



# Graft versus host disease in a patient with chronic granulocytic leukemia who received an hematopoietic progenitor cell transplant

Marta Zapata-Tarrés<sup>1</sup>  
Eduardo López-Corella<sup>2</sup>  
Martín Pérez-García<sup>3</sup>  
Roberto Rivera-Luna<sup>4</sup>

<sup>1</sup>Oncology Service.

<sup>2</sup>Department of Pathology.

<sup>3</sup>Transplant Service.

<sup>4</sup>Hemato-Oncology Division.

Instituto Nacional de Padiatría, México, D.F.

## SUMMARY OF CLINICAL HISTORY

Male patient age 6 years 9 months, native of the State of Mexico, without relevant antecedents. Parents age 32 years, with high-school level education, merchants; subject has a brother age 10 years and a sister age 4 years. His government vaccination plan is complete. Two months ago he presented onset of fever, epistaxis, and splenomegaly. At Hospital Materno Infantil de Toluca the blood biometry reported: hemoglobin 8.8 g/dL, hematocrit 24%, leukocytes 230,500/mm<sup>3</sup>, neutrophils 21%, bands 34%, lymphocytes 9%, monocytes 7%, blasts 14%, basophils 11%, eosinophils 4%. An analysis found: serum creatinine and liver function tests normal.

Physical examination detected splenomegaly of 15 cm. A peripheral blood smear showed data compatible with chronic granulocytic leukemia. The bone marrow aspirate found hypercellularity, 5% blasts and proliferation of all series, BCR/ABL in ABC 27,253 copies. The patient was initially treated with imatinib (tyrosine kinase inhibitor) at doses of 450 mg/m<sup>2</sup>. The patient was referred to the National Institute of Pediatrics for evaluation by physicians in the Hematopoietic Stem Cell Transplant service.

On arrival, the patient was observed with: pallor of teguments and facial dermatitis secondary to imatinib. The day of the evaluation at the Hematopoietic Stem Cell Transplant service the patient was receiving 300 mg of imatinib daily; there are no data on tumor activity. He was considered suitable for hematopoietic stem cell transplant if a compatible donor was available and extension and HLA studies were planned.

Received: November, 2013

Accepted: January, 2014

### Correspondence

Dr. Roberto Rivera Luna  
Hematology-Oncology.  
National Institute of Pediatrics  
riveraluna@terra.com.mx

### This article must be quoted

Zapata-Tarrés M, López-Corella E, Pérez-García M, Rivera-Luna R. Enfermedad injerto contra huésped en un paciente con leucemia granulocítica crónica con trasplante de células progenitoras hematopoyéticas. Acta Pedit Mex 2014;35:45-54.

The HLA result was 100% compatible with the healthy 10-year old brother. The RT-PCR result of the bone marrow aspirate t (9.22) was positive. The patient continued receiving imatinib therapy.

Admission to the Hematopoietic Stem Cell Transplant service and start of preparatory treatment with busulfan. A catheter was placed in the left subclavian artery. On the eighth day graft was infused (cellular dose  $1.6 \times 10^6/\text{kg}$  of body weight); however, due to a blood clot the number of cells was insufficient, and the patient was treated again with cells harvested from a 100% compatible donor, without complications ( $\text{CD}34+ 7.7 \times 10^6/\text{kg}$  of body weight). Ten days after the transplant, the patient had myeloid graft, in view of which he was taken off neupogen. It was decided to release the patient due to his improvement.

On day +20 the patient was asymptomatic and in good general health, alert and reactive; treatment was continued with cyclosporine, fluconazol, TMP/SMZ, acyclovir, and omeprazole.

Eight days after consultation, the patient came in reporting a peak temperature of  $38^\circ\text{C}$ . He mentioned that two days before he had skin lesions on his neck and underarms, with no other symptoms. At admission he had heart rate of 120 beats per minute; temperature  $38.2^\circ\text{C}$ ; generalized skin erythema, scaling, without pruritus; scabs from ulcers on the occipital region; distal pulses, capillary filling two seconds, with no apparent evidence of infection.

