Disseminated aspergillosis due to *Aspergillus flavus* in a pediatric patient with a recent diagnosis of acute lymphoblastic leukemia

Martha J. Avilés Robles¹, Fredenet Oviel Mendoza Camargo², Bertha Lilia Romero Baizabal³, Carlos Alberto Serrano Bello⁴ and Fernando Ortega Riosvelasco¹

¹Departament of Infectology; ²Departament of Oncology; ³Imaging Services; ⁴Departament of Pathology. Hospital Infantil de México Federico Gómez, Mexico City, Mexico

Summary of the clinical history

Eight-year-old male patient with a diagnosis of acute lymphoblastic leukemia (ALL).

Background

Twenty-nine-year-old mother, primary school education, cleaning assistant, single, denied drug use. Father outside of family nucleus, medical history unknown. A nine-year-old brother, healthy. Two half-brothers on the mother's side of 6 and 12 years of age, healthy.

Resident of Tultitlán, State of Mexico. He lived in a house with five rooms with running water, electricity and drainage system, with 20 cohabitants. He was exposed to lamb, chickens, cats, a dog, and a rabbit. His diet consisted of four meals a day, with a high intake of simple carbohydrates. Psychomotor development according to age. He attended the third grade and had good academic performance. Immunizations: the fourth dose of the pentavalent vaccine and the first and second doses of the triple viral vaccine were missed.

Product of the third pregnancy. Irregular prenatal control from the second trimester. The mother began with hematinics since the second trimester. She attended five consultations, and four obstetric ultrasounds were performed (OUS) which were referred as normal.

She had repeated episodes of cervicovaginitis from the second trimester, without any treatment. The product was delivered vaginally at 42 weeks of gestation (WOG). He breathed and cried at birth. Apgar 8/9, Capurro 42 WOG, a weight of 2700 g, and 47 cm of height. Advanced resuscitation maneuvers were not required. Both were discharged after 24 hours without any complications. Allergies, previous surgical history, traumas, transfusions and previous hospitalizations were denied.

Final illness

He attended the Hospital Infantil de México Federico Gómez (HIMFG) for the first time for paleness, asthenia, adynamia of three months of evolution, daily fever, nocturnal, of 15 days of evolution. An infiltrative syndrome was documented by the presence of cervical, axillary and inguinal adenopathies. A complete blood count showed hemoglobin (Hb) of 3.6; hematocrit of 12.5%; leukocytes of 88,100; neutrophils of 16%; lymphocytes of 5%; monocytes of 2%; blasts of 76%; platelets of 52,000; lactate dehydrogenase (LDH) of 440; phosphorus of 5.1. A bone marrow aspirate (BMA) was done with the following results: blasts 91%; L2 morphology (FAB). Immunophenotype: CD19, 98.7%; CD2 98.9%; CD7, 94.2%; CD13, 89.6%; CD15, 96.7%;

Correspondence:

*Martha J. Avilés Robles

E-mail: marmtom@hotmail.com

Received: 18-04-2017

Accepted: 11-05-2017

DOI: 10.24875/BMHIME.M17000008

Available online: 18-06-2018

Bol Med Hosp Infant Mex. 2017;74:370-380

www.bmhim.com

2444-3409/© 2018. Hospital Infantil de México Federico Gómez, published by Permanyer México SA de CV, all rights reserved.

myeloperoxidase, 0.2% (T immunophenotype). Translocation panel: negative. Cerebral spinal fluid (CSF) with lymphocytes and monocytes. IV fluids and hyperhydration were started 2500/50 mEq HCO_3^- and allopurinol 300 mg/m²SC/day and steroid window with dexamethasone 6 mg/m² for eight days.

Complete blood count on the eight days of the steroid window showed a poor response to treatment. Absolut blast count was 44,190, so induction into remission was started. He evolved with a leukocyte count of up to 154,800, remaining hospitalized for observation. He received chemotherapy with vincristine 2 mg/m²/day, daunorubicin 25 mg/m²/day, L-asparaginase 10,000 U/m²/day, dexamethasone 6 mg/m²/day. He was discharged without further complications. Afterward, a BMA was performed, and chemotherapy with vincristine was administered. A day after, chemotherapy with L-asparaginase was also administered.

He was admitted two days later with a 38.2°C fever, accompanied by diaphoresis. He presented with abdominal pain of 8 h of evolution after feeding, of sudden onset, progressive, of colic-type, generalized, with an intensity of 8/10, without any mitigating and aggravating factors. He had six episodes of vomiting, preceded by nausea and bowing, of moderate quantity. Bowel movements were loose: five times, in moderate quantity, with abundant mucous, semiliquid.

