Case report

Invasive fungal infection in a child with Ewing's sarcoma

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ABSTRACT

Invasive fungal infection (IFI) in patients with malignant solid tumours is rare, but it highly increases the risk of cancer recurrence because of prolonged discontinuation of cancer treatment.

This paper presents a case of IFI induced by *Candida glabrata* in a 14-year-old girl with advanced Ewing's sarcoma and metastases to the bone marrow. She was intensively treated with chemotherapy (CWS, CEVAIE, EWING 2008 + VIDE) and radiotherapy. As the treatment was ineffective, the tumour was surgically removed from the sacrum, and VAI chemotherapy was administered. Due to the symptoms of infection, antifungal treatment was initiated with caspofungin, followed by prophylaxis with posaconazole. In terms of anti-cancer treatment, the patient received megatherapy with auto-HSCT. Signs of infection and gastrotoxic complications developed, which is why broad-spectrum antibiotics and amphotericin B lipid complex were administered. Even so, a multisystem IFI appeared, causing the patient's death.

In multidrug chemotherapy with extended periods of agranulocytosis, primary prevention should be considered, similar to the one offered to patients with haematological malignancies.

Key words: invasive fungal infection, *Candida glabrata*, Ewing's sarcoma, children

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INTRODUCTION

Invasive fungal infections (IFI) are systemic infections of fungal aetiology. Out of the 7 types, 4 are most frequently diagnosed: candidiasis/candidemia, aspergillosis, cryptococcosis and mucormycosis [1]. IFI occurs in patients with primary or secondary immune deficiency. It is diagnosed, inter alia, in patients undergoing oncological treatment, in patients with diabetes or HIV, and in preterm infants.

Incidence of IFI in paediatric oncology depends on the type of neoplastic disease. A significant number of IFI cases are reported in patients with haematological malignancies. The risk of IFI infection is particularly high in patients who undergo megatherapy with HSCT (*hematopoietic stem cell transplantation*), and during the induction phase of therapy for acute myeloid leukaemia [2, 3].

Cases of IFI in patients with malignant solid tumours are described only sporadically, which is why the exact IFI incidence has not yet been established. It is believed that even a single episode of *Candida* dissemination in the bloodstream may result in a systemic infection [3]. The incidence of mycoses increases considerably along with therapy intensification. As in the group of patients with haematological malignancies, patients undergoing megatherapy with HSCT are particularly vulnerable to it. It has been demonstrated that IFI incidence in children with neuroblastoma, undergoing intensified therapy with autologous HSCT, is significantly higher than in patients who are subjected to conventional chemotherapy (2% vs. 0.8%) [4]. The high-risk group also includes patients with central nervous system tumours, as their therapy involves multi-drug chemotherapy as well as radiotherapy and glucocorticosteroids, which increases IFI risk [5]. IFI is also diagnosed in children diagnosed with solid tumours, who require particularly intensive chemotherapy regimens, leading to several-week-long periods of profound myelosuppression.

Cases of IFI have been described in the course of paediatric tumours such as neuroblastoma, Ewing's sarcoma or soft tissue sarcomas [4, 6, 7]. Additionally, it has been indicated that the risk of fungal infections is higher in individuals with specific gene polymorphisms, e.g. TLR4, dectins [5].

Clinical factors that are conducive to IFI development include: prolonged neutropenia ($< 0.1 \times 10^9$/L), and in particular one that lasts over 3 weeks, glucocorticosteroid therapy (over 1 mg/kg for more than 10 days), fungal colonisation in multiple locations or significant colonisation of a single site, radiotherapy and in the case of some fungal infections also the presence of central catheter [3].

An increase in the incidence of *Candida non-albicans* infections (and *Candida parapsilosis* infections in particular), observed over the recent years, appears to be related to the long-term use of central catheters and their colonisation [4]. The majority of the *Candida* infections involve an opportunistic endogenous infection, as the mucous membranes of the GI tract, vagina and urethra are a natural reservoir of the fungus [8]. It has been demonstrated that even up to 10% of catheter-related infections in patients with haematological malignancies are fungemias [9].