The patient was evaluated by physicians of the infectology service and it was decided to prescribe antibiotics: ceftriaxone and dicloxacillin. Cyclosporine was resumed at doses of 6 mg/kg/day, methylprednisolone 2 mg/kg/day, and topical tacrolimus. He was hospitalized due to clinical signs of graft versus host disease on the skin and to confirm the diagnosis.

The dermatology service diagnosed a disseminated dermatosis affecting primarily neck folds, underarms, and the infraclavicular region, consisting of scaling, thick whitish scales, which detached easily, macular pruriginous rash, predominantly on the legs, with 5 cm maculas mm. This problem evolved in three days; a skin biopsy was taken. Patient was diagnosed with clinically acute graft versus host disease.

Virology studies showed: Epstein Barr Virus (EBV), VCA IgM and IgG negative, EBV EA negative, EBV EBNA negative, herpes simplex virus IgM E IgG negative, parvovirus B19 IgM E IgG negative.

The next day, after eating, patient reported colic-like epigastric pain, without evacuations; a labstix showed blood. Abdominal ultrasound revealed inflammation of ileum, colon, and appendix with less than 10 mL of free fluid; intestinal loops with thickness even of 6 mm. It was concluded that the study suggested colitis. Patient was left fasting and with analgesic. Metronidazole was added and vitamin K given due to the patient's presenting prolonged prothrombin time. He continued fasting due to abdominal pain. He presented edema on the face and on both hands. Uresis below normal values. Prednisone and sertraline were added to his treatment.

Six days later, patient continued to report abdominal pain, nausea, vomiting, and increased fecal flow with evacuations of diminished consistency. Hypoalbuminemia of 1.5 was detected and general urinalysis found proteinuria. A stool parasite examination was positive for *Blastocystis homini* and *Aspergillus* antigen negative. Parenteral nutrition was started. Patient was given dose-response doses of infliximab.

Three days later the patient had a seizure characterized by clonic motions, supraversion of eyes and sucking lasting 30 seconds. A study of



serum electrolytes showed hyponatremia, hypocalcemia, and hypomagnesemia. Subsequently the patient had further partial complex seizures, with neurological deterioration, Glasgow score 7. For this reason, orotracheal intubation was performed and diphenylhydantoin was prescribed. Meropenem and liposomal amphotericin B were added. Brain tomography ruled out edema and cerebral hemorrhage. Brain magnetic resonance showed corticosubcortical zones of vasogenic edema in the parieto-occipital regions, raising suspicion of a syndrome of reversible posterior encephalopathy and corticosubcortical cerebral atrophy. Diphenylhydantoin and methylprednisolone were discontinued.

The dose of cyclosporine was increased and hydrocortisone was added at doses of 50 mg. Blood culture was positive for gram positive cocci, in view of which vancomycin was added. One day before his death the patient had hypoalbuminemia, generalized edema, and hypothermia. He had hypocalcemia of 2.4. The electrocardiogram showed flattening of T waves. Because he had hyperglycemia as high as 367 mg he was given an infusion of insulin. Hydrocortisone was discontinued and methylprednisolone started.

The day of his death the patient had 60% desaturation, he was aspirated and given ventilation with positive pressure, which improved saturation and the ventilatory pattern. Gasometry reported pH 7.43, pCO<sub>2</sub> 30, pO<sub>2</sub> 61, HCO<sub>3</sub> 20, CO<sub>2</sub>T 21. The patient had hematuria, bleeding from the orotracheal cannula, and melanic evacuation. He continued to present episodes of desaturation and hemodynamic instability with bradycardia leading to asystole, with no response to resuscitation maneuvers.

### CLINICAL COMMENTS

The analysis of the case, for academic purposes, can be separated in the manner of approaching

the diagnosis of chronic granulocytic leukemia, the description of TCPH, and the final episode of acute graft versus host disease which poses difficulties in establishing diagnosis and administering treatment.

The patient's condition started with febrile and hemorrhagic syndrome. The association of the two syndromes necessitated a blood biometry. An abdominal mass in the left hypochondrium was added to this clinical pattern.