Physical examination revealed the following: weight of 21 kg, height of 120 cm, heart rate of 194/min, respiratory rate of 30/min, blood pressure of 80/23 mmHg, temperature of 38.6°C, capillary refill of 3 s. Male patient of apparent age similar to chronological age, normocephalic, isochoric and normally reactive pupils, permeable external auditory canals, intact tympanic membranes, permeable nares, mildly dehydrated oral cavity, normochromic pharynx, without any cervical adenopathies, thorax with adequate ventilatory movements, well-ventilated pulmonary fields, rhythmic cardiac sounds and of adequate intensity.

He presented with a globose abdomen, with an abdominal perimeter of 62 cm, with voluntary resistance, with abdominal tenderness at middle and deep palpation. He had decreased peristalsis, tympanic to percussion, without organomegaly, phenotypically male genitalia, Tanner 1, cold extremities, weak pulses, capillary refill of 3 s. During the neurological examination, he was alert, active, reactive, without any signs of focalization or involuntary movements.

Laboratory results showed a Hb of 8.2 g/dl; hematocrit of 23.2%, leukocytes of 800/μl; neutrophils of 0.4%, lymphocytes of 98%; monocytes of 0.6%; platelets of 9,000.

Arterial blood gases showed the following values: pH of 7.35; PO_2 of 45.8 mmHg; PCO_2 27.5 mmHg; HCO_3^- of 14.9 mmol/l; lactate of 5.1 mmol/l; saturation of 73%. Management in the emergency department included *nil per os*, three crystalloids boluses at 20 ml/kg/dose, adrenaline at 0.1 μg/kg/min, meropenem at 100 mg/kg/day, and vancomycin at 40 mg/kg/day. He persisted with signs of shock, so orotracheal intubation was decided. The patient presented with hypotension during intubation, so norepinephrine (0.1 μg/kg/min), albumin at 55 (10 ml/kg/dose), hydrocortisone (100 mg/m² SC/day) were started. A nasogastric tube (NGT) was placed to drain fecaloid and biliary material.

The patient had abdominal distention with abdominal wall involuntary resistance, positive fluid wave test, intraabdominal pressure of 19 mmH₂O, so a Tenckhoff catheter and a femoral central venous access were placed. One-thousand milliliters of citrine fluid was drained. Afterward, the patients persisted with signs of mixed shock, for which an exploratory laparotomy (EX-LAP) was performed, with right hemicolectomy, placement of a Bogotá bag and an ileocolostomy. The following findings were reported: generalized dilatation of the ileum and colon, mild peritonitis, cecum with areas of necrosis and ischemia. The patient was admitted to the intensive care unit and presented with left pleural effusion; a chest tube was placed which drained 350 ml of clear discharge, along with an orogastric tube, that drained a greenish biliary discharge. He presented with signs of complicated disseminated intravascular coagulation. Plasmapheresis was indicated due to thrombocytopenia and multiple organ failure.

Three days after his admission, both norepinephrine and adrenaline support decreased. The patient was administered an infusion of furosemide at 200 μg/kg/h. He had a uresis of 2.7 ml/kg/h, a fluid balance of +39 ml, fluid overload index of 19%, abdomen without peristalsis. He received empiric antifungal therapy with amphotericin B lipid complex at 3 mg/kg/day; a diagnostic approach was requested.

Days after, the patient had an accumulated balance of 2696 ml, without aminergic support, but with fluid overload, for which extended hemodialysis was indicated. An increase in ventilatory parameters was reported, along with increased secretions with changes in their characteristics, crepitant crackles, radiography with a predominantly right alveolar infiltrate and a complete blood count with a hemoglobin of 10 g/dl; leukocytes of 1000/μl, neutrophils of 70%; lymphocytes of 28%, platelets of 39,000. Nosocomial pneumonia

was diagnosed, and trimethoprim/sulfamethoxazole (TMP/SMX) was added at a dose of 20 mg/kg/day.

Four days later, ventilatory parameters and aminergic support were decreased. The patient presented with fever of 38.5°C, tachycardia, bleeding through both the orotracheal cannula and the colostomy. On physical examination, he presented with a whitish plaque on the palate, upper and lower extremities with indurated violaceous lesions of approximately 0.5 cm. The radiography showed a diffuse alveolar infiltrate in the left hemithorax and right apical atelectasis. Extension of antibiotic coverage was decided to cover multiresistant microorganisms with colistin 5 mg/kg/day. A 4-mm punch biopsy was done and, on the next day, a computerized axial tomography (CAT), which showed three round peripheral lesions in the right hemithorax. Galactomannan antigen was reported positive at 2.2 (positive >0.5). A diagnosis of probable invasive aspergillosis was integrated, and treatment with voriconazole was started at a dose of 6 mg/kg every 12 hours (impregnation) and 4 mg/kg every 12 hours (maintenance). Treatment with TMP/SMX was suspended.

