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INVASIVE ASPERGILLOSIS IN PATIENTS WITH ACUTE LEUKEMIA

State Institution "National Research Center for Radiation Medicine"
of the NAMS of Ukraine, Kiev

I.S.Dyagil,Z.V. Martina, A.O.Tovstohan, S.M.Kravchenko, D.R. Afanasiadi, N.G. Romanenko, V.F. Kuzmenko, O.Y. Mishcheniuk

Summary. *The article presents clinical cases that serve as evidence that invasive aspergillosis the leading ones in the structure of mortality of patients with acute leukemia. Risk stratification of invasive fungal infections and adequate use of modern diagnostic methods provide timely verification of invasive aspergillosis. In turn, the optimized management of patients is a prerequisite for early appointment of a specific therapy improves prognosis.*

Keywords:*invasivefungalinfection, invasiveaspergillosis, acuteleukemia, diagnostic, antimycotictherapy.*

The Development of experimental and clinical hematology, also the improvement of chemotherapy and maintenance therapy led to patients with acute leukemia (AL) had an increase of the number of remission and improvement of survival. According to most international studies of AL high-dose chemotherapy is most effective and requires the use powerful broad-spectrum antibiotics, which results in increase invasive fungal infection (IFI). The latter is the leading one in causes of mortality in AL patients.

The species Candida and Aspergillus occupy a leading position in the spectrum of IFI pathogens in patients with hematological diseases. The prevalence of invasive candidiasis (IC) ranges from 8% to 18% in a cohort of patients with AL [20].

Aspergillus species are the next most common fungal pathogen after the species Candida. A few species of Aspergillus are in population of patients

encountered. Aspergillusfumigatus is the most common species recovered from cases of invasive aspergillosis (IA), frequency of it is reaching 66%, the second – A. flavus, it has been identified in 14% of cases, the third and fourth position is belong of A. niger and A. terreus and they are represent in 7% and 4% cases respectively [13]. Invasive Aspergillus occurs from 4% to 15% patients with AL [10].

The risk of IA in AL patients initially correlates with the duration and depth of neutropenia. During the first weeks of neutropenia the risk of invasive pulmonary aspergillosis is 1% per day, but increases to 4.3% per day from 24 days of granulocytopenia [1]. Association between the risk of IA and form of AL also has been detected. Patients with refractory and recurrent form of acute myeloid leukemia (AML) have higher risk of IA, compared with patients with AML de novo [21].

The recent development of new azole antifungal agents has led to growth the incidence of systemic fungal infections caused by azole-resistant species of Fusarium and Zygomycetes in the past two decades. According to the epidemiological studies GIMEMA, the Zygomycetes are the second most common mold pathogen of IFI in AL patients [12]. However, the absolute number of invasive fusariosis and zygomikozis cases remains low. According to the results of a multicenter study SEIFEM-2004, which included 11,802 patients, the Zygomycetes and Fusarium species represented only 0.1% of the total IFI cases [11].

Diagnosis of IFI still remains a challenge for clinicians. In particular, IA verification complicated by heterogeneity and nonspecific clinical picture, low pathognomonic of radiological symptoms, the failure to use the full range of mycological resource of the diagnostic.

According to the diagnostic criteria for IA in immunocompromised patients with cancer, the definitions assigned three levels of probability to the diagnosis of invasive fungal, namely, “proven,” “probable,” and “possible”. Note, that in the

world literature and practice, the term "IA" will assume a diagnostic certainty of proven or probable IA.

The definition for proven IA requires histopathological or cytological confirmation of infection and/ or a positive result of culture of a specimen obtained under sterile conditions from a normally sterile site (without Aspergillus or another fungal pathogens), that clinically or radiologically consistent with infection. That is, the implementation of microbiological criteria for the diagnosis of IA with confidence level "proven", requires for invasive traumatic procedures, that is often impossible in this population of patients. The definition of probable aspergillosis requires also requires mycological confirmation. However, with some important exceptions for diagnosis, at the first - microbiological evidence can be obtained from non-sterile material (sputum, aspirate from the sinuses, bronchoalveolar lavage) and secondly - as mycological criterion can be used surrogate marker, exactly positive galactomannan (GM) and 1-3-βglucan (1-3-βH) test [19].