Clinical symptoms of IFI depend on the type of infection. In the case of candidiasis they may be revealed in an acute form involving prolonged fever, drop in arterial pressure, septic shock, myalgia, and papular non-painful skin lesions. Systemic candidiasis may also be of chronic character, involving persistent febrile episodes, with elevated levels of C-reactive protein, abdominal pain, escalating activity of alkaline phosphatase in blood serum, and symptoms of progressive cachexia. In the case of a mould fungus infection, the symptoms include lung involvement, nasal sinus congestion, persistent cough, chest pain, and possibly haemoptysis. They usually set in at a late stage of disseminated mycosis. There might also be other symptoms depending on the involvement of other internal organs such as the liver, spleen, kidney and lungs [3].

It is not easy to diagnose an IFI infection, as the diagnosis is based on the confirmation of the presence of fungal fragments in a histopathology examination or on culturing the fungus from a sterile biological material under appropriate conditions. The criterion of proven IFI diagnosis often requires invasive harvesting of the altered tissue, which is not always possible, especially in children with an actively treated neoplastic disease. Each invasive procedure is an additional risk factor in that case. Thus, in most cases, the diagnosis of IFI is classified as "probable", determined on the basis of positive fungal antigen tests as well as the characteristic imaging features. When positive fungal markers are revealed in a patient with clinical symptoms and/or characteristic imaging features, one is authorized to initiate the so-called pre-emptive antifungal therapy [3, 4]. In patients without specific clinical symptoms or imaging lesions, who are neutropenic and febrile despite the use of broad-spectrum antibiotics, empirical treatment is administered involving antifungal agents [3, 5, 10].

Recommendations on pre-emptive and empirical therapy adopted by ECIL [5] stem from the poor prognosis of immunosup-

pressed patients with IFI. Despite the introduction of new and efficacious antifungal drugs over the past decade, IFI-related mortality is still high. As demonstrated, it mainly results from the difficult early diagnosis and delayed initiation of effective antifungal therapy. Patient prognosis in IFI also depends on the type of fungus that caused the infection, and on the risk factors involved, which may pile up in a single individual [1]. The severe patient condition and development of fungal sepsis or respiratory failure often require the treatment to be carried out at an intensive care unit (ICU), where ventilation therapy is possible, which frequently interrupts anti-cancer therapy.

All cases of IFI require intensive and long-term treatment. It usually begins from anti-fungal monotherapy. The choice of drugs depends on the type of fungal infection. The most frequently used antifungals in candidiasis include: echinocandins, liposomal amphotericin B or amphotericin B lipid complex, and voriconazole, especially recommended in *Candida krusei* infections. In *Candida parapsilosis*-related IFI, fluconazole and liposomal amphotericin B have been proven efficacious. In each IFI case one should consider central catheter removal [3, 9].

In patients with solid tumours, IFI may develop at different stages of oncological treatment, but they are most often reported in patients with refractory cancer or in the course of disease progression (65%), and in those who undergo chemotherapy, following which most patients suffer from prolonged periods of neutropenia [3]. In an attempt to counteract the threat in high-risk groups, anti-fungal prophylaxis has currently been implemented. Recommendations on prophylaxis in children with neoplasms cover patients after allogeneic transplantation of hematopoietic stem cells, patients diagnosed with acute myeloid leukaemia and myelodysplastic syndrome, as those are patient groups characterised by long-lasting periods of neutropenia. It is also recommended that anti-fungal prevention be considered in all other patients subjected to chemotherapy in whom neutropenia $< 0.1 \times 10^9$/L lasts longer than 7 days [6].

CASE REPORT

A 14-year-old girl was admitted to the Clinic of Paediatrics, Oncology and Haematology in Gdansk due to an MRI scan revealing a large tumour of the small pelvis ($6.5 \times 9 \times 12$ cm) infiltrating the S1 and S2 spinal segments, and penetrating the vertebral canal at the level of L5/S1.

The first symptoms of the disease emerged one month before the hospitalization in the form of lumbosacral pain, radiating to the lower right extremity. Neurological examination revealed flaccid paralysis, primarily distal, of the lower right extremity, reduced right ankle jerk and plantar reflexes, gait disturbances with a right limp, and painful lumbosacral region. Abdominal CT confirmed the presence of a tumour sized $10.9 \times 7.4 \times 9.5$ cm located in the small pelvis. Based on the examination of the tumour specimen, extraskeletal Ewing's sarcoma/PNET was diagnosed. Additionally, bone marrow metastases were revealed, with other distant metastases excluded.

