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SOLVING CLINICAL PROBLEMS IN BLOOD DISEASES

A physician or group of physicians considers presentation and evolution of a real clinical case, reacting to clinical information and data (boldface type). This is followed by a discussion/commentary.

Isolated congenital asplenia: An overlooked cause of thrombocytosis

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1 | CASE PRESENTATION

We herein report on a 26-year-old Caucasian female who was referred to our outpatient clinic with a history of abnormal blood count, including thrombocytosis and monocytosis known for the last 7 years. No family history of hematological disorders was reported. Her past medical history was significant for a papillary thyroid carcinoma that was treated with surgery and radioiodine therapy, obtaining a complete remission; the patient is currently taking levothyroxine. Her physical exam was normal and no signs and/or symptoms of infection were noticed.

Thrombocytosis, defined as a platelet count >450 $\times$ 10^9/L, is a common finding in routine blood tests and a common cause of referral to hematologists for further investigation. Causes of thrombocytosis

Luca Arcaini and Elisa Rumi contributed equally to this study.

can be grouped into primary, secondary, and spurious (Table 1).[1] To find out the etiology of the thrombocytosis, the clinical hematologist should take into consideration data from familial and medical history, physical examination, results of hematological exams, clonal genetic abnormalities and bone marrow aspirate and trephine biopsy for morphological features, flow cytometric data, and cytogenetic and molecular markers of clonality.

During the visit, we performed additional blood tests. A complete blood count revealed the following values: Hemoglobin 150 g/L, hematocrit 42.8%, mean cell volume 88 fL, white blood cell count 10.23 $\times$ 10^9/L, and platelet count 582 $\times$ 10^9/L. Differential white blood cell count revealed a normal absolute neutrophil count and a slightly increased number of monocytes (1.2 $\times$ 10^9/L). Morphological examination of peripheral blood smear confirmed the higher-than-normal platelet count with no significant platelet clumping and also showed several target cells, acanthocytes, and Howell-Jolly bodies within red blood cells (Figure 1A). C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) results within the normal range. Serum levels of iron, transferrin, ferritin, and transferrin saturation were normal. Serum level of lactic acid dehydrogenase (LDH) was within the normal range. A chest X-ray revealed no abnormalities attributable to pulmonary infection. Abdominal ultrasound showed no evidence of infection and reported limited visualization of the spleen. The *BCR-ABL* rearrangement was absent in peripheral blood. No mutations of *JAK2*, *CALR*, and *MPL* genes were found in DNA from peripheral blood granulocytes.

During the assessment of a new-onset thrombocytosis, it is of the upmost importance to distinguish secondary (or reactive) thrombocytosis from true hematological diseases. First of all, for practical purposes, we searched for causes of secondary thrombocytosis. The most common causes of reactive thrombocytosis are iron deficiency, inflammation, infection, hemolysis, and other causes that trigger an acute-phase response (Table 1). Thus, we performed serum tests to check for the iron status (iron, transferrin, and ferritin) and for an

TABLE 1 Causes of thrombocytosis

Primary	Secondary	Spurious
Essential thrombocythemia	Iron deficiency	Cryoglobulinemia
Polycythemia vera	Acute-phase response due to:	Microspherocytes
Primary myelofibrosis	• Infection	Schistocytes
Chronic myeloid leukemia, *BCR-ABL1*-positive	• Inflammation	Bacteria
Myelodysplastic syndrome with isolated del(5q)	• Recent surgical intervention (post-operative thrombocytosis)	Neoplastic cell cytoplasmic fragments
Chronic myelomonocytic leukemia	Hemorrhage	Pappenheimer bodies
Atypical chronic myeloid leukemia, *BCR-ABL1*-negative	Hemolysis	
Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis	Malignancy	
Myelodysplastic/myeloproliferative neoplasm, unclassifiable	Hyposplenism	
	Drug therapy with:	
	• Corticosteroids	
	• Adrenaline	
	• Cytokines (e.g., thrombopoietin)	