At 48 hours, a neutrophilic hypodermatitis with septic vasculitis was found on the skin biopsy, probably due to aspergillosis. Diagnosis of disseminated aspergillosis to lung and skin was integrated. The patient completed a 14-day course of meropenem/vancomycin. Colistin was suspended given the absence of multiresistant microorganism. He continued with voriconazole and amphotericin B.

Therapy with norepinephrine was restarted, and aminophylline was added. The patient continued with phase III mechanical ventilatory support, furosemide infusion and hydrocortisone. He required platelet transfusion due to thrombocytopenia. Two days after he once again presented with fever spikes, tachycardia, new violaceous necrotic lesions in the left palpebral region and anterolateral face of the right thigh. The level of galactomannan was 1.1. On direct examination of the bronchial aspirate, macrosiphonated, hyaline, and septated hyphae were observed. Change to combined therapy with voriconazole and caspofungin and amphotericin B was suspended. No bacteria were isolated from four central and two peripheral blood cultures.

A week later, the patient persisted with a fluid overload of 10%, phase III mechanical ventilatory support, with an increase in ventilatory parameters. Chest radiography showed full left lung consolidation, abundant thick secretions through the cannula and mucous plugs with traces of blood, with a permissive hypercapnia strategy due to extensive lung damage. Liver renal

Figure 1. Chest radiography where diffuse radiopacities with nodules in the right basal region can be observed.

function tests were normal. The patient was found to be afebrile, with multiple skin nodules which ulcerated when exposed. Norepinephrine was suspended, and he was maintained without aminergic support.

Twenty-five days after his admission, the patient presented with massive pulmonary hemorrhage with abundant bleeding through the orotracheal cannula and immediate hemodynamic deterioration: he presented with asystole and cardiorespiratory arrest which didn't revert with resuscitation maneuvers. A day after the patient died, the culture of the endotracheal aspirate reported the identification of *Aspergillus flavus*.

Case report

Chest radiography showed ground glass opacities in the right lung, with pleural drainage projected to the left hemithorax, and the distal tip in the ipsilateral pulmonary apex as well. Also, an endotracheal cannula, a right jugular venous catheter with the distal tip in the ipsilateral atrium, as well as a left subclavian catheter with the distal tip in the same location of the atrium. A radiopacity in the right pulmonary apex was also identified, suggestive of atelectasis, which persisted with obliteration of the ipsilateral costodiaphragmatic recess. Perihilar and right basal nodules were also identified, as well as findings of pulmonary congestion (Figure 1). A contrast-enhanced computed tomography (CT) with a mediastinal window was indicated which showed areas of alveolar occupation with air

Figure 2. Chest CT with a mediastinal window where nodules in the right basal region with zones of alveolar occupation in the left lung can be observed.

Figure 3. Lung window in which nodules in the right basal region with a hypodense halo and zones of alveolar occupation in the left lung can be observed.

bronchogram. In the more inferior slices, nodules referred in the chest radiography were seen projected over the left basal area with air bronchogram (Figure 2). In the lung window, bronchoalveolar occupation in both lungs was observed and confluent zones or nodules, many over the same region of alveolar occupation. A hypodense halo and a vascular structure leading to it were detected, which likely correspond to the formation of thrombi around this nodular lesion, probably of mycotic origin (Figure 3). The left basal bronchoalveolar occupation also persisted. CT slices of the abdomen showed thickening of the intestinal mucosa, intestinal and colonic stomata; mesenteric fat stranding, which could correspond to an infectious or inflammatory process (Figure 4). Right renal pelvic dilatation and mesenteric fat stranding in the pelvic cavity were also detected.

Based on the radiologic findings, a pneumonic process of possible fungal origin was concluded, probably aspergillomas. Pulmonary superinfection in these consolidation areas could not be discarded; also, there was fluid overload and an intestinal inflammatory process.

Discussion

The present case concerns an 8-year-old male patient, who attended the HIMFG a month before his death by integrating a diagnosis of acute lymphoblastic leukemia. This disease is the most frequently identified cancer in pediatric patients, with an incidence of up to 40%. Initial symptomatology is insidious; nevertheless, there are clinical and laboratory data that allow for a suspicion of this diagnosis, which is a reflection of neoplastic cell infiltration of the bone marrow and the organism.

Figure 4. Thoracoabdominal tomography with the presence of pulmonary nodules and signs of generalized thickening of the intestinal mucosa.

At diagnosis, the patient debuted with an anemic syndrome by presenting asthenia, adynamia, Hb of 3.6 g/dl, as well as an infiltrative syndrome by presenting with cervical, axillary and inguinal adenopathies. A BMA reported the presence of 91% of blasts. He was classified as high risk by presenting a leukocyte count greater than 100,000 (according to the Rome criteria), T immunophenotype (according to the immunologic classification), in addition to the poor response to the steroid window by reporting over 1000 blasts in peripheral blood at the eight day of steroid treatment. Therefore, chemotherapy was started with the HIMFG 2003 protocol for high-risk leukemias. This protocol consists of four phases; an induction to remission phase, an intensification phase, a consolidation phase and a maintenance phase. For the induction to remission phase four drugs during four

weeks are used: a vinca alkaloid (vincristine), an anthracycline (daunorubicin), an enzyme-like drug (L-asparaginase) and steroids.