Despite, the implementation of new instrumental and laboratory methods in diagnostic algorithm, and using the recommended criteria for IA verification, about 30% episodes of disease, diagnosed only after the autopsy [18].

Taking into account all of the above, the question of early diagnosis and adequate treatment strategy is an actual in AL patients who receive high-dose chemotherapy which increased the risk of infectious complications, including fungal. Therefore, consider it appropriate to bring to the discussion two clinical cases of AL patients with IA.

Case №1.

The patient C, a 25-year-old man, was first admitted to the hematology department of ICR "NSCRM of the NAMS of Ukraine" and was diagnosed with acute monoblastic leukemia, M5a. The patientfelt sick during the last month, when he began feel the weakness in the "normal" physical activity and the "heartbeat" at rest. The last three days began to bother a sore throat which accompanied by fever over than to 39.5ºC. The patient entered to a regional hospital, where general blood

test was performed, the latter had demonstrated pancytopenia. The bone marrow aspiration was performed also, blast cells totally replaced the bone marrow. Patient received symptomatic therapy and then he was transferred to the hematological department of ICR "NSCRM NAMS of Ukraine." for specific therapy.

At the time of admission the patient in poor general condition with ECOG 3. He had complaint of general weakness even during moderate physical activity, increase the temperature till 38.5-39.5ºC, sore throat. The consciousness is clear. Anemic (pale skin, tachycardia), hemorrhagic (a few ecchymoses was on the skin of the trunk) and infectious toxic syndrome (hyperemic of the throat, pyrexia, tachypnea) were present.

Given the fact that the patient was in febrile neutropenia state ceftazidime (2000 mg three times a day) therapy has been started immediately, which led to a normalize body temperature on the third day of treatment. Two days later the patients has been started the protocol of induction the remission "7+3" in standard doses. Decontamination of the digestive tract and prevention of mycosis was carried out of ciprofloxacin (500 mg 2 times a day) and itraconazole (400 mg 1 time per day), respectively. The patient received granulocyte colony stimulating factor (5 mg/kg) after chemotherapy during sixteen days until to recovery of granulocytes more than 500 in 1 mm^3.

The second wave of febrile neutropenia arouses two days after the end of induction therapy. In general clinical blood test: white blood cells (WBC) - 0.4×10^9/L, red blood cells (RBC)- 2.4×10^{12} /L, platelets - 12×10^9/L, erythrocyte sedimentation rate (ESR) -65 mm/h. A venous blood sampling for bacteriological study and sensitivity to antibiotics was performed; nose-throat swab specimen has been collected from the patient. Imipenem therapy (1000 mg 3 times daily) in combination with amikacin (15 mg/kg three times a day) has been started, the prevention of fungal complications continued with itraconazole. Despite the prescribed treatment, the patient in poor general condition: body temperature was 38.4ºC, it also had been present the tachypnea and tachycardia. In general clinical

analysis was blood leukopenia - 0.05×10^9/L, erythrocytopenia - 2.0×10^{12}/L and profound thrombocytopenia - 0.5×10^9/L, ESR -55 mm/h.

On the third day of febrile wave, the patient began to complain of dry cough, the sore over the course of the esophagus, the nausea, and the latter ended twofold vomiting with impurity of the blood. During examination of the surgeon was diagnosed erosive esophagitis which complicated by bleeding. On the skin of the trunk and extremities revealed multiple hemorrhages. In the blood test was third degree anemia (hemoglobin - 60 g/l), thrombocytopenia (platelets - 2×10^9/L) and fourth degree leukopenia (leukocytes - 0.1×10^9/L). All of the above resulted in the need for replacement platelet therapy (PT) and fresh-frozen plasma (FFP), against hemostatic agents.

The next day the patient continued to withhold high body temperature to 39ºC (fourth day of febrile neutropenia) and he was with progression of hemorrhagic syndrome despite to administration of the antihemorrhagic therapy. During the physical examination of the lungs of patients was revealed crepitus and blunting pulmonary sound above the bottom of fate left lung. Given the severe physical condition of the patient, neutropenia, febrile temperature to 39ºC and IV degree thrombocytopenia (platelets - 4×10^9/L), chest X-ray was not performed. Additionally the patient was scheduled linezolid (600 mg twice a day).

