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MEDICAL AND BIOLOGICAL MEASUREMENTS

PRINCIPAL SOURCES OF ERRORS IN NONINVASIVE MEDICAL SPECTROPHOTOMETRY. PART 2. MEDICOBIOLOGICAL FACTORS OF ERRORS

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Early results of complex experimental investigations on instrumental and methodological errors of diagnostics in noninvasive medical spectrophotometry are described. Biomedical sources and factors of errors are considered for natural measurements under clinical conditions. It is shown that the greatest contribution to the total measurement error is made by the interactive component, due to the interaction of the measurement instruments and objects.

Keywords: *noninvasive medical spectrophotometry (NMS), back-scattered radiation, laser Doppler flowmetry (LDF), optical tissue oximetry (OTO), laser fluorescence diagnostics, oxyhemoglobin saturation.*

Investigation and development of metrological provision for the methods and instruments in modern noninvasive medical spectrophotometry (NMS) are the most important and key problems, which must be solved for the successful introduction of this new diagnostic direction in practical medicine. The basic theoretical situations, formulated in [1], enable us at the present time to convert directly to systemic and complex experimental investigations in this area. In Part 1 of this study [2], we considered the physicotechnical factors and phenomena that have the strongest effect on the metrological characteristics and are the principal sources of errors and scatter of measurement results for different methods and NMS instruments for test measurements in the laboratory for nonbiological simulation measures (SM). The second stage of the investigation, reflected in the present article, is devoted to searching for and analyzing fundamental biomedical and organizational-clinical factors and phenomena, which are the most important sources for the occurrence of measurement mistakes and errors when carrying out natural diagnostic procedures in the clinic.

All the biomedical investigations were carried out just as for the first stage, on the basis of three diagnostic technologies of NMS: optical tissue oximetry (OTO), laser Doppler flowmetry (LDF), and laser fluorescence spectroscopy (LFS). In the investigations, we used three Spektrotest tissue oximeters from one experimental group of instruments, two laser Doppler instruments of the LAKK series with channels of red (632 nm) and of infrared (805 nm) ranges of wavelength, a multifunctional LAKK-M laser diagnostic complex, and the LESA-01 Biospek system of laser fluorescence diagnostics. All the indicated instruments are output in series, have registration certificates issued by the Federal Service for Inspection in the Sphere of Public Health and Social Development, and can be used to solve problems in medical practice. Investigations were carried out with the participation of medical personnel having different qualifications, and also actual patients, and public-service, healthy test subjects from various clinics and divisions of Vladimirskii Moscow Regional Research Clinical Institute (MONIKI) in endoscopy, radiology, laboratories of medicophysical research, etc. (with solutions coming from the Independent Committee on Ethics of MONIKI and the corresponding license for carrying out such scientific-research studies).

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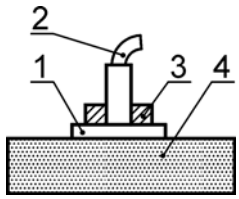

Fig. 1. Modeling of different pressures of a fiber gage on a biological tissue with the use of test loads: *1*) bearing of fiber probe; *2*) optical fiber probe; *3*) test load; *4*) biological tissue.

The principal part of the experiments was carried out by the method of repeated statistical tests (measurements) in the same anatomic-topographical region of each of the test subjects with the aim of detecting the scatters in the results, and the subsequent analysis of the scatters in order to reveal the methodological or instrumental errors (both random and systematic). For this, based on test results, we estimated, in each series of s identical (in the opinion of the doctor) measurements, the arithmetic mean value M_s of each recorded diagnostic parameter, the empirical root-mean-square deviation σ, and the scatter or coefficient of variation $\delta = (\sigma/M_s)\cdot 100$ of the measurement results according to the level σ in the percent of the arithmetic value of the quantity being measured. The method of investigation included the following statistical analysis based on the scheme indicated above, both for all the principal, resulting, medicobiological, diagnostic data, calculated in instruments programmatically, and also for all the principal primary and secondary physical data (photodetector voltages), being measured by instruments directly on a person [1, 2]. Since all the medicobiological tests require greater time and effort and are unlike the physicotechnical investigations, it was proposed that a person be used as the test biological object (BO), and the number of tests in certain series be minimized (no more than 5–10). In a first approximation, all the measurements in a series were assumed to have equal scatter and equal precision for comparison of the results from different series [2].

