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IM - CASE RECORD

Less is not always more: complex interactions in blood clot mysteries

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A 52-year-old man was admitted in autumn 2022 to the emergency department (ED) of San Raffaele Hospital because of mesogastric abdominal pain of severe intensity, exacerbated by the meals, lasting since 2 weeks and gradually increasing. He also reported meteorism and constipation. Upon examination at the arrival to the ED, the patient's body temperature was 36.7 °C, blood pressure was 145/100 mmHg, heart rate was 97 beats per minute (no additional information is available since an EKG was not performed in the ED), and oxygen saturation was 96% while breathing ambient air. The weight was 80 kg, and the body mass index (the weight in kilograms divided by the square of the height in meters) was 26.1 kg/m^2. Laboratory tests performed at the ED were within normal range except for elevated mean corpuscular volume (MCV) of 104.4 fL (reference range 80–100 fL; hemoglobin 13.8 g/dL) and increased serum lactate dehydrogenase (LDH) (354 U/L; reference range: 125–220 U/L). Abdominal ultrasound showed severe meteorism, mild hepatic steatosis, and hypoechoic material in the portal vein. Contrast-enhanced computed tomography (CT) of the abdomen demonstrated massive splanchnic vein thrombosis (SVT) involving the superior and inferior mesenteric veins, the splenic vein, and the portal vein. Mesenteric thrombosis spread to the distal branches and to the splenic vein and completely occluded the vessels. Thrombosis of the portal vein was partial and included the intrahepatic left and right branches. Edema of the mesentery and reduced enhancement of the intestinal wall at the jejunum–ileum, both in the arterial and venous phase and compatible with tissue injury from venous stasis, were also described. The patient started anticoagulant therapy with low-molecular-weight heparin (enoxaparin 1 mg/kg every 12 h, total dose 80 mg every 12 h) and was transferred to the ward of general medicine. The patient's medical history was apparently unremarkable, and he was taking no chronic medication. He was an occasional smoker, reported a 15-alcohol unit per week consumption in the last 6 months and a carbohydrate-prevalent diet. He lived with the wife and two sons and worked as a violinist. His family history included type 2 diabetes mellitus and cardiovascular diseases. The laboratory tests performed the day of the admission confirmed macrocytosis (MCV 105 fL; hemoglobin 13.0 g/dL) with normal counts of platelets and white blood cells, and increased LDH (357 U/L). A manual optical complete blood count performed at that moment revealed no morphological alterations of blood cells. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and homocysteine serum levels were increased: AST 85 U/L (reference range 5–35 U/L), ALT 72 U/L (reference range 6–59 U/L), and homocysteine 58.5 µmol/L (reference range 6.0–15.6 µmol/L). On the same day, we ordered an extensive workup to assess the risk factors for SVT including C protein, S protein, antithrombin deficiency and factor V Leiden, Prothrombin G20210A, JAK2V617F mutations, all of which yielded negative results. Eight days after the start of anticoagulation, the patient reported substantial improvement of his symptoms with complete resolution of fasting abdominal pain and major decrease of postprandial pain (*claudicatio abdominis*). A chest CT scan, performed to exclude lung malignancies, showed at the thorax–abdominal scan nearly complete resolution of portal vein thrombosis while thrombosis of mesenteric and splenic veins was unchanged. Imaging and additional laboratory tests excluded liver cirrhosis, solid cancer, myeloproliferative neoplasm, antiphospholipid syndrome and autoimmune, hematologic and inflammatory bowel diseases. For the evidence of hyperhomocysteinemia and macrocytosis, serum vitamin B12 and folate were dosed, and methylenetetrahydrofolate reductase (MTHFR) mutations were investigated. Vitamin B12 was indeed found to

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be low (74 pmol/L; reference range 145–567 pmol/L), while folate was in the normal range (21 ng/mL); 1298C > V polymorphism of MTHFR was detected in our patient. An esophagogastroduodenoscopy (EGDS), performed after 13 days of anticoagulation therapy, showed hypotrophy of the gastric mucosa, a single F1 esophageal varix, and a reticulum of varicose vessels at the gastric fundus spread to the greater curve (GOV2). Biopsies taken in the antrum/angulus showed mild chronic inflammation (1+) without glandular atrophy and intestinal metaplasia. Biopsies of the body and fundus showed moderate chronic inflammation (2+) with intestinal metaplasia (2+) and discrete glandular atrophy (2+). In both biopsies, no pathogen attributable to *Helicobacter pylori* was found. Anti-parietal cell antibodies resulted positive (1:180 titer), while anti-transglutaminase antibodies were negative. A diagnosis of autoimmune atrophic gastritis (AAG) determining vitamin B12 deficiency, macrocytosis, and hyperhomocysteinemia was made. Accordingly, the patient received an intramuscular injection of 5000 µg of cyanocobalamin. Organ-specific autoantibodies detected in other autoimmune conditions such as Hashimoto's thyroiditis, type 1 diabetes mellitus, and Addison's disease that have been described in association with AAG resulted negative. Five days after vitamin B12 supplementation, laboratory tests showed normalization of LDH (204 U/L), AST (29 U/L), ALT (57 U/L) and hemoglobin (14.0 g/dL, MCV 104.3 fL) and the decrease of homocysteine to 16.1 µmol/L. The patient was discharged completely asymptomatic on warfarin anticoagulation, as at that time there was scarce and discordant evidence on the use of direct oral anticoagulants in the context of SVT, even if suggested by various authors, and the treating physicians preferred to prescribe vitamin K antagonists [1]. He also received the advice to receive 5000 µg of cyanocobalamin by intramuscular administration every 3 months for the rest of his life. Finally, the patient was referred to the coagulation service of our hospital for the follow-up but never came back (Fig. 1).