The patient was qualified for treatment in accordance with the 2006 CWS protocol for soft tissue tumours at stage IV of clinical advancement [11]. Positive response to CEVAIE chemotherapy regimen was reported. MRI revealed tumour mass reduction by 75%. From the very beginning, the treatment was complicated with grade 4 myelosuppression and gastrotoxic symptoms, including peristalsis abnormalities and emesis upon oral feeding attempts. Due to the latter, periodic parenteral nutrition was provided. Despite the positive response to chemotherapy, the girl continued to suffer from lower right extremity pain, making it difficult for her to walk.

At the next stage of treatment, radical radiotherapy was initiated, at the total dose of 50.4 Gy, and a follow-up imaging assessment revealed the residual tumour mass sized $6.3 \times 5.4 \times 6.0$ cm, located in the small pelvis anterior to the sacrum. The tumour was shown to infiltrate the obturator muscle. Additionally, signs of sacral osteolysis were revealed at the S2–S3 level on the right side, with a suspicion of segmental extension of the tumour into the spinal canal. The tumour was assessed as inoperable, and decision was taken to continue chemotherapy, changing the previous treatment protocol for the VIDE regimen used in the skeletal type of Ewing's sarcoma (EWING 2008) [11].

The follow-up imaging tests, performed 3 months later, revealed a stable disease. The altered chemotherapy was complicated with periods of persistent neutropenia and febrile neutropenia. The lack of further tumour regression led to another change of chemotherapy to the regimen including vincristine, temozolomide and irinotecan.

The consecutive examinations failed to demonstrate tumour reduction, which is why it was decided that the tumour would be removed, in spite of the risk of irreversible neurological complications. 13 months into the anti-cancer treatment, the tumour was surgically removed together with part of the sacrum at the S1–S2 level, the sacral plexus nerves and the sciatic nerve, infiltrated by the tumour mass, were resected, and L5–S1 laminecto-

my was performed. The procedure was assessed as macroscopically radical. Its consequences included neurological deficits in the form of neurogenic bladder, neurogenic rectum and lower right extremity paresis. Suprapubic cystostomy was created for urinary drainage.

Following the surgery, VAI adjuvant chemotherapy was administered [11]. On day 4 after the completion of chemotherapy, agranulocytosis was reported as well as signs of infection with fever and elevated CRP levels. The patient's condition deteriorated, and nausea and anorexia set in. Despite the use of broad-spectrum antibiotics, inflammatory markers continued to rise. The mannan antigen test came in positive, indicating a possible fungal infection. Imaging tests (abdominal ultrasound) and microbiological tests failed to locate the site of infection. Empirical treatment with caspofungin was administered, triggering clinical improvement and normalization of the CRP levels. Following one month of anti-fungal treatment, the patient was started on posaconazole as secondary prevention.

Further oncological treatment included megatherapy with auto-HSCT, performed 3 months after the surgical procedure. It was complicated with gastrointestinal mucositis (WHO grade 4), recurrent fever, persistent thrombocytopenia and agranulocytosis lasting for 11 days.

The patient's condition remained unstable for a long time, mainly due to the gastrotoxic complications (abdominal pain, diarrhoea, malabsorption). In spite of the parenteral nutrition, gradual cachexia was observed. During the 2-month-long post-transplantation period, broad-spectrum antibiotic therapy as well as antiviral and antifungal therapy was initiated. Afterwards, the patient was transferred for further treatment to the Clinic of Paediatrics, Haematology and Oncology in Gdansk. Gastrotoxic symptoms, including anorexia, abdominal pain, periodic diarrhoea, and signs of significant malnutrition persisted. Antimicrobial treatment with broad spectrum antibiotics was continued, as was the antifungal treatment with amphotericin B lipid complex. Still, the patient's general condition failed to improve, and a week later haematuria was observed. Ultrasound and CT revealed the presence of a pathological mass in the bladder, rendering urine drainage from the right kidney difficult. As IFI was suspected, urine culture was taken, confirming the presence of *Candida glabrata*. Urinary tract candidiasis was diagnosed. Due to the earlier diagnosis of invasive candidiasis caused by *Candida glabrata* and due to the long-term antifungal treatment involving multiple drugs, intravenous combined therapy was provided at that stage, including amphotericin B lipid complex and caspofungin. Additionally, local antifungal therapy was initiated, involving bladder irrigation with the same amphotericin B product, administered 4 times daily, using a solution containing 6 mg of amphotericin B in 25 ml of saline. In order to decompress the right pyelocalyceal system, pyelostomy and vesicostomy were performed. The histological and microbiological examinations of the tissue harvested surgically from the urinary tract confirmed the presence of *Candida glabrata*. Additionally, right renal pelvis irrigation was initiated once daily with the use of amphotericin B.

