

Persistent cytopenias in a patient transplanted for myelodysplastic syndrome

Citopenias persistentes en paciente trasplantado por síndrome mielodisplásico

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Abstract

The case describes a 53-year-old male diagnosed with myelodysplastic syndrome (MDS), who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) from his HLA-identical sister. Two years after the allo-HSCT, the patient developed persistent cytopenias in all hematopoietic lineages, along with dysplastic signs in bone marrow and complete donor chimerism, ultimately labeled as donor cell-derived MDS. Molecular analysis revealed the presence of various pathogenic variants in the DDX41 gene, suggesting a genetic predisposition to myeloid neoplasms. This case underscores the urgent need for a comprehensive approach in the diagnosis and management of hematologic malignancies, and how significant it is to identify germline predispositions that may influence disease progression. It also emphasizes the necessity of continuous monitoring and proper management of post-transplant complications, advocating for careful consideration of family history and genetic aspects in the overall management of these clinical conditions.

Keywords: Myelodysplastic syndrome. Cytopenias. Germline predisposition. DDX41.

Resumen

El caso describe a un varón de 53 años diagnosticado de síndrome mielodisplásico (SMD), que fue sometido a un trasplante alogénico de progenitores hematopoyéticos (alo-TPH) de su hermana HLA-idéntica. Dos años tras el alo-TPH, el paciente desarrolló citopenias persistentes en todas las líneas hematopoyéticas, acompañadas de signos displásicos en médula ósea y quimerismo completo del donante, siendo finalmente etiquetado de SMD en células del donante. El análisis molecular reveló la presencia de distintas variantes patogénicas en el gen DDX41, sugiriendo una predisposición genética a neoplasias mieloides. Este caso subraya la imperante necesidad de un enfoque integral en el diagnóstico y tratamiento de las neoplasias hematológicas, así como la importancia de identificar una predisposición germinal que pueda influir en la evolución de la enfermedad. Asimismo, recalca la urgencia de una vigilancia continua y una gestión adecuada de las complicaciones post-trasplante, y aboga por considerar detenidamente los antecedentes familiares y aspectos genéticos en el manejo global de estas condiciones clínicas.

Palabras clave: Síndrome mielodisplásico. Citopenias. Predisposición germinal. DDX41.

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Case report

Medical history

A 53-year-old male was diagnosed in October 2015 with myelodysplastic syndrome (MDS) at low risk and normal karyotype. Due to the lack of response to stimulating factors (erythropoietin/EPO, granulocyte colony-stimulating factor/G-CSF), the development of transfusion dependence, and progression to MDS with excess blasts type 1, an allogeneic hematopoietic stem cell transplant (allo-HSCT) from his HLA (human leukocyte antigen)-identical sister was performed back in July 2016 as the only potentially curative treatment. As the main post-transplant complication, two years after the transplant, the patient developed cytopenias in all three hematopoietic lines:

- Thrombocytopenia: leading to several episodes of upper GI bleeding requiring hospitalization. After ruling out other causes and considering the patient's context, autoimmune thrombocytopenia was assumed, prompting the patient to receive the following lines of treatment: dexamethasone, prednisone, romiplostim, IV immunoglobulins, and rituximab.
- Neutropenia: requiring G-CSF administration and resulting in infectious complications, such as neutropenic fever, esophageal candidiasis, and herpes simplex on the hard palate. In all cases, hospitalization was required.
- Anemia with EPO requirements and transfusion of blood products.

Due to the lack of response to these treatments, a comprehensive assessment of the origin of cytopenias was conducted on multiple occasions from 2018 through 2022.

