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Review article
Pregledni članak

DILEMMAS AND CONTROVERSIES IN THE INTERPRETATION OF LABORATORY RESULTS

DILEME I KONTROVERZE U TUMAČENJU LABORATORIJSKIH NALAZA

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Summary: Concentration of many substances in blood is a good indicator of the physiologic state of a patient. It is usual that results obtained represent the real concentration of tested substances in a patient, that is, represent his physiological state. The influence of some factors indicates that this assumption is not always true. Mistakes owing to analytic factors are reduced to the least possible rate by using the quality control. Also, many nonanalytic factors can change the concentration of one or more substances in the sample, so the results obtained are not an indicator of the physiological state of the patient. Results of clinical-biochemical determination are interpreted by comparing with the reference values and so the conclusion is made by a comparison method. In order to perform this process properly, reference values for each specific parameter are necessary. Cyclic variations, physical activity, stress, and other factors significantly affect the obtained result analysis. In the interpretation of results, these specificities have to be considered, otherwise they will be interpreted as pathologic, which leads to wrong conclusions. Inadequate preparation of a patient for a certain analysis and disrespect for rules regarding preparation and sample analyzing can lead to drastic deviation of results from the real values. For those reasons there are certain dilemmas and controversies in the result interpretation.

Key words: result interpretation, preanalytical factors, tumor markers, variations.

Kratak sadržaj: Koncentracija mnogih supstanci u krvi je dobar odraz fiziološkog stanja pacijenta. Uobičajeno je da dobijeni rezultati predstavljaju stvarnu koncentraciju ispitivane supstance kod pacijenta, odnosno da predstavljaju njegovo fiziološko stanje. Uticaj nekoliko faktora ukazuje da ta pretpostavka nije uvek tačna. Greške zbog analitičkih faktora svode se na najmanju moguću meru primenom kontrole kvaliteta. Takođe, mnogi neanalitički faktori mogu menjati koncentraciju jedne ili više supstanci u uzorku, tako da dobijeni rezultati nisu odraz fiziološkog stanja pacijenta. Rezultati kliničko-biohemijskih određivanja interpretiraju se poređenjem sa referentnim vrednostima, pa se i zaključak donosi metodom poređenja. Da bi taj proces mogao pravilno da se izvede potrebno je da postoje referentne vrednosti za svaki određivani parametar. Ciklične varijacije, fizički napor, stres i drugi faktori imaju značajan uticaj na dobijene vrednosti analiza. Pri tumačenju rezultata te specifičnosti moraju biti uzete u obzir, jer će u protivnom biti protumačene kao patološke, što navodi na pogrešan zaključak. Neadekvatna priprema pacijenta za određenu analizu i nepoštovanje pravila koja se odnose na pripremu i analiziranje uzorka mogu dovesti do drastičnog odstupanja rezultata od stvarne vrednosti. Iz tih razloga pri tumačenju rezultata nastaju određene dileme i kontroverze.

Ključne reči: tumačenje nalaza, preanalitički faktor, tumor markeri, varijacija.

Introduction

It is widely known that 60% of information that doctors use to set the diagnoses are the information obtained in laboratories. A lot of researches have the aim to improve the diagnostic system, that is, to find the possibility for more complex analyses that give us more information about the patient's state.

The improvement of laboratory diagnostic refers to the standardization of laboratory procedure, simplification of laboratory procedure, reduction of time necessary for analyses and application of computer sample processing. All of the above mentioned have the aim both to reduce the possibility of mistakes caused by human factor and to protect the staff during the work (1, 2).

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Using automation a palette of 25 to 30 analyses can be done in short period of time. In everyday routine work, such big demands can be justified only in some special cases (to receive patients with special pathology, when there are special protocols in treatment, in periodical controls of patients in some chronic program). However, it frequently occurs that such big and unjustified demands, not for specific parameters but for 'all and everything', are sent to laboratory and on the basis of the results obtained conclusions are made. In some, less clear situations, this way ca help, but it can-not become the diagnostic model. For some doctors it is usual practice and the most frequent pattern. Many doctors beginners on duty beside the necessary often ask for numerous unnecessary analyses, probably red by the idea »not to miss something«. However, doctors with more experience and knowledge ask for fever but specific analyses, that is, those analyses that will give him useful information. When you ask for an analysis, there should be, clearly defined question and the analysis should give you the answer. For example: erythrocyte sedimentation, or a more specific analysis: C-reactive proteins, or more specific: procalcitonin, should give the answer if it is a viral or bacterial infection (screening diagnostic).