On the fifth day of the fever, after stabilization of the general condition, the patient was performed the chest X-ray. The X-ray revealed multiple infiltrative shadows in lungs, latters ranging from 2 to 6 cm in size, and darkening of the left costal-phrenic angle. Given the presence of fever, lack of response to antibiotic therapy was prescribed broad-spectrum antifungal drug- Amphotericin B (0.3 mg/kg on the first day with a increasing of 5 mg daily).

The next day (the sixth day of febrile neutropenia) in the patient's left shoulder appeared 3 cm of diameter necrotic zone with a clear smooth path. He had been coughing up sputum mixed with blood (biological material sent for microbiological and morphological study), there was also increasing of pulmonary

insufficiency, the latter manifested by shortness of breath, increased respiratory rate and decreasing the blood oxygen saturation.

Bases on the general analysis of blood (IV degree thrombocytopenia) and coagulogram (was detected the extension of prothrombin time, the increasing products of fibrin degradation, decreased fibrinogen level) the development of disseminated intravascular coagulation (DIC) syndrome has been diagnosed.

Thus, all of the arguments served to transfer the patient to the intensive care unit, where was been started intensive therapy and continued therapy support. Bronchoscopy for a diagnostic aid was not performed because of IV degree thrombocytopenia (platelets - 5×10^9/L) and hypoxia (the partial oxygen tension in arterial blood amounted to 70 mm Hg).

It should be noted that the microbiological analyses of patient blood samples, which repeatedly conducted during the period of the entire hospital stay, were sterile. Bacteriuria was not found and the throat and nose swab culture showed only low titer of group symbiotic bacteria.

On the eighth day from the beginning of febrile neutropenia was held normalization of body temperature and was observed improvement of respiratory function of the patient.

Computed tomography of the patient’s chest cavity (CT) was performed on the next day (the ninth day of febrile neutropenia), the results of which revealed multiple foci of the type of "frosted glass" - the “halo” sign and a few cavities – the air “crescent” sign (Figure №1). Given the radiological picture, which was typical for IFI, a blood sample for a galactomannan (GM) test by enzyme immunoassay (ELISA) was taken.

On the same day, with the development of toxic nephropathy, Amphotericin B was replaced to voriconazole (6 mg/kg twice a day per os during the first day, followed by reduction of the dose to 4 mg/kg twice a day). Therapy continued during the last 16 days.

Within the next two weeks against the background of intended antimycotic and antibiotic therapy was observed improve the patient overall condition with

regression of laboratory and clinical symptoms of sepsis: it has been slowing ESR and reducing C-reactive protein. The period of granulocytopenia was completed on the fifteenth day after the course of chemotherapy. It was observed normalization of the body temperature during the day with subfebrile temperature maintaining in the second half of the day. It was remained moderate shortness of breath, dry cough with release of a small amount of sputum mixed with blood, the wheezing was absent.

On the eighteenth day after chemotherapy needle biopsy of the bone marrow was performed, the latter resulted the diagnosis of remission of AL.

From the date fixed radiological changes in the lungs control X-ray was performed every five days. On the twentieth day after the end of remission induction the X-ray examination revealed enlargement of the heart shadow despite clinical stabilization of the patient. With echocardiogram help exudative pericarditis was diagnosed. Given the primary diagnosis, severity of condition, the patient is transferred to the Institute of Cardiovascular Surgery, where pericardial puncture with aspiration of 600 ml of sero-hemorrhagic fluid was performed. After one day the patient returned to the hematology department, where he continued to receive anti-infective and symptomatic therapy.

The next four days the patient was guarded and complained to cough with small amount of sputum mixed with blood. Auscultation of lungs revealed vesicular breath sounds with hard touch, percussion determined the lung sounds with tympanitis.

On the twenty-fourth day, at the night, in the patient occurred profuse pulmonary hemorrhage with expectoration of lung pieces during an attack of coughing, which led to asphyxia, acute cardiovascular collapse and death of the patient.

Histological study of the lungs and liver tissues revealed a septate hyphae of Aspergillus. The GM test result, which was received after the death of the patient, was negative.

Case №2.

