

ORIGINAL ARTICLE

AN INTEGRATED APPROACH TO THE DIAGNOSIS OF BACTERIAL AND FUNGAL BLOODSTREAM INFECTIONS IN CANCER PATIENTS

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ABSTRACT

Purpose of the study. To evaluate the diagnostic significance of accelerated and affordable verification of a bloodstream infection pathogen using biomarkers: procalcitonin and the Platelia™ Candida Ag Plus mannan antigen.

Patients and methods. 349 cancer patients with febrile fever were examined from 6 medical and diagnostic oncological hospitals in the Southern Federal District of the Russian Federation during 2019. Patients aged from 1 to 85 years were hospitalized in intensive care, pediatric oncology and hematology oncology departments. Patient informed consent for the study was obtained. The diagnostic algorithm included: a blood test using an automatic BacT /ALERT 3D analyzer and a parallel study of the level of biomarkers with enzyme immunoassay. Identification of strains and determination of sensitivity to antimicrobial agents was determined on a Vitek 2 automatic analyzer (BioMerieux, France). Procalcitonin levels greater than 10 ng/ml were registered to determine the development of bacterial inflammation. Procalcitonin was determined with Procalcitonin – ELISA-BEST kits (Russia). Mannan antigen was determined using Platelia Candida Ag kits (France). The result was considered positive at the antigen concentration of ≥ 125 pg/ml. Candida mannan antigen allowed us to decide on the involvement of *Candida spp.* in the infectious process.

Results. An integrated approach to the diagnosis of bloodstream infections increased the percentage of detection of pathogens up to 58.7%. Bacterial infection testing both with the blood culture method and the procalcitonin determination in blood serum revealed similar diagnostic values. Candida mannan antigen testing significantly improved the early diagnosis of Candidal infection, despite negative blood culture, which was probably associated with prolonged cultivation of *Candida spp.* in the blood (from 2 to 5 days). The inclusion of biomarker testing in the diagnostic algorithm in cases of suspected bloodstream infection allowed early pathogen identification and starting an adequate antibacterial or antifungal therapy.

Conclusion. An integrated approach to the diagnosis of bloodstream infections improved and, just as importantly, significantly accelerated the pathogen verification. Bacterial infection cases showed comparable results of hemoculturing and biomarker testing; however, in case of candidal infection, determination of Candida mannan antigen appears critical, as it was significantly more sensitive than the result of blood culture and allowed to identify the etiology of fever of unknown origin in many patients.

Keywords:

procalcitonin, mannan, bloodstream infection, *Candida spp.*, diagnosis of sepsis, biomarkers.

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КОМПЛЕКСНЫЙ ПОДХОД К ДИАГНОСТИКЕ БАКТЕРИАЛЬНЫХ И ГРИБКОВЫХ ИНФЕКЦИЙ КРОВОТОКА У ПАЦИЕНТОВ ОНКОЛОГИЧЕСКОГО ПРОФИЛЯ

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РЕЗЮМЕ

Цель исследования. Оценить диагностическую значимость ускоренной и доступной верификации возбудителя инфекции кровотока с помощью биомаркеров: прокальцитонина и маннанового антигена Platelia™ Candida Ag Plus.

Пациенты и методы. Обследовано 349 онкологических больных в течение 2019 года с фебрильной лихорадкой из 6 лечебно-диагностических стационаров онкологического профиля Южного Федерального округа Российской Федерации. Пациенты в возрасте от 1 года до 85 лет находились в отделениях реанимации, детской онкологии и онкогематологии. Информированные согласия пациентов на исследования получены. Диагностический алгоритм включал исследование крови с помощью автоматического анализатора BacT/ALERT 3D и параллельное исследование уровня биомаркеров иммуноферментным методом. Идентификацию штаммов и их чувствительность к антимикробным препаратам проводили на автоматическом анализаторе Vitek 2 (BioMerieux, Франция). Одновременно определяли уровень прокальцитонина с помощью наборов Прокальцитонин – ИФА-БЕСТ (Россия) и маннанового антигена *Candida spp.* – Platelia Candida Ag (Франция) соответственно. Учитывали значения прокальцитонина более 10 нг/мл для определения развития бактериального воспаления. Результат определения маннанового антигена расценивался как положительный при концентрации ≥ 125 пг/мл. Маннановый антиген *Candida spp.* позволил судить о причастности к инфекционному процессу грибов *Candida spp.*

Результаты. Комплексный подход к диагностике инфекций кровотока увеличил процент выявления патогенов до 58,7%. Следует принять во внимание, что тестирование бактериальной инфекции как методом гемокультивирования, так и методом определения прокальцитонина в сыворотке крови выявило их почти одинаковое диагностическое значение. Тестирование маннанового антигена *Candida spp.* значительно улучшило раннюю диагностику кандидозной инфекции несмотря на отрицательную гемокультуру, что, вероятно, связано с длительным культивированием *Candida spp.* в крови (от 2 до 5 суток). Включение тестирования биомаркеров в алгоритм диагностики при подозрении на инфекцию кровотока позволило выявить возбудителя как можно раньше и начать адекватную антибактериальную или противогрибковую терапию.