What could be suspected in this patient? The pediatrician, faced with a patient with an abdominal mass (albeit asymptomatic) should refer him to a pediatric oncologist to determine the least invasive sequence of studies needed to establish a diagnosis. The blood biometry showed anemia of 8.8 mg/dL, leukocytes 230,500, blasts 14%; platelets are not mentioned.

Before performing the bone marrow aspirate, oncological emergencies should be ruled out; in this case, they were primarily tumor lysis syndrome, a mediastinal mass, and complications from hyperleukocytosis, such as: hypoxemia and seizures. The latter are infrequent in children with chronic granulocytic leukemia; however, the most likely diagnosis in a child with hyperleukocytosis is an acute leukemia. Basophilia is a common finding in chronic granulocytic leukemia.

With such findings a bone marrow aspirate should be performed. However, in chronic granulocytic leukemia analysis of a peripheral blood smear may be sufficient for an initial diagnosis. At first, treatment should focus on cytoreduction, which can be achieved with hydroxyurea and busulfan. Today, imatinib, a direct tyrosine kinase inhibitor, produces complete remission in more than 95% of patients.

Taking into account the patient's antecedents, some risk factors for leukemias can be described.

The parents' age has been related to cancer, mainly leukemia. In the father, age over 40 years has been associated with leukemia, with a relative risk above 3. Conditions during pregnancy and maternal age have been studied, without finding any specific association.

Given that the patient had chronic granulocytic leukemia, the only factor described at pediatric age is ionizing radiation. It has not been associated with infection, and very rarely appears as a second neoplasm.

Chronic granulocytic leukemia is a very rare disease in children: 2 cases per 100,000 inhabitants, occurring in the fourth and fifth decades of life. In children, cases have been reported in infants, but 80% are diagnosed after 4 years and 60% after 6 years.

Chronic granulocytic leukemia was first described in 1845 by Bennett. It was the first report of malignant neoplasm associated with a specific chromosomal abnormality. Daley showed that the malignant phenotype of the disease is caused by the BCR-ABL fusion gene, whose transcripts give rise to the production of an active tyrosine kinase (PM 210kD), which influences cell adhesion in the stroma of the bone marrow and also inhibits apoptosis.

In the past no effective treatment had been found for the disease, and patients died around three years after diagnosis, as a result of a blastic crisis. In the 1990s, with the development of tyrosine kinase inhibitors (imatinib mesylate), patients started to achieve complete cytogenetic remission; seven-year follow ups mention disease free survival of 83%, and 92% free of blastic crisis.

Today there is a dilemma, even in patients with 100% compatible donors, as to whether or not to perform a hematopoietic stem cell transplant. In favor is the high index of episode free survival

and against the fact that imatinib is a drug for which phase III and phase IV clinical trials have not yet yielded results in children.

For more than 15 years, hematopoietic stem cell transplant has been indicated for this disease; access to imatinib is more recent, raising the question of whether the prognosis changes if it is given before or after imatinib therapy, which actually delays the procedure and we do not know whether or not it increases morbidity and mortality. The European Bone Marrow Consortium has stated that there are no differences at 5 years follow up. Also, at present it is not known at what time the medication can be discontinued, an issue that arises especially in children.

The definitive treatment of this patient was with an allogenic hematopoietic stem cell transplant, which was performed without complications. The patient was released on day +20. At 8 days after his release from the hematopoietic stem cell transplant unit, the patient returned with fever and lesions of the skin. The lesions, mainly in skin folds, are described as scaling, without pruritus, detachable. The classic lesions described also include bullae.

Differential diagnoses should be drug reactions and viral rash. Biopsy is definitive in this diagnosis, and the principal findings described are perivascular lymphocytic infiltration, and in advanced cases, separation of the dermo-epidermal junction. Abdominal pain is added. The semiology is not described. The EICH describes colic and diarrhea. The differential diagnosis is intestinal infection by *Clostridium difficile* or by cytomegalovirus.

Prophylactic treatment is based on cyclosporine, steroids, and tacrolimus. In cases where a steroid is indicated in large doses, such as 2 mg/kg, and there is no response, monoclonal antibodies are prescribed, such as: daclizumab, alemtuzumab,



or infliximab, as used in this patient, at three weeks from his admission. The antibiotic scheme indicated was within established criteria, and the patient had no signs of sepsis at the time.

