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Notas clínicas

Impact of the homozygous mutation in Nudix hydrolase 15 on myelosuppression with 6-mercaptopurine in a European girl with acute lymphoblastic leukemia: A case report

Impacto de la mutación homocigota en Nudix hidrolasa 15 sobre la mielosupresión con 6-mercaptopurina en una niña europea con leucemia linfoblástica aguda: A propósito de un caso

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Conflicto de intereses

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Resumen

Una paciente pediátrica de 6 años, diagnosticada de leucemia linfoblástica aguda (LLA) de riesgo intermedio, presenta mielotoxicidad grave y múltiples infecciones durante la fase de inducción IB del tratamiento con 6-mercaptopurina (6-MP). En las siguientes fases del protocolo de tratamiento, que incluía también 6-MP, la paciente continúa mostrando aplasia de médula ósea y neutropenia, requiriendo numerosos ajustes de dosis e interrupciones. La dosis recomendada de 6-MP se reduce entonces al 5 %. El análisis farmacogenético, realizado en la fase de inducción IB, detectó tres polimorfismos de nucleótido único (SNPs) en el gen que codifica para la enzima tiopurina S-metiltransferasa (TPMT), observándose un fenotipo de metabolizador normal para esta enzima. Como consecuencia, se requirió de un segundo análisis farmacogenético más completo, que reveló polimorfismos patológicos en el gen de la hidrolasa Nudix 15 (NUDT15), explicaría la mielotoxicidad observada en esta paciente. Por ello, un análisis farmacogenético completo debería llevarse a cabo con anterioridad al inicio de 6-MP y de manera rutinaria en la práctica clínica, para conseguir prevenir los efectos adversos graves y/o el fracaso terapéutico.

Palabras clave: farmacogenética; 6-mercaptopurina; leucemia linfoblástica aguda.

Abstract

A 6-year-old girl diagnosed with intermediate-risk acute lymphoblastic leukemia (ALL) presented with severe myelotoxicity and multiple infections during phase IB induction treatment with 6-mercaptopurine (6-MP). In the subsequent treatment phases, which included 6-MP, the patient continued to show bone marrow aplasia and neutropenia, necessitating numerous dose adjustments and interruptions. The recommended dose was eventually reduced to 5 %. A pharmacogenetic analysis, conducted in induction phase IB, detected three single-nucleotide polymorphisms (SNPs) of the thiopurine S-methyltransferase (TPMT) gene, and the phenotype of a normal metabolizer was observed. As a result of a second pharmacogenetic analysis, pathological polymorphisms were revealed in Nudix hydrolase 15 (NUDT15), which may explain the patient's myelotoxicity. Hence, a pharmacogenetic analysis performed in advance would have been able to prevent her from suffering severe toxicity and/or treatment failure.

Keywords: pharmacogenetics; 6-mercaptopurine; acute lymphoblastic leukemia.

Highlight

Hasta la fecha son pocos los casos descritos de la presencia del alelo *3 del gen NUDT15 en europeos. Se relaciona con un fenotipo metabolizador lento, lo que supone acumulación de, por ejemplo, la 6-mercaptopurina, con los efectos tóxicos que esto conllevaría.

Este caso clínico complementa y completa la evidencia disponible hasta la fecha sobre la presencia del alelo *3 del gen NUDT15 en pacientes de origen no asiático.

La principal implicación práctica de nuestro caso clínico es la necesidad de avanzar en la implementación de la farmacogenética en los hospitales españoles, de forma que se consiga predecir la predisposición a eventos adversos graves e impedir su aparición. Del mismo modo que permita individualizar la posología a las condiciones de cada paciente.

Introduction

Acute lymphoblastic leukemia (ALL) accounts for 30 % of all childhood cancers and is the most common malignancy in children of developed countries⁽¹⁾. Higher survival rates and better quality of life

have been observed as treatment protocols with cure rates that exceed 80 % have been established. Yet the development of toxicity in some patients remains a cause for concern⁽¹⁾.

6-mercaptopurine (6-MP) functions as an antimetabolite and is normally administered for prolonged periods in the consolidation and maintenance treatment phases (2 to 3 years). It acts through the incorporation into DNA of its metabolite, thioguanosine triphosphate (TGTP), preventing proper repair of the molecule and triggering apoptosis⁽²⁾.