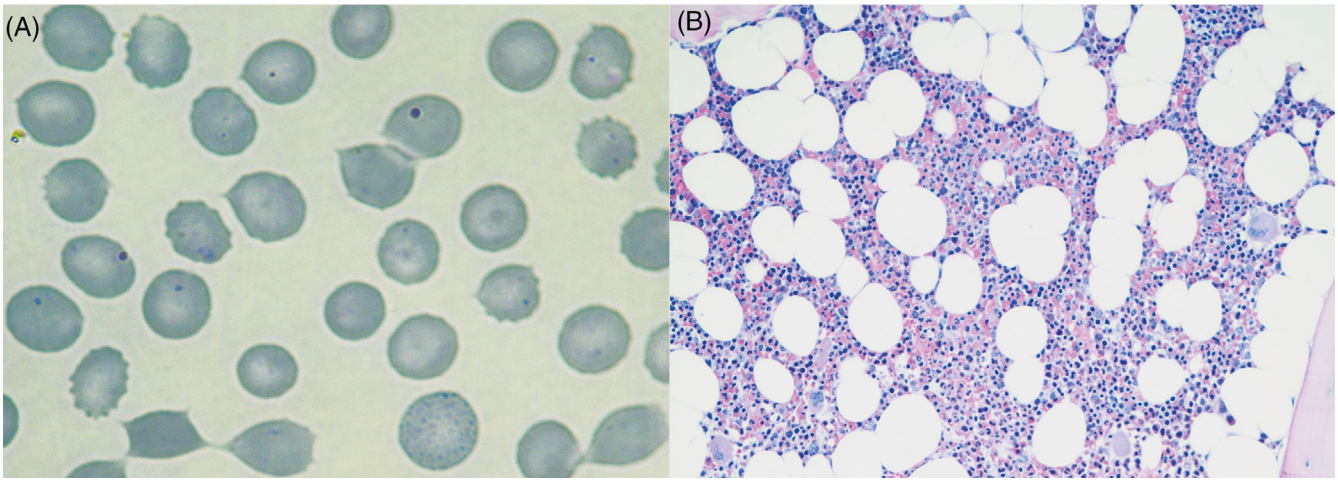

FIGURE 1 (A) Peripheral blood smear. The peripheral blood smear shows morphological abnormalities typically described in hyposplenism (e.g., Howell-Jolly bodies, Heinz bodies, spiculated cells, target cells) (Hematoxylin–eosin $\times$100). (B) Bone marrow biopsy. The bone marrow biopsy shows normal cellularity for the age, normal myelo-erythroid ratio and maturation; megakaryocytes are scattered and without the typical cytologic modifications of myeloproliferative neoplasms (Hematoxylin–eosin, $\times$20) [Color figure can be viewed at wileyonlinelibrary.com]

acute-phase reaction (CRP, ESR): all of them were normal. A normal LDH serum level, together with a normal hemoglobin concentration and reticulocyte count, excluded hemolytic processes. Despite the absence of symptoms and signs of an underlying infection, we performed a chest radiograph that ruled out a pulmonary infection, and an abdominal ultrasound that revealed no signs of abdominal infection and reported a poorly assessable spleen. The most common mutations associated with myeloid neoplasms (*BCR-ABL* rearrangement for chronic myeloid leukemia and *JAK2*, *CALR*, and *MPL* mutations for Philadelphia-negative myeloproliferative neoplasms) were not found.[2–4]

In view of the persistent thrombocytosis (platelet count was 532 × 10^9/L at the next blood count), we performed a bone marrow aspirate and trephine biopsy to search for hematological causes. Histological examination (Figure 1B) revealed a cellularity of 40% and a slightly reduced number of erythroid precursors with a preserved ratio of myeloid to erythroid precursors and a normal number of megakaryocytes without significant abnormalities. The number of CD34+ precursors was also normal (2%–3% of the total nucleated cells). No significant bone marrow reticulin fibrosis was detected (grade 0 according to the WHO grading system).[5] Bone marrow flow cytometric analysis and cytogenetic analysis were also normal.

Bone marrow biopsy is not usually required to investigate a secondary thrombocytosis. However, in absence of a reactive etiology, we performed a bone marrow aspirate and trephine biopsy to search for hematological disorders associated with thrombocytosis.

The most common hematological malignancies associated with thrombocytosis are chronic myeloid leukemia, *BCR-ABL1* positive,[4] and the so-called Philadelphia-negative myeloproliferative neoplasms (i.e., polycythemia vera, essential thrombocythemia, and myelofibrosis).[2] In our case, all of these entities were ruled out because of the absence of their typical molecular markers (searched on peripheral blood, see the previous paragraph) associated with normal findings on bone marrow aspirate and trephine biopsy examination. The diagnosis of a myelodysplastic/myeloproliferative neoplasm (e.g., atypical chronic myeloid leukemia, *BCR-ABL1*-negative, chronic myelomonocytic leukemia, and myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis) was also excluded by cytomorphological examination of the bone marrow aspirate. The absence of a clonal marker at cytogenetics also supported the nonclonal etiology of the thrombocytosis.

