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LASER FLUORESCENCE ANALYSIS METHOD USE FOR EXPRESS-DIAGNOSIS OF ANTIBIOTIC RESISTANCE AMONG ABDOMINAL SEPSIS PATIENTS POST-OPERATION

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The adequate method of antibiotic choice is reported using the laser fluorescence analysis of blood plasma with the laboratory diagnostic device "Spectrolux-MB". The method is based on the ability of micro-organisms and products of their vital functions (metabolism) to fluoresce under laser radiation. The method of fluorescence analysis has great prospects in urgent surgery and other medicine brunches due to its high information content, low cost (compared to other methods of express-diagnostics) and high speed of information acquisition.

Keywords: Fluorescence; antibiotic; express-diagnosis; sepsis.

1. Introduction

One of the most difficult surgery problems is the treatment of abdominal suppurative diseases and their complication. In the structure of the surgery diseases, the peritonitis and the destructive lesions of abdominal organs are the dominant causes. Moreover, recently we have had to stuck with the neglected forms of these diseases.[1] Local inflammation, sepsis, heavy sepsis and multiorgan deficiency are the one chain links in the organism reaction on the inflammation because of microbe infection.[2]

The abdominal surgery infection can be considered as a set of the nosological forms in clinical

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medicine which reflects complicated urgent surgery diseases and lesions at the position of the general clinical problem of sepsis. As it is known, the abdomen responds with the inflammation to any pathological processes induced by infectious and inflammatory or traumatic destruction of abdominal organs and pelvis. In this case, the whole abdominal surface area (nearly 2 sq m), its complex structure, reactivity, and the efficiency of physiological functions — exudative, resorptive and barrier — are in danger of expanded abdomen inflammation for organism life.[3]

Despite achievements in intensive therapy and pharmacology, the lethality from the peritonitis and pancreonecrosis has leveled down to 30−40% but not shown to further decrease in the last 20 years.[2]

Compulsory requirements of complex treatment of abdominal sepsis are based on the principles of etiotropic, pathogenetic direction, whose aim is the elimination of abdominal infection, removal of endotoxin, and multiorgan deficiency.

A destructive lesion of abdominal organs is an abdominal infection disease, often regarded as incurable sepsis. Due to this, the role of the efficient antibacterial therapy is preferred as it is able to prevent the (a) infection generalization, and (b) development of different complications after operation and fatal multiorgan deficiency.[3] Conception of modern surgery of patient treatment with abdominal sepsis demands either adequate surgery allowance or the early rational antibiotic therapy, its monitoring, level of endotoxicosis identification, and microbiological monitoring of abdominal surgery infection. Microbiological sepsis diagnosis is defined in the choice of the adequate regimes in antibacterial therapy.

The results of the etiotropic sepsis therapy are much better than the empirical one.[2] One of the fundamental moments in patient treatment with abdominal sepsis is the rational antibacterial therapy during the early stages post-operation. Traditional methods of antibiotic selection are quite long and sometimes allow valuing antibacterial therapy *post factum*. At the post-operation period we are stuck with the problematic choice — the empirical appointment of antibacterial therapy without sensation confirmation by microbiological service. Providing antibacterial therapy is not often adequate and in its turn can cause post-operation complications either systematic or localized, high expenses, increase in stay period in the hospital, and lethality increase. Antibacterial sepsis therapy is provided to achieve stable positive dynamic of the patient condition and disappearance of main infection symptoms. Due to the lack of bacterial infection and pathognomonic signs, it is problematic to define absolute termination criterions of antibacterial therapy. As a rule, the decision of therapy termination is taken individually after complex observations on the patient condition. Adequate antibiotic therapy should provide the following requirements:

— positive dynamics of main infection symptoms;
— the absence of inflammation reaction signs;
— normalization of digestive tract function;
— leucocytes number normalization in blood and leucocytic formula; and
— negative microbiological culture.

The existence of the only one bacterial infection sign is not the absolute reading to continue antibacterial therapy. Isolated low-grade fever (maximum day temperature within 37.9°C) without chills and changes in peripheral blood can occur after infection asthenia manifestation or nonbacterial inflammation after operation, and does not demand antibacterial therapy as well as preservation of a moderate leukocytosis at the lack of leucocytic formula shift into the left and some other bacterial infection signs. Traditional duration of antibacterial therapy is from 5 to 10 days. Much longer antibiotic therapy is not appropriate because of some possible treatment complications, risk selection of resistant strains and development of super-infection.