Despite its effectiveness, 6-MP has a very narrow therapeutic range and thus, the risk of adverse reactions is high^(2,3). Myelotoxicity is one of the most severe toxic effects that may lead to the interruption or even discontinuation of treatment. This toxicity is commonly associated with polymorphisms in the thiopurine S-methyltransferase (TPMT) and Nudix hydrolase 15 (NUDT15) genes⁽³⁾. The main elimination pathway of thiopurines is S-methylation mediated by TPMT. NUDT15, in turn, is responsible for the dephosphorylation of active thiopurine metabolites⁽⁴⁾. Genetic variants involving reduced function of these enzymes result in an accumulation of nucleotides within cells and consequently high toxicity, even with conventional doses⁽⁵⁾.

In this clinical case, we examine the impact of genetic polymorphisms in 6-MP and the importance of knowing the patient's genetic profile in advance, which will allow us to adjust doses from the start and avoid severe toxicity.

Results

Case Description

A 6-year-old girl was diagnosed with B-cell ALL in April 2017, with intermediate risk due to hyperdiploidy⁽¹⁾, and was treated according to the SEHOP PETHEMA 2013 protocol⁽⁶⁾. After induction phase IB the patient was admitted to the pediatric intensive care unit (PICU) with sepsis as a result of prolonged aplasia. A first pharmacogenetic analysis was ordered, including only the *2, *3B and *3C polymorphisms of the TPMT gene; the results indicated that the patient was a normal metabolizer. During the consolidation phase, which began in August 2017, the patient again developed aplasia and febrile neutropenia, so a reduction to 10 % of the 6-MP total standard dose was performed. However, in the reinduction phase her myelotoxicity reappeared.

In March 2018, the patient started the maintenance phase in month 10, with dose of 6-MP reduced to 10 % (2.5 mg QD) and dose of oral methotrexate (MTX) reduced to 85 % (7.5 mg QW). A month later, she presented with low leukocyte and platelet counts, as well as with hemoglobin level reduction, as shown in Figure 1. Therefore, treatment was temporarily interrupted. During this period, the patient was hospitalized due to febrile neutropenia and the need of platelet transfusion. When her blood parameters had returned to normality, 6-MP at 10 % of dose and MTX at 50 % were resumed.

In month 18, the patient showed grade 4 hematologic toxicity and was admitted to the PICU. Subsequently, the fourth intrathecal dose of MTX was administered and maintenance oral chemotherapy restarted.

Up to the date of the end of treatment, in August 2019, numerous dose adjustments of both drugs were necessary. In addition, many transfusions of packed red blood cells and platelets were needed to avoid further bone marrow aplasia and hospitalization for the resulting complications. Finally, the patient received 6-MP at 5 % of the dose and MTX at 35 % until treatment completion.

An extensive pharmacogenetic study including the TPMT genetic variants (*2, *3B, *3C and *4) and also the variant in NUDT15 (*3), was conducted by real-time polymerase chain reaction (RT-PCR) using TaqMan® probes (ABI Applied Biosystems, 7300 Real-Time PCR System) in accordance with the manufacturer's instructions. Sanger sequencing (Figure 2) confirmed the NUDT15 results.