The Patient T a 30-years-old male, was first admitted to the hematology department of ICR "NSCRM of the NAMS of Ukraine" and was diagnosed with first period of acute monoblastic leukemia, M5.

From the history of the disease we had known, that the patient considered himself sick during the last month, when he began observe raising the body temperature to 39ºC, and appeared dry cough. In general clinical blood test, the latter was performed by place of residence, was found anemia (hemoglobin – 108 g/L), leukopenia (leukocytes - 2.0×10^9/L), accelerated ESR (54 mm/h), blast cells - 42%. The patient has been diagnosed AML in the hematology department of ICR "NSCRM of the NAMS of Ukraine" (according to result of bone marrow aspiration - myeloblasts were 81.5%).

At the time of admission the patient in poor general condition with ECOG 3, he was complained on periodic attacks of coughing with the release of a small amount of sputum, fever up to 37.5-38.1ºC, sweating, general weakness, feeling "heartbeat" and shortness of breath on moderate exertion. There was recurrent bleeding gums and nasal mucosa during the last month.

During the physical examination of the patient showed signs of anemia (the skin and visible mucous membranes was pale, tachycardia was present), hemorrhagic syndrome (the hemorrhagic rash on the skin and mucous membranes of the mouth) and signs of enteropathy (defecation 3-4 times a day). The palpation of lymphatic nodes showed enlargement of submandibular and axillary lymph nodes. The lower edge of the liver was approximately by 2 cm below the costal margin. The physical examination of other organs and systems of the body did not identified changes.

Given the myeloid type of AL, the patients has been started the protocol of induction the remission "7+3" in standard doses with the therapy of support. The prevention of contamination by the intestinal tract flora was carried by ciprofloxacin (500 mg twice a day) and the prevention of fungal complications - itraconazole (200 mg twice a day).

Granulocytopenia (WBC - 0.6×10^9/L) developed in the last (7) days of chemotherapy and persisted for the next twenty days. The patient received granulocyte colony stimulating factor (5 mg/kg) during all granulocytopenic period until to recovery of granulocytes more than 500 in 1 mm^3.

On the second day after completion of induction therapy in a patient temperature rose to 38.7ºC, the patient complained of pain in the left half of the chest, which was reinforced during the act of breathing. The auscultation of the lungs was determined vesicular breathing, the percussion - clear lung sound. After collection of the venous blood for microbiological research, the patient was treated by cefepime (1000 mg twice daily) in combination with amikacin (500 mg twice a day). The X-ray revealed infiltration of the left upper-lobe lung parenchyma.

Despite the administration of active antimicrobial therapy the body temperature was maintained on the level of 38.0-38.5ºC, and on the fourth day of the fever linezolid (600 mg twice a day) was added to the therapy, the prevention of mycosis was continued by itraconazole.

On the fifth day of the febrile neutropenia the patient's condition continued to remain extremely difficult: the evidence of the lungs lesion was escalated - above the surface of the left lung was respiratory depression and crepitantrales were heard. The lungs CT examination was revealed the consolidation of the upper left lung parenchyma by the "frosted glass" type - the "halo" sign (Figure №2) and 20 mm and 23-25 mm in size lesions in the liver and spleen, respectively (Figure №3 and №4). This gave a reason to think about the development of invasive fungal infections (IFI). Amphotericin B (the starting dose was 0.25 mg/kg, followed by an increase in the dose of 5 mg daily) was started at the same day. However, 48 hours after initiation of the antimitotic therapy the patient creatinine clearance increased greater than 25% from baseline, which necessitated Amphotericin B dose reduction to the maximum tolerated.

In spite of prescribed treatment (fourteenth day of febrile neutropenia) it was observed in negative radiological dynamics: increasing the size and number of lesions in the left lung and the appearance of new "frosted glass" like lesions in the

upper right lung. The patient was consulted in the center of sepsis. The bronchoalveolar lavage was performed (BAL), and the resulting material was sent for histological, microscopic and culture studies. Results of studies showed Candida species. Given the lack of response to the given therapy and worsening of the patient condition, cefepime with amikacin were replaced to meronem (1000 mg three times daily) and amphotericin B was changed to caspofungin (70 mg once a day for the first day, followed by reduction of the dose to 50 mg).