For scientific purposes, a total of about 200 patients from MONIKI were investigated, with an age range of 20–60. Of these, about 100 were found to be under constant observation over the entire course of medical treatment. For different methods of NMS, we carried out 28 complete series of experiments with patients according to the method of repeated statistical tests for estimation of the distribution laws, mathematical expectation, and scatter of the measurement results. In addition, about 30 people were invited to be voluntary, arbitrary, healthy test subjects. Most of the measurements were carried out on the external body surfaces of the people (the skin of the face and of the fingers) and tissues of the oral cavity (mucous membranes, tongue, etc.) both for the case of normal tissues and also for pathological (e.g., malignant) processes, i.e., in places that are sufficiently convenient to access and position instrumental probes. However, in order to estimate the effect of the measurement results on the scatter, we carried out more-labor-intensive inter-cavity investigations in which we measured values in the mucous membranes of the upper branch of the gastrointestinal tract (esophagus and stomach) and the mucous membrane of the colon with the use of endoscopic technology. This technology substantially complicates the examination process, makes it uncomfortable for the patient, and, hence, can lead to additional errors and mistakes in the diagnosis. Comparative inter-cavity examinations by the LFS method, with observation of fluorescence in the green spectral range (excitation I_L at wavelength 532 nm, recording and estimate of the fluorescence signal I_F at 560 nm) were carried out for two different inter-cavity procedures – gastroscopy and colonoscopy.

Another important stage is the investigation of the interactive component of the instrumental error [1]. It was estimated in parallel to the LAKK-M diagnostic complex, the LAKK-01 laser Doppler analyzer, and two Spectrotest instruments for the example of the investigation of the effect of the pressure of the optical gage of the instrument for the tissue of the BO being tested. Different pressures of the gage on the biological tissue were modeled by test loads for which we used calibrated lead plates applied to a bearing of the optical probe for operation on the LAKK-M diagnostic complex and the LAKK-01 instrument (Fig. 1), and also standard weights for the case of the operation of the Spektrotest oximeter, positioned freely on the rear surface of the optical tip of the instrument.

TABLE 1. Statistical Measurement Results by the OTO Method for Single (brief) Measurements of a Biological Object (BO)

Object of investigation (BO)	Statistical parameters	Recorded signals of photoreceiver, mV			Calculated medical parameters, arb. units	
		U_G	U_R	U_{IR}	S_tO_2	V_b
Healthy tissue	M_5	821.9	2052	1358	0.81	0.13
	σ	65.71	73.95	47.13	0.09	0.02
	δ, %	**7.99**	**3.59**	**3.47**	**11.1**	**15.3**
Malignant process (cancer)	M_5	774.5	2678	1594	0.93	0.16
	σ	39.76	83.18	40.51	0.06	0.01
	δ, %	**5.13**	**3.11**	**2.52**	**6.45**	**6.25**

The absence of a standardized method and procedure for the examination of the patients has an effect on the reliability and reproducibility of results of diagnosis in NMS. To estimate this effect, the patients being tested were examined by medical staff having different qualifications. A portion of the measurements was assumed to be carried out by medical personnel having many years of experience in the operation of similar instruments and methods at MONIKI, including carrying out dissertation-level scientific research on the subject of NMS, and a portion was carried out by young physicians and postgraduate students, who had been given the minimal necessary and possibly unclear instructions. Here we estimated the comparative reproducibility, information content, and reliability of interpretation of the results obtained by the first and second groups of investigators. A portion of the results was obtained by postgraduate students who first completed unregulated research and then research based on earlier, accurately written and recorded diagnostic algorithms. In this case, we determined the effect of inexact fixing of the gage of the instrument at the same anatomic-topographical point on the body of the patient for scatter in the data for multiple repeated measurements.