At admission, the patient was in the third week of induction to remission, which has the objective of reducing tumor burden in a percentage of 99%. Also, to restoring hematopoiesis and improving the functional state of the bone marrow, objectives that very probably were not achieved because they were not reported in the clinical summary of weekly evaluations. Therefore, the state of the disease at admission was unknown. It cannot be ruled out that pancytopenia on this patient was associated with disease activity and was not secondary to a cytotoxic effect of the chemotherapeutic drugs that were administered.

The patient presented in shock (according to the emergency department), which can be classified as a distributive septic type due to the clinical conditions at admission. According to the American Heart Association, this is characterized by the poor distribution of blood flow, causing a state of tissue hypoperfusion and anaerobic metabolism. The clinical manifestations of the patient at admission were tachycardia and hypotension with wide pulse pressures and arterial blood gases showing metabolic acidosis, with a lactate of 5.1 and a base excess (BE) of -9.4 mmol/l. Crystalloid boluses were administered, with the objective of improving preload which was found to be decreased due to the initial vasodilatation. As a compensatory reflex mechanism, and as the shock progressed, there was a decrease in cardiac output and sepsis-induced myocardial dysfunction.

In this case, after the administration of three boluses of crystalloids, treatment with adrenaline and norepinephrine was deemed necessary, due to the cardiovascular dysfunction the patient presented.

The source of infection was identified in the abdomen. A diagnosis of neutropenic colitis was integrated, which is considered an infectious oncological urgency and presents in 65-75% of the patients with hematological neoplasms. It is characterized by the presence of abdominal pain (90%), fever (84%), and diarrhea (72%), which were present in this patient. This disease is conditioned by a combination of factors such as the presence of mucositis, bacterial overgrowth, edema of the intestinal wall, and decreased perfusion. It can present up to two weeks after beginning cytotoxic chemotherapy or at the nadir of chemotherapy. In this case, the patient was in the third week of cytotoxic treatment.

According to the Schabarger criteria, which predict the need for surgery in patients with neutropenic colitis,

the patient persisted with progressive hemodynamic deterioration despite the use of amines. As this translated in amine refractory septic shock, exploratory laparotomy was necessary, which allowed for the detection of ileum and colon dilatation and the presence of areas of ischemia and necrosis in the cecum, for which a hemicolectomy and ileostomy was performed.

When fever and signs of inflammatory response persisted after 72 hours of the beginning of the antibiotic administration, it was decided empirically to add antifungal treatment on a critical route. The identified risk factors in our patient included previous surgery, the use of broad-spectrum antibiotics, and profound neutropenia. Taking into account that the main agent causing fungal infections and that lives in a saprophyte manner in mucous membranes is *Candida*, treatment with amphotericin B was started in accordance with fever and neutropenia guidelines proposed by the Infectious Diseases Society of America in 2010. By presenting with crepitant crackles on physical examination, a right alveolar infiltrate in the chest radiography and, by meriting an increase in ventilatory parameters, a diagnosis of nosocomial pneumonia was integrated. *Pneumocystis jirovecii* is among the etiologies to consider in an immunocompromised patient. This is characterized for presenting a subacute course with insidious symptoms, such as a non-productive cough, dyspnea and fever, associated with hypoxemia and LDH elevation >500, which are common indicators, although not specific, and are associated with radiological characteristics such as the presence of an alveolar and diffuse interstitial infiltrate. Nevertheless, the precise diagnosis merits a bronchoalveolar lavage, with the objective of demonstrating the presence of cysts of trophozoites. This study was not performed in this patient, so empiric coverage with TMP/SMX was decided. Afterwards, herpes simplex virus infection was documented, so treatment with acyclovir was started. According to the aforementioned guidelines, treatment for viral infections is only indicated if there is evidence of disease activity, as in this patient's case, in whom it was documented by the Tzank test and the characteristic skin lesions. Five days after the onset of empiric treatment for *P. jirovecii*, the patient persisted with fever and signs of systemic inflammatory response, identifying the presence of a whitish lesion in the palate and violaceous lesions in the extremities on physical examination. These lesions forced to rule out a systemic mycosis being that the patient had a severe and prolonged immunosuppression under intense antibiotic pressure. A diagnostic approach was necessary, having

