Acquired protein C deficiency in a child with acute myelogenous leukemia, splenic, renal, and intestinal infarction

Roula A. Farah[a], Khalil S. Jalkh[b], Hussein Z. Farhat[c], Paul E. Sayad[d] and Adel M. Kadri[e]

We report the case of a 6-year-old boy diagnosed with acute promyelocytic leukemia (AML-M3V) when he presented with pallor, abdominal pain, anorexia, and fatigue. Induction chemotherapy was started according to the AML-BFM 98 protocol along with Vesanoid (ATRA, All-trans retinoic acid). On the sixth day of induction, he developed splenic and gallbladder infarcts. Splenectomy and cholecystectomy were performed while chemotherapy induction continued as scheduled. Four days later, he developed ischemic areas in the kidneys and ischemic colitis in the sigmoid colon. Hypercoagulation studies showed severe deficiency of protein C. Tests showed protein C 16% (reference range 70–140%), protein S 87% (reference range 70–140%), antithrombin III 122% (reference range 80–120%), prothrombin time 13.6 s (reference = 11.3), INR (international normalized ratio) 1.21, partial thromboplastin time 33 s (reference = 33), fibrinogen 214 mg/dl, D-dimer 970 μg/ml, factor II 98%, and that antinuclear antibody, antiphospholipid antibodies, mutation for factor II gene (G20210A), and mutation for Arg506 Gln of factor V were all negative (factor V Leiden). There was no evidence of clinical disseminated intravascular coagulation (DIC). He was treated with low molecular weight heparin and did well. He continues to be in complete remission 7 years later with normal protein C levels. Acquired protein C deficiency can occur in a variety of settings and has been reported in acute myelocytic leukemia. However, clinically significant thrombosis in the absence of clinical DIC, such as our case, remains extremely rare. *Blood Coagul Fibrinolysis* 22:140–143 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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[a]Department of Pediatrics, [b]Department of Medicine, Saint George Hospital University Medical Center, [c]Department of Laboratory Medicine, [d]Department of Surgery, Rizk Hospital University Medical Center, Beirut and [e]Department of Medicine, Tel Chiha Hospital, Zahle, Lebanon

Correspondence to Roula A. Farah, MD, Division of Hematology and Oncology, Department of Pediatrics, St George University Hospital, Beirut, Lebanon
Tel: +961 3681309; fax: +961 1582560; e-mail: roula.fs@dm.net.lb

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Case report

We report the case of a 6-year-old Lebanese boy who presented with pallor associated with abdominal pain, anorexia, and fatigue. Physical examination revealed ecchymoses on his right lower calf, abdominal tenderness over the epigastric region, and slight hepatosplenomegaly. A complete blood count was performed upon admission and showed a hemoglobin level of 10.2 g/dl, hematocrit of 30.8%, platelet count of $32 \times 10^3/\mu l$, and white blood cell count of $36.55 \times 10^3/\mu l$. Peripheral smear showed 5% neutrophils, 8% lymphocytes, 81% promyelocytes (1% are hypergranular and 80% are hypogranular), and 6% blasts. Liver function studies showed serum glutamic pyruvic transaminase (SGPT) of 50 U/l, serum glutamic oxaloacetic transaminase (SGOT) of 11 U/l, direct bilirubin of 0.3 mg/dl, and total bilirubin of 0.5 mg/dl. Abdominal ultrasound at diagnosis showed mild hepatosplenomegaly. Bone marrow aspiration showed a hypercellular marrow massively invaded by atypical hypogranular promyelocytes (93% of the counted cells). Immunophenotyping showed positivity for the following markers: CD13, CD33, CD45, CD64, and myeloperoxidase. Cells partially expressed CD14 and were negative for the expression of HLADR and CD34. Results are consistent with acute promyelocytic leukemia (AML-M3V). Molecular studies were positive for t(15;17) PML/RAR-α fusion transcript and negative for t(8;21) AML1/ETO, inv16 RBF-β/MYH11, and t(9;11) MLL/AF-9 fusion transcripts. Bone marrow karyotype at diagnosis showed t(15;17) and complex chromosomal abnormalities of chromosomes 1, 4, and 7 in all cells studied: [46, XY, del(1)q(11), der(4)t(?1q;4p), der(7)t(?1q;7q), t(15;17)(q22;q21)]. DNA index was 1.08. Cerebrospinal fluid was negative for malignant cells.