Separate estimates of the scatter of the results of multiply repeated measurements by the LFS method in a radiological clinic are represented in [3–5]. It was shown that the scatter δ is found to be within the limits ±10% for the initial signals and data and ±30% for the calculated coefficient of fluorescent contrast K_f assumed to be the final medicobiological diagnostic criterion in LFS [3]. A similar situation was discovered earlier also for the OTO method [4, 5]. Within the framework of the described investigations, these results were repeated and reproduced in a large number of tests and a variety of diagnostic apparatus. In Table 1, we show statistical data for single (brief) measurements by the OTO method, which enables us, as the final medical parameters, to determine the tissue saturation of oxyhemoglobin of mixed blood S_tO_2 and volume blood-filling (vascularization) V_b of the microcirculation channel of the investigated section of biological tissue. In the present example, we examined a patient with a malignant process on the skin. With comparatively small scatter of 5–7% of the recorded initial physical signals U_i of the photodetectors, the scatter of the final calculated medical parameters S_tO_2, V_b increases up to ±15% and greater. In a series of experiments for different copies of the Spektrotest instrument, we obtained δ = ±35%. This is considerably greater than values recorded earlier for nonbiological SMs [2], i.e., the biological nature of the object of investigation, its physiological variations and fluctuations of the measured parameters during the experiment can be, apparently, sources of sufficiently large random errors for instantaneous (single) measurements in the clinic [4, 6]. Also, the computational algorithms of the instruments, in which a simplified, physicomathematical model of the BO is established, ignoring its complex structure and functional organization (the presence, e.g., of sweat detection, different chromophores in tissues, etc.), constant for each measurement, lead to the appearance of systematic methodological errors (distorted estimates) in the final medicobiological data. Therefore, the scatter in the values of the calculated parameters increases.

The results of multiply repeated inter-cavity examinations of five patients by the LFS method with the use of an endoscopic technique is represented in Table 2 (the LESA-01 recording unit, excitation of fluorescence at 532 nm, recording in the region of fluorescence of lipofuscin at 560 nm). From Table 2, it follows that, on average, for endoscopic procedures, the scatter of the data increases by 10–15% in comparison with the results of external examinations [3, 5], where healthy tis-

TABLE 2. Data from LFD of Patients for Inter-Cavity Procedures

Object of investigation (BO)	Statistical parameters	Physical signals, arb. units		Calculated K_f
		I_L/β	I_F	
Patient 1, gastritis, gastroscopy	M_{10}	655.0	250.0	0.545
	σ	64.33	66.67	0.067
	δ, %	**9.82**	**26.67**	**12.23**
Patient 2, gastritis, gastroscopy	M_{10}	420.0	190.0	0.627
	σ	63.25	31.62	0.121
	δ, %	**15.06**	**16.64**	**19.36**
Patient 3, inflammation, colonoscopy	M_{10}	522.0	202.0	0.567
	σ	99.98	17.51	0.076
	δ, %	**4.75**	**11.75**	**7.61**
Patient 4, ulcerous colitis, colonoscopy	M_{10}	743.0	206.0	0.436
	σ	69.77	13.50	0.035
	δ, %	**9.39**	**6.55**	**8.11**
Patient 5, standard, colonoscopy	M_{10}	448.0	245.0	0.706
	σ	70.05	62.41	0.150
	δ, %	**15.64**	**25.47**	**21.17**

sues (patient 5) also give large scatter in comparison with varying mucous membranes (patients 1–4). The scatter of the values of K_f also in the general case fit within the range obtained earlier $\delta = \pm 30\%$, i.e., the maximum errors of 30% are not specific for a particular method of examination in NMS and can appear both for endoscopic procedures and also for external examinations, and hence are due to other types of causes.