Supplementary tests after allogeneic hematopoietic stem cell transplantation

The following cytomorphological findings were observed in peripheral blood and bone marrow (**Fig. 1**):

- Peripheral blood: initially thrombocytopenia ($10\,000$ platelets/ mm^3), followed by leuko-neutropenia (1620 leukocytes/ mm^3 , 600 neutrophils/ mm^3), and macrocytic anemia (hemoglobin 11g/dL , mean corpuscular volume 108fL) on the blood count. Smear findings included anisocytosis and anisochromia of erythroid cells, dacryocytes, granulocytic series degranulation and hyposegmentation, chromatin clumping, and absence of blast cells.
- Bone marrow study:
 - Cytomorphology: normal cellularity with inverted myelo/erythroid ratio, cellular gigantism, blastosis of

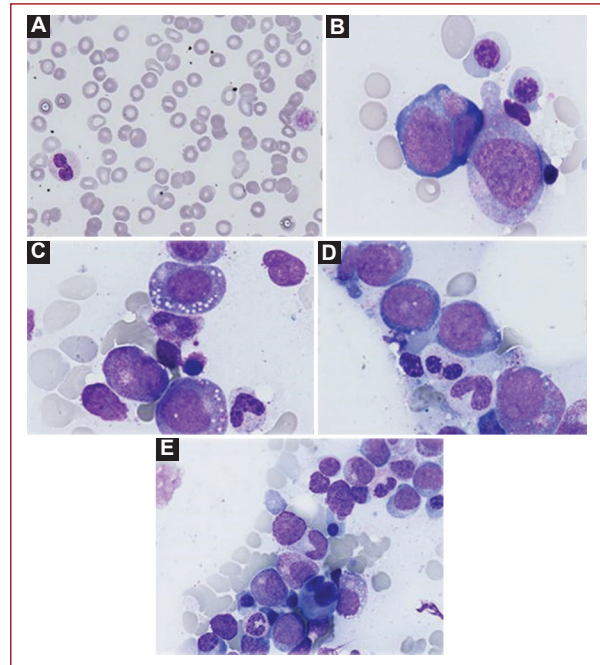


Figure 1. Morphological study after allo-HSCT (May Grünwald-Giemsa, 100x). **A:** peripheral blood smear: granulocyte with pseudo-Pelger nucleus, degranulation, clumping, and a megathrombocyte. **B:** bone marrow, large multinucleated erythroblast. **C:** bone marrow, cytoplasmic vacuolization in progenitors. **D:** bone marrow with pseudo-Pelger anomaly, clumping, and agranular blasts. **E:** bone marrow, small binucleated megakaryocyte, vacuolization of myeloid progenitors, dysgranulopoiesis with nuclear hyposegmentation, and blasts.

6% to 10%, increased histiocytes-macrophages with hemophagocytosis images, and evident dysplastic signs (around 30% in each of the lines): dyserythropoiesis (cytoplasmic vacuolization, megaloblastosis, nuclear cariorrexis), dysmegakaryopoiesis (small mononuclear and binucleated megakaryocytes), and dysgranulopoiesis (predominance of immature forms, striking cytoplasmic vacuoles in progenitors with normal copper levels, Döhle bodies, nuclear hyposegmentation, and hypogranulation).

- Flow cytometry: an increase in myeloid progenitor cells corresponding to the blasts observed in the morphological study (4-6%) was observed, as well as indirect signs of dysplasia, which are detailed in **figure 2**.
- Cytogenetic study: $46,XX20$.
- Molecular study: no variants were detected by NGS, and complete donor chimerism was confirmed.

Based on the results obtained with a donor graft, the evidenced dysplasia, and the blast increase could be associated with the administration of growth factors.