Induction chemotherapy was administered according to the AML-BFM 98 protocol consisting of continuous infusion for 48 h (days 1, 2, and 3) of cytarabine 100 mg/m^2 and then 100 mg/m^2 every 12 h for 12 doses (days 3 through 8) as well as etoposide 150 mg/m^2 (days 6, 7, and 8), idarubicin 12 mg/m^2 per day (days 3, 5, and 7), and intrathecal cytarabine (days 1 and 8) along with vesanoid 20 mg orally twice daily from day 1 of induction.

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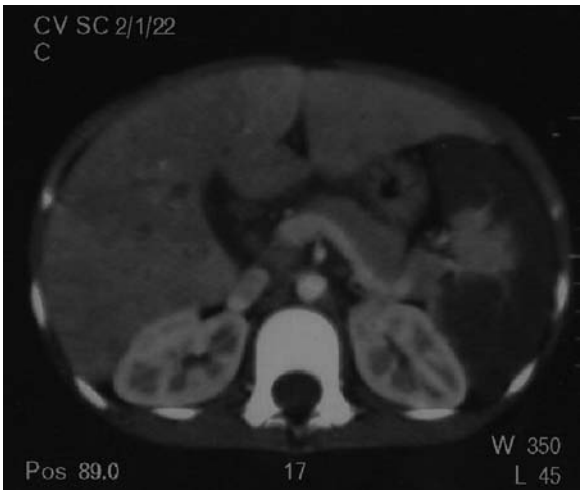
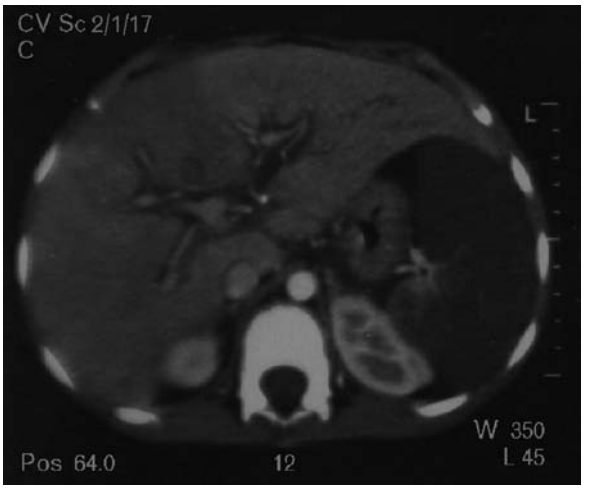
Fig. 1



The figure shows an enlarged spleen and hypodensities are seen in the spleen except for a focus around the hilum. This implies that it is the only focus that is irrigated, and the rest of the spleen is infarcted. Large areas of decreased intensity are seen in the liver, which can signify irregular fatty liver infiltrates.

Fig. 2



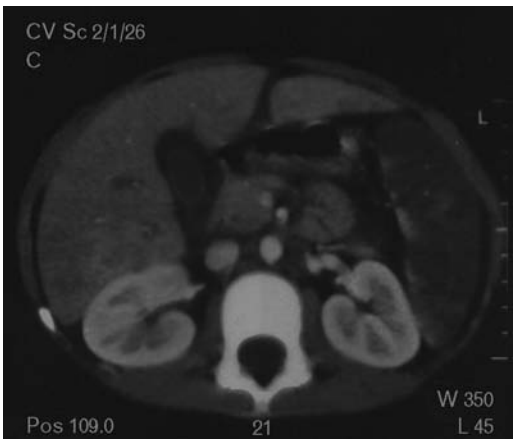
The figure shows evidence of an infarcted spleen. Severe edema of the porta hepatis is also identified.