We are concerned that effective medicine and express diagnostic protocols of purulent surgery infection, its clinical treatment, rational antimicrobial medicine usage allow decreasing the frequency of purulent-inflammatory diseases and in turn leads to a decrease in the duration of hospital service (the highest expenses for medical help) and decreased illness and lethality rate.[4] The microorganisms resistance to antibiotics is more efficient to the microbes groups with the antibiotic resistant strains. On the other hand, their spread causes the decrease of efficiency of antimicrobial medicines.[5]

The stream line is the problem of antibiotic resistance. In many cases, last generation of antibacterial medicine becomes ineffective because of developed resistance to them, which is frequently related to unprofessional therapy choice.

The results of purulent-inflammatory diseases treatment are often unsatisfactory: slow course, relapses, super-infections, resistance to antibiotics, and, sometimes, lethality, for example, severe sepsisof pancreasand others. The medicine is found empirically when it comes to antibiotic resistant strains formation. It is, therefore, important to develop a new clinical microbiological method for choosing antimicrobial medicine.[6]

2. Materials and Methods

The choice of adequate antibiotic treatment suggested in the present paper is based on the fluorescence of blood plasma acquired with the use of the laboratory diagnostic device "Spectrolux MB" developed in Scientific Industrial Center of Medical and Industrial Biotechnologies "Spectrolux", Moscow.[7,8] He−Ne laser radiation with wavelength of 633 nm is directed to the investigated sample with the dual-channel light-guide. Laser radiation is either reflected from the sample surface or causes the laser-induced fluorescence(LIF). This secondary radiation is captured by the adoptive light-guide fiber and is directed to the dispersion knot and multi-channel photodetector. Data are displayed on the computer monitor as an LIF spectrum. To trace the sensitivity to antibiotics, we use the microorganisms' and their life products' ability to fluoresce under laser irradiation with dependence on their peculiarities and physiological processes activity. Patients have various sensitivities to the same antibiotic and to the same type of microbes. This is one of the advantages of fluorescence analysis to mark the reaction of organisms with the adequate antibacterial treatment and its influence on microflora. It allows estimating the treatment which starts with the early antibiotic sensitivity determination for each individual patient.

The method includes the blood sampling from a patient vein and antibacterial therapy assignment that requires a volume of 25 ml. After that, the patient is provided by essential operative assistance. The purulent center is sanitized.

The intensity of fluorescence spectrum depends on the number of live micro-organisms in blood plasma. It is the peculiarity of this diagnosis method. When the blood plasma is irradiated by the red light with the wavelength of 633 nm, the fluorescence feedback is given by the porphyrins, which are the products of metabolism of live microorganisms. This fact is confirmed by the results of microbiological analysis.

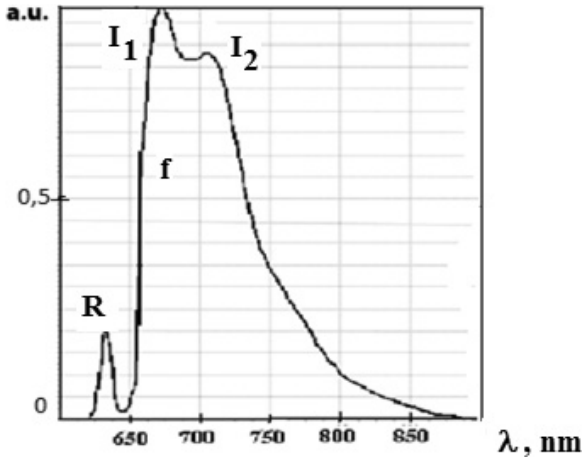

Fig. 1. Typical spectrum of LIF of the blood sample. Excitation with a He−Ne laser at wavelength of 633 nm.

Typical fluorescence spectrum is presented in Fig. 1.

Fluorescence spectrum consists of two peaks I_1 and I_2 with some fraction of reflected laser radiation R. The spectrum shown in Fig. 1 allows identifying the following fluorescence indicators

- $F = I_f$, integral intensity of fluorescence.
- $K_f = F/R$, fluorescence normalized indicator.
- $k_a = (I_1 - I_2)/(I_1 + I_2)/2$, fluorescence intensity proportion of I_1 and I_2 (aerobic), where:
- I_1, integral intensity of peak 1; I_2, integral intensity of peak 2.