When you make conclusions, it is necessary to use the method of induction or deduction, and if it is necessary to make more analyses which will help to confirm or to exclude certain opinions. That is why we should use protocols, wherever it is possible. It is the right and rational diagnostic way (3, 4).

The Use of Tumor Markers in Diagnostics

The mortality caused by cancer has increased during the last century and malignant tumors take the second place after cardiovascular diseases as the more frequent cause of death. Serious efforts have been made to find the substances, that is, markers that will detect malignant cell transformation as soon as possible. Tumor markers are the results in this field in the last 60 years. Tumor markers are the compounds (proteins, enzymes, receptors, and other cellular products) that make and excrete tumor cells in more quantity than normal cells. Generally, a tumor marker is any compound whose concentration can be increased in the presence of malignant cells. In this sense, the sedimentation of erythrocytes and the activity of LDH are classical tumor markers. These markers are also increased in inflammation and the necrosis process, and that is why they are highly nonspecific for malignancy. In some sense, tumor markers are macromolecules and their concentration is related to the presence and diffusion of a malignant tumor. Actually, tumor markers are very important to follow the treatment efficiency, as well to predict the response to therapy. The application is limited in the field of screening, in the diagnostic of localization and stage, tracking and prognoses of malignancy. It means that most tumor markers are significant for therapy tracking, and only few of them are useful for early detection of cancer (5, 6).

An ideal tumor marker from the clinical point of view (7) should satisfy the following criteria:

- that it is specific for the tested tumor (malignant process),
- that it is in correlation with tumor mass,
- that the level in the tested sample is increased in the presence of metastases, that is, in the stage when neither physician nor methods currently available can detect its presence,
- that if it is present in the plasma of healthy people its concentration is lower than in a certain stage of cancer,
- to indicates the therapy effect,
- that its concentration is in accordance with prognosis stage
- that for its determination in body fluids methods that are simple and not expensive are used.

The criteria for ideal tumor marker, which would have 100% sensitivity and specificity, have not been fulfilled by any known tumor marker. Although today there are many tumor markers, their use can be helpful to diagnose the cancer but it can not be the only criteria (6–8).

The problem of specificity and sensitivity of tumor markers, as well as any other substance which is to be determined, is connected with the chosen upper limits of the reference range. Lower cut-off value, higher sensitivity, »positive« results will be noticed in the initial stage of illness. Unfortunately, this sensitivity will be connected with lower specificity – a small increase would lead to a great number of false positive diagnoses in the benign stage. Contrary to this, the choice of high cut-off values leads to higher specificity and lower sensitivity and to a great number of false negative results in the presence of tumor spread. The explanation is probably in the simple response of an organism to pathological change in its tissues. During the inflammation or necrosis process, there is an increased cell production of tumor markers. The immune system recognizes them as »extraneous« and eliminates them, which leads to normalization of passing concentration increase of an tumor markers in the serum (5).

If we want to estimate the value of a tumor marker, it should be applied to an adequate population of healthy people as well to patients with benign diseases and patients with different types of cancer. For example, there should be:

– patients with chronic gastritis when we define an reference values of tumor markers for stomach cancer, or

– patients with chronic hepatitis or cases with active liver cirrhosis when we define an reference values of tumor markers for primary cancer of liver cells.

If we examine patients with a specific cancer, the various stages of cancer should be included. If tumor markers are used to follow the therapy, a set of samples should be taken to estimate the efficiency of tumor markers. If we want to value markers, it is necessary to determine reference values, to calculate predictive values, distribution of the marker's values and significance in disease estimation (8).

This discovery leads to better results in patient tracking after radical surgery, early detection of relapse or metastases, adequate treatment or better results in an illness.

Tumor markers, with few exceptions, are not adequate for a primary diagnosis of a malignancy. They do not show enough sensitivity or specificity for their purpose. Positive results have to be interpreted carefully because some tumor markers are increased in nonmalign diseases, as well in a small number of healthy people. Besides that, false negative results may arise because a tumor does not create markers and the values found in healthy people may overlap with those found in an early phase of cancer. False positive values appear when values are increased even when there is no cancer. False negative values occur when values are normal and there is a cancer (9).

False positive results in cancer diagnosis can lead to a lot of psycho-trauma while false negative results postpone the therapy, which can be fatal. In some rare cases, the increased values of tumor markers in serum can help to make an exact diagnosis, if the increase is connected with adequate clinical data. For example: CA 125 in suspected ovarian cancer, HCG in suspect of choriocarcinoma (5, 9).

