfunctions. The method can be used to evaluate the effectiveness of individually used treatments. Antigens contained in the Kit are the following:

1. **NF-200.** Protein NF-200 is an axon specific protein wherein the growth of antibodies (AB) in it accompanies the degeneration of nervous fibers.

2. **Antigen S100** is one of the regulators of apoptosis. It is a specific trophic factor for serotoninergic neurons of the brain. One of the most common reasons for excessive production of such auto-Abs is the human papilloma virus infection by molecular mimicry mechanisms. Excess of auto-Abs against this antigen may lead to emotional disturbances (e.g. aggressiveness, phobias, depression) and be related to papillomatosis and some forms of cancer.

3. **Antigen GFAP** is the specific antigen of intermediate filaments of the cytoskeleton of the astrocytes (glial cells). Excess of auto-Abs against this antigen indicates reactive gliosis (abnormal proliferation of astrocytes). Gliosis is usually induced by mechanical trauma of the brain, ischemic or hemorrhagic events, brain tumors, or neuroinfections. Excess of auto-Abs against this antigen may be a prognostic sign of epilepsy or other disturbances in the bioelectrogenesis of the brain.

4. **Antigen Myelin Basic Protein (MBP)** is the specific antigen of myelinated nervous fibers. Excess of auto-Abs against this antigen is a marker sign of pathological changes in nervous fibers (i.e. radiculitis, neuritis, plexites, etc.). More rarely, it may be a marker sign of demyelinating disease (i.e. multiple sclerosis).

5. **Voltage-gated Ca-channel** is the specific antigen associated with neural-muscular synaptic contacts. Excess of auto-Abs against this antigen is a marker sign of amyotrophic lateral sclerosis, cerebellar ataxia, syndrome of Lambert-Eaton, and other forms of myopatic syndromes.

6. **n-Acetylcholine receptors** is the main regulating component of the central nervous system's memory and learning mechanisms. Excess of auto-Abs against this antigen can be related to certain dysfunctions, such as Alzheimer's disease.

7. **NMDA-receptors** (excitotoxic receptors). Excessive stimulation of NMDA-receptors causes excitotoxicity, a phenomenon implicated in both acute and chronic neurodegenerative diseases (e.g. ischemic stroke, Huntington's disease, amyotrophic lateral sclerosis). Excess of auto-Abs against this antigen is a marker sign of respective forms of neuropathology.

8. **GABA-receptors** are the main regulating components of the central nervous system's balance between neuronal excitation and inhibition. Excess of auto-Abs against this antigen is typical for seizure syndromes and some cognitive dysfunctions.

9. **Dopamine receptors.** 1) D1-receptors are the main components of the central system's regulation of fine motor movements. Excess of auto-Abs against this antigen can be related to Parkinson's disease. 2) D2-receptors are the main components of the central

system's regulation of motivation. Excess of auto-Abs against this antigen can be related to schizophrenia and some forms of autism.

10. **Serotonin (5-HT) receptors** are the main components of the central system's regulation of mood and emotional state. Excess of auto-Abs against this antigen can be related to mood disorders, bipolar disease, and some forms of pathological addictions.

11. **Opiate μ-receptors** are the main components of the endogenous system of reinforcement and satisfaction. Excess of auto-Abs against this antigen is a marker sign of anomalies in the endogenous opiate system, which is typical for mood disorders, bipolar disease, and some forms of pathological addictions.

12. **β-endorphin** is endogenous to opiate peptide. Excess of auto-Abs against this antigen is a marker sign of anomalies in the neuron systems related to control of motivations and sense of reinforcement and satisfaction (e.g. typical for mood disorders, bipolar disease, some forms of pathological addictions, etc.).

