IMPORTANCE OF VARIABILITY OF LABORATORY PARAMETERS AND UNCERTAINTY OF LABORATORY EXAMINATION METHODS IN CLINICAL PRACTICE

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Abstract
The methodology of laboratory examination methods is also influenced by the biological variability of the determined parameter and by the uncertainty of the methodology used for its determination in the laboratory. At the model example of the dynamics of the concentration of the Ca15-3 tumor marker, we have demonstrated the limits of the informational contribution of laboratory analyzes. We note that the value of laboratory examination methods is significantly multiplied with other diagnostic methods, especially imaging techniques. Their common informational power thus makes it possible to reach the right diagnostic decision at an earlier time, which increases the success rate of patient therapy.

Key words: Variability. Reference limit. Coefficient of variation. Tumor marker.

1 Introduction

Laboratory examination methods in healthcare must be a reliable source of data, which are necessary for correct differential diagnostics and the whole treatment-preventive process. In the recent decades, we have witnessed the tremendous development of this branch of biomedical industry. It has been resulted in hundreds of different laboratory parameters that allow us to control and monitor a large variety of biochemical-metabolic processes. However, there is also great variability in the methodological palette of examinations. A given parameter can be determined using enzyme immunoassay, chemiluminescence, photometry, radioimmunoassay, electrochemi-luminescence, etc. However, this variability also poses a risk and challenge to further improve laboratory investigative techniques and methodologies [1].

2 Requirements of physicians

From the point of view of patient´s care management, it is crucial for the physician and nursing staff to have a reliable and veracious outcome of the ordained parameter. Their clear requirement is that the results are to be delivered in a explicit format and, in the case of quantitative numerical data, with a clearly defined biological reference interval for the parameter. The patient sample must be received and laboratory processed within the appointed time limit. In fact, this simple set of conditions conceals the complex machinery of processes [2].

3 What physicians don't see?

However, there is also information that doctors do not see in the patient's result sheet. They are not given, because they mainly indicate the degree of variability and inaccuracy of the laboratory results.
They belong here:

Parameters of a particular examination method: detection limit, determination limit, linearity region, analytical specificity of the method, measurement accuracy, measurement preciseness, and robustness of the laboratory method.

Above all, *quality control* should be mentioned. It is a process where a sample with a known concentration of the analyzed parameter is added to a series of unknown samples. If a laboratory determination shows a result within a tolerated interval around the indicated value, we assume that this parameter is determined correctly also in unknown samples. External quality control and interlaboratory comparative tests have a similar control function [3, 4].

Interferences: The presence of some substances and molecules affects the test results. In the case of bio-chemical parameters it is usually icterus, hemolysis and lipid content. Therefore, interferences must be taken into account and their presence and influence monitored.

Measurement uncertainty: The laboratory investigative methods of each parameter consist of a sequence of steps, each of which contributes a certain error to the final value. We try to quantify the sum of all possible inaccuracies and errors by calculating the so-called extended combined uncertainty U_c, which quantifies the contribution of all the uncertainties of all individual steps and actions [5].

Biological variability: is caused by the characteristics of the living systems themselves in combination with the environment in which they are found and includes factors such as age, race, pregnancy, menstrual cycle, menopause, genetic predisposition, eating habits, etc. Biological variability from the individual's point of view is divided into intraindividual and interindividual [1 ,6].

All these factors, to a greater or lesser extent, affect the specific outcome of the patient's laboratory examination. The question is to what extent this information is also important for physicians.

3 Aim

On the results of selected laboratory parameter Ca15-3 verify the significance of information on its variability for the decision-making process in clinical practice.

4 Material and Methods

We had a time series of examinations of the Ca15-3 tumor marker in a patient who was treated with a breast cancer diagnosis and subsequently followed up with regular mammography and tumor marker determination intervals. No other co-morbidities were present in the patient. All processed data have been de-identified in order to guarantee patient privacy.

The tumor marker Ca15-3 was determined by the electrochemiluminescence method based on ruthenium complex excitation. The biological cut-off value of Ca15-3 for the Caucasus population of the catchment area of the laboratory was 35 kU/l. Intraindividual variability of the determined parameter is given at the coefficient of variation CVi=6.1%. The expanded combined laboratory method uncertainty was U_c=5.2%.

5 Results and Discussion

In our patient, the Ca15-3 parameter was determined 17 times in total. The results are shown in Table 1. Tumor extirpation was performed in the period after the second tumor marker determination. This was followed by a gradual decrease in its concentration, which confirmed the causal relationship between its concentration and the removed neoplasm mass. The results are shown in Figure 1. The fifth assay has already verified the achievement of a biological limit of 35 kU/l, followed by a further decrease (Table 1, Figure 1). The development shown is a typical example of a laboratory image of successful tumor therapy.

Table 1 *Results of the tumor marker Ca15-3 examinations.*

Examinations sequence	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Ca15-3 (kU/l)	101.2	107.3	60.4	40.2	30.9	27.1	21.1	20.5	19.8	15.6	14.9	19.9	15.4	14.7	16.4	15,2	27.9

However, the following dynamics of change is no longer as simple as it would seem. The patient was clearly in remission, with an average Ca15-3 concentration of $\bar{x}$=17.35±2.62 kU/l over the 7th to 16th determination period, with a coefficient of variation of Vk=15.12% (Figure 2). This coefficient of variation is higher compared to the given parameter of intra-individual variability of Ca15-3 (CVi=6.1%). The reason may be the biometabolic constitution of the organism, as well as the fact that it is not a healthy individual but an individual in whom the cancer has been treated. Measurement values no. 7-16 are an example of an ideal state of tumor marker concentrations in a long-term monitored patient in remission. Extended combined uncertainty of laboratory method U_c=5.2% guarantees accuracy and feasibility of provided data.

