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Letter to the editor

Role of next-generation sequencing in acquired amegakaryocytic thrombocytopenic purpura

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Acquired amegakaryocytic thrombocytopenic purpura (AATP) is a rare bone marrow disorder characterized by severe thrombocytopenia associated with megakaryocytic aplasia or hypoplasia and preservation of other cell lineages [1–3]. The pathophysiology underlying the condition is uncertain with several studies suggesting heterogeneous etiologic mechanisms [4–6]. The main differential diagnosis of AATP is immune thrombocytopenia (ITP) and distinction between the two entities is crucial because steroids and intravenous immunoglobulin (IVIG), the mainstay of treatment for ITP, are largely ineffective in AATP, which usually responds to immunosuppressive agents such as cyclosporine and anti-thymocyte globulin (ATG) [3]. However, to date, no guidelines are available for the management of AATP [1,3–8]. Prognosis remains unclear and ranges from a chronic relapsing-remitting course to rapid evolution into aplastic anemia (AA) or myelodysplastic syndrome (MDS) [1,3,7]. Here, we present the case of a patient diagnosed with AATP and its subsequent evolution into MDS over the course of approximately two years, highlighting the importance of genomic data in the diagnosis and follow-up of this condition.

A 65-year-old man presented with a history of mild bruising and petechiae accompanied by isolated severe thrombocytopenia (platelet count [PLT], 24 $\times$ 10^9/L). Past medical records included ischemic cardiomyopathy, type 2 diabetes, essential hypertension, and non-alcoholic fatty liver disease. On assessment, serologies for HIV, HBV, HCV, and Helicobacter pylori were negative; CMV and EBV serologies were positive for IgG only. Autoimmunity markers, including anti-platelets antibodies, were negative. Iron, folate, vitamin B12, copper, and lead levels were within the normal range, and there was no hepatosplenomegaly on abdominal ultrasound. The peripheral blood smear showed no signs of platelet clumping or dysplastic features, but an increase in mean corpuscular volume (MCV, 105 fL) was observed [6]. A diagnosis of ITP was made and initial treatment with prednisone 1 mg/Kg/day was started. Two weeks later, the patient was hospitalized with worsening thrombocytopenia (PLT, 5 $\times$ 10^9/L) and treated with high-dose dexamethasone (40 mg/day for 4 days) and IVIG (0.4 g/Kg/day for 4 days), but no improvement in platelet count was obtained. Bone marrow aspirate and trephine biopsy were then performed after a platelet transfusion, which resulted in a normal platelet increase after one hour (PLT, 42 $\times$ 10^9/L), and treatment with the thrombopoietin receptor agonist (TPO-RA) eltrombopag was promptly started pending results. Contrary to our initial suspicion of MDS [8], bone marrow smear and biopsy showed normal cellularity with a slight reduction in megakaryocyte count and no signs of dysplasia (Image 1A–B). Based on these findings and the lack of response to eltrombopag after 6 weeks of treatment at the maximum dose of 75 mg, we discontinued the TPO-RA and repeated marrow analysis after one month. Trephine biopsy showed age-adjusted hypercellularity, expansion and slight left shift of the erythroid series with decreased myeloid/erythroid ratio and severely reduced number of megakaryocytes; no increase in blast or lymphoid cell counts was observed (Image 1C–D). Cytogenetic analysis demonstrated a normal male karyotype, and the next-generation sequencing (NGS) panel (QIAseq Targeted DNA Custom Panel CDHS-33828Z-1177) was negative for myeloid-associated mutations. Flow cytometry on peripheral blood revealed a population of 0.06 % GPI-deficient erythrocytes by FLAER assay. After careful review of both marrow specimens, a diagnosis of AATP was established. Since the patient was considered unfit for ATG treatment, oral cyclosporine monotherapy at a dose of 5 mg/Kg/day was started. Unfortunately, six months after initiating calcineurin inhibitor therapy and following the addition of rituximab (375 mg/m^2 weekly for 4 doses) [9], only a minimal response was observed as PLT never exceeded 40 $\times$ 10^9/L. Due to the progressive increase in MCV followed by the appearance of a transfusion-dependent macrocytic anemia, marrow analysis was repeated after discontinuation of cyclosporine. Morphological evaluation highlighted dysplastic features of the granulocytic and megakaryocytic lineages without an excess of blasts (Image 2), and NGS revealed a mutation of unknown clinical significance in DNMT3A (c.926G>C/p.R309P; VAF 27 %). A diagnosis of MDS with multilineage dysplasia was made and the patient was started on erythropoietin stimulating agents.