ELI-V-Test Method

The ELI-V-Test method is intended to analyze specific serum immune reactivity profiles of 6 auto-Abs of the IgG class, related to or reflecting dysfunctions in the immune system. The method can be to evaluate risks of complications before preventive vaccinations in children and adults. Antigens contained in the Kit are the following:

1. **Antigen dsDNA** is the main component of cell nucleus. Excess of respective auto-Abs is a marker sign of the virus-induced activation of apoptosis, in most cases. Rarely, this increase may indicate a systemic autoimmune process or malignant tumor growth.

2. **Antigen Interferon gamma** is one of the T-lymphocytes secreting cytokines. Excess of auto-Abs against this antigen should be considered indicative of chronic activation of the T-lymphocytes.

3. **Antigen Interferon alpha** is one of the macrophagal secretory antigens. Excess of auto-Abs against this antigen should be considered indicative of the inflammatory process of any region.

4. **Antigen β2-Glycoprotein I** is a phospholipid-binding protein of blood. Excess of auto-Abs against this antigen is a marker sign of the anti-phospholipid syndrome, which may cause thrombosis in different organs.

5. **Antigen Fc-fragment of IgG.** Excess of auto-Abs against Fc-fragment (Rheumatoid factor) is an indicator of chronic inflammation of any region.

6. **Antigen Collagen II** is the main protein of the intercellular matrix. Excess of auto-Abs against this antigen is a marker sign of an active adhesive process of any region. Furthermore, it may be a sign of pathological changes in connective tissue.

ELI-Pulmo-Test Method

The ELI-Pulmo-Test method is intended to analyze specific serum immune reactivity profiles of 6 auto-Abs of the IgG class, related to or reflecting lung dysfunctions. The method can be used to evaluate the effectiveness of individually used treatments. Antigens contained in the Kit are the following:

1. **LUS-06-80** is one of most common cytoplasmic antigens of lung cells. Excess of auto-Abs against this antigen should be considered indicative of inflammatory processes in the lungs.

2. **LUS-06-300** is one of most common cytoplasmic antigens of lung cells. Excess of auto-Abs against this antigen should be considered indicative of inflammatory processes in the lungs.

3. **LUM-02-250** is one of most common antigens of lung cell membranes. Excess of auto-Abs against this antigen should be considered indicative of degenerative (destructive) processes in the lungs.

4. **Collagen IV α3-chain.** Excess of auto-Abs against this antigen should be considered the marker sign of Goodpasture syndrome, an autoimmune disease in which antibodies attack the lungs and kidneys and lead to bleeding from the lungs and kidney failure. data below -20 (*NB: with "minus"*) may indicate previously active adhesive processes in the lungs / kidneys.

5. **Elastin** is a protein in connective tissue that is elastic and allows many body tissues to resume their shape after stretching or contracting. Elastin is very important in the lungs, elastic ligaments, the skin, the bladder, and elastic cartilage. Excess of auto-Abs against this antigen is especially typical for emphysema and other degenerative processes in lungs.

6. **gamma-INF.** Excess of auto-Abs against this antigen should be considered the marker sign for chronic activation of T-lymphocytes (e.g. tuberculosis in the lung).

7. **Fc-fragment of IgG.** Excess of auto-Abs against this antigen should be considered the marker sign of chronic inflammation of any region.

8. **Antigen dsDNA** is the main component of cell nucleus. Excess of respective auto-Abs is a marker sign of virus-induced activation of apoptosis, in most cases. Rarely, this increase may indicate systemic autoimmune processes or malignant tumor growth.

Method ELI-Nephro-Test

The Method ELI-Nephro-Test is intended for the analysis of specific profiles of the serum immune reactivity of 6 auto-Abs of the IgG class, related to or reflecting dysfunction in the kidneys. The method can be used to evaluate the effectiveness of individually used treatments. Antigens contained in the kit are the following:

1. **Antigen KiM-05-300** is one of the most common antigens of kidney cell membranes. Excess of auto-Abs against KiM-05-300 should be considered an indication of degeneration in renal tissue.