Despite the combined systemic therapy and the local antifungal treatment, the patient's condition continued to deteriorate. Signs of intestinal ileus emerged. Ultrasound and CT revealed the presence of perihepatic and interloop fluid in the abdominal cavity. The fluid was also reported in the pleural cavities. As fungal peritonitis was suspected, surgical drainage of the peritoneal cavity was performed, irrigating the peritoneal cavity with a solution of amphotericin B lipid complex, containing 1 mg of amphotericin B/2 l of saline, based on the published findings [12]. In order to determine the cause of the patient's worsening condition, cytological and microbiological analysis of peritoneal fluid was performed. The harvested fluid revealed no cancer cells, and the microbiological tests failed to identify a pathogen responsible. Over the following few days, the patient's condition deteriorated radically, with signs of increasing mechanical ileus. The CT scan indicated intestinal impaction in the adhesions. No unequivocal signs of cancer recurrence were reported, and it was only a solid hepatic lesion revealed in CT that required differentiation between a focus of fungal infection and a metastatic lesion. Due to the mechanical ileus, another surgical intervention was necessary, even though the patient's condition was poor. Following the surgery, complicated with intestinal perforation, the girl's condition was extremely severe, and further treatment was offered at the ICU. In spite of the intensive antibacterial and antifungal therapy, the patient's condition failed to stabilize, and on day 3 after the surgery, the patient died of multi-organ failure.

DISCUSSION

Ewing's sarcoma belongs to a group of tumours which develop in the bones or, more rarely, in the soft tissues. It is a small round cell neoplasm, diagnosed in children at developmental age and in young adults. Presently, thanks to intensive and comprehensive treatment, many patients diagnosed with the disease accomplish long-lasting remission periods [11]. However, prognosis is still uncertain at the more advanced stages of the disease. In the skeletal type of Ewing's sarcoma, the EWING 2008 protocol provides for neoadjuvant multi-drug chemotherapy, tumour resection, high-

-dose chemotherapy with auto-HSCT, adjuvant chemotherapy, and radiotherapy [11]. All of the stages of such intensive treatment are burdened with complications, and in particular with long periods of agranulocytosis and severe infections.

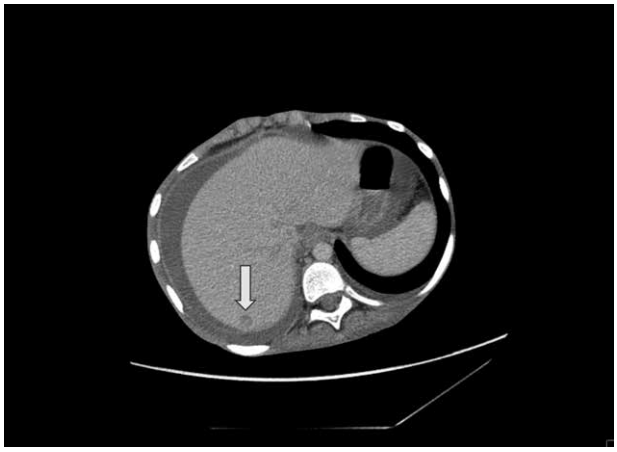
In the case discussed above, the tumour was diagnosed at an advanced stage. Location of the primary tumour within the small pelvis and the sacrum was especially unfavourable due to the infiltration of the spinal canal and the nerves at the lumbosacral level. As a consequence, the patient suffered from significant pain and neurological deficits. To minimize permanent neurological damage, treatment had to be modified, extending the preoperative chemotherapy, and delaying the surgical procedure. Consecutive lines of multi-drug chemotherapy were administered in order to receive a maximum response with reduced disease burden. From the very start of the treatment, several-week-long periods of grade 4 myelosuppression were observed with accompanying episodes of infection resistant to antimicrobials. Therefore, as IFI was suspected, empirical echinocandin therapy was initiated, initially leading to a regression of the symptoms. Despite the secondary prevention, following auto-HSCT, the patient developed signs of a systemic fungal infection, caused by the *Candida glabrata* species. The infection was confirmed by the microbiological tests performed (positive urine culture from suprapubic cystostomy and from the anal smear), by the positive mannan antigen test, and first and foremost by the histological test of the specimen collected from the urinary tract mycetoma. The gastroenterological symptoms affecting the child indicated a chronic and disseminated hepatosplenic candidiasis. Cachexia, immobility, and urinary stasis with compromised renal function were some of the additional aggravating factors. In spite of the systemic combined antifungal treatment and the local amphotericin B therapy, the infection continued to develop with signs of fungal peritonitis, which was the cause of the patient's death.