Nevertheless, the patient had seizures, initially considered of metabolic origin. Hemorrhage was ruled out, despite being a possibility to be considered because the patient had 26,000 platelets. The patient did not have fever; however, it would have been advisable to perform a lumbar puncture.

The patient was tested for cytomegalovirus, a viral infection which occurs in up to 70% of child transplant recipients; this problem appears, characteristically, in the first 100 days. In this patient diagnostic studies were negative.

The patient did not react to treatment. In such cases it should be considered that other problems may be occurring, for example: uremic-hemolytic syndrome or cyclosporine-associated thrombotic microangiopathy. The latter is an entity clinically and anatomopathologically characterized by: anemia, acute renal involvement, and thrombocytopenia predominantly caused by a renal microangiopathy, but which may affect other parenchymas, such as the central nervous system and the gastrointestinal tract. In this case, the patient had compatible alterations, as well as heightened urea concentrations of 7 to 31, although creatinine was unaltered.

Microangiopathy is caused by damage to microvasculature due to calcineurin inhibitors, chemotherapy or total body irradiation, or by infections. At this point we can also consider the antibiotic regimen based on the clinical evidence of deterioration and the lack of evidence of infection.

Finally, the patient suffered hemodynamic deterioration possibly due to an undetected infection.

Final diagnoses

1. Chronic granulocytic leukemia in remission.
2. Post 100% compatible allogenic transplant.
3. Probable thrombotic microangiopathy.
4. Grade 4 Acute intestinal and skin graft versus host disease.
5. Grade 4 graft versus host disease *versus* meningitis.
6. Septic shock-graft versus host disease secondary to a non-bacterial infection.

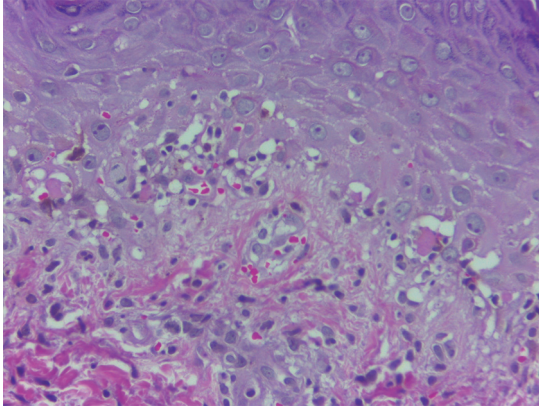
Cause of death: pulmonary hemorrhage.

## PATHOLOGY

Four hours after the patient's death an autopsy was performed in the Department of Pathology, with exit diagnoses of pulmonary hemorrhage, graft versus host disease in skin and intestine, and chronic granulocytic leukemia. The patient presented extensive skin lesions with eschars and scaling; we had access to histological documentation on the skin biopsy, performed one month before, containing findings compatible with graft versus host disease, with necrotic keratinocytes and vacuolar changes in the basal portion of the epidermis and lymphocyte infiltrates in the dermoepidermal junction and intraepithelial. Although these changes are not specific or reactions to medications, they can produce similar alterations; in the clinical context of this patient, a diagnosis of graft versus host disease can be considered (Figure 1. Pathology). This complication is explained because the autopsy found good cell repopulation in the bone marrow post-transplant. Leukemic infiltration was not found in any organ.

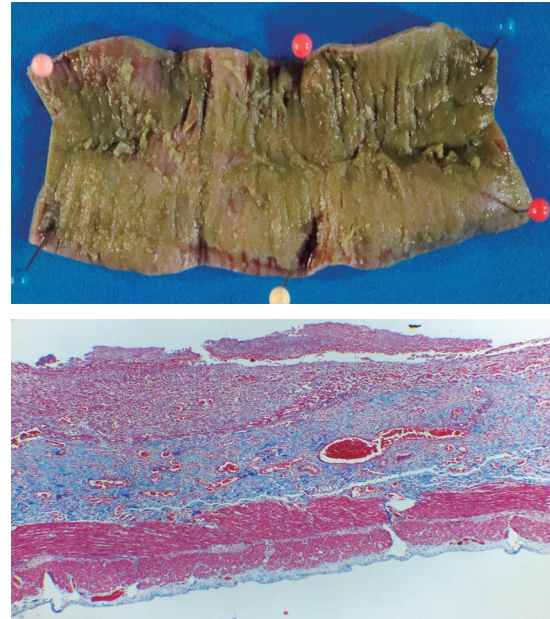
The final clinical manifestation was gastroenterological. In the stomach there were some focal changes suggestive of graft versus host disease, but the most notable damage was in the small intestine, with an extensive lesion affecting the jejunum and the ileum but not the colon. There