2. **Antigen KiM-05-40** is also one of the most common antigens of kidney cell membranes. Excess of auto-Abs against KiM-05-40 should be considered an indication of degeneration in renal tissue

3. **KiS-07-120** is one of the most common cytoplasmic antigens of kidney cells. Excess of auto-Abs against this antigen should be considered an indication of inflammation in renal tissue.

4. **Collagen IV α3-chain.** Excess of auto-Abs against this antigen should be considered the marker sign of Goodpasture syndrome—an autoimmune disease in which antibodies attack the lungs and kidneys, leading to bleeding in the lungs and kidney failure. data below -20 (*NB: with "minus"*) may indicate a previously active adhesive process in the lungs / kidneys.

5. **Antigen ANCA.** Expression of this antigen increases during systemic or local small vessel vasculitides (inflammation of an internal wall of blood vessels by any cause). Excess of such auto-Abs is a typical sign of different forms of vasculitides. Elevation of such auto-Abs combined with elevation of auto-Abs against specific antigens may indicate ANCA-associated vasculitides with injury of the kidneys.

6. **Antigen dsDNA** is the main component of the cellular nucleus. Increase of auto-Abs is a marker sign of virus-induced activation of apoptosis, in most cases. Rarely, this increase may indicate a systemic autoimmune process or malignant tumor growth.

For additional information please see the following publications in English:

1. **Shoenfeld Y., Poletaev A.B**. Natural autoimmunity in physiology and pathology 1st Moscow International Conference Moscow, Russia, September 15–17, 2005. A meeting review. **Autoimmunity Reviews**, 2006, 5, 5, 357-363.
2. **Poletaev A.B., Maltseva L.I., Zamaleeva R.S., Nukhnin M.A., Osipenko L.G.** - Application of ELI-P-Complex Method in Clinical Obstetrics. American Journal of Reproductive Immunology (2007), 57, 294–301.
3. **Poletaev A.B., Stepanyuk V.L., Gershwin M.E.** - Integrating immunity: The immunculus and self-reactivity, 2008, Vol. 30, Issue 1-2, pp 68-73.
4. **Poletaev A.B., Churilov L.P.** - Immunophysiology, natural autoimmunity and human health. Anosia, 2010,6,1,11-18.
5. **Poletaev A., Boura P.** The immune system, natural autoantibodies and general homeostasis in health and disease. HIPPOKRATIA, 2011, 15, 4: 295-298
6. **Moiseeva O., Mitrofanova L., Karelkina E., Zverev D., Lebedev D., Skurydin S., Poletaev A.** - Serological diagnostics of myocardium diseases based on multivariate analysis of cardiotrophic autoantibodies' profiles Open J. Immunology, 2012, 2, 1, 48-58.
7. **Poletaev A.B.** - Maternal Immunity, Pregnancy and Child's Health. In: From Preconception to Postpartum, ISBN 978-953-51-0353-0, S. Sifakis, N. Vrachnis (Eds). 2012, InTech - Open Access Publisher. Rijeka, Croatia. 41-57.
8. **A.B. Poletaev, L.P. Churilov, Yu.I. Stroev, M.M. Agapov** - Immunophysiology versus immunopathology: Natural autoimmunity in human health and disease. Pathophysiology. 2012, 19, 221-231.
9. ***Physiologic Autoimmunity and Preventive Medicine*** (Ed. A.B. Poletaev), Bentham Science Publishers, Sharjah - Oak Park - Bussum, 2013.

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Technical Documentation

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herewith confirms that the manufacturer

Limited Liability Company «Medical Research Center "Immunculus»
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Russia

has prepared a technical documentation for the medical devices:

ELI-P-Complex, ELI-Viscero-Test,

ELI-Viscero-Test (pediatrics),

ELI-Viscero-Test (short panel)

A review of the technical documentation has shown that it contains the relevant product-related aspects according to the requirements of Annex III of the medical devices Directive 98/79/EG.

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Darmstadt, 12 Oktober 2009

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