Заключение. Комплексный подход к диагностике инфекций кровотока позволил улучшить и, что не менее важно, существенно ускорить верификацию возбудителя. Если в случае бактериальной инфекции результаты гемокультивирования и тестирования биомаркеров были сопоставимы, то в случае кандидозной инфекции ключевым в диагностическом плане представляется исследование маннанового антигена *Candida spp.*, т.к. именно оно оказалось значительно чувствительнее, чем результат посева крови, и позволило идентифицировать этиологию лихорадки неясного генеза у существенной части пациентов.

Ключевые слова:

прокальцитонин, маннан, инфекции кровотока, *Candida spp.*, диагностика сепсиса, биомаркеры.

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INTRODUCTION

Worldwide, cancer is the second leading cause of death and it kills about 8.8 million people every year, according to statistics from the World Health Organization (www.who.int/mediacentre/factsheets/fs297). Severe bloodstream infections and sepsis complicate treatment and the outcome of recovery, antitumor treatment, and have a significant negative impact on the life expectancy and cost of treatment of cancer patients. Rapid diagnosis of sepsis and initiation of treatment are key factors in reducing mortality in cancer patients [1–5]. Infection-mediated mortality during chemo-induced immunosuppression is an urgent issue that requires studying risk factors and developing strategies to reduce mortality by optimizing diagnostic methods and accompanying therapy [6]. In the etiology of bloodstream infection, both gram-positive and gram-negative bacteria are most common. Yeast-like fungi of the genus *Candida* play a significant role. Using modern equipment to get the pathogen from the blood is still problematic. In addition, it takes from a day to several days. In this situation, biomarkers are an objective and reliable way for a clinician to quickly respond to the possible development of an infectious complication [7–8].

It was discovered by chance that bacterial infection increases the concentration of procalcitonin in the blood. This contributed to the procalcitonin usage as a marker of bacterial infections. In contrast to all known markers of inflammation, the method for determining procalcitonin is more sensitive and highly specific for severe bacterial infection [9–14]. Yeast fungi of the genus *Candida spp* are one of the most common pathogens of invasive mycoses. Diagnosis of invasive candidiasis is difficult due to the non-specific clinical symptoms and insufficient sensitivity of the hemocultivation method. One of the available biomarkers of invasive candidiasis that allow us to judge the presence of invasive candidiasis is mannan, one of the *Candida spp.* antigens, which is a soluble polysaccharide bound to the walls of yeast cells [15].

So, the development and application of new and improvement of existing methods for determining the pathogen, which allow to accelerate and clarify the etiological factor of bloodstream infection for the earliest possible start of specific treatment, is an urgent task of modern medicine.

The purpose of the study: to evaluate the diagnostic significance of accelerated and accessible verification of the bloodstream pathogen using biomarkers: procalcitonin and mannan antigen.

PATIENTS AND METHODS

349 cancer patients with febrile fever from 6 medical and diagnostic hospitals of the southern Federal district of the Russian Federation were examined during 2019. Patients (men and women) aged from 1 to 85 years were in intensive care units, pediatric oncology and oncohematology departments. There are informed patient consents for research.

The diagnostic algorithm included a blood test using an automatic bact/ALERT 3D analyzer. Two sets of vials were used for one septic episode. Each set included: for patients with a body weight of more than 36 kg: a bottle for aerobic and anaerobic cultivation and a volume of 10 ml of blood in each bottle. For children with a body weight of up to 36 kg (inclusive), two pediatric vials and a volume of blood from 0.5 to 5.0 ml per vial, also depending on body weight. Each blood culture was accompanied by a parallel study of the level of biomarkers by the immune-enzyme method. Identification of strains and their sensitivity to antimicrobial agents was performed using an automatic Vitek 2 analyzer (BioMerieux, France). Simultaneously with the seeding, a sample was taken into a vacuum tube for an enzyme-linked immunoassay (determination of the level of procalcitonin and the mannan antigen *Candida spp*). Procalcitonin values of more than 10 ng/ml were taken into account to determine the development of bacterial inflammation. The *Candida spp.* manann antigen, as one of the available biomarkers of invasive candidiasis, allowed us to judge the involvement of *Candida spp* fungi in the infectious process. The result was considered positive at an