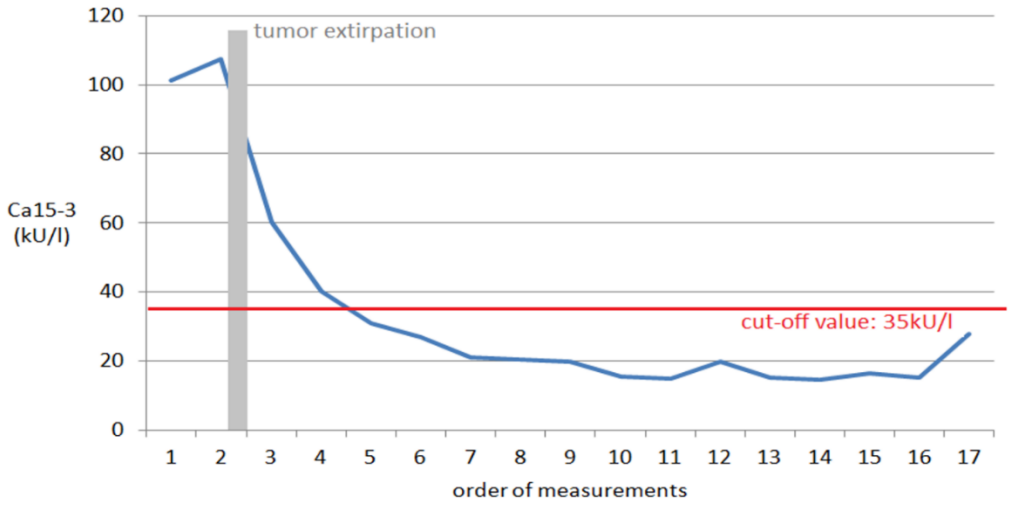

Fig. 1 *Time dynamics of decrease of Ca15-3 tumor marker concentrations*

The last 17th value of Ca15-3 determination (27.91 kU/l) appears to be very problematic, although it is still below the 35 kU/l limit value. Its magnitude already exceeds the interval of three times the standard deviation value (±3*sd*=7.66) of the observed arithmetic mean of the stabilized values of Ca15-3 concentrations in remission. And exactly at this moment, laboratory diagnostics alone is becoming insufficient. The question is what factor caused the observed increase in the concentration of the monitored parameter. If we would to rely solely on the monitoring of the laboratory parameter, further examination would necessarily follow at a defined time interval. If this following examination also showed a tendency to increase Ca15-3 concentration, it would most likely be a recurrence of the disease. If the following examination showed a return to the original stabilized range of numerical values, other effects (mastitis, trauma, etc.) would probably be the cause. In this respect, therefore, the focus is shifted to imaging techniques, especially to the mammography and ultrasonography, which are able to eliminate non-malignant processes immediately after detection of increased concentrations of the endpoint and thus speed up the diagnostic conclusion, which is crucial for further development of the patient's health condition.

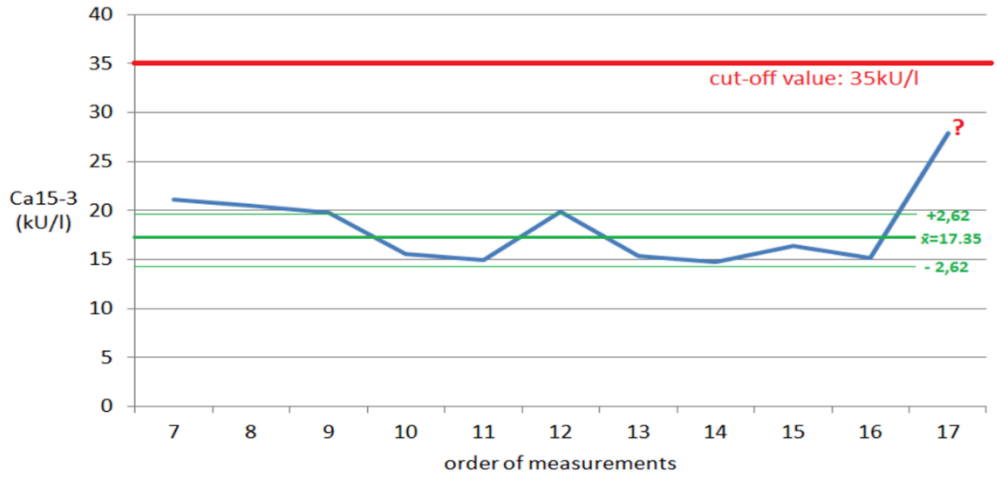

Fig. 2 *Variability of Ca15-3 tumor marker during remission*

6 Conclusion

In clinical practice, the multispectral informational palette of data variability of laboratory parameters and uncertainty of laboratory examination methods is significantly reduced. The result is a final value of the parameter concentration along with the indication of its biological reference interval. Other information, in particular data on the uncertainty of the laboratory examination of a particular parameter by a particular method, is not included in the results sheets. While they represent an information benefit, from the point of view of the dichotomy of decision algorithms in the process of differential diagnostics, they mean some confusion of information. Therefore, the partial results of laboratory diagnostics should be compared and aligned with the other results of other biomedical and laboratory components.

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