On the sixth day of induction, he developed increasing abdominal pain. A computed tomography (CT) scan of the abdomen and pelvis with intravenous contrast revealed an infarct involving most of the spleen and evidence of fatty infiltrates in the liver (Fig. 1). Severe edema of the gallbladder and porta hepatis was also identified, which is suspicious of a gallbladder infarct (Figs 2 and 3). The splenic infarct was confirmed by nuclear scan, which showed no uptake in the spleen. Liver function studies at that time were within normal limits; however, albumin levels were found to be mildly decreased to 2.9 g/dl. A splenectomy and a cholecystectomy were performed. The spleen was completely infarcted and the gallbladder had multiple areas of necrosis. He received one dose of pneumococcal vaccine 1 day before the operation. The postoperative course was uneventful and he improved considerably.

On postoperative day 10 (day 16 from the start of induction), the patient experienced new onset of postprandial abdominal pain associated with fever, and severe diarrhea. A repeat CT scan of the abdomen showed an area of wedge infarct in the left kidney (Fig. 4) and evidence of ischemic colitis in the sigmoid colon (Fig. 5). A colonoscopy was performed showing edema of the mucosa with superficial ulceration in the sigmoid colon. The patient was treated conservatively. He was started on low molecular weight heparin (LMWH), 40 mg q24h on day 21 from the start of induction for a duration of 10 days. The dose was then augmented to 60 mg q24h and was maintained for another 10 days. Factor Xa levels were followed. He was then discharged home on LMWH subcutaneously for a total of 3 months.

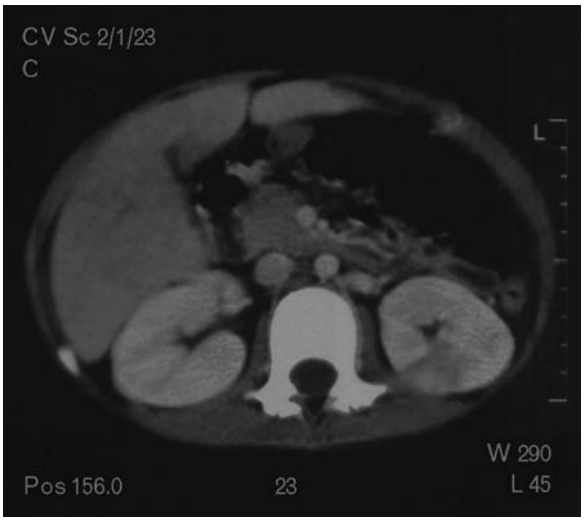
Hypercoagulation studies were performed (on day 23 after the start of induction, 8 days after end of induction) and revealed a notable deficiency in protein C activity. Tests yielded the following results: protein C 16% (reference range 70–140%), protein S 87% (reference range 70–140%), activated protein C (APC) ratio 1.76 (normal = 2–3.5, heterozygote = 1.7, homozygote = 1.2), antithrombin III 122% (reference range 80–120%), prothrombin time (PT) 13.6 s (reference = 11.3), international normalized ratio (INR) 1.21, partial

Fig. 3



The figure shows evidence of an infarcted spleen as well as severe edema of the gallbladder.

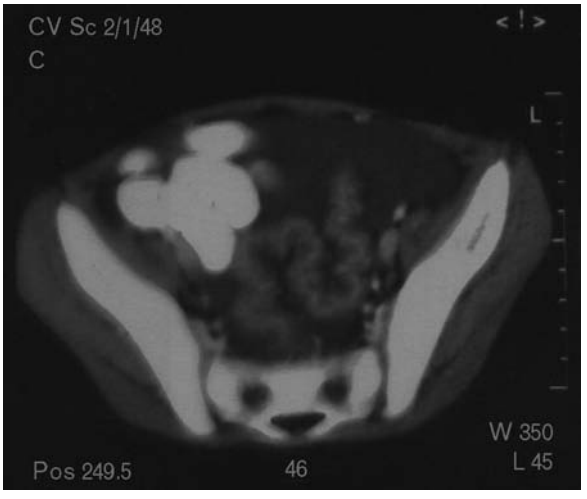
Fig. 4



The figure shows a wedge infarct in the posterior part of the left kidney.