These fluorescence indicators allow rapid tests on the efficiency of various antibiotics. The test was performed in the following way.

The tube with test blood is centrifuged within 5 min, and then the obtained plasma is allocated into 10 cuvettes. Antibiotics, which are often used in treatment, are diluted by the prescription in calculated min and max concentrations, are added to the investigated samples. One cuvette remains without any additions and serves as areference. After that, the fluorescence spectrum indicators are measured within 2−4 h with 30 min break in automatic regime. The temperature was kept at 37°C with the help of special thermostat.

The device "Spectrolux" automatically generates the analysis protocol of antibiotics efficiency after the measurement procedure of the fluorescence response. The protocol includes the patient's data:

gender, age and preliminary diagnosis. Moreover, there are the dependence graphs of F, K_f, and K_a of the time, which are used to make the conclusions. The idea is as follows: the most efficient antibiotic causes the highest decrease of LIF intensity in the probed plasma.

As an example in Figs. 2–4 these functions are presented for six different antibiotics for one patient.

The largest decrease of integral intensity of fluorescence is observed under action of both abactal and gent.

The same conclusion is supported by the dependence of normalized fluorescence on time. Finally, the dependence of fluorescence intensity proportion k_a shows much higher efficiency of abactal compared to other tested antibiotics.

Logarithmic calculations of the spectrometric curve allow concluding over the degree of intoxication and sensitivity to the certain antibacterial medicine. We consider that the most effective drug is abactal after results of this graph. Indeed, the addition of this antibiotic decreases the LIF signal. This means

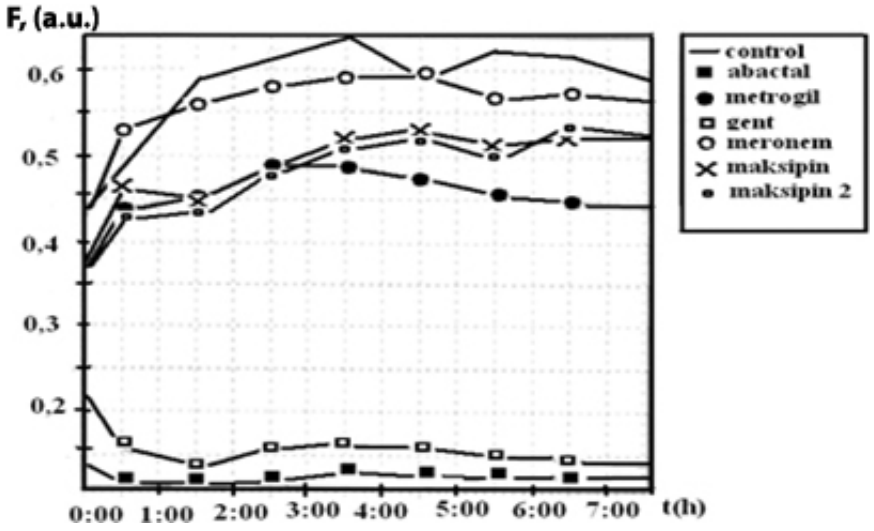

Fig. 2. Dependence of integral intensity of fluorescence F on time. The inset shows the symbols corresponding to different antibiotics.