Given the inability of the previous abdominal ultrasound to assess the spleen, we ordered a new ultrasonographic examination of the abdomen. No signs of infection were detected. The spleen was described as poorly visualized with an estimated longest dimension of 3 cm and no focal abnormality. In view of the inconclusive result of the ultrasound, abdominal magnetic resonance imaging (MRI) was performed. It revealed a very small spleen, approximately measuring 7 × 45 × 24 mm and was conclusive for splenic hypoplasia (Figure 2). Thus, a diagnosis of reactive thrombocytosis related to hyposplenism was made. We, therefore, suggested a complete vaccine cycle for encapsulated bacteria.

The protein-coding region of the Ribosomal Protein SA (*RPSA*) gene was analyzed by Sanger sequencing but no private or rare variants were found. Abdominal ultrasound of both the mother and sister of the patient revealed the presence of a normal size spleen.

Splenic hypoplasia and asplenia, respectively, refer to the partial or complete lack of splenic tissue. Causes of asplenia include a heterogeneous group of conditions. Surgical asplenia is the most common cause of asplenia. Congenital asplenia can be part of a complex clinical picture involving multiple congenital abnormalities,[6] or can be an isolated abnormality (isolated congenital asplenia, ICA),[7] which is an extremely rare condition. In the presented case, hyposplenism was initially suggested by the altered blood count (thrombocytosis associated with monocytosis) accompanied by the presence of morphological

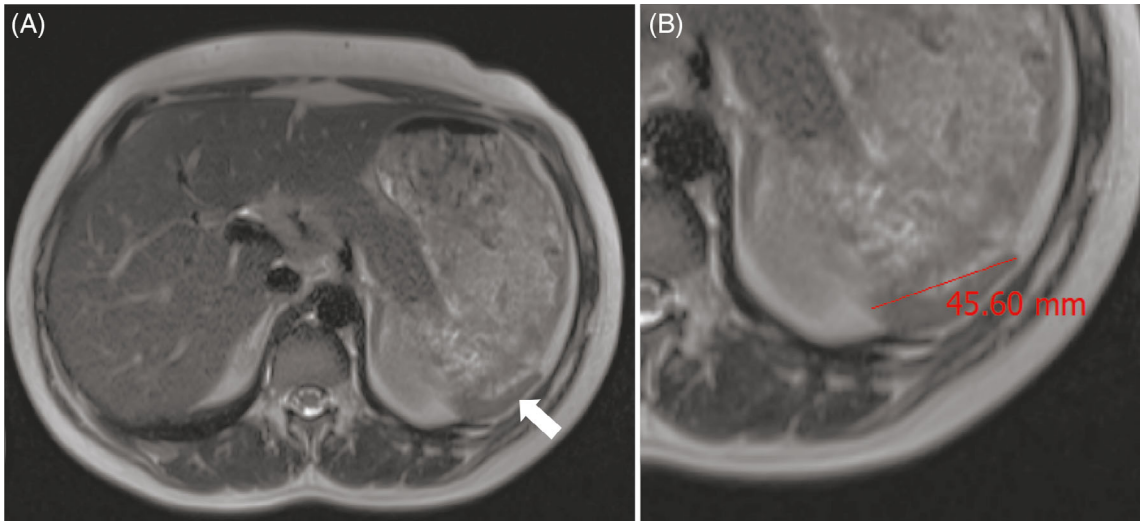

FIGURE 2 (A) Abdominal magnetic resonance imaging. Axial T2 weighted image of the upper abdomen reveals a crescent-shaped hypoplastic spleen (white arrow), within the posterior aspect of a dilated stomach. (B) Detail of the spleen. Magnified view of the left upper quadrant demonstrates spleen measurement of 45 mm in longest dimension [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Causes of anatomical or functional hyposplenism