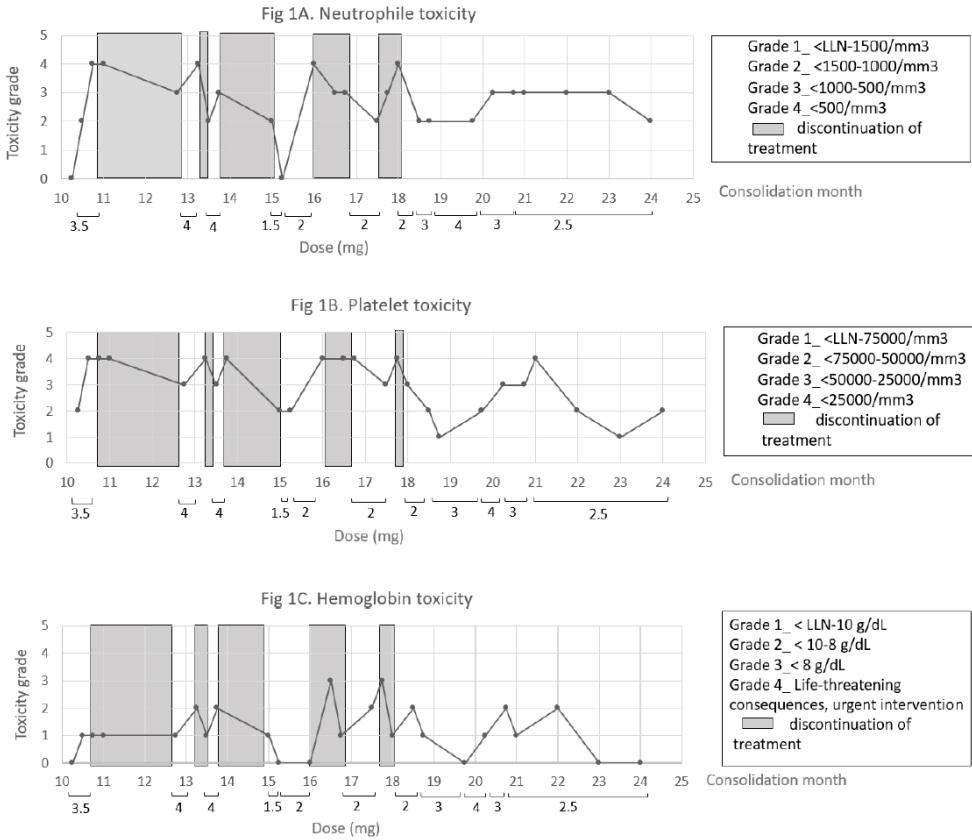


Figure 1. Evolution of hematologic parameters involved in toxicity: neutrophils (1A), platelets (1B), hemoglobin (1C), and 6-MP doses during the consolidation phase of PETHEMA 2013.

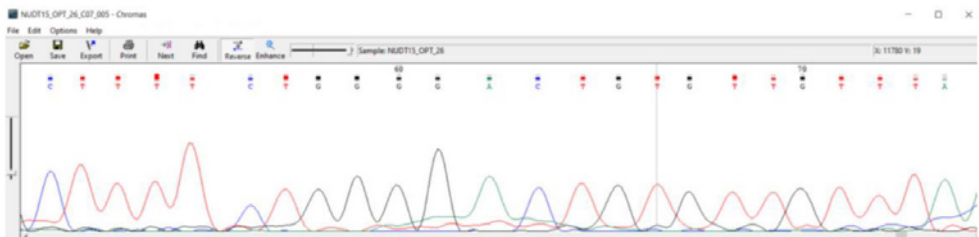


Figure 2. Results of the analysis of the NUDT15*3 homozygous rs116855232-TT polymorphism.

Discussion

The pharmacogenetics of 6-MP has been extensively described. In 2015, Yang et al. first reported the relationship between the development of myelosuppression during treatment with 6-MP and variants of NUDT15⁽⁷⁾. Their observations were based on two prospective clinical trials in an East Asian population. A correlation was observed between the patients with TT genotype at rs116855232 were exquisitely sensitive to 6-MP, with an average dose intensity of 8.3 %, compared with those with TC and CC genotypes, who tolerated 63 % and 83.5 % of the planned dose, respectively. They also observed that this genetic variant was rare in Europeans and absent in Africans⁽⁷⁾. A multiethnic meta-analysis demonstrated that the T allele of rs116855232 contributed to a 7.86-fold ($P < 0.0001$, 95 %CI = 6.13–10.08) higher risk of developing leukopenia⁽⁸⁾.

In accordance with the foregoing, the Clinical Pharmacogenetics Implementation Consortium (CPIC[®]) developed pharmacogenetic guidelines for TPMT polymorphisms⁽⁹⁾, including determination of allele *3 of the NUDT15 gene, with the main SNPs being at level of evidence of 1A. Consequently, the Spanish Agency for Medicines and Medical Devices (AEMPS) considers performing the genetic test before starting treatment with this drug⁽¹⁰⁾. However, it proves difficult to transfer this into clinical practice, as observed in our clinical case, where the pharmacogenetic analyses were conducted only after the occurrence of severe toxicities.

Our patient manifested prolonged myelosuppression, entailing repeated dose reductions and treatment interruptions, as well as numerous admissions to the PICU. At this point, the first TPMT genotyping was carried out, revealing normal metabolizing activity. Following the CPIC[®] recommendations⁽⁹⁾, a second analysis, including determination of NUDT15 allele *3, was conducted. The results showed the rs116855232-TT genotype, related to a slow metabolizer phenotype for NUDT15. Thus, explaining the severe toxicity observed when 6-MP was administered at standard and reduced doses. The frequency of these polymorphisms varies between different populations. For the NUDT15 gene, allele *3 is most common in Asians (6.7 %). In those of European and Latin origin, however, its frequency is markedly lower: 0.2 % and 0.8 %, respectively⁽⁹⁾.