Invasive forms of candidiasis develop primarily in patients suffering from acute myeloid leukaemia and in the post-HSCT patients [3, 13]. Currently, disseminated forms of candidiasis emerge much less frequently in haematological malignancies thanks to the inclusion of primary antifungal prophylaxis in the treatment algorithms dedicated to the high-risk patients [2, 6, 13]. It should be emphasised that in the above discussed case, once IFI had been suspected, empirical antifungal therapy was implemented, followed by the secondary prevention with posaconazole, administered after the subsidence of clinical symptoms and normalization of the CRP levels [2, 6]. Still, during the period of profound immunosuppression following auto-HSCT, multi-organ candidiasis developed, refractory to the antifungal agents in use.

Undoubtedly, in the case discussed, what was conducive to the development of IFI was that several unfavourable prognostic factors collided, including intensive anti-cancer treatment, multiple prolonged periods of agranulocytosis, long-term broad-spectrum antibiotic therapy, consecutive surgical procedures, immobility, and cachexia. It is worth emphasising that the number of fungal strains resistant to antifungal medications has been on the increase [5], leading to therapeutic failure at times. Some epidemiological studies on yeast infections have demonstrated a higher mortality amongst patients infected with the *Candida glabrata* species. Its special properties lead to a quick escalation of resistance to the existing antifungal drugs [8, 10, 14].

Diagnostic tests performed at the final stage of disease revealed no signs of cancer relapse. No cancer cells were reported in the fluids harvested repeatedly from the peritoneal cavity and from the pleural cavities. What was found suspicious was a solid lesion found in the liver CT scan (fig. 1).

FIGURE 1.
Abdominal CT – a visible solid lesion of non-defined character in the left hepatic lobe.



It was necessary to determine whether the lesion was due to the fungal infection process or cancer relapse. Similar doubts arise in every case of a new solid lesion revealed in imaging tests during an active chemotherapy. Its character may only be determined beyond any doubt by a histological examination [5]. It is, however, not always possible to collect the tissue for examination, when the patient's condition is severe, as in the above described case. Presently, new diagnostic imaging techniques, including FDG-PET/CT, may be helpful, when differentiating neoplastic lesions from infectious ones [15]. As there were no other metastat-

ic foci, and in the light of the multi-organ IFI, one has to conclude that in the above discussed case fungal hepatic abscess was a more probable diagnosis.

Treatment of IFIs caused by the *Candida glabrata* species often ends in failure. The related mortality amounts to 62% [14]. It is also worth mentioning that the number of new fungal pathogens has been on the rise, as has the problem of strains resistant to multiple antifungals, including azoles and echinocandins [1, 14].

CONCLUSIONS

In each patient with a malignant solid tumour, who undergoes intensive multi-drug chemotherapy with periods of prolonged agranulocytosis, one should consider primary antifungal prevention, similar to the one administered to patients with haematological malignancies. If IFI is diagnosed, one should also consider secondary prevention after symptom resolution, and in particular in those patients in whom multiple therapeutic methods have been applied, including radiotherapy, surgery and auto-HSCT.

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