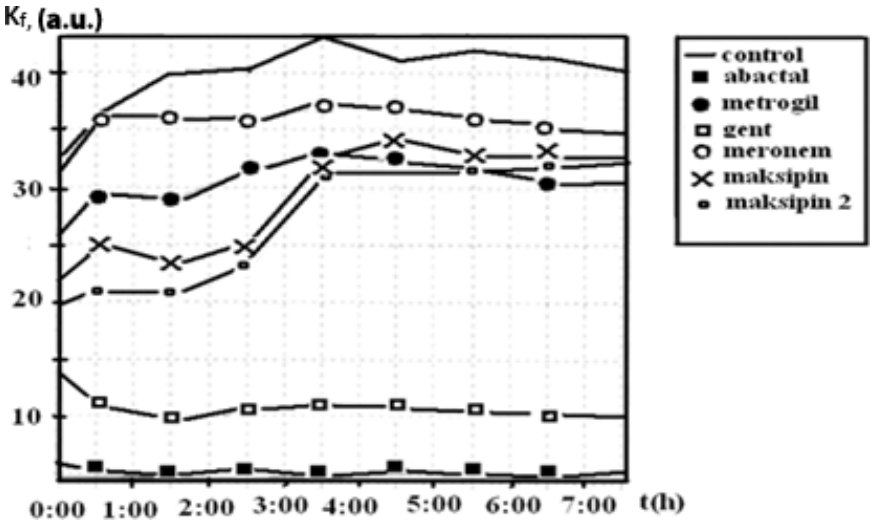

Fig. 3. Dependence of normalized fluorescence indicator $K_f = F/R$ on time. The inset shows the symbols corresponding to different antibiotics.

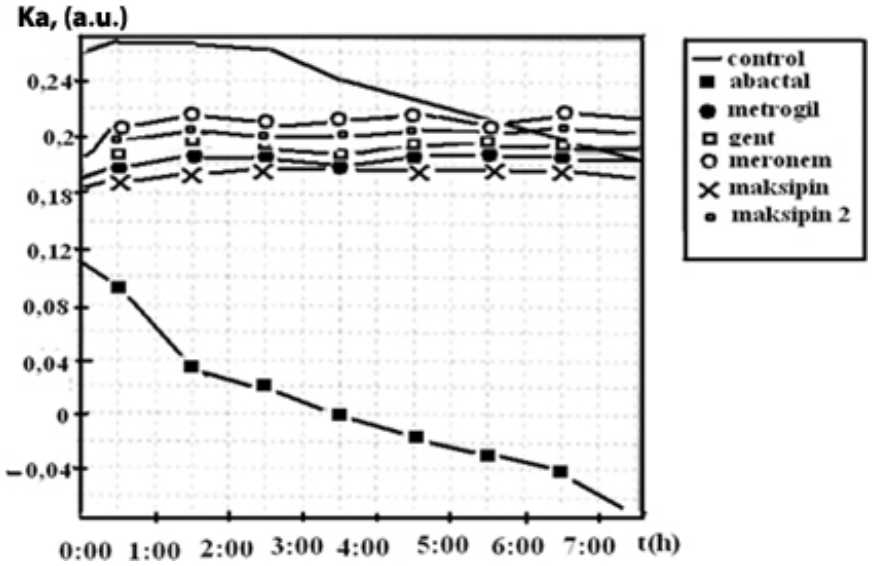

Fig. 4. Dependence of fluorescence intensity proportion $K_a = (I_1 - I_2)/(I_1 + I_2)/2$ on time. The inset shows the symbols corresponding to different antibiotics.

that both the microbes and the products of their metabolism disappear from the sample under treatment with this antibiotic.

The decrease in fluorescence level indicates the adequacy of chosen antibacterial drug, while the lack of changes in LIF or its increase with time points its resistance to tested antibiotics. Obtained results are confirmed clinically and by a microbiological research.

3. Results and Discussion

Based on the Department of General Surgery (City Clinical Hospital N23 by "Medsantrud"), the prospective multifactorial analysis of fluorescence method for antibiotic resistance express-diagnosis among peritonitis and abdominal sepsis patients was obtained. There were in total 53 operated patients with various surgery pathologies in the investigated group.

All patients were selected by gender, age, associated diseases, surgery pathology, and number of transfer surgeries. Condition of severity (assessment of organ and systemic disorders) on admission was evaluated on the scale SOFA. Estimating the patient population with sepsis is heterogeneous either by character and severities of homeostasis disorders or by age and comorbidities (it depicts on the pathological process course). It is necessary to mark the general condition severity (evaluated on the scale APACHE III).

It was found that on the first day after operation, the average APACHE III was 38.4% among patients with acute appendicitis; with pancreonecrosis — 61.6%; gastric perforation — 48.5%; acute destructive cholecistis — 59.2%; segmental mesenteric thrombosis — 78.4%; intestinal obstruction — 90.4%; perforation of diverticulum with abscess — 52.4%.

The method was tested on patients of different ages and genders with abdominal sepsis signs of varying severity (conciliation conference of 1992 ACCP/SCCM), demanded operation treatment, adequate antibacterial therapy appointment.