Congenital disorders	Isolated congenital asplenia (ICA) Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome Stormorken's syndrome Ivemark's syndrome Fetal hydantoin syndrome Congenital cyanotic heart disease Normal and premature neonates
Autoimmune disorders	Primary vasculitis syndromes (e.g., granulomatosis with polyangiitis, polyarteritis nodosa) Secondary vasculitis associated with systemic disease (Lupus vasculitis, rheumatoid vasculitis, sarcoid vasculitis) Goodpasture's syndrome and other glomerulonephritis Sjögren's syndrome Thyroiditis
Gastrointestinal disease	Autoimmune conditions (e.g., celiac disease, dermatitis herpetiformis) Inflammatory bowel diseases (Crohn's disease and ulcerative colitis) Gastrointestinal infections (e.g., Whipple's disease) Other (e.g., intestinal lymphangiectasia, idiopathic chronic ulcerative enteritis)
Liver disease	Viral chronic hepatitis Alcoholic liver disease Primary biliary cirrhosis Hepatic cirrhosis leading to portal hypertension
Hematological and neoplastic disorders	Myeloproliferative neoplasms Hemoglobinopathies (e.g., hemoglobin H diseases) Hematopoietic stem cell transplantation Chronic graft-versus-host disease Acute leukemia Solid neoplasms (e.g., sarcoma) and metastases
Sepsis/infectious diseases	Bacterial infection (e.g., pneumococcal meningitis) Viral infection (e.g., Human Immunodeficiency Virus) Parasitic infection (e.g., malaria) Fungal infection (e.g., candidiasis, histoplasmosis)
Splenic artery and vein thrombosis	Thrombosis of splenic vein Thrombosis of splenic artery Thrombosis of celiac artery
Iatrogenic conditions	Spleen radiation, surgical splenectomy

abnormalities of red blood cells. However, these morphological alterations are often described during the routine morphological evaluation of peripheral blood smear and, due to their low specificity, are frequently overlooked. Given the suspect of splenic hypoplasia arose from abdominal ultrasounds, a technique with the higher soft-tissue resolution was performed and splenic hypoplasia was confirmed. In the absence of another congenital abnormality suggesting a heterotaxy syndrome,[8] we hypothesized an ICA. Sanger sequencing of the translated and untranslated exonic regions at the *RPSA* locus to search for mutations previously shown to underlie approximately half of all ICA cases did not reveal any pathogenic variant.[9] Because of the high proportion of familial cases,[7] we checked both the mother and sister of our patient: No spleen abnormalities were detected with an abdominal ultrasound. Given that patients with hyposplenism have high mortality caused by superimposed infection by encapsulated bacteria, a complete cycle of vaccination against *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* was performed soon after diagnosis of ICA was made.[10]

2 | DISCUSSION

Thrombocytosis is a common finding in routine blood exams and a common cause of referral to the hematologist for further investigation. From a physiopathological point of view, thrombocytosis can be categorized into primary (i.e., depending on the true hematological disease), secondary (i.e., reactive), and spurious (Table 1).[1] As reactive thrombocytosis is more frequent than primary thrombocytosis, it is widely accepted in common clinical practice to search, at first, for causes of secondary thrombocytosis. Thus, blood tests and any other complementary examinations to search for reactive causes (e.g., iron deficiency, infection, inflammatory disease, and hemolysis) should be performed. In addition, a detailed medical history should be obtained, with particular emphasis on the present and past illness and recent surgical intervention (thrombocytosis secondary to malignancy, postoperative thrombocytosis). In addition, a comprehensive and updated list of current medications (including prescription medications, herbals, and over-the-counter drugs) should be noted (thrombocytosis caused by administration of corticosteroids, adrenaline, and thrombopoietin mimicking drugs).

After exclusion of all causes of reactive thrombocytosis, a new platelet count should be repeated to confirm the persistence of unexplained thrombocytosis. Thus, investigations to search for hematological disorders should be performed. Molecular markers for the most common myeloid neoplasms associated with thrombocytosis can be searched on peripheral blood: *BCR-ABL* rearrangement for chronic myeloid leukemia and *JAK2*, *CALR*, and *MPL* mutations for Philadelphia-negative myeloproliferative neoplasms.[2,4] Subsequently, according to the clinical suspicion, a bone marrow aspirate and trephine biopsy, together with cytogenetic and other molecular analysis (including a myeloid next-generation sequencing panel), should be carried out. Bone marrow biopsy is particularly useful to exclude triple-negative myeloproliferative neoplasms.

Hyposplenism, defined as an abnormally low rate of activity of the spleen,[11] is one of the most overlooked causes of thrombocytosis. Irrespective of the underlying cause, hyposplenism is suggested by typical morphological abnormalities of circulating red blood cells, including acanthocytes, spiculated spherocytes, stomatocytes, target cells, pitted erythrocytes, and Howell-Jolly bodies.[12] Pitted red cells are erythrocytes whose membrane appears to contain the so-called "pits." It has been shown by electron microscopy that these "pits" are large vacuoles attached to or beneath the plasma membrane. Within