One of these causes, as subsequent investigations showed, is the placement of the gage with multiply repeated measurements not exactly at the initial position, but with a small shift in the position. The microcirculation channel of the majority of the soft tissues of humans is very inhomogeneous. Therefore, a small shift of the instrument gage in the examination zone can lead to a considerable change in the number and hierarchy of microvessels with various degrees of filling with blood, which has a strongly favorable effect on the measurement results. The distribution of the recorded fluorochromes in tumors and inflamed mucous membranes of organs and in many normal tissues is also inhomogeneous. Table 3 shows more detailed conclusions on the basis of experimental data obtained by the Spektrotest instrument in the Physiotherapeutic and Radiological Branches of MONIKI. In this fragment of the investigations with each test we carry out three series (with five measurements in each) of multiply repeated measurements for minimization and averaging of the effect of other random errors, and the scatter was estimated from series to series. Here, test 1 was carried out by a young postgraduate, who did not indicate the aim and method of the research – to position most accurately the instrument gage for each new measurement. Test 2 was carried out by a different, more experienced and accurate postgraduate, and his attention was focused especially on the need for accurate control of the positioning of the instrument gage on the body of the patient. Test 3 was found to be under the control of the experienced investigators. A clear decrease in the scatter of the data was recorded from experiment to experiment, i.e., accurate control of the positioning of the instrument gage on the body of the patient, and also an increase in the qualifications of the investigators and an exactly formulated diagnostic algorithm (procedure for patient examination), are important measurement components for decreasing errors in NMS.

TABLE 3. Results of Statistical Tests Using the Spektrotest Oximeter Performed by Medical Personnel Having Different Classifications

Parameter (measurement series)	Values of medical parameters, arb. units, for tested BO					
	1		2		3	
	V_b	S_tO_2	V_b	S_tO_2	V_b	S_tO_2
M_5 (1)	0.119	0.876	0.209	0.730	0.280	0.840
M_5 (2)	0.085	0.973	0.166	0.852	0.275	0.826
M_5 (3)	0.177	0.776	0.166	0.855	0.274	0.827
M_3	0.127	0.875	0.180	0.812	0.276	0.831
σ	0.046	0.099	0.025	0.071	0.003	0.008
δ, %	36.3	11.3	13.8	8.7	1.1	0.9

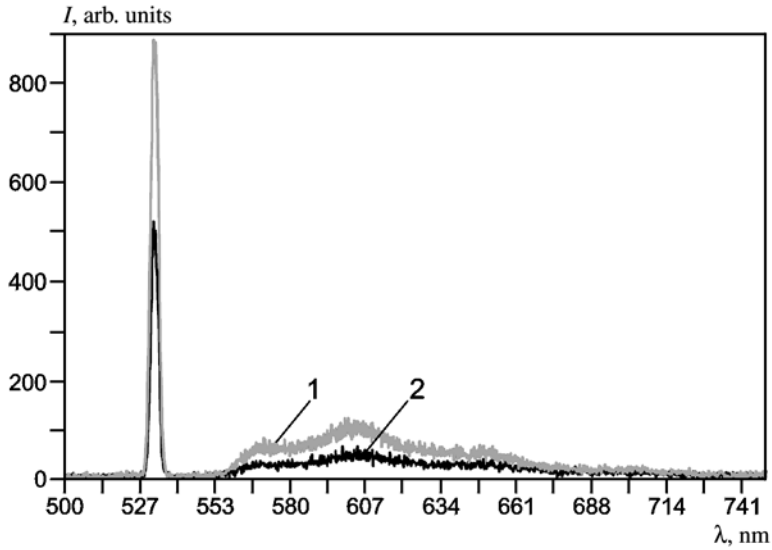

Fig. 2. Effect of gage pressure on fluorescence signal in the wavelength range 530–700 nm: *1*) for pressure 10^4 Pa, $K_f = 0.2$; *2*) in the absence of pressure, $K_f = 0.09$.

