ELISA IMMUNO ASSAY IN DISEASE DIAGNOSIS

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ИММУНОФЕРМЕНТНЫЙ АНАЛИЗ В ДИАГНОСТИКЕ ЗАБОЛЕВАНИЙ

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Annotation. The article shows the capabilities of a modern serological research method - enzyme immunoassay in the diagnosis of infectious, endocrine and oncological diseases. Practical examples of diagnosing individual pathological conditions, factors influencing the result, and the diagnostic value of the markers used are considered.

Key words: enzyme immunoassay, markers, diagnostic value, infectious and endocrine diseases.

Аннотация. В статье показаны возможности современного серологического метода исследования - иммуноферментного анализа в диагностике инфекционных, эндокринных и онкологических заболеваний. Рассмотрены практические примеры диагностики отдельных патологических

состояний, факторы, влияющие на результат, диагностическая ценность используемых маркеров.

Ключевые слова: иммуноферментный анализ, маркеры, диагностическая ценность, инфекционные и эндокринные заболевания.

Introduction. Enzyme-linked immunosorbent assay (ELISA) is a highly sensitive and highly specific laboratory research method based on the specific binding of antigens and antibodies in a sample with their further detection by an enzyme label. Enzyme immunoassay is the most informative in diagnosing infectious diseases. Studies performed to detect pathogen antigens and specific antibodies to them during infections are available laboratory diagnostic methods [2, 13]. ELISA is used to diagnose viral, bacterial, fungal and parasitic infections. The method is especially indispensable in the diagnosis of viral diseases, where direct methods for detecting the pathogen are difficult. In addition, in some cases, serological tests remain the only method for screening diagnosis of infections, for example, toxoplasmosis, toxocariasis, trichinosis. ELISA is used in two directions: detection of antibodies in the blood serum of the subject for diagnostic purposes and determination of pathogen antigens to establish its genus or species. Taking into account the dynamics of the synthesis of individual classes of immunoglobulins in the immune response, the presence of class 1gM antibodies indicates a primary acute infection, while the detection of only IgG marks a long-standing process or the presence of immunological memory without an active disease. IgG also includes post-vaccination antibodies. Determination of specific 1gA is informative for further monitoring of the cure of the disease, because 1dA, having a short half-life, disappears from the circulation after successful treatment for two weeks. The avidity of IgG antibodies allows us to judge the duration of infection, which is especially important when screening pregnant women for intrauterine infections.

Let us examine the use of enzyme immunoassay using the example of diagnosing an infection caused by the Epstein-Barr virus (EBV). To date, groups of EBV antigens have been identified, the identification of antibodies to which allows not only to determine the presence of infection, but also to differentiate the stages of the disease, predict its development and monitor the effectiveness of treatment measures.

The greatest significance for the serodiagnosis of EBV infection is the determination of antibodies to capsid antigens ICA). Antibodies to the viral capsid antigen (VCA) are divided into classes and IgG. Antigens are detected at an early stage of infection and are determined within 4-6 weeks. IgG is detected in the acute phase of infection, and its level decreases to undetectable within 3-6 months. Antibodies to early antigen (anti-EA) mark active infection, but may be present for years in 20% of healthy

individuals. The presence of antibodies to an early antigen may also indicate reactivation of an existing infection several years after the initial infection.

Antibodies to the nuclear antigen EBLA do not indicate an acute phase, but slowly increase within 2-4 months from the onset of the disease and persist for life [3, 14]. To diagnose EBV, the CDC recommends testing for anti-VCA IgM and IgG antibodies and EA-D early antigen to diagnose current or recent infection.

To diagnose endocrine diseases, a study of hormone levels is used. In this case, a quantitative enzyme immunoassay is used. To quantify the antigen content, the enzyme immunoassay test system contains a number of standard solutions of the analyte with known concentrations. Based on the results of the analysis of standard solutions, a calibration curve is constructed, reflecting the dependence of optical density on antigen concentration. Using the calibration curve, knowing the optical density of the test sample, the concentration of the antigen being determined can be calculated. When using purified antigen, its content can be expressed in concentration units - mass unit/volume unit [1, 12].