On the twenty-fourth day after the induction course there was the granulocytes more than 500 in 1 mm^3 of blood recovery. As a result of the control bone marrow punction - the boon morrow remission was achieved. According to the control CT was kept negative dynamics: in both lungs were radiological signs of infiltrates with a necrotic content - the air “crescent” symptom (Figure №5). The blood sample for the GM ELISA test was taken. On the thirty-fifth day after the end of chemotherapy, despite the antibacterial, detoxification, blood components replacement therapy, there was significant deterioration of the general state: the sopor and a multiple organ failure were developed. The gastrointestinal bleeding was started on the basis of the disseminated intravascular coagulation syndrome. The intensive therapy was ineffective and biological Death been ascertained. Autopsy was not carried out, due to the refusal of the patient’s relatives.

The GM test result was obtained 10 days after the collection of material, there was after the patient death. The GM titer was equal to 1.5 OD, the latter indicated the presence of Aspergillosis.

Discussion.

The aim of the review of these clinical cases, which were complicated by the IA development, is to analyze the characteristics of the disease, mistakes in the process of diagnosis and treatment tactics. The IA is increasingly found in patients with AL due to a broader use of high-dose chemotherapy, and is one of the main reasons of the high mortality even when a marrowy remission was achieved. The level of mortality from IA correlates with the term of the start of the antimycotic therapy.

In the first clinical case, the patient empirically assigned amphotericin B. Due to the development of renal toxicity and increase the level of confidence in the IA (typical radiological symptoms, appearance of necrotic foci shoulder) amphotericin B was replaced to voriconazole. Note that both drugs are recommended by most international guidelines for IFI and IA in Immunocompromised Patients with Cancer and for empirical treatment of the febrile neutropenia. Although, given the fact that the patient at the time of the fever episode was in a state of neutropenia, as least ten days, specific therapy was delayed. This is confirmed, in particular, by the "crescent" radiological sings, the latter appears in the third week of aspergillosis and is an unfavorable prognostic marker [2, 16]. On average, this is about two weeks from the moment of profound neutropenia until clinical or radiological symptoms that make you think about IA, due to the nonspecific clinical picture and a late manifestation of the latter [16]. Note that the use of X-rays, as instrumental method for identification of radiological symptoms IA absolutely pointless. Results obtained M. Subira and colleagues show that in 32% of patients with the pulmonary form of IA on X-rays are no symptoms of lung lesions, and only 8% of cases detected changes were pretext that allowed suspected pulmonary form of IA [18].

Also, note that the majority of patients with pulmonary form, which occurs in 90% of cases of IA, die from massive pulmonary hemorrhage just after granulocyte recovery, due to the release of elastase from neutrophils, the latter are migrate to inflammation. The elastese breaks down components of connective tissue of the vascular wall. Thus, the early administration of antifungal drugs, probably, on the second-line of antiinfection therapy, potentially would reduce the dissemination of Aspergillus, micotic load on the body and the degree of invasion of large vessels.

The empirically antifungal therapy with amphotericin B which was described in the second clinical case, probably, was started at an earlier stage of infection in comparison with the firs patient. Recall, that at the time of the first CT of lungs the second patient has the "halo" signs already, which is identified in 30-

60% of cases of IA, and in 75% of patients disappears during the first week of the disease [8].

The above radiological signs although described in IA, are not absolutely pathognomonic, and found in other IFI. It should be noted, that involvement in the infectious process of the spleen and liver are not specific for IA. In particular lesion in the liver found only 15% of cases [5].So uncharacteristic lesions, detection of Candida in BAL and development of nephrotoxicity, induced a replacement of amphotericin B to caspofungin. The letter is a drug with a higher level of specificity regarding yeasts. Although, it should be noted, that the cultural method of diagnosis in patients with oncohematologic pathology is answered at 50-57% of cases if BAL is used as material for planting, and - 20-22% cases, when the sputum culture is analyzed [18]. Therefore, in the clinical case number 2 was no growth of Aspergillus.