To the best of our knowledge, this is the first time in which NGS methods have been integrated in the diagnostic process of AATP: the absence of myeloid mutations at diagnosis helped us differentiating AATP from other causes of thrombocytopenia and can be a useful aid also in the follow-up of this condition. Furthermore, an initially elevated erythrocyte MCV and its gradual increase over time may represent early predictive factors of progression towards MDS and potentially define a distinct disease subtype [6]. The pathophysiological mechanism

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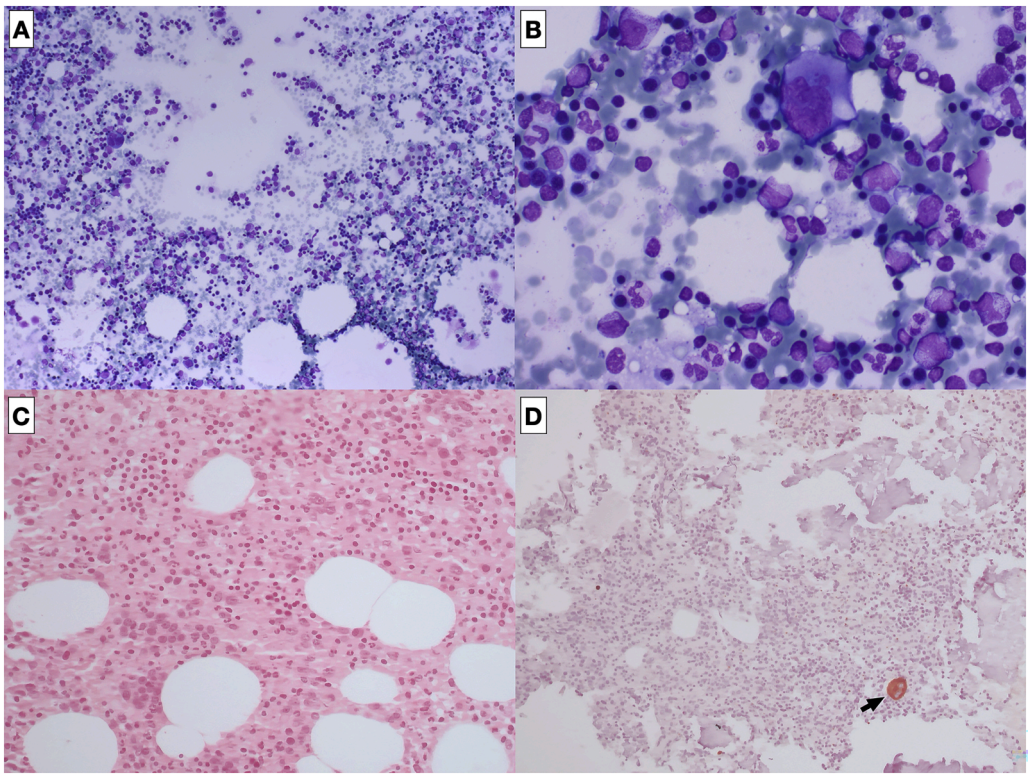

Image 1. A) Bone marrow (BM) aspirate smear (Wright-Giemsa, x100) shows a normocellular marrow with few megakaryocytes. **B)** High magnification (Wright-Giemsa, x400) of the same BM smear showing a normal megakaryocyte with no signs of dysplasia. **C)** Histological analysis from the second BM biopsy shows hypercellular marrow, with expansion and slight left shift of the erythroid series and severe reduction of megakaryocytes (H&E, x400). **D)** At low magnification (x100), immunohistochemical staining for CD61 confirms the severe reduction of megakaryocytes (*black arrow*) within the BM biopsy.