aspergillosis as the main suspicion and, as differential diagnosis, the presence of *Fusarium*, *Zygomycetes*, *Scedosporium*. Currently, *aspergillosis* is considered as the most common cause of death by infectious pneumonia in immunocompromised patients. The most frequently identified species is *Aspergillus fumigatus*^{1,2}. According to the cooperative group of the European Organization for Research and Treatment in Cancer, three levels of certainty for invasive aspergillosis were established: proven, probable and possible. These definitions have been developed with the objective of maintaining consistency in clinical and epidemiological studies, and to drive therapeutic decisions. Proven aspergillosis requires histopathological evidence of the infection and a positive culture of a sample taken from a usually sterile site. Probable aspergillosis requires that certain signs be present in three categories: factors related to the host, clinical manifestations and microbiological data³. Given that a sample of a bronchoalveolar lavage for culture, percutaneous aspiration, or biopsy are needed to establish the diagnosis, alternative methods such as galactomannan detection have been used. Galactomannans are a component of the *Aspergillus* cellular wall that are released during angioinvasion and are detected in peripheral blood, reaching a sensitivity of 61% and a specificity of 93%. Imaging studies are used to determine the site of infection, the number, and size of the lesions. CAT is the study of choice that allows distinction of typical but not specific characteristics, such as wedge-shaped lesions or infarctions, a halo sign defined as a nodule great than 1 cm surrounded by an area of opacity and, the air crescent sign⁴. This patient was catalogued as having a proven invasive fungal infection supported by suggestive radiological images, a direct examination of the endotracheal aspirate with the presence of hyaline macrosiphonated and septated hyphae, positive galactomannans and a skin biopsy reporting vasculitis due to aspergillosis.

Voriconazole is the initial therapy of invasive aspergillosis. Combined therapy is suggested as rescue therapy in patients not responding to monotherapy with voriconazole or amphotericin B liposomal⁵. In these patients, the addition of an echinocandin, such as caspofungin, is suggested. This combination of antifungals has translated into a positive impact on survival, compared to monotherapy with triazoles. Duration of antifungal therapy depends on the location of the infection, the underlying disease of the patient, the need for additional immunosuppression and response to treatment. Antifungal therapy is maintained until all signs

Figure 5. Histological slices showing neutropenic colitis with extense necrosis and hemorrhage of the colonic mucosa, edema of the submucosa, lymphocytic inflammatory infiltrate, without evidence of neutrophils (hematoxylin-eosin (HE) 10x).

and symptoms of the infection have resolved, as well as when imaging findings have disappeared.

Pulmonary aspergillosis presents mortality of 75-92%, with massive hemoptysis as the main direct cause of mortality in up to 80% of the cases. Poor prognosis factors that have been identified include prolonged immunocompromise, the use of steroids, the site and extension of the infection and the use of mechanical ventilation; these factors were present in the patient described in this study⁶.

In conclusion, this patient had a torpid evolution due to severe immunosuppression, which can be attributed to previous treatment or to disease activity, which determined an increased risk of infections. Among these infections, invasive aspergillosis is found, which led to pulmonary hemorrhage and, secondarily, to a mixed shock (hypovolemic and septic).

According to the evolution of this patient, the following final diagnosis can be integrated:

1. High-risk ALL by T immunophenotype, leukocyte count and poor response to steroids
2. Neutropenic colitis
3. Refractory septic shock
4. Pulmonary invasive aspergillosis

As a direct cause of death: pulmonary hemorrhage and hypovolemic shock.

Pathology

Regarding pathology findings, the patient had an an-temortem study that corresponded to an intestinal segment measuring 20 cm. Of these, 6 cm corresponded to the terminal ileum and the rest to the cecum and ascending colon. The macroscopic image was of a

Figure 6. Percentage of cases that presented with neutropenic colitis (C) and the percentage of neutropenic colitis associated with different diseases (SC). ALL, acute lymphoblastic leukemia.

necrotic-hemorrhagic colon, with an edematous mucosa with a thickness of 0.4 cm (Figure 5).

The pathophysiology of neutropenic colitis is related to severe neutropenia which allows the growth of enteropathogenic bacteria, which produce toxins that damage the epithelium of the mucosa causing necrosis, adding to the cytotoxic effect of the chemotherapeutics. These changes translate histologically as extense necrosis with recent hemorrhage, significant submucosal edema and variable grades of wall damage that can lead to microperforations or complete perforations of the intestinal wall.

In the pathology service, a study of more than 240 autopsies of patients having any immunodeficiency was conducted, in which it was observed that up to 25% of these immunocompromised patients had neutropenic colitis. Of these, almost 60% were associated with ALL, 22% with acute myeloid leukemia (AML) and the rest to sarcomas, lymphomas or nephroblastomas (Figure 6).

In the post-mortem study, the patient presented with an abdominal scar of 25 cm, erythematous-violaceous lesions, mainly in the fingers of the hands and feet, in the arms and the left cheek; some of these were caviated with fibrin and hemorrhagic background (Figure 7).