The ambiguity in the fixing of the position of the gage on the examined object is a source of considerable random errors, and for investigations on nonbiological SMs [2], it has an especially favorable effect on the ambiguity and random angular position of the optical-fiber probe without a screening bearing. These errors, to a large degree, also appear for endoscopic procedures (see Table 2), since when carrying them out it is impossible to use the indicated bearing.

However, under clinical conditions and the biological nature of the object of the diagnosis, one of the important and large components of the instrumental error increases up to this random (and sometimes also systematic) methodological error, due to the interaction of the measurement device and the BO – an interactive component of the error [1]. As a result of such an interaction, there is a change in the properties of the BO, which does not occur with a nonbiological SM. The clearest manifestation of this error source, in the opinion of the authors, is the situation of uncontrolled pressure of the instrument gage for the test region of the BO, which leads to partial bleeding in the microcirculation channel and a considerable change in all the opticophysical properties of tissues in the examination zone, beginning with the density and ending with linear (transport)

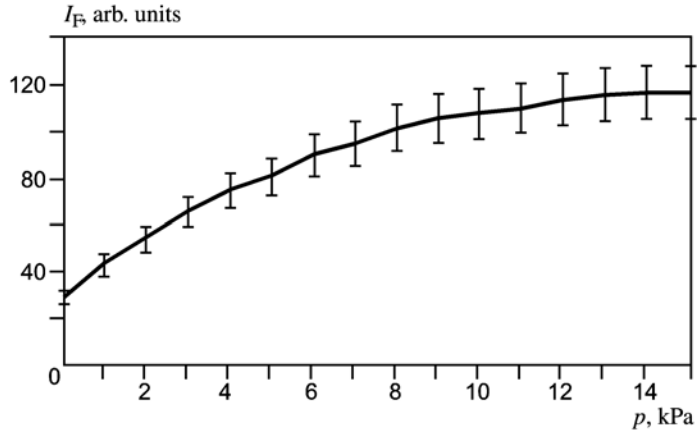

Fig. 3. Dependence of recorded amplitude of signal IF of finger skin at the 460-nm line on the external pressure p applied to the instrument gage; excitation at the 375-nm line.

optical absorption coefficients, scattering, and fluorescence of biological tissue. In all the experiments carried out, we noted a large effect of the gage pressure on all the physical and medicobiological indices being tested that were recorded by NMS methods. The nature of this effect is approximately the same for different instruments and diagnostic NMS methods. As an example, in Fig. 2 we present curves of the fluorescence of the skin of the finger cushion in two cases: without induced external pressure (the probe is fastened to the mounting) and with a sufficiently strong, induced pressure of 10^4 Pa. Excitation of fluorescence was initiated by a laser with wavelength 532 nm. An increase in the fluorescence signal by a factor of 2–3 can be clearly seen with the application of an external pressure. Synchronized with the amplitude of the fluorescence signal I_F there is an increase in the amplitude of the back-scattered radiation I_L in the fluorescence excitation lines. However, the expected distortion of the form of the fluorescence signal contour in these experiments was bearly noticeable. Nevertheless, normalization of the fluorescence lines I_F to the amplitude of I_L for calculation of the coefficient K_f all led to significant differences, almost by a factor of two, by the numerical values of K_f. This indicates that distortion of the spectrum (see Fig. 2) still occurred but mainly in the I_L lines, which increased slightly less than the fluorescence lines I_F.

We compare the results obtained with similar known results, e.g., those presented in [7, 8]. This is interesting from the point of view of not only the reliability of the results obtained but also their reproducibility in different setups and different associations of researchers, since interlaboratory comparison and reproducibility of measurement results are also one of the most important and indeterminate metrological problems in NMS. In order to carry out such a comparison we constructed the amplitude of the signal of fluorescence I_F of the skin of the finger cushion recorded on a LAKK-M unit as a function of the applied external pressure on the gage for the fluorescence line 460 nm with excitation of fluorescence at the 375-nm line (Fig. 3). Similar data are represented in [7]. We observe a practically complete agreement of the results, except for the absolute value of the recorded signal. In the experiments carried out, we recorded the amplitude of the signal I_F , which was several times greater than the results of [7], which is evidently a consequence of the different sensitivity of the apparatus used. The LAKK-M unit used in the investigations described above has a sensitivity that is an order of magnitude greater. The general tendency of the distortions of the results with an increase of the applied pressure is similar, but differs by a factor of 5–6 in comparison with the initial levels of I_F.