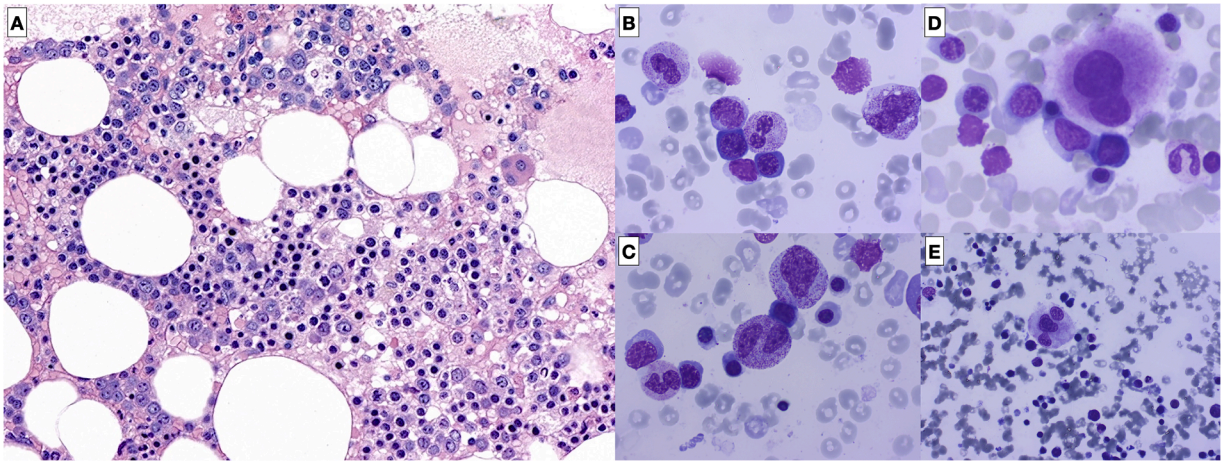

Image 2. A) Histological analysis from bone marrow (BM) biopsy shows severe dyserythropoiesis and a dysplastic megakaryocyte (*upper right*) with widely-separated nuclei (H&E, x200) **B)** BM aspirate smear at high magnification (Wright-Giemsa, x400) shows asynchronous maturation of myeloid lineage cells with irregular distribution of cytoplasmic granules and abnormal condensation of chromatin. **C)** Dysgranulopoiesis with nuclear fragmentation (Wright-Giemsa, x400). **D)** High magnification (Wright-Giemsa, x400) of the same BM aspirate smear showing a dysplastic megakaryocyte with hypolobulated nuclei. **E)** Another example of dysmegakaryocytopoiesis (Wright-Giemsa, x400) with a single megakaryocyte with multiple dispersed nuclei ("pawn ball megakaryocyte") and hypogranular cytoplasm.

implicated in our case, given the lack of response to numerous immunosuppressive therapies, could be related to an undetected intrinsic defect in the stem or progenitor cells compartment, which possibly led to the subsequent myelodysplastic evolution [2,6]. Another possibility could be that AATP is a bone marrow failure syndrome that lies in the spectrum between acquired AA and hypocellular MDS: an initial immune attack against megakaryocyte autoantigens may be followed by immune escape and expansion of a pre-existing or de novo acquired hematopoietic clone leading to secondary MDS [10]. The initial absence of clonal myeloid mutations in combination with evidence of a small paroxysmal nocturnal hemoglobinuria (PNH) clone, as typically found in AA, and the subsequent appearance of myelodysplastic features in conjunction with a DNMT3A mutation may be proof of this concept. Prolonged exposure to immunosuppression in our case may have played a role in the clinical progression from AATP to MDS, highlighting the need to better define the pathogenesis and genomic profile of this

condition and abandon the current empirical therapeutic approach.

In conclusion, genomic studies using NGS, along with morphological and cytogenetic analyses, are mandatory in patients with unexplained and isolated thrombocytopenia who are refractory to standard initial treatments for ITP but demonstrate an adequate response to platelet transfusions. AATP should be strongly considered whenever the decrease or absence of megakaryocytes occurs selectively without overall marrow hypocellularity; the lack of acquired mutations in myeloid-related genes could serve as an additional diagnostic criterion for this condition. The presence of a PNH clone, as also reported by other authors [5,11], further support the diagnosis. Multidisciplinary collaboration between hematologists, hematopathologists, and other disease specialists is essential to reach a timely diagnosis and avoid delays in treatment, including allogeneic stem cell transplant for those eligible for the procedure [12].

Ethical and data availability statement

Patient approval for publication was obtained and no personal identifier information is presented here. Data supporting the findings of this study are original and available from the corresponding author upon reasonable request.

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Author contributions

Lazzari Lorenzo, Bergonzi Gregorio Maria, Gariazzo Camilla, Diral Elisa, Ciceri Fabio, and D'Alessio Andrea were involved in the treatment of the patient. Lazzari Lorenzo designed and wrote the manuscript. Bongiovanni Lucia, Ronchi Paola, and Ponzoni Maurilio analyzed the marrow and peripheral blood samples. All authors were involved in the diagnostic process, reviewed the article, and gave their final approval.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Lorenzo Lazzari[a,*], Lucia Bongiovanni[b,c], Paola Ronchi[d], Gregorio Maria Bergonzi[a,c], Camilla Gariazzo[a,c], Elisa Diral[a], Fabio Ciceri[a,c], Andrea D'Alessio[e], Maurilio Ponzoni[b,c]

[a] *Hematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Scientific Institute, via Olgettina 60, Milan 20132, Italy*

[b] *Hematopathology Diagnostic Area, Pathology Unit, IRRCS San Raffaele Scientific Institute, Milan, Italy*

[c] *Vita-Salute San Raffaele University, Milan, Italy*

[d] *Immunohematology and Transfusion Medicine Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy*

[e] *Medical Oncology and Internal Medicine, Policlinico San Marco, Zingonia, Italy*

[*] Corresponding author.
E-mail address: lazzari.lorenzo@hsr.it (L. Lazzari).