**Figure 1.** Extensive skin lesions compatible with graft versus host disease. The skin biopsy from one month before shows necrotic keratinocytes and basal vacuolar changes compatible with that alteration.

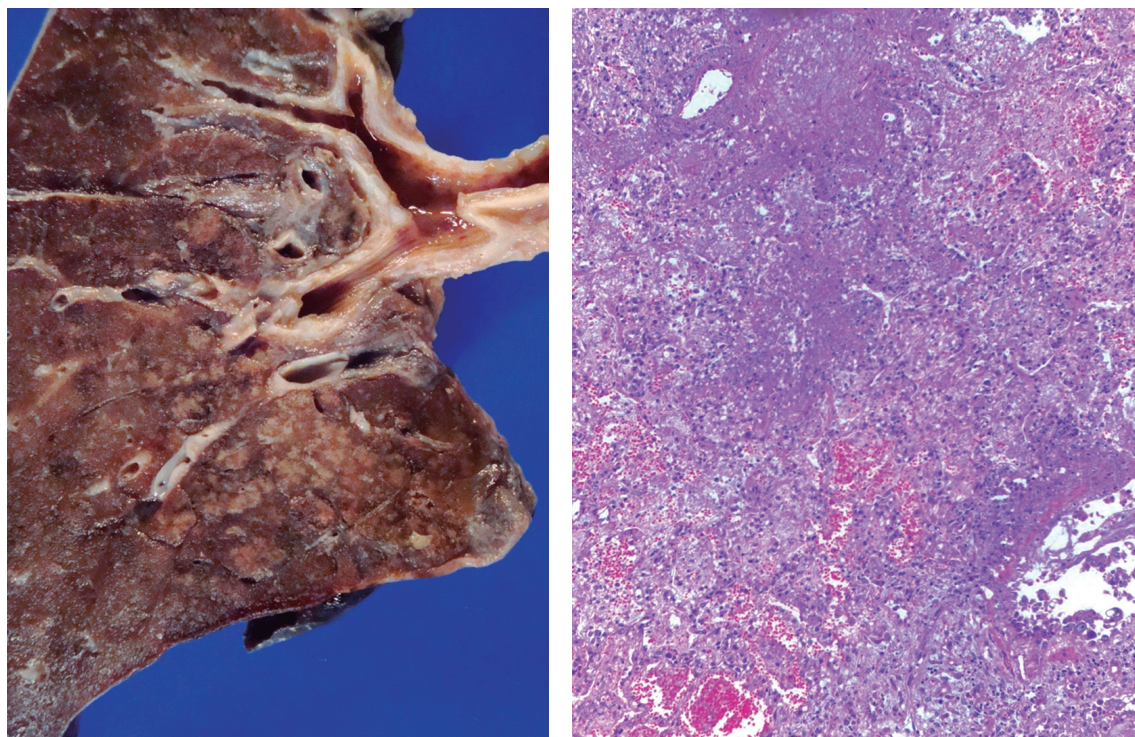
was total necrosis of the mucosa, with formation of pseudomembranes, fibrin, and scarce mononuclear inflammatory cells. (Figure 2. Pathology) Given that there was no acute exudate with neutrophils, although the bone marrow was reasonably populated, or mobilization of a neutrophil population, it did not behave like a neutropenia. Therefore we have a neutropenic enteritis, which in children is commonly caused by *E. coli* or *Pseudomonas*. In this case, the postmortem culture isolated *Pseudomonas aeruginosa* in the lung, liver, spleen, blood, and Peyer's patch. In the left lung there was multifocal hemorrhagic pneumonia with extensive consolidation. The histology of this lesion showed limited inflammation and extensive necrosis with deposit of a bluish pigment, characteristic of *Pseudomonas* infection, formerly known as "piecianic bacillus" (Figure 3. Pathology). The patient had a septicemia caused by *Pseudomonas* with the expected cohort of complications. We cannot venture an opinion on the involvement of graft versus host disease in the intestinal pathology because, when the mucosal epithelium is absent, the diagnostic signs of that alteration disappear.