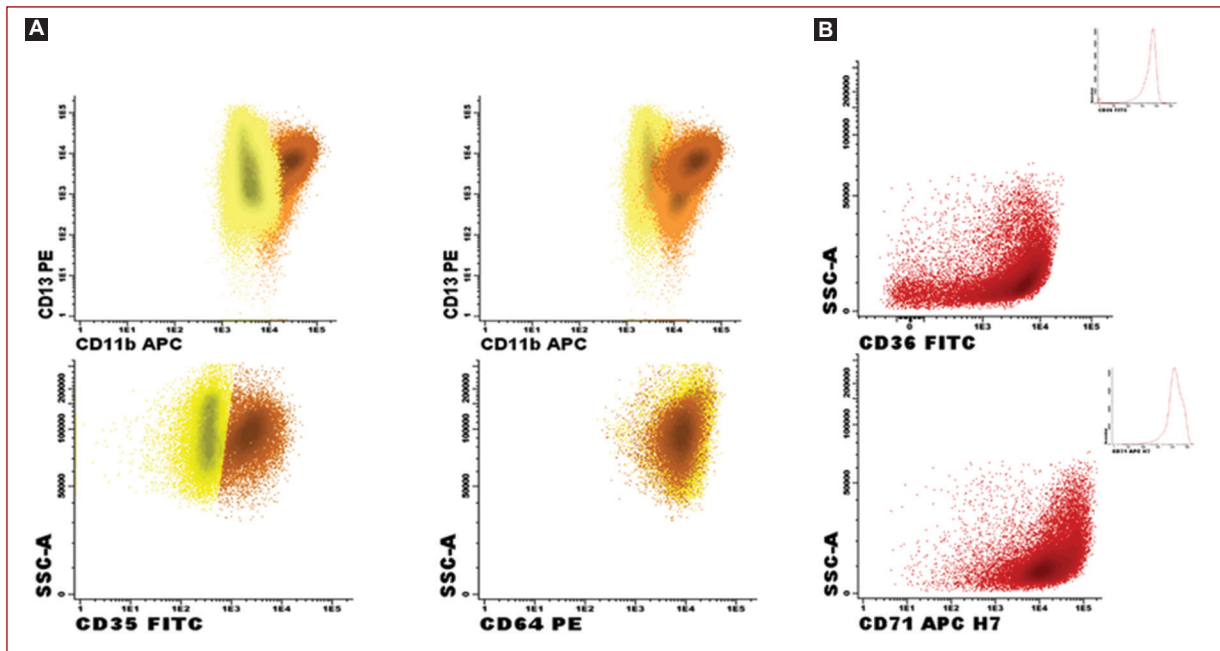


Figure 2. Flow cytometry study in granulocytic and erythroid series. **A:** in the granulocytic series, changes to the immunophenotypic and maturation CD13/CD11b pattern, as well as overexpression of CD64. **B:** in the erythroid series, heterogeneous patterns in the expression of CD36/CD71. All these changes are suggestive of dysplasia.

The genetics unit was consulted in May 2022 to perform exome sequencing, finding no relevant variants in the UBA1 gene, thus ruling out VEXAS syndrome. However, two pathogenic variants were detected in the DDX41 gene (NM_016222): c.536C>T; p.Pro179Leu and c.1574G>A; p.Arg525His, with variant allele frequencies (VAF) of 50% and 23%, respectively.

We should mention that the DNA analyzed belonged to his HLA-identical sister, as the patient had complete donor chimerism. Following the discovery of these two pathogenic variants in DDX41 six years after allo-HSCT, other samples from the patient were retrospectively evaluated. At the diagnosis of MDS, two pathogenic variants in DDX41 (NM_016222): c.536C>T; p.Pro179Leu (VAF 50%) and c.1412G>C; p.Arg471Pro (VAF 41%) were detected, as well as another pathogenic variant in SRSF2 (VAF 8%). Six months after the allo-HSCT, the patient remained well without cytopenias, complete chimerism, and normal female karyotype. At that time, the same DDX41 mutation (NM_016222): c.536C>T; p.Pro179Leu was still observed, with the absence of mutations in DDX41 c.1412G>C; p.Arg471Pro and SRSF2. The germline origin of the variant in the DDX41 gene p.Pro179Leu was confirmed by germ line study of the culture of skin fibroblasts, with a VAF of 50%, and both siblings shared it.