Thyroid diseases are very common and occupy second place after diabetes mellitus among all endocrine diseases. The advent of enzyme immunoassay methods was an important milestone in endocrinology. ELISA methods allow one to obtain important information about the development of pathology at the preclinical stage and thereby significantly increase the effectiveness of treatment.

Today, the arsenal of laboratory methods for diagnosing in vitro thyroid diseases includes nine most frequently performed tests [3, 19]. This is a determination of the concentration in the blood serum of thyroid-stimulating hormone of the pituitary gland (TSH), total and free thyroxine (total T4 and free T4), total and free triiodothyronine (total T3 and free T3), and thyroxine-binding proteins. In addition to the determination of thyroid hormones, the most significant in laboratory diagnosis is the determination of autoantibodies to thyroid tissue: antibodies to thyroglobulin (Ab-TG), antibodies to thyroid peroxidase (Ab-TPO), antibodies to TSH receptors.

The key hormonal markers of thyroid diseases are TSH and fT4. The level of TSH in the blood serum is a strategic marker of the functional state of the thyroid gland. The first level test, TSH, is necessary to differentiate the state of euthyroidism from hypo- and hyperthyroidism.

Indications for the purpose of determining the TSH content in the blood are: a TSH screening test (it is recommended to be carried out not only in pregnant women and newborns, but also in adults over the age of 35 years (women) and 50 years (men) with an interval of 5 years); diagnosis of thyroid function disorders; confirmation of the diagnosis and differentiation of forms of central and peripheral hypo- or hyperthyroidism; screening for congenital hypothyroidism [1, 18].

The blood level of TSH in healthy individuals ranges from 0.30 to 4.0 mIU/l (euthyroid range).

The clinical significance of determining the level of TSH in the diagnosis of diseases of the thyroid status: with primary hypothyroidism, there is a decrease in the concentration of thyroid hormones T3 and T4 and pathological secretion of TSH; with secondary hypothyroidism, the secretion of TSH by the pituitary gland stops, the thyroid gland receives a small amount of stimuli for the synthesis of T3 and T4; to differentiate primary and secondary hypothyroidism, a TRH-stimulating test is used (determining thyrotropin-releasing hormone) or TSH kits with a sensitivity of at least 0.01 mIU/l are used; with thyroid hyperfunction, TSH synthesis is partially or completely suppressed; Pregnant women and women taking contraceptives have normal TSH levels and elevated T3 and T4 levels.

A pathological TSH level (below 0.1 and above 10 mIU/l) in parallel with elevated levels of T4 and (or) T3 clearly indicates hyperthyroidism, a reduced level of T4 confirms hypothyroidism [2, 4].

The second level test, fT4, is necessary to confirm the presence of hypo- and hyperthyroidism. Thyroxine (T4) is produced only by thyroid cells. Only a small part (0.03% of T4) is in free form, but it is this that determines the biological activity of the hormone.

Indications for the purpose of determining the content of free T4 in the blood are: diagnosis of hyper- or hypofunction of the thyroid gland; monitoring the patient's condition during treatment.

Clinical significance of determining the level of free T4 in the diagnosis of thyroid diseases: in hyperthyroidism, the concentration of free T4 is increased, the concentration of TSH is decreased; at the initial stage of hypothyroidism, the concentration of free T4 decreases before the concentration of total T4. The diagnosis is confirmed if the TSH concentration increases [2, 5].

The following factors can affect the concentration of total and free T4:

- An increase in the binding capacity of thyroxine-binding globulin (TBG), which can be observed when taking oral contraceptives, pregnancy, in the neonatal period, active form of hepatitis, and with a rare (1:40000) genetic pathology.
- Decreased TSH binding capacity, which occurs in liver cirrhosis or a rare genetic pathology.
- Changes in albumin concentration with glomerular protein loss.
- Presence of binding inhibitors: diabetic ketoacidosis, fasting, treatment with heparin, acetylsalicylic acid, amiodarone, phenytoin, phenobarbital, carbamazepine.