SVT is a rare yet potentially life-threatening manifestation of venous thromboembolism with an estimated annual incidence of 0.5–1 cases per million per year. SVT encompasses portal vein thrombosis, mesenteric vein thrombosis, splenic vein thrombosis, and the Budd–Chiari syndrome [1]. Risk factors for SVT have been classified as (i) persistent acquired: liver cirrhosis, solid cancer, myeloproliferative and other hematologic diseases (e.g., nocturnal hemoglobinuria), autoimmune diseases (e.g., Bechet's), inflammatory bowel diseases, and antiphospholipid syndrome; (ii) transient acquired: intraabdominal inflammation/infections, abdominal surgery, hormonal therapy, pregnancy or puerperium; and (iii) inherited: factor V Leiden, prothrombin G20210A and JaK2V617F mutations, and protein C, protein S and antithrombin deficiency [2]. It must be noted that, in our patient, the evaluation of inherited SVT risk factors has been performed while under anticoagulation therapy, which may have altered the results. Repetition of such tests in outpatient settings in an anticoagulation-free interval is warranted. The most common symptom of SVT is abdominal pain, reported in about half of SVT patients, followed by gastrointestinal

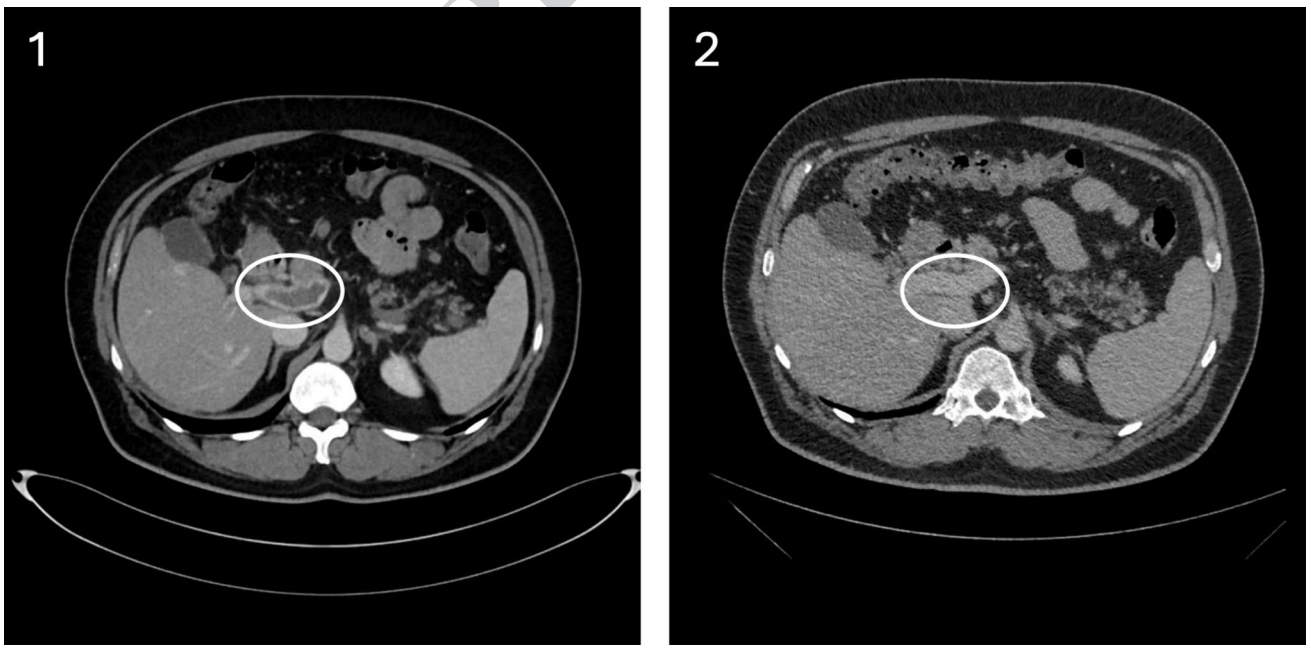

Fig. 1 CT scan of the abdomen before (1) and after (2) anticoagulation, showing the nearly complete resolution of the portal vein thrombosis (circle)

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bleeding and ascites. Additional nonspecific symptoms are nausea, vomiting, anorexia, diarrhea or constipation, and fever. In one third of patients, SVT can be asymptomatic and detected incidentally during imaging performed for other reasons.