Conclusion

Pharmacogenetics could be an important factor in responding to this patient's high-grade myelotoxicity. If testing results had been known prior to 6-mercaptopurine administration, a dose tailored to the patient's genetic profile would have been started, which might have prevented the multiple dose adjustments and toxic reactions. Future actions should be focused on facilitating the introduction of pharmacogenetics into everyday clinical practice to validate our results on a larger sample of patients.

References

1. Lopez-Lopez E, Gutierrez-Camino A, Bilbao-Aldaiturriaga N, Pombar-Gomez M, Martin-Guerrero I, Garcia-Orad A. Pharmacogenetics of childhood acute lymphoblastic leukemia. *Pharmacogenomics*. 2014 Jul;15(10):1383-98. DOI: 10.2217/pgs.14.106
2. Lennard L, Cartwright CS, Wade R, Vora A. Thiopurine dose intensity and treatment outcome in childhood lymphoblastic leukaemia: the influence of thiopurine methyltransferase pharmacogenetics. *Br J Haematol*. 2015;169(2):228-40. DOI: 10.1111/bjh.13240.
3. Maamari D, El-Khoury H, Saifi O, Muwakkat SA, Zgheib NK. Implementation of Pharmacogenetics to Individualize Treatment Regimens for Children with Acute Lymphoblastic Leukemia. *Pharmgenomics Pers Med*. 2020;13:295-317. DOI: 10.2147/PGPM.S239602
4. Bernsen EC, Hagleitner MM, Kouwenberg TW, Hanff LM. Pharmacogenomics as a Tool to Limit Acute and Long-Term Adverse Effects of Chemotherapeutics: An Update in Pediatric Oncology. *Front Pharmacol*. 2020;11:1184. DOI: 10.3389/fphar.2020.01184.

- 5.** Dorababu P, Nagesh N, Linga VG, Gundeti S, Kutala VK, Reddanna P, Digumarti R. Epistatic interactions between thiopurine methyltransferase (TPMT) and inosine triphosphate pyrophosphatase (ITPA) variations determine 6-mercaptopurine toxicity in Indian children with acute lymphoblastic leukemia. *Eur J Clin Pharmacol.* 2012;68(4):379-87. DOI: 10.1007/s00228-011-1133-1.
- 6.** Schmiegelow K, Nielsen SN, Frandsen TL, Nersting J. Mercaptopurine/Methotrexate maintenance therapy of childhood acute lymphoblastic leukemia: clinical facts and fiction. *J Pediatr Hematol Oncol.* 2014;36(7):503-17. DOI: 10.1097/MPH.0000000000000206.
- 7.** Yang JJ, Landier W, Yang W, Liu C, Hageman L, Cheng C, Pei D, Chen Y, Crews KR, Kornegay N, Wong FL, Evans WE, Pui CH, Bhatia S, Relling MV. Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. *J Clin Oncol.* 2015;33(11):1235-42. DOI: 10.1200/JCO.2014.59.4671.
- 8.** Yin D, Xia X, Zhang J, Zhang S, Liao F, Zhang G, Zhang Y, Hou Q, Yang X, Wang H, Ma Z, Wang H, Zhu Y, Zhang W, Wang Y, Liu B, Wang L, Xu H, Shu Y. Impact of NUDT15 polymorphisms on thiopurines-induced myelotoxicity and thiopurines tolerance dose. *Oncotarget.* 2017;8(8):13575-13585. DOI: 10.18632/oncotarget.14594.
- 9.** Relling MV, Schwab M, Whirl-Carrillo M, Suarez-Kurtz G, Pui CH, Stein CM, Moyer AM, Evans WE, Klein TE, Antillon-Klussmann FG, Caudle KE, Kato M, Yeoh AEJ, Schmiegelow K, Yang JJ. Clinical Pharmacogenetics Implementation Consortium Guideline for Thiopurine Dosing Based on TPMT and NUDT15 Genotypes: 2018 Update. *Clin Pharmacol Ther.* 2019;105(5):1095-1105. DOI: 10.1002/cpt.1304.
- 10.** Centro de Información de Medicamentos (CIMA) de la AEMPS [Internet]. Ficha Técnica Mercaptopurina - [cited 2023 Mar 30]. Available from: <https://cima.aemps.es/cima/publico/home.html>