these vacuoles, there is waste material (e.g., ferritin, hemoglobin, and residual material of mitochondria and membranes) that confers a low optical density. Normal splenic function is characterized by pitted red cells less than 4% of total erythrocytes. A pitted erythrocyte count >4% has been suggested as the gold standard for a diagnosis of hyposplenism.[13] Howell-Jolly bodies that consist of basophilic DNA remnants within erythrocytes, also strongly support a diagnosis of hyposplenism if elevated, but are considered a less sensitive morphological feature than pitted red cells.[11] Finally, radio-isotopic methods (e.g., 99mTc-labeled heat-altered autologous erythrocyte scintigraphy) allow a splenic morpho-functional assessment by studying the uptake and clearance of an injected radiolabeled tracer. However, given their high costs, their use in clinical practice is limited. This underlies the remarkable importance of morphological examination of peripheral blood smear when assessing a patient with a new-onset thrombocytosis. New MRI techniques have increased the detection of splenic diseases so that, nowadays, MRI is an excellent tool for diagnosis and characterization of both focal and diffuse splenic abnormalities.[14]

Splenic dysfunction can be the result of either anatomic (e.g., post-splenectomy hyposplenism, congenital asplenia/splenic hypoplasia) or functional hyposplenism (Table 2).

Congenital asplenia can be part of a complex clinical picture involving multiple congenital abnormalities or can be an isolated abnormality, which is an extremely rare condition.[6,7] ICA is the only known human developmental alteration exclusively involving a lymphoid organ.[15] A French national retrospective study involving pediatric patients with ICA declared that this condition is more common than previously reported.[7] In another survey on pediatric patients with primary immunodeficiencies, an autosomal dominant inheritance in at least some kindreds was described.[16] Thus, it is suggested that relatives of patients diagnosed with ICA are also evaluated for the same condition.

RPSA encodes for the ribosomal protein SA, a component of the small subunit of the ribosome. It belongs to a larger family of ribosomal protein-encoding genes, each of them characterized by numerous pseudogenes that can hinder their sequencing.[17] Mutations in the coding and 5′ untranslated region at the *RPSA* locus have been described in about half of all ICA cases.[9] Exome sequencing of 33 patients with ICA revealed heterozygous mutations in RPSA in 18 (54%) of them. Expression studies suggested that, despite the heterogeneity of *RPSA* mutations (which includes nonsense mutation, frameshift duplication, and different missense mutations), these variants cause autosomal dominant ICA by haploinsufficiency.[18] *RPSA* mutations (consisting in 7 exons) are either in the translated or 5′ untranslated region (5′UTR) of the gene and have a variable penetrance.[9] More recently, impaired ribosome production and function have been hypothesized as cause of several different tissue-specific inherited disorders. Mechanistically, studies on animal models revealed that disruption of *RPSA* in early embryonic development impairs pre-rRNA processing and ribosome biogenesis, thus resulting in impairment of normal expression of key spleen patterning genes (e.g., *pod1*, *nkx2-5*, *bapx1*).[19,20]

In conclusion, ICA is a rare disease that the clinical hematologist should not forget when approaching a patient with a new-onset thrombocytosis. Morphological evaluation of peripheral blood smear is still the first and most important exam that could suggest a diagnosis of ICA, and should always be performed. Demonstration of asplenia or hypoplastic spleen by abdominal ultrasound (or other imaging techniques) should be followed by a search for other congenital malformations (which, if present, suggest that the asplenia/hypoplastic spleen is part of a more complex syndrome) and, if absent, by Sanger sequencing of the coding and UTR region in *RPSA*. Relatives should also be checked with abdominal imaging techniques since ICA can be inherited as autosomal dominant trait in at least some kindreds. Finally, given the high mortality related to infection with encapsulated bacteria, all patients with ICA (or other diseases causing hyposplenism) should receive a complete vaccination cycle against the most important encapsulated bacteria (*S. pneumoniae*, *H. influenza*, and *N. meningitidis*).[10]

AUTHOR CONTRIBUTIONS

Oscar Borsani and Elisa Rumi conceived the study and wrote the manuscript; Sara Fraticelli provided histopathological data; Marta Braschi-Amirfarzan provided MRI data; Daniela Pietra screened for driver mutations; Ilaria Carola Casetti, Daniele Vanni, Chiara Trotti collected clinical data; Takaki Asano, Bertrand Boisson, and Jean-Laurent Casanova did Sanger sequencing of *RPSA* gene; Alessandro Borghesi and Luca Arcaini finalized the manuscript. The study was approved by the local ethics committee.

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CONFLICT OF INTEREST

No competing financial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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