Two years after the allo-HSCT, when the patient presented cytopenias, he had acquired a second hit in the

DDX41 gene: p.R525H different from the second somatic hit in DDX41 acquired at the diagnosis of MDS. This acquisition triggered MDS in donor cells (Fig. 3).

Several aspects should be highlighted in this history:

- At the diagnosis of MDS in October 2015, valuable information provided by family history was ignored, as the patient's father had died at 57 years old due to MDS.
- DDX41 was included in our NGS panels starting in 2022, and therefore, during the subsequent molecular studies conducted during the cytopenia period (2018), no pathogenic variants were ever detected.
- In the family segregation study: the patient's daughter and a nephew (son of the donor) are also carriers of the pathogenic variant in DDX41 (NM_016222): c.536C>T; p.Pro179Leu. They, along with the patient's sister, have initiated clinical follow-up.

Diagnosis

Myeloid neoplasm with germline DDX41 mutation and development of a new neoplasm with germline DDX41 mutation on donor cells after allo-HSCT.

Course of the disease

Patient progression was torpid, as the MDS progressed into acute myeloid leukemia. Unfortunately, the patient died in May 2023.

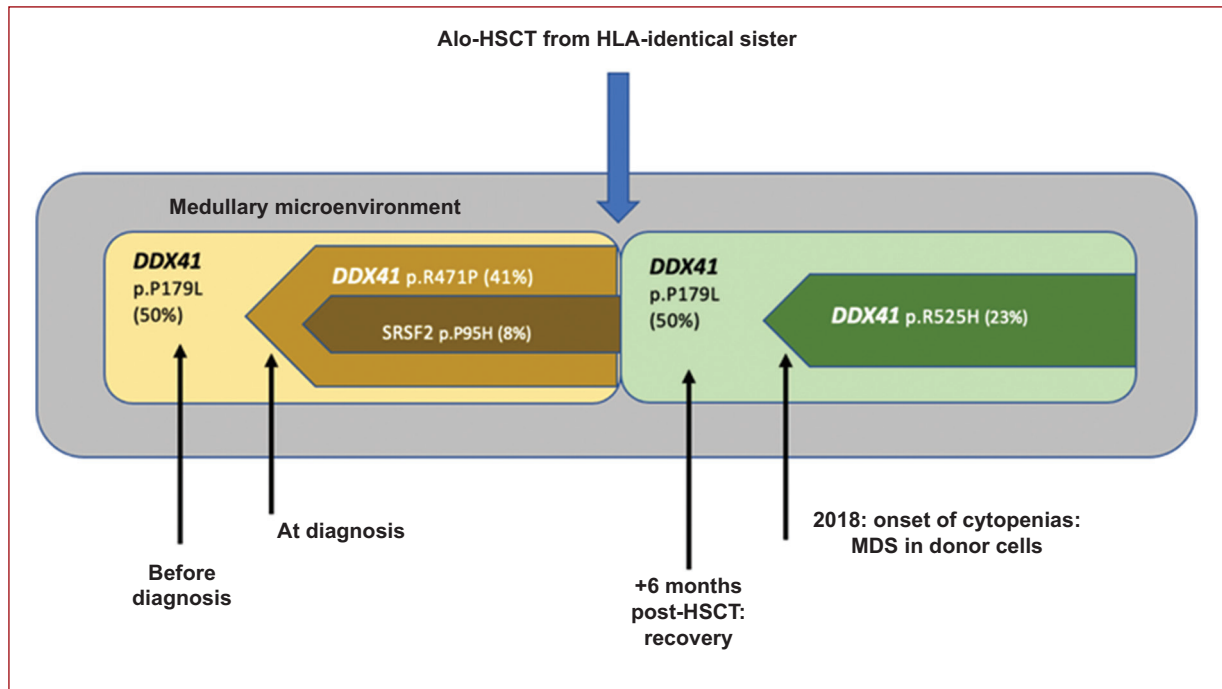


Figure 3. Patient's clonal evolution. In gray, the medullary environment. In yellow, the mutations found before allo-HSCT, and in green after the infusion of hematopoietic progenitor cells from the patient's sister.