For various kinds of cancer, tumor markers are used as markers of first choice, that is, second order. A combination of markers may increase their diagnostic sensitivity.

Insufficient specificity of a tumor marker sometimes exclude its use in detection of tumor location, while sometimes it can be useful, for example: an increased level of CA 125 in cases of ovarian cancer and CA 19-9 in pancreas cancer. Sometimes the high price of analyses makes it heavier to use the whole palette of routine tumor markers in the diagnosis of an unknown primary tumor with clinically obvious metastases.

As for the state and prognosis, a persistently extremely high concentration indicates the progress of illness and bad prognosis. There are significant individual variations in value level, so the values in the reference range do not exclude definitely the malignancy.

Tumor markers that are most frequently used in routine work are: enzymes, tissue receptors, antigens, oncogenes, and hormones (9).

Many enzymes that are found in tissues with cancer can be found in serum in a greater concentration. Enzymes are usually measured by the photometrical method, while most of other types of tumors are measured by the immunochemical method. The example of some enzymes whose activity increases in cases of malign diseases are: acid phosphatase, alkaline phosphatase, amylase, creatine kinase, gamma glutamyl transferase, lactate dehydrogenase, enolase, and terminal deoxynucleotid transferase.

The second type of tumor markers are tissue receptors, that is, proteins connected with cell membranes. These substances are connected with hormones and the growth factor and that is why they affect the tumor growth. Some tissue receptors have to be measured in samples of the tissue obtained by biopsy, while the rest are excreted in extracellular fluid and can be measured in the blood. Some important receptor tumor markers are estrogen receptor, progesterone receptor, interleukin-2-receptor and epidermal growth factor receptor.

Oncofetal antigens are proteins, gene products which are very active during fetal development, but after birth their function is very limited. Genes are activated when malign tumor increase and produce great quantities of proteins. Antigens comprise the largest group of tumor markers including the glycoprotein antigen. Important tumor markers from this group are: alfa-fetoprotein (AFP), carcinoembrionic antigen (CEA), prostate specific antigen (PSA), cathepsin-D, HER-2/neu, CA-125, CA-125, CA-19-9, CA-15-3, nuclear matrix protein and bladder tumor-associated antigen.

Some important oncogenes are BRAC-1, myc, p53, RB (retinoblastoma) gene, and Ph (Philadelphia chromosome).

Hormones are the fifth type of tumor markers. These are hormones excreted by tissues where a malign process progresses, while they are not excreted normally. (ectopic production). In this group belong as follows: adrenal corticotropic hormone (ACTH), calcitonin, catecholamines, gastrin, human chorion gonadotropin (hCG) and prolactin.

From the above mentioned we can conclude that the most frequently used tumor markers are substances which only tumors excrete in different body parts. In the second group there are markers excreted by both malign and benign tissues (PSA). Third category are tumor markers whose effect we track through receptors increase (HER-2/neu) (10).

Beside the influence of different factors referring to tumor markers and in that way to result interpretation, other factors that influence the results of other analyses and in that way their interpretation have to be considered.

In order to estimate the state of a patient, laboratory tests which determine the concentration of some substances in a blood sample or other body fluid are used. The concentration of many substances in the

blood is a good indicator of the physiologic state of a patient. Usually, the results obtained represent the real concentration of the tested substances in a patient, that is, they represent his physiological state. The influence of some factors indicates that this assumption is not always true (11–14).

Influence of Preanalytic Factors

Mistakes owing to analytic factors are reduced to the least possible rate by using the quality control. Also, many nonanalytic factors can change the concentration of one or more substances in the sample, so the results obtained are not an indicator of the physiological state of the patient. In order to avoid the effects of preanalytical factors and to reduce the mistakes at work, it is necessary to abide by the standard procedure regarding the preparation of patients, taking and processing of the samples for analyses (12). Results of clinical-biochemical determination are interpreted by comparison with the reference values, and so the conclusion is made by a comparing method. In order to perform this process properly, reference values for each specific parameter are necessary (14).