Results repeating what was indicated above and the a priori logical thesis about the squeezing of blood from the examination region with application of an external pressure on the gage, based on which we can estimate the approximate volume of squeezed blood, were also obtained using Spektrotest oximeters (Fig. 4). The practically completely identical results of two repeated measurements confirms the objectivity of the process being observed, its reproducibility, and the possible complete loss of blood in the examined volume of the BO with application of a pressure of 10^4 Pa. The smooth drop in the indices over 5–10 sec confirms the inertial and dynamic nature of this process. Parallel detailed results were also obtained

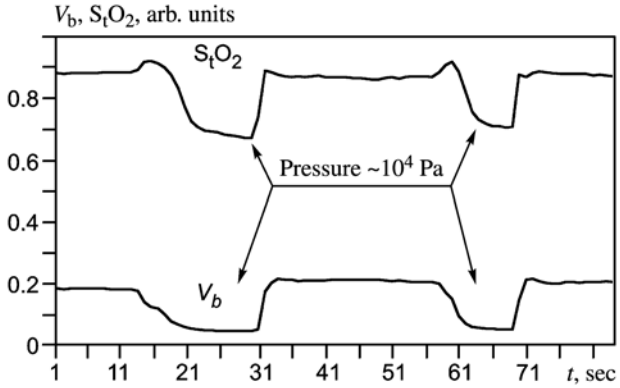

Fig. 4. Experimental data obtained by the OTO method, with external pressure of order 10^4 Pa at the instrument gage.

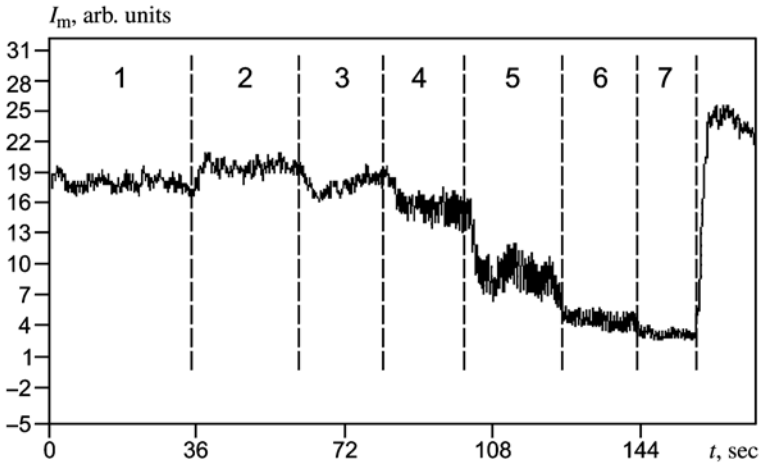

Fig. 5. Experimental data obtained by the LDF method for different external pressure p at the instrument gage: *1*) initial level, pressure absent; *2–7*) for p equal to $5.8 \cdot 10^3$, $1.1 \cdot 10^4$, $2.3 \cdot 10^4$, $4.4 \cdot 10^4$, 10^5, $1.5 \cdot 10^5$ Pa, respectively.