The lungs had a diffuse thickening of the visceral pleura with areas of extense fibrosis and granulomatous nodular lesions. In serial slices of the lung parenchyma, extense areas of necrosis and hemorrhage were observed practically in 100%, with multiple pseudocysts and caviated areas; some with a necrotic background, others with a hemorrhagic background. In histological slices, these corresponded to secondary

Figure 7. Excavated skin lesions. Skin microscopy with necrosis of the dermis and epidermis, lymphocytic inflammatory infiltrate (HE 5x), associated with septated hyphae with dichotomous budding, which are morphologically compatible with *Aspergillus* (Grocott 40x).

Figure 8. Top: lung with pleural thickening, parenchymal necrosis and cystic lesions at the lung bases. Bottom: histological images with emboli of *Aspergillus* hyphae (HE and Grocott 10x).

Figure 9. Significant thickening of the pericardium is shown, perforated abscesses in the cardiac septum; microscopically, lymphocytic inflammatory infiltrates, and *Aspergillus* hyphae are observed (HE 10x).

Figure 10. Subcapsular lesions in the liver; microscopically, granulomas with *Aspergillus* hyphae are observed (Left: HE 10x; Right: Grocott 40x).

infarctions provoked by septic emboli of the previously described hyphae (Figure 8).

In another area, in which the parenchyma was better conserved, extensive regions of hemorrhage were observed, with thickening of the interalveolar septa and formation of hyaline membranes.

The heart presented with fibrinous pericarditis with areas of hemorrhage and necrosis at the apex. In the interventricular septum slice abscesses defined by a clear halo were observed; in histological slices, a lymphocytic inflammatory infiltrate with evidence of *Aspergillus* hyphae was observed (Figure 9).

The abdominal cavity presented with multiple adherence; the stomach, with thickened folds, edematous, with areas of hemorrhage and two perforations, the largest of 1.4 cm at the fundus and the smallest of 0.4 cm. Histological slices evidenced granulomas with *Aspergillus* hyphae.

The small intestine, colon, and stomata presented with focal loss of the mucous folds, areas of mucinous aspect with a greenish color. In histological slices, the

Figure 11. Nodular lesion at the corticomedullary junction; microscopically emboli of *Aspergillus* hyphae are observed (HE and Grocott 10x).

mucosa was partially conserved; a fibrin deposit, inflammatory infiltrate, and the presence of *Aspergillus* hyphae was observed in the serosa.

The diaphragm presented with multiple areas of necrosis that microscopically corresponded to areas invaded by the fungus.

The liver had a brown-yellow color. In the subcapsular region, the color was violaceous-greenish; histological slices corresponded to necrosis with the formation of giant cells related to *Aspergillus* hyphae. The rest of the parenchyma presented signs of shock, characterized by micro and macrovesicular steatosis, dilatation and sinusoidal congestion (Figure 10).

The pancreas presented histological signs of damage secondary to chemotherapy, characterized by chronic pancreatitis and bands of fibroconnective tissue that formed nodules of pancreatic acini.

The right kidney presented with a nodular lesion of approximately 1 cm at the corticomedullary junction; in histological slices, these corresponded to zones of infarction secondary to thrombosis by *Aspergillus* hyphae (Figure 11).

The bone marrow had approximate cellularity of 80%, with the presence of the three hematopoietic lines with adequate maturation; neoplastic infiltration or granuloma formation related to *Aspergillus* hyphae were not observed.

Lymph nodes were observed to be depleted of lymphoid tissue with numerous macrophages with hemophagocytosis by macrophage activation secondary to the fungal infection.

The brain presented signs of edema, wide circumvolutions, congestive vessels; at the base of the brain, congestion and opacity of the meninges were

Figure 12. Lesion in the frontal cortex with a necrotic-hemorrhagic background; microscopically, *Aspergillus* hyphae are observed (Grocott 10x).

observed. In histological slices, mainly in the frontal, parietal and temporal region, partially defined areas with a greenish mucinous material and areas of hemorrhage were observed, that corresponded to granulomas with necrosis and the presence of hyphae (Figure 12).

Final diagnosis

The main diagnosis was T-immunophenotype ALL, associated with disseminated aspergillosis with septic emboli to the lung, heart, diaphragm, peritoneum, liver, stomach, brain and skin. In addition, acute ulcerated tracheitis, neutropenic colitis in post-colectomy status, active chronic peritonitis, macrophage activation syndrome in the lymph nodes and chronic pancreatitis, probably secondary to treatment.

Histological signs of shock were also diagnosed (visceral myopathy, acute tubular necrosis, hypoxic encephalopathy and diffuse alveolar damage). The cause of death was a septic shock.