Discussion

Myeloid neoplasms with predisposition in the germline or hereditary myeloid neoplasms (HMN) have been associated with well-defined pediatric syndromes as traditionally considered very rare neoplasms in adults. Additionally, clinical and family history may be absent in up to 40% of patients. Thanks to the implementation of NGS, hereditary mutations are being identified in individuals older than 60 years. Moreover, currently, we know that approximately 4% up to 13% of pediatric patients and 5% to 10% of adult patients with MDS or AML have an HMN¹⁻³. HMN are defined as myeloid neoplasms with a germline genetic basis, including familial predisposition syndromes to MDS/AML and hereditary syndromes with bone marrow failure. Despite the scarcity of descriptions of these entities in the last decade, there is sufficient evidence for the World Health Organization (WHO) Classification of 2016⁴ to incorporate myeloid neoplasms with predisposition in the germline as provisional categories. In its 2022 updates, both the WHO⁵ and the International Consensus Classification (ICC)⁶ consolidate these entities and make it clear the need to identify, diagnose, and properly manage patients with hereditary syndromes.

Myeloid neoplasms with germline variants in *DDX41*

It is estimated that they represent 2% to 3% of all cases of MDS-AML. Phenotypically, Myeloid neoplasms are an indolent condition that may begin with cytopenias years before the diagnosis of myeloid neoplasm or remain asymptomatic until then⁷, accompanied by a hypocellular or normocellular bone marrow associated with a usually normal karyotype and a good prognosis. The median age of onset is the 6th-7th decades of life, being more frequent in males^{8,9}. Beyond AML and MDS, germline mutations in *DDX41* appear to predispose to other hematologic neoplasms, such as chronic myelomonocytic leukemia, chronic myeloid leukemia, MDS/myeloproliferative syndromes, lymphoproliferative neoplasms, and non-hematopoietic neoplasms^{7,10}. Carriers of the pathogenic variant in *DDX41* have a risk (penetrance) of developing a neoplasm of 50% over their lifetime, with this risk being negligible before the age of 40. However, risk increases from that age to reach 25% and 50% by 70 and 90 years, respectively¹¹. Late onset, incomplete penetrance, and absence of family history, present in up to 70% of cases, hinder the recognition of these cases of germline predisposition⁸.

DDX41 is a tumor suppressor gene located on the long arm of chromosome 5 (5q35), which appears to be part of the spliceosome and is involved in the differentiation and maturation of hematopoietic progenitors¹². More than 60% of patients with germline mutations in DDX41 exhibit loss of gene function (non-sense, frameshift, splicing, start codon disruption), with the most common variants being p.M1? or p.D140fs^{13,14}. These germline mutations predispose to the occurrence of somatic hits in the healthy allele of the same gene, thus providing a greater clonal advantage; therefore, most patients (50-70%) have more than one variant in the DDX41 gene, especially in the hotspot (p.R525)^{8,9,10,12,13}. In fact, the acquisition of a somatic pathogenic variant in DDX41 is a criterion of causality for the interpretation of germline variants^{9,10}.

Finally, we should mention the role played by DDX41 in immune activation through STING signaling, which implies that recipient cells of an allogeneic transplant with the germline variant in DDX41 may present an inflammatory environment that could lead to aberrant immune activation after transplantation^{15,16}. Adding to this, the donor cells also harbored a pathogenic variant in the same gene, and within the context of allogeneic transplantation, there is a significant inflammatory environment (intense cell cycle activity, likely altered differentiation program, elevated oxidative stress, immunocompromised patient). This suggests that the interplay between cancer and inflammation triggered the development of a second somatic pathogenic hit in DDX41 and the consequent clonal hematopoiesis leading to MDS/AML in donor cells.