From each patient blood sampling was taken three times: at the checking in before the operation, after the operation, and in two days, to estimate the provided antibacterial therapy efficiency. If necessary there was an extra blood sampling for the antibacterial therapy correction.

There was the parallel microbiological research of the peritoneal exudate. It was obtained at the perforation of gastric ulcer with limited serosal peritonitis — enterobacterias, pseudomonadas, aerobic coccus; at the acute gangrenous appendicitis and perforation of the colon — staphylococcus, streptococcus, enterococcus, colon bacillus, enterobacterias; at the pancreonecrosis — pseudomonadas, intestinal colic, enterococcus. Microbial associations were marked among 20% patients.

When the patient with clinical-instrumental picture of pyoinflammatory diseases is admitted into the surgical department, he is involved into the research with his agreement. The blood sampling of

25 ml is done. After centrifugation plasma is allocated into the spectrometric cuvette. One is kept as control. The solution of antibacterial preparation is estimated by the data of pharmacokinetics and pharmacodynamics. The concentration of antibacterial preparation in the solution does not increase maximal concentration in blood plasma (*in vivo*). Then we carry out the research and analysis of spectral efficiency graphs. It is obligatory to carry out prolonged monitoring (24 h) — measurement of fluorescence data in equal time intervals. With the measurement results, we make a conclusion of the antibacterial preparation efficiency for the patient under treatment.

The antibiotic choice by the fluorescence method analysis is based on the change in monitoring of fluorescence efficiency of blood plasma by antibiotic activity in comparison to the control plasma sample without antibiotic. In urgent surgery pathology (estimating use antibiotic recommendations in pyoinflammatory diseases) the antibacterial preparation choice for the research is done by one of the following groups: beta-lactams, aminoglycosides, nitroimidazols, fluoroquionolones. The antibiotic choice by the fluorescence method analysis is recommended with pharmacokinetics features estimation, the length of partial ejection T of more than 1 h, less blood protein binding, maximal accumulation in blood plasma within 2−24 h, parenteral mode of introduction, wider action spectrum (especially for frequent causative agents of hospital infection). To accept the influence on the fluorescence spectrum of antibacterial preparations, we carry out the antibiotic solution measurement in the given concentration with the saline at the same exposure (an extra control).

The choice of the effective antibiotic is obtained by the following way: microflora is supposed to be sensitive to the antibacterial preparation if there is normalized fluorescence intensity decrease K more than 20% under its activity. For antibiotic resistant microflora, the fluorescence intensity of blood plasma does not change within the time or increases with respect to the control spectrum. In this case, it is necessary to correct the concentration of input preparation or to replace it.

All researched patients were taken for the interoperation blood sampling of peritonial solution for the microbiological seeding to define the etiological factor and to define the antibacteriogram by the results of the seeding. The informative seeding results were in 54% cases. The microflora increase was not defined in other cases. By the analysis of spectral fluorescence characteristics 86% cases of mixed-infection was obtained. The clinical efficiency of antibacterial therapy by the fluorescence method and antibacterial preparation prescription was achieved in 74% cases. In 26% cases it was necessary to repeat the analysis and antibacterial therapy.

4. Conclusion

The express method for the antibiotics efficiency determination by laser fluorescence diagnosis of blood plasma has been offered. We have described the analysis method and the device scheme for measurement. The clinical trials of the method have been carried out. The conducted prospective research with fluorescence diagnosis method allowed confirming the mixed infection in 86% cases; the microbiological exudate seeding results were not informative in 46% cases among these patients. The clinical efficiency of conducted antibacterial therapy with the fluorescence plasma results was achieved in 74% cases (excluding the patients with developed complications). This allows considering the method as the promising direction in the optimal antibacterial therapy development among patients with abdominal sepsis and demands to carry on some researches for the further increase of efficiency.

The fluorescence analysis method of antibiotic efficiency has shown its productivity and significance among the patients with pyoinflammatory infection in the abdominal surgery. It was confirmed microbiologically and clinically. The method allows matching adequate and effective chemotherapy within 3−4 h since the patient checking-in the hospital. Standard microbiological analysis usually requires 2−4 weeks. Therefore, LIF of blood plasma has enormous advantage over classical method. It improves the treatment results and decreases the patients' stay in the hospital.

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