Summarizing the discussion of empirical antimycotic therapy was appointed to the second patient, we note that, probably, the amphotericin B effectless was explained by insufficient concentration of the drug to inhibit the growth of fungi of the genus Aspergillus. Recall, that a purpose amphotericin B often accompanied by the development of nephrotoxicity and the use of the effective dose of the drug was impossible. Another possible reason for the unsatisfactory therapy answers - is likely infecting strains of fungi A. terreus or A. nidulans, which are resistant to amphotericin B [17]. Appointment caspofungin that although has mycostatic effect on Aspergillus, held on the stage of full-scale infection that could not affect the course of infection.

Therefore, given on our experience, a way to reduce mortality of AL patients with high risk of IA - is a strict following of the algorithm for anti-infective therapy in febrile neutropenia.

Recall, that monotherapy or combination antibiotic regimens on the first line should has the bacteriostatic coverage for both Gram-positive and Gram-negative bacteria, including Pseudomonas aeruginosa. The second line antimicrobial therapy aimed at multi-drug-resistant strains of gram-positive bacteria, and the

third - on molds and yeasts of fungal species. Timely appointment of empirical antibiotics and adequate assessment of the therapy answer allows starting antifungal treatment at an earlier stage of the infection. Given that, the timely modifications of the first-line therapy, which, according to the majority of the world's recommendations, is performed in the range of 2 to 5 days, and for the next line in the range of 5 to 7 days from the date of appointment of the chosen treatment regime, may be more appropriate at an earlier period. This is will ensure the appointment of empirical antifungal therapy in the early development of the infectious process.

Empirical antifungal treatment, that is, without microbiological confirmation, as the third-line therapy, as recommended by IDSA, is appointed by 4-7 day of febrile neutropenia in the absence of the response for antibiotic adequate therapy. The first-line antifungal drugs include liposomal amphotericin B, lipid colloidal dispersion of amphotericin B, and lipid complex of amphotericin B, itraconazole, voriconazole and caspofungin [6, 7]. A guideline on the management of invasive fungal infection during therapy for haematological malignancy of the British Committee for Standards in Haematology UK (BCSH) has limited IFI empirical therapy with liposomal amphotericin B and caspofungin. Vorikonazol has been used only in case of "proven" or "probable" IHI [15]. Recall, that therapy of verified IA has been regulated by a recommendation of the International Association of Immunologists (ECIL) for the use of antifungal drugs in patients with leukemia, which was updated in 2009, and a recommendation of the American Association for Infectious Diseases (IDSA). In accordance with the recommendations of ECIL the first line drugs were vorikonazol, which has the highest level of evidence, liposomal amphotericin B, lipid complex of amphotericin B and caspofungin (with a lower standard of proof) [3]. IDSA recommends the use as a first-line drug vorikonazol. Posaconazole and itraconazole are a second-line therapy drugs [19].

In discussing methods of IA verification, beside of already analyzed diagnostic approaches in the examples above, we used indirect mycological

method - the determination of the GM level of ELISA. The latter and is the most common method in the mycological world. Its advantage is the speed of the method, and also high sensitivity and specificity of the test. According to the majority of clinical studies sensitivity and specificity of the method reaches 90% for patients with oncohematologic pathology [14]. Though, there is no algorithm for monitoring the level of GM in blood or BAL, but according to international practice the test performing is recommended twice a week since the moment development of neutropenia for screening, or immediately if a patient has symptoms that are clinically or radiologically consistent with IA [4]. This strategy reduces the percentage of false negative test results. The level of circulating fungus antigen the directly correlated with mycoticload, and the use of empirical antifungal therapy suppresses the secretion of GM and directly inhibits the growth of mycelium of Aspergillus. That explains subthreshold level of GM in the patient number 1, which the test was performed after a long period of antifungal therapy [9]. Take cognizance of, that the testing performance was significantly delayed in both patients, due to the inability of its implementation in Ukraine. Well-established screening GM test would optimize the clinical management of patients receiving high-dose chemotherapy. In the absence of or delays in the use of screening methods for diagnosis IFI such as GM or 1-3-β-D-glucan, which allow verified "probable" IA therapy since the early days of the infection unfortunately almost impossible.

These clinical cases demonstrate nonspecific clinical and diagnostic features of IA, controversy that accompanies the disease in patients receiving high-dose chemotherapy. Delayed diagnostic and practical approaches on suspicions of IA dramatically reduce the survival rate of patients with AL and other diseases in which develops deep immunodeficiency.

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