by the LDF method. Figure 5 demonstrates a typical dependence of the recorded index of microcirculation I_m on the applied external pressure in one experiment with one using a LAKK-01 instrument ($\lambda = 632$ nm). As follows from the curve, with an initial small external pressure, the index I_m first increases by 10% (zone 2). With close examination, this effect can also be seen on the graph for the parameter of tissue saturation S_tO_2 (see Fig. 4). This result can be combined with the fact that from the compressed volume of the tissue there is primarily a displacement of venous blood, which has a large absorption coefficient in comparison with arterial blood for the range 630–640 nm. Accordingly, there is an increase in the mean parameter S_tO_2 and a scattered radiation component of moving blood, which the LAKK-01 instrument identifies as an increase in the number of erythrocytes in the examination zone and a proportional increase in the calculated I_m. Furthermore, in pressure zones 4 and 5, we observe a sharp increase in the rhythmic component of the blood flow. Thus a change in the blood filling owing to the applied external pressure can have a considerable effect on all the indices of blood microcirculation in the examination zone, being recorded by NMS methods, i.e., regulation and standardization of the gage pressure on the tissue of the patient is very important for attaining uniformity in the measurements and minimization of the errors in the diagnosis.

As a result of the investigations carried out, we established the following principal medicobiological factors and phenomena – the sources of errors in the diagnosis, most strongly affecting the measurement results in NMS and their scatter:

1) factors and phenomena due to the interaction of the measurement agents and the BO, as a result of which there is a change in the properties of the BO and there arises an interactive component of the error, which can total 85–90% of the total value of the combined instrumental and methodological errors in the diagnosis;

2) nonuniformity of the examined object at the level of the anatomic-morphological structure of soft tissues and the system for the microcirculation of blood, which for a small shift of the instrument gage often leads to considerable scatter of the measurement results, not owing to the instrumental errors but rather owing to the different opticophysical properties of the BO for distances equal to the shift of the instrument probe;

3) the complex structure and functional organization of the real BO (including the presence of mucus, bile, fatty deposits, and perspiration, and of various natural chromophores and fluorochromes in the tissues), incomplete agreement of which in the assumed simplified physicomathematical model of the BO, in each specific measuring process determine systematic errors (shifted estimates) for operation of the computational algorithm of the instruments; and

4) the subjective error and operator (physician) mistake, connected with such individual features of medical personnel as the degree of professional preparedness, attentiveness, concentration, accuracy, and satisfaction of all procedures.

The general indeterminacy of the measurement results in the clinic, if it is estimated according to the obtained scatter of the results of multiply repeated statistical tests, can attain ±(30–40)% based on the level of the root-mean-square deviation σ from the mean value of each measured medicobiological parameter in NMS. If we assume that the confidence probability of the error-less result is 95%, i.e., the result is estimated according to level 2σ, then the scatter of the indices in the norm increases up to ±(70–80)%, which superimposes definite constraints on the interpretation of the results of the differential diagnosis in terms of the norm – the pathology for each separate patient.

The effect of other biomedical or organizational-clinical factors and phenomena, e.g., involuntary movements of the patient during the examination, external, strong lighting in the zone of the operational field, etc., was deemed unimportant at the given stage of the investigations. All these factors can easily be removed by the physician-operator when the gage is prepared for the measurements or directly at the stage of the examination by repeated measurements, if, e.g., the patient involuntarily twitches at the moment when the index is recorded. The given phenomena either are unimportant and contribute to the general measurement error less than 1–2% or are connected with the qualifications and professional preparation of the medical personnel.

As a general conclusion based on the results of all the investigations, we can note the following: A key problem was a maximally broad search and analysis of all the principal physicotechnical and biomedical factors and phenomena, most strongly affecting the metrological characteristics of the methods and instruments in the new area of medical diognostics – NMS. At the first stage of investigations, we considered physicotechnical factors and phenomena for carrying out model measurements on nonbiological SMs. The second stage of the investigations was devoted to search and analysis of the principal medicobiological and organizational-clinical factors and phenomena for natural measurements in the clinic. A comparative analysis of the stages enables us to separate, systematize, and analyze all the principal factors and phenomena, and also estimate the approximate levels of indefinite measurement results in the clinic, which, based on the research results, can reach the level 70–80% of the measured quantity. Such a complex metrological problem in modern NMS, in the opinion of the article authors, has been formulated and solved for the first time in the world. Its solution expands knowledge in this area of metrological science and medical techniques and serves to further the scientific-methodological foundations for development of concrete metrological provision of medical diagnostic methods, instruments and devices of the given class.

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