Final comments

Infectology

Aspergillus is a ubiquitous, hyaline, filamentous fungus. It constitutes the first cause of fungal infection by filamentous fungus and the second cause (only after *Candida*) of invasive fungal infection⁶.

Invasive aspergillosis has emerged in the last couple of years as an important cause of morbidity and mortality in immunocompromised patients; pediatric immunocompromised patients, particularly those with a

profound and prolonged decrease in neutrophils, are the most affected, with reported mortality between 50-100% if no treatment is given⁷⁻⁹.

Once the diagnostic possibility of invasive aspergillosis has been established, there are several diagnostic studies available. It must remember that the most reliable samples will be obtained from sterile or profound sites. Direct microscopic examination notably increases the sensitivity of the culture. While global sensitivity is not that high, in around 50%, visualization of septated hyphae, macrosiphonated and with acute-angle branching in a patient at risk, will be highly suggestive of invasive aspergillosis¹⁰.

Galactomannan is a polysaccharide, and a component of the fungus cellular wall, which is released mainly during angioinvasion, and its detection in serum and bronchoalveolar lavage through an enzyme-linked immunosorbent assay (ELISA) is helpful^{11,12}. In bronchoalveolar lavage, it has a sensitivity greater than 70% and in serum, of 57-71%, and a variable specificity of 66-89%. In 2008, in the journal *Anales de Oncología*, it was reported that two consecutive tests increased the accuracy of the test significantly, with a 92.1% sensitivity and a 97.5% specificity.

The gold standard will always be the identification of the fungal infection by filamentous fungus in culture from sterile sites.

Before first-line therapy with voriconazole, mortality by invasive pulmonary aspergillosis was greater than 60%¹³. Voriconazole has increased survival. In several analyses that have been conducted after treatment with voriconazole, it has been observed that disseminated disease increases, at least, three times the probability of death (from 66 to 90% of mortality)¹⁴. It has been observed that when patients with cancer have an invasive fungal disease, it can lead to a decreased mortality. Epidemiological studies have not demonstrated that double antifungal therapy is superior to monotherapy¹⁵. There is a randomized clinical trial in adults in which treatment with an echinocandin (anidulafungin, still not approved in children) combined with voriconazole against monotherapy with voriconazole. Said study did not demonstrate that double therapy was better than monotherapy, not even in epidemiologic studies. Therefore, the niche for double therapy would be patients that have a disease that is refractory to treatment⁴, which are considered to be who have received at least seven days of adequate treatment and evidence of worsening. One must be careful with this definition, given that there could be a similar syndrome as

the one observed with HIV immune reconstitution, where the fact that there is a recovery of neutrophils during an infectious disease could potentially have a paradoxical effect. Once the disease is established as refractory, rescue or salvage therapy must be administered. There are two possibilities: changing the type of medication to monotherapy or indicating combined therapy. Regarding monotherapy as a rescue therapy, amphotericin B is inferior; it offers a survival of barely 30-39% in refractory disease. The new azoles, such as posaconazole have an efficacy of around 45% in refractory disease. In a meta-analysis published three years ago, it was reported that combined therapy decreases mortality in comparison to monotherapy.

Facing the logical question of which medications to administer in combination, the two options are an azole with amphotericin or an azole with echinocandins. In animal models, an antagonism and synergism have been described with voriconazole and amphotericin B; although they are mentioned as an option in clinical guidelines, it is probably not the best therapy. Apparently, a combination of voriconazole and caspofungin is the best therapeutic option, and the niche would be the patient that already has a documented disease by *Aspergillus*, and that is refractory to treatment with voriconazole monotherapy⁴.

Intensive care unit

Without a doubt, children with cancer admitted to the intensive care unit (ICU) are a challenge. For the general population, there is a 10% mortality in the ICU due to septic shock, which is similar to international goals. However, in oncological patients, there is a mortality of 20-22%, which is perfectible given that in other centers, like St. Jude Children's Research Hospital, the percentage of oncological children with these characteristics dies in 15-17% of the cases.

The identified factors for a greater risk of mortality upon admission to the pediatric intensive care unit (PICU) are profound and prolonged neutropenia, as well as inotropic support from admission. Also, mechanical ventilation and vasopressor concomitant support, acute renal failure from admission, a high pediatric risk of mortality (PRISM III score), fungal infections, and bone marrow transplant. In addition to abdominal sepsis, this patient presented seven of these criteria. In case of admission to the ICU, the treatment must be very aggressive but fluid resuscitation very cautious.

The consensus states that two to three boluses should be administered, but it is not a rule. Caution is needed with the fluid administration since radiological and pathological evidence can show the consequences of excessive fluid resuscitation. It is very important that in these children all resuscitation efforts be conducted in the ICU because sometimes they present with hepatomegaly. To this end, invasive monitoring is used to decide upon the number of fluids, pulse variability, Pico systems, passive leg-raising tests and echocardiographic measurements of inferior vena cava collapsibility. All these help to guide management; in this case, the final options of management would be plasmatic exchange therapy and slow plasma exchange. Plasmapheresis can also be done, with the objective of improving the cytokine profile, inflammation and, above all, improving thrombotic microangiopathy that can be present in these children and that is the cause of organic dysfunction.