antigen concentration of ≥ 125 PG/ml. Given the low specificity of the study of the *Candida spp.* manann antigen, the results were compared with risk factors for the development of invasive candidiasis (perforation or surgery of the gastrointestinal tract, infected pancreonecrosis, Central venous catheter, broad-spectrum antibiotics, diabetes mellitus, complete parenteral nutrition, severe patient condition, steroids, immunosuppressors, acute renal failure, colonization of *Candida spp.* more than 2 loci) [16]. The level of procalcitonin was studied using Procalcitonin-ELISA-BEST (Russia). Determination of manann antigen – using kits Platelia Candida Ag (France). Statistical data processing was performed using the statistical package STATISTICA 13.3 (StatSoft Inc., USA). Pearson's Chi-square test was used to compare the data.

The results of the study and their discussion

As a result of the microbiological study, positive blood cultures were obtained in 84 patients, which was 24.1%. Pathogens were distributed as follows: bacteria made up 73.8% (65 strains), yeast-like fungi of the genus *Candida spp.* they made up 22.6% (19 isolates). Bacterial-Candida associations were detected in 3 (3.6%) cases in particularly severe

patients, which significantly worsened the condition of patients.

In a parallel study of biomarkers (procalcitonin and manann antigen *Candida spp.*), an increased level of one of them was found in 205 (58.7%) patients (Fig.1). Procalcitonin values of 10 ng/ml or more were observed in 68 (33.2%) patients, which indicated in favor of severe bacterial inflammation. A positive result of the *Candida spp.* manann antigen was found in 118 (57.6%) patients. The results allowed us to suggest Candida infection of the bloodstream in the presence of appropriate clinical signs and risk factors for the development of invasive candidiasis. In 19 (9.2%) patients, two biomarkers were elevated, indicating a possible mixed infection. The results obtained when comparing the informative characteristics of the two methods for diagnosing blood flow infection showed statistically significant indicators ($p < 0.0001$).

The results obtained made it possible to optimize treatment tactics and start adequate etiotropic therapy in a timely manner.

In a comparative analysis of the study using biomarkers and hemocultivation for bacteria, almost comparable values were obtained: positive hemoculture in 65 (73.8%) patients and 68 (33.2%) patients with procalcitonin levels of 10 ng/ml or

Table 1. Characteristics of patients

Characteristics		Abs. numbers	Percentage
Sex	Male	140	40.1
	Female	209	59.9
Age, years	1-7 years	5	1.4
	8-12 years	12	3.4
	13-17 years	42	12.0
	18-40 years	40	11.4
	41 and more	250	71.6
	oncohematological	212	60.7
Leading disease	tumors of the gastrointestinal tract	89	25.5
	Lung Tumors	48	13.8
The management	Surgical	122	35.0
	polychemotherapy	227	65.0

more. According to Pearson's Chi2 criterion, the difference was significant, $p=0.0001$. Slightly different results were obtained for the yeast-like fungi *Candida*. *Candida* from the blood was isolated in only 19 (9.2%) patients, at the time, as the level mannopova antigen R. *Candida* was elevated in 118 (57.6%) patients (according to Pearson's Chi2 criterion, the difference was significant, $p=0.0006$). Taking into account the clinical manifestations and risk factors, despite the negative result of blood seeding, which was probably due to long-term cultivation of *Candida spp.* antifungal therapy was prescribed to all patients, with the positive dynamics. Positive results with the use of biomarkers suggested the presence of bacterial-Candida associations in 19 (9.2%) patients, while associations were obtained only in three (according to Pearson's Chi2 criterion, the difference in this case was not significant, $p=0.12$). All these patients were in the departments of anesthesiology and intensive care after surgery. Their condition was assessed as extremely serious. In this case, the measurement of the mannan anti-

gen *Candida spp.* compared with the risk factors for the development of invasive candidiasis, allowed suggesting an invasive candidiasis infection and prescribing an adequate timely therapy. The data obtained are shown in figure 2.

When diagnosing a bacterial pathogen of blood flow, the culture method showed an advantage and amounted to 73.8%. The use of the biomarker in this study was 33.2%. The advantage of the culture method was the identification of the pathogen and the determination of antibiotic sensitivity. However, the culture method for diagnosing the bacterial pathogen was significantly comparable with the results of the study of the level of procalcitonin ($p=0.0001$).

When diagnosing *Candida* infection, the best result was obtained when using the *Candida spp* mannan antigen and was 57.6%, and in hemoculture – 22.6%, which was a statistically reliable indicator ($p=0.0006$). The result can be explained by the complexity of fungal cultivation in hemoculture.