The clinical picture of our patient was serious with significant abdominal pain, claudicatio abdominis and laboratory evidence of cell necrosis (increased LDH, AST and ALT) due to severe venous stasis and, possibly, to ineffective hemopoiesis with hemolysis. The patient showed macrocytosis without clinical and hematologic signs of pernicious anemia (PA). Only the finding of hyperhomocysteinemia and vitamin B12 deficiency prompted us to consider the presence of atrophic gastropathy. Vitamin B12 deficiency is causally associated with hyperhomocysteinemia because it is a required cofactor of methionine synthase in the homocysteine remethylation pathway [3]. Deep vein thrombosis in subjects with hyperhomocysteinemia and vitamin B12 deficiency due to PA have been previously reported and some of the characteristics of these cases are summarized in Table 1. Noteworthy, venous thromboembolism appeared independent from the severity of the anemia, being observed also in patients with slight decrease in hemoglobin levels, as in our case. In the other case reports, only two out of 12 patients had SVT, while the majority had venous thrombosis in the legs or lung.

Mutations that decrease the enzymatic activity of MTHFR can increase serum homocysteine, especially if associated with folate deficiency. However, the positive correlation between serum homocysteine, MTHFR polymorphism, and venous thrombosis is somehow controversial. It was suggested by studies conducted in the 1980s but it was definitely excluded by studies performed in the next decade that documented the lack of relationship between venous thromboembolism and the polymorphism of the MTHFR gene [4]. In light of this evidence, the American College of Medical Genetics and Genomics (ACMG) Professional Practice and Guidelines Committee in 2013 made the statement that serum homocysteine and MTHFR polymorphism should not be included in the diagnosis of thrombophilia [5]. More recently, however, the same ACMG published the addendum that "*this document no longer meets the criteria for an evidence-based practice guideline by the College, it has been reclassified as a Clinical Practice Resource*". Besides, the MTHFR 1298C > V polymorphism detected in our patient and found in 8–15% of the general white population, causes a less pronounced reduction of MTHFR activity than the 665C > T mutation and is not associated to the deficiency of B12.

In conclusion, we believe that the massive SVT occurred in our patient probably due to the combination of acquired and inherited risk factors, with hyperhomocysteinemia caused by vitamin B12 deficiency in the context of AAG playing the main role. Interestingly, in this peculiar case, the thrombosis was actually the first manifestation of B12 deficiency, possibly mediated by hyperhomocysteinemia, even before the development of overt anemia. The MTHFR 1298C > V polymorphism might have given only a minimal contribution, especially considering the high folate level found in our patient. The present report questions the advice against the determination of homocysteine in the evaluation of the causes of venous thrombosis. In fact, not assessing serum homocysteine and vitamin B12 levels would have not allowed the diagnosis of PA in our patient and, most importantly, the recognition of the necessity of life-lasting vitamin B12 supplementation to prevent the occurrence of hematological, neurological, and thrombotic complications due to vitamin B12 deficiency. Therefore, less is not always

Table 1 Subjects with venous thromboembolism and hyperhomocysteinemia as first manifestation of vitamin B12 deficiency in pernicious anemia, from literature review. References are reported below

	Site of venous thrombosis	Hemoglobin (g/dl)	Serum homocysteine (µmol/l)	Serum vitamin B12 (pmol/l)	Age	Sex	References
Case 1	Superior cava	5.7	66	66	24	M	[6]
Case 2	Tibial posterior	12.8	42	37	35	M	[6]
Case 3	Popliteal	5.8	33	63	60	M	[7]
Case 4	Tibial	4.7	56	68	74	M	[7]
Case 5	Portal, superior mesenteric, splenic	8.7	70	44	60	M	[8]
Case 6	Femoral	9.0	131	41	71	F	[9]
Case 7	Pulmonary	9.0	50	44	34	M	[10]
Case 8	Ileofemoral, popliteal	9.5	125	44	60	M	[10]
Case 9	Femoral, popliteal	8.6	200	44	58	M	[10]
Case 10	Popliteal	12.0	167	15	47	F	[10]
Case 11	Pulmonary, superior mesenteric	12.0	65	22	58	M	[11]
Case 12	Cerebral	11.6	26	54.79	45	F	[12]

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more in the everyday life of our wards. A limitation to this case report is the lack of a follow-up for the patient, as the management was continued in an outpatient clinic outside our institution due to the current visit booking system and management characterizing our healthcare organization. Therefore, we unfortunately have no data about the duration of anticoagulation and about the outcomes of treatment to discuss, speculate about and add to the experience on the use of VKA vs DOAC for the treatment of SVT.

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Author contributions Alberto Davalli, Giacomo Leo Paolazzi: conceptualization, data curation, writing—original draft. Marco Montagna: data curation, writing—review and editing. Armando D'Angelo, Patrizia Rovere Querini: conceptualization.

Declarations

Ethical approval Written informed consent was obtained from the patient for the publication of this case report, including any associated images.

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