On the other hand, up to 25% of fluid overload was documented in the history of the patient. It has been reported that a value greater than 10% at initial stages of resuscitation in septic shock is a factor associated with mortality, which is important because this overload prevented the rapid extubation of the patient. Thus, triggering all the consequences that presented later. The initial diagnosis (abdominal sepsis) was treated with surgery, and the patient improved later and came to be in favorable conditions, but the sum of all the previously mentioned perfectible factors was what led to this unfortunate outcome.

Nephrology

For the Acute Kidney Injury Network, patients in the ICU have the possibility of developing renal failure in 30-80% of the cases; this increases morbimortality and the time in the ICU. To assess renal failure, clinical as well as metabolic criteria are available. In this patient, it was very clear that the metabolic criteria did not apply. He never had an increased creatinine, he had metabolic acidosis but no significant metabolic derangements. In addition, despite having an adequate urinary output, he presented with a significant percentage of fluid overload. Development of all the criteria is not strictly necessary to decide upon renal replacement therapy, but with fluid overload, renal replacement therapy is indicated.

Among slow and continuous renal replacement therapies, the patient required hemofiltration to remove volume as soon as possible. The kidney was depurating despite having the damage already commented in the pathology description, but it needed intense hemodynamic support.

Oncology

Overall survival in patients with ALL is 65-70%. The expected mortality percentage in the induction to remission phase is about 1%. In the HIMFG, mortality of 1.2-2% has been described in this phase. If mortality is greater than 1%, there is an opportunity for improvement.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Tragiannidis A, Roilides E, Walsh TJ, Groll AH. Invasive aspergillosis in children with acquired immunodeficiencies. *Clin Infect Dis*. 2012;54:258-67.
2. Groll AH, Schrey D, Tragiannidis A, Bochennek K, Lehrnbecher T. Invasive aspergillosis in children and adolescents. *Curr Pharm Des*. 2013;19:3545-68.
3. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis*. 2008;46:1813-21.
4. Patterson TF, Thompson GR, Denning DW, Fishman JA, Hadley S, Herbrecht R, et al. Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2016;63:e1-60.
5. Walsh TJ, Anaissie EJ, Denning DW, Herbrecht R, Kontoyiannis DP, Marr KA, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis*. 2008;46:327-60.
6. Braham Segal H. Aspergillosis. *N Engl J Med*. 2009;360:1870-84.
7. Anugulruengkitt S, Trinavarat P, Chantarnuwat P, Sritippayawan S, Pancharoen C, Thanyawee P, et al. Clinical features and survival outcomes of invasive aspergillosis in pediatric patients at a medical school in Thailand. *J Med Assoc Thai*. 2016;99:150-8.
8. Burgos A, Zaoutis TE, Dvorak CC, Hoffman JA, Knapp KM, Nania JJ, et al. Pediatric invasive aspergillosis: a multicenter retrospective analysis of 139 contemporary cases. *Pediatrics*. 2008;121:e1286-94.
9. Sipsas N, Kontoyiannis DP. Invasive fungal infections in patients with cancer in the Intensive Care Unit. *Int J Antimicrob Agents*. 2012;39:464-71.
10. Del Bono V, Mikulska M, Viscoli C. Invasive aspergillosis: diagnosis, prophylaxis and treatment. *Curr Opin Hematol*. 2008;15:586-93.
11. Chal LY, Kullern BJ, Johnson EM, Teerenstra S, Khin LW, Vonk AG, et al. Early serum galactomannan trend as a predictor of outcome of invasive aspergillosis. *J Clin Microbiol*. 2012;50:2330-2336.
12. Penack O, Rempf P, Graf B, Blau IW, Thiel E. Aspergillus galactomannan testing in patients with long-term neutropenia: implications for clinical management. *Ann Oncol*. 2008;19:984-989.
13. Chen J, Yang Q, Huang J, Li L. Risk factors for invasive pulmonary aspergillosis and hospital mortality in acute-on-chronic liver failure patients: a retrospective cohort study. *Int J Med Sci*. 2013;10:1625-31.
14. Karthaus M, Buchheidt D. Invasive aspergillosis: new insights into disease, diagnostic and treatment. *Curr Pharm Des*. 2013;19:3569-94.
15. Blyth C, Gilroy N, Guy S, Chambers T, Cheong E, Gottlieb T, et al. Consensus guidelines for the treatment of invasive mould infections in haematological malignancy and haemopoietic stem cell transplantation, 2014. *Intern Med J*. 2014;44:1333-49.