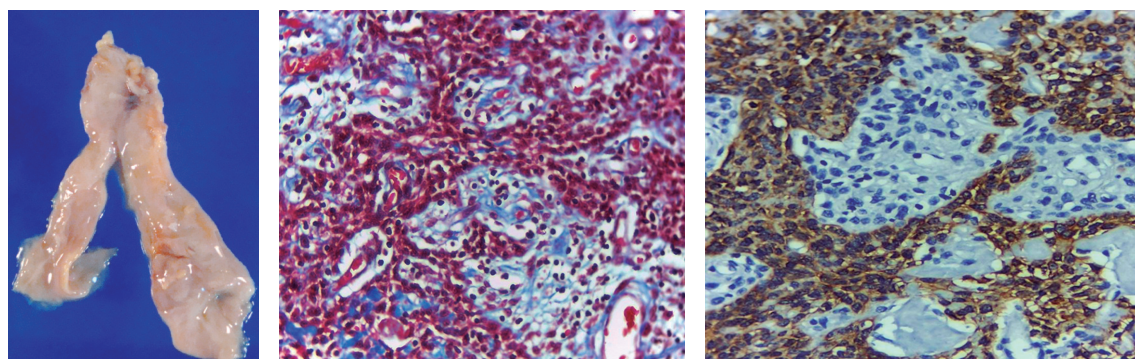
**Figure 2.** Segment of jejunum. Complete necrosis of the mucosa covered by fibrinous pseudomembranes. No inflammatory cellular exudate is seen.

The central nervous system was studied in detail, bearing in mind the neurological manifestations in life. Involvement was limited, with some atrophy, very minor anoxic damage, and without finding vasculitis, demyelination or gliosis, based on which we assume that the cause of the seizures could have been metabolic or angiospasm.

In the thymus there was an interesting finding: it was small and without histological signs of atrophy, but with signs of dysplasia with lymphoid loss and without epithelial maturation to Hassall's corpuscles. (Figure 4. Pathology) This is seen in the primary severe combined immunodeficiency, but has also been described in graft versus host disease due to transfusion or transplant, and in acquired immunodeficiency syndrome. In animal models it appears to be a reversible phenomenon. Its physiopathological meaning is unclear.



**Figure 3.** Basal consolidation in the left lung, with necrotic and hemorrhagic pneumonia with scant inflammatory exudate. The blue tone of the necrosis is characteristic of *Pseudomonas* infection.



**Figure 4.** The thymus is small with a histology of dysplasia. The stroma is fibrous, with scant lymphocytes, and the epithelial cells stained with Masson's trichrome stain (Photograph 1) and with anti-keratin antibody (Photograph B) are arranged in trabeculae without formation of Hassall's corpuscles.



In summary, this patient with chronic granulocytic leukemia t(9:22), who underwent successful hematopoietic stem cell transplant subsequently presented graft versus host disease with severe lesions in the skin, and possibly in the digestive tract, which was complicated by a pseudomembranous neutropenic enteritis in the context of a sepsis due to *Pseudomonas*.

#### Comment by the hematopoietic stem cell transplant service

In the evolution of hematopoietic stem cell transplant there are three decisive phases: the first is pre-transplant, in which there must be an indication to perform it. In this case the patient suffered chronic granulocytic leukemia; however, to be eligible for transplant the indication of the underlying condition does not suffice, an organic evaluation that does not contraindicate the procedure is also necessary, and naturally a source of stem cells from peripheral blood, umbilical cord, bone marrow, all to offer greater possibility of success.