thromboplastin time (PTT) 33 s (reference = 33), fibrinogen 214 mg/dl (normal range = 200–400 mg/dl), D-dimer 970 μg/ml, antinuclear antibody (ANA) was negative, antiphospholipid antibodies were negative, factor II 98%, mutation for factor II gene (G20210A) was negative, and mutation for Arg506 Gln of factor V was negative (factor V Leiden). There was no family history of thrombotic diseases. Family studies revealed normal protein C levels in both parents. Fibrinogen levels were repeated on several occasions and had shown steady levels throughout the course of the disease (Table 1).

Fig. 5



The figure shows thickening of the bowel walls. The wall of sigmoid colon is thickened, and evidence of dense fat is seen, which is suggestive of mesenteric infarct.

Table 1 Serial measurements of fibrinogen and D-dimer

	9/13/2002	9/27/2002	11/26/2002	21/23/2002
D-dimer (μg/ml)	970	200	200	220
Fibrinogen (mg/dl)	214	247	302	391

The protein C activity assay was performed on a STA coagulation analyzer using the STA-staclot Protein C kit (Diagnostica Stago, Asnieres-sur-Seine, France). The protein S activity assay was performed on a STA coagulation analyzer using the STA-staclot Protein S kit (Diagnostica Stago).

Determination of antithrombin III was done using chromogenic tests, COAMATIC Antithrombin (Chromogenix Instrumentation Laboratory, Milan, Italy).

Antiphospholipid screen was performed using ORGENTEC Anti-Phospholipid Screen IgG/IgM assay (ORGENTEC Diagnostika GmbH, Mainz, Germany).

Determination of possible factor V mutation was done using *HemosIL* factor V Leiden [(APC Resistance V) – 0020008700 (Instrumentation Laboratory Company, Lexington, Massachusetts, USA)].

He went into complete remission and continued his chemotherapy as scheduled. Protein C levels returned to normal 33 days after the end of induction.

The patient was followed up on a yearly basis for 7 years after his initial treatment. Results showed that he was still in complete remission with normal protein C levels throughout the 7 years.

Discussion

Protein C is a vitamin K-dependent protein synthesized in the liver. It circulates as a zymogen and exerts its anticoagulant function after activation to the serine protease, APC. The primary effect of APC is to decrease the coagulation activity of factors Va and VIIIa, which are necessary for efficient factor X activation and thrombin generation [1]. Low plasma APC levels have been associated with an increased risk of venous thromboembolism [2,3]. Acquired deficiency can occur in a variety of settings, including liver disease, severe infection, septic shock, disseminated intravascular coagulation (DIC), acute respiratory distress syndrome, and breast cancer patients receiving cyclophosphamide, methotrexate, and 5-fluorouracil, and in association with L-asparaginase therapy [4–7].

Thromboembolic events are common in leukemia patients and are usually attributed to a concurrent state of DIC. Tsumita *et al.* [8] reported a patient with acute myelocytic leukemia (AML), multiple thrombophlebitis, and DIC accompanied with lower protein C activity and antigen levels. All parameters had returned to normal after complete remission. As such, protein C deficiency

was attributed to consumption during DIC and from unknown factors released from leukemic cells. Cases of patients showing thrombotic events in the absence of DIC were also reported in whom coagulation studies were performed showing a decrease in free protein C levels. Maureen *et al.* [9] described a patient with chronic lymphocytic leukemia, AML, and protein C deficiency, who developed deep vein thrombosis of the calf. The patient had no evidence of liver dysfunction, DIC, or family history of protein C deficiency; however, there was a strong family history suggestive of hypercoagulability. Low protein C, S, and antithrombin III have also been reported in a series of leukemia patients, mainly with AML-M3. In this series, thrombotic complications coinciding with