Conclusions

- The recognition of signs of dysplasia allowed for the detection of a new neoplasm and further molecular study.
- Myeloid neoplasms with germline mutations in DDX41 are a recognized subtype within myeloid neoplasms according to the new classifications.
- The identification of these entities is crucial for the proper management and follow-up of patients and their families.

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Conflicts of interest

None declared.

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 no patient data appear in this article. Furthermore, they have acknowledged and followed the recommendations as per the SAGER guidelines depending on the type and nature of the study.

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

Use of artificial intelligence for generating text. The authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

References

1. Akpan IJ, Osman AEG, Drazer MW, Godley LA. Hereditary myelodysplastic syndrome and acute myeloid leukemia: diagnosis, questions, and controversies. *Curr Hematol Malig Rep*. 2018;13(6):426-34.
2. Yang F, Long N, Anekpuritanang T, Bottomly D, Savage JC, Lee T, et al. Identification and prioritization of myeloid malignancy germline variants in a large cohort of adult patients with AML. *Blood*. 2022;139(8):1208-21.
3. Drazer MW, Kadri S, Sukhanova M, Patil SA, West AH, Feurstein S, et al. Prognostic tumor sequencing panels frequently identify germ line variants associated with hereditary hematopoietic malignancies. *Blood Adv*. 2018;2(2):146-50.
4. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-405.
5. Khoury JD, Solary E, Abal O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia*. 2022;36(7):1703-19.
6. Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. *Blood*. 2022;140(11):1200-28.
7. Sébert M, Passet M, Raimbault A, Rahmé R, Raffoux E, Sicre de Fontbrune F, et al. Germline DDX41 mutations define a significant entity within adult MDS/AML patients. *Blood*. 2019;134:1441-4.
8. Cheah JJC, Hahn CN, Hiwase DK, Scott HS, Brown AL. Myeloid neoplasms with germline DDX41 mutation. *Int J Hematol*. 2017;106:163-74.
9. Li P, White T, Xie W, Cui W, Peker D, Zeng G, et al. AML with germline DDX41 variants is a clinicopathologically distinct entity with an indolent clinical course and favorable outcome. *Leukemia*. 2022;36:664-74.
10. Li P, Brown S, Williams M, White T, Xie W, Cui W, et al. The genetic landscape of germline DDX41 variants predisposing to myeloid neoplasms. *Blood*. 2022;140:716-55.
11. Makishima H, Saiki R, Nannya Y, Korotev S, Gurnari C, Takeda J, et al. Germ line DDX41 mutations define a unique subtype of myeloid neoplasms. *Blood*. 2023;141(5):534-49.
12. Quesada AE, Routbort MJ, DiNardo CD, Bueso-Ramos CE, Kanagal-Shamanna R, Khoury JD, et al. DDX41 mutations in myeloid neoplasms are associated with male gender, TP53 mutations and high-risk disease. *Am J Hematol*. 2019;94:757-66.
13. Rio-Machin A, Fitzgibbon J. DDX41: the poster child for familial AML. *Blood*. 2022;140:667-9.
14. Qu S, Li B, Qin T, Xu Z, Pan L, Hu N, et al. Molecular and clinical features of myeloid neoplasms with somatic DDX41 mutations. *Br J Haematol*. 2021;192:1006-10.
15. Saygin C, Roloff G, Hahn CN, Chhetri R, Gill S, Elmariah H, et al. Allogeneic hematopoietic stem cell transplant outcomes in adults with inherited myeloid malignancies. *Blood Adv*. 2023;7(4):549-54.
16. Zhang Z, Yuan B, Bao M, Lu N, Kim T, Liu YJ. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells published correction appears in *Nat Immunol*. 2012 Feb;13(2):196. *Nat Immunol*. 2011;12(10):959-65.