Cyclic variations reflect on changes in the concentration of the substance in a certain period of the day, week and month. Rhythmic variations are typical for many biological functions (for example: daily variations in metabolism of the medicines and incidence of myocardial infarction). The example of circadian variation is melatonin, hormone of the epiphysis which is excreted as a response to the darkness and affects a lot of processes between the hypothalamus and hypophysis. Because of that, the concentration of the hormone of hypophysis increases during the night and reduces during the day. It is considered that sleeping and activity affect the daily variations more than changes regarding the hour (12). Several other substances, like iron and acid phosphatase, also show noticeable circadian variation. If we determine the concentration of most electrolytes (Na, K, P) in urine in samples taken in different periods of the day the differences can be up to 50%. In these cases there is a dilemma: if the difference in results arose due to circadian variation or it is the result of metabolic disturbance. Standardization of sample procedure obviates the dilemma (15, 16).

Many hypophysis hormones are not excreted in the circulation in a constant quantity but periodically. The concentration in certain periods may be several times higher than the usual level. The individual sample cannot be representative sample of the hormonal production. Cyclic variations referring to more than one day also affect the results of laboratory testing. Menstrual cycle is connected with significant changes in the concentration of ovary hormones. Thus it is connected with the monthly fluctuation in the concentration of other parameters (Ca, Mg, cholesterol, PTH, rennin, aldoserone, antidiuretic hormone) (17). Cyclic annual variations are connected to seasonal changes in nutrition and climatic variations. For example, the concentration of serum 1,25 dihydroxiholecalciferol is higher in summer than in winter, and concentration of oxalates in urine is higher in summer than in other seasons. The above mentioned random variations can cause clear changes in concentration from day to day. Some parameters (electrolytes, proteins, ALP) show changes in concentration lower than 5% during the day, opposite to others (bilirubin, CK, triglycerides and most of the steroid hormones) where that difference can be by 20% higher from day to day (18, 19). Secretion of creatinine in the urine may fluctuate by 10% in a given sample, while most of the other substances excreted in urine can fluctuate from 25% to 50% in a relatively short period of time.

There are certain parameters whose values are significantly higher after physical activity. Individuals who exercise regularly (aerobic) have constantly higher values of the muscle enzymes (CK, LDH, AST, ALT) than people who are not so active (20). Hard exercises are related to higher values of muscle enzymes, uric acid, and bilirubin. With people who exercise periodically, the concentrations of gonadotropin and sex hormones in serum are significantly lower, while the concentration of prolactin is higher. In interpretation of results, these specificities have to be considered, otherwise they will be interpreted as pathologic, which leads to wrong conclusions (21).

Changes in the concentration of certain parameters are the result of nutrition. After the meal, the concentration of substances taken in with food (proteins, glucose, triglycerides) is higher. The concentration of Na, uric acid, iron, and hormones: gastrin and insulin, as well as the activity of LDH is significantly higher after a meal (postprandial growth). Substances existing in food can also interfere with results of a test. Vanillin interferes with method for the determination of vanillymandelic acid, and serotonin taken in with food can increase the concentration of 5-hydroxyindoleacetic acid (5-HIAA) in urine. Test of occult bleeding in faeces is positive after ingesting meat and in some cases after ingesting iron and horse radish.

Stress of any kind (mental or physical) can affect the results of laboratory testing (22). It has been known for a long time that stress induces the production of ACTH, cortisol and catecholamines. Even moderate stress (after pinprick, tremor before the exam, planned admission into hospital) can be sufficient to cause changes in values of hormones. Total cholesterol can be increased as a result of moderate stress, and the value of HDL cholesterol reduces to 15%. Stronger stress causes considerable changes. After acute myocardial infarction, cholesterol starts to decrease during 24 hours and can reduce by 60%, and it returns to the usual value of a patient in approximately 3 months.

Besides the above mentioned, important preanalytical factors that have to be considered during the result interpretation are as follows:

a) Variations that are the result of sample collecting:

- the technique of blood collecting
- the type of the sample (whole blood, serum, plasma),
- mistake as a result of use of preserving agents and anticoagulants,
- mistakes due to inadequate identification of sample and patient,
- variations during urine collecting (biological, time of collecting, stability of sample),
- sample collecting in infants (sample collecting for metabolic mistakes, gathering of capillary blood);

b) Mistakes that can occur after sample collecting:

- transport of samples,
- preparation of samples (centrifuging),
- sample keeping.

The given examples indicate that inadequate preparation of a patient for a certain analysis and disrespect for rules referring to preparation and sample analysing can lead to a drastic deviation of results from the real values. For those reasons there are certain dilemmas and controversies in the result interpretation. Thus, obtained results of analyses can be badly interpreted or abused in several ways and for different purposes, whether by the doctor who asked for them or by the patient himself.

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