In the second, transplant phase, the conditioning regimen is important. In this patient it was with busulfan and cyclophosphamide. There are several factors to take into account for the onset of graft versus host disease. In our population, the statistically significant factors are: the source of peripheral blood, infections 3 months prior to transplant, cytomegalovirus infection in the recipient, and cell dosage above  $8.3 \times 10^6/\text{kg}$ . There is greater probability of graft versus host disease at higher cell dosage, due to the number of lymphocytes which are also infused. The other is the source from which the stem cells are obtained. The risk is greatest when the cells are obtained from peripheral blood; after, when the source is bone marrow and least when cells obtained from umbilical cord blood are used. In this case there were none of these factors, except the fact that hematopoietic stem cells were ob-

tained from peripheral blood. In this patient the graft was performed in the expected time, with evidence of recuperation in peripheral blood between 12 and 14 days.

In the third phase, post-transplant, follow up focuses on three decisive aspects: monitoring of infections, sustaining the transplant, and start of graft versus host disease, which may be acute or chronic. This patient had a splicing syndrome. The sites affected by acute graft versus host disease were the skin and the intestine. The greater the immunosuppression may be the greater the risk of infectious complications. The more immunodepressants are indicated the greater the risk will be, especially of bacterial and viral infections: adenovirus, cytomegalovirus, Epstein-Barr, VK, or some caused by atypical agents. In this patient, due to the duration of immunosuppression, the possibility of an atypical manifestation, such as aspergilosis or a virus that could, even, cause neurological alterations, was considered. This case appears to be a graft versus host disease in control with a mixed bacterial and viral or bacterial and mycotic infectious process secondary to immunosuppression.

The fact that a patient recovers hematologically does not mean that he does so immunologically. The latter recovery can be documented after 12 or 18 months, which may explain the characteristics of the thymus. The T-lymphocytes already produced by the transplanted bone marrow migrate to the thymus and repopulation occurs; follicles are formed again.

In the three phases of transplant it is essential to consider that the patient and his family must undergo a very intensive process of health education, because early detection and timely referral, as well as post-transplant care and compliance with immunosuppressant treatment, are factors in the potential appearance of complications and the final prognosis.





## FINAL COMMENT

Today, in Mexico, the second leading cause of death in patients between 4 and 18 years of age is cancer. Also, Mexico has one of the highest rates of pediatric cancer in the world. The National Institute of Pediatrics has the country's largest hematopoietic stem cell transplant service. Consequently, the number of complications documented is high. The case reported shows not only that it does not suffice for a child to receive a successful transplant in an experienced unit, but also the lack of education of parents regarding the potentially fatal effects that may appear if the patient is not given cyclosporine, which conditions graft versus host disease, and eventually also death.

In summary, we report the case of a child with chronic granulocytic leukemia who was initially treated with imatinib, then with hematopoietic stem cell transplant, and then developed graft versus host disease, which led to his death, an outcome potentially avoidable with closer social monitoring.

## REFERENCES

1. Castro-Malespina H, Schaison G, Briere J, Passe S, Pasquier A, et al. Philadelphia chromosome-positive chronic myeloid leukemia in children: survival and prognostic features. *Cancer* 1983;52:721.
2. Gartner JG. Thymic involution with loss of Hassall's corpuscles mimicking thymic dysplasia in a child with transfusion-associated graft-versus-host disease. *Pediatr Pathol* 1991;11:449-456.
3. Hughes TP, Hochhaus A, Bradford S, Muller MC, Kaeda JS, et al. Long-term prognostic significance of early molecular response to imatinib in newly diagnosed chronic myeloid leukemia. *Blood* 2010;116:3758-3765.
4. Lichtman MA. Is There an entity of chemically induced BCR-ABL-positive chronic myelogenous leukemia? *The Oncologist* 2008;13:645-654.
5. Ortiz-Hidalgo C. Notas sobre la historia de la leukemia. *Patologia* 2013;51:58-89.
6. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 1973;1:290-293.
7. Vigneri P, Wang JY. Induction of apoptosis in chronic myeloid leukemia cells through nuclear entrapment of BCR-ABL tyrosin kinase. *Nat Med* 2001;7:228-234.