In bacterial-Candida Association, diagnosis using biomarkers was 9.2%, and in blood culture

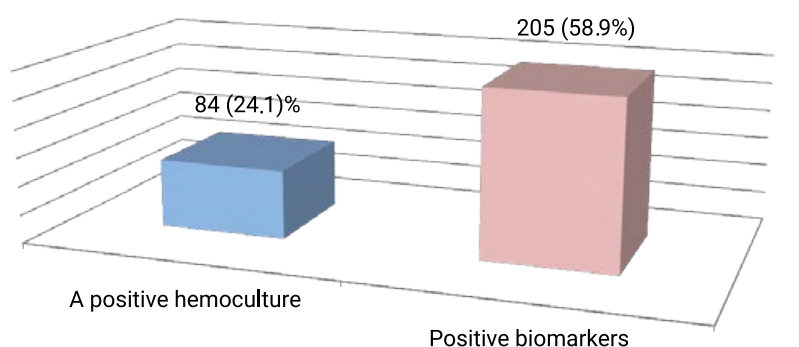


Fig. 1. Comparative characteristics of the information content of two methods for diagnosing bloodstream infection.

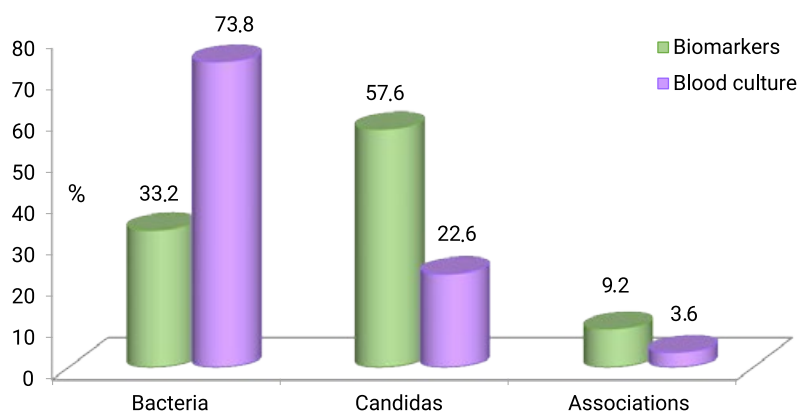


Fig. 2. Comparative values of diagnostics of bacteria and *Candida* by two diagnostic methods in percent.

– 3,6%. During statistical processing, the difference was not significantly significant ($p=0.12$). The result can be explained by a small number of cases of bacterial-Candida Association.

Additional and/or repeated clinical and laboratory tests were performed for 60 (17.2%) patients with negative hemoculture and biomarker levels within the normal range.

As a result of these studies, much later, but the pathogen was verified in 10 more patients. All 10 patients had a positive level of biomarkers, of which 4 had an increased level of procalcitonin, and 6 had positive values of the *Candida spp* mannan antigen. When repeated blood cultures were sown in 6 patients, the growth of hemoculture was also obtained (bacteria were isolated from 4 samples, and *Candida* from 2 samples).

In 26 patients with persistent febrile fever, the condition was regarded as a manifestation of cancer.

24 patients were diagnosed with fever of unknown origin (no infectious agent was detected in additional studies, and no data were found for the progression and recurrence of the cancer process).

Thus, the culture method of hemoculture research allowed us to verify the causative agent of blood flow infection in 24.1% of cancer patients during the initial study and with additional repeated

blood cultures in 6 more patients. Multiple (dynamic) studies of the level of biomarkers in this diagnosis improved the result to 61.6% with a significant difference of $p<0.0001$.

CONCLUSION

In case of bacterial infection of the bloodstream, the culture method showed an advantage in identifying the pathogen and allowed to determine antibiotic sensitivity in comparison with the use of a biomarker, but the determination of procalcitonin allowed to reduce the time for obtaining the result, which is extremely important for determining the direction of the pathogen of bloodstream infection in cancer patients.

The use of the mannan antigen *Candida spp*. in diagnostics demonstrated a significantly higher sensitivity than the result of hemocultivation, which is probably due to the extended period of cultivation of *Candida* in the blood.

An integrated approach with the study of the level of biomarkers in the diagnosis of bloodstream infections in cancer patients allowed to improve and significantly accelerate the verification of the pathogen, which in turn contributed to timely adequate antibacterial or antifungal therapy.

Authors contribution:

Kutsevalova O.Yu. – research concept and design, text writing, material processing.

Kozel Yu.Yu. – scientific editing.

Dmitrieva V.V. – collection, analysis and interpretation of data.

Kozyuk O.V. – collection, analysis and interpretation of data.

Lysenko I.B. – research concept and design, material processing.

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