Enzyme immunoassay is also indispensable in the immunodiagnosis of cancer using tumor markers. Tumor markers are substances produced by tumor cells and secreted into biological fluids, in which they can be quantified by non-invasive

methods. Measuring the level of tumor markers is widely used in diagnosis, treatment, monitoring the condition of cancer patients and preclinical detection of relapses. Tumor markers include a large group of factors, the concentration of which in biological fluids depends on the development of the malignant process. Tumor markers differ from compounds produced by normal cells either qualitatively (tumor-specific) or quantitatively (associated with a tumor, but present in low concentrations in normal cells). Some tumor markers are secreted into the blood, so their concentration can be determined using enzyme immunoassay. There are a number of substances known that can be considered as tumor markers for various localizations of the malignant process. These include tumor-associated antigens or antibodies to them, hormones, enzymes, metabolic products, and cytokines. In clinical practice, about two dozen substances are used that have sufficient diagnostic significance and are recommended for use as tumor markers [2, 8].

Indications for the use of tumor markers

- Screening — it is generally accepted that at the moment none of the known tumor markers has sufficient specificity and sensitivity to recommend it for screening.
- Diagnostics - tumor markers can be an effective and cost-effective method in the complex of diagnostic procedures for malignant neoplasms. A combination of several markers can be used to identify the primary location of the tumor during metastasis. Also, tumor markers can be used in the differential diagnosis of benign and malignant diseases. The degree of increase in the concentrations of many markers can be used to assess the stage of the disease [3, 10].
- Prognosis - the marker level before treatment or the concentration and rate of change after primary therapy correspond to the prognosis. This follows from the fact that the level of the tumor marker usually corresponds to the tumor mass. An aggressive, rapidly growing tumor with multiple metastases produces a very high level of marker, indicating a poor prognosis. A well-differentiated tumor, less aggressive, produces less marker [3, 12].
- Assessing the effectiveness of therapy and monitoring is the most important area of application of tumor markers. The marker concentration profile most quickly and clearly reflects the effectiveness of the surgical operation performed, various treatment regimens, indicates complete or partial remission, and allows relapses to be identified long before their clinical manifestation. A rapid decrease in the concentration of a tumor marker to a normal level after surgery or other treatment indicates the success of the therapy. Failure of a decrease in marker levels to normal after first line therapy may indicate that treatment was unsuccessful or was only partially successful. A long-lasting low level of the marker indicates a period of remission. A consistent increase in concentration indicates a relapse of the disease. The increase may precede clinical tumor progression confirmed by other methods by 3-12 months. A decrease in the level

of a tumor marker after a period of increase associated with the development of relapse indicates a response to second-line therapy. An increased concentration of the marker after treatment most likely means that the tumor is resistant to the treatment methods used and the prognosis is unfavorable, or the treatment regimen needs to be changed [1, 13].

The dynamics of the marker level is of more interest than a single value. It is very important to observe the analysis time intervals correctly. It is essential to have samples taken before starting treatment. As a rule, recommended sampling intervals for analysis are at least once a month during the first year after treatment, then once every 2 months during the second year after treatment, then once every 3 months during the third year of observation.

The level of almost any of the tumor markers increases in various benign diseases. The influence of concomitant diseases must be taken into account when interpreting test results.

A very important component that facilitates the interpretation of results and increases the diagnostic value of tumor markers is the determination method. Reproducibility of results is especially important when observing dynamics. The coefficient of variation should not exceed 5%. Reference intervals for measured concentrations of tumor markers may vary depending on the manufacturer of the test kits. Values obtained using reagents from different manufacturers cannot be interchanged. The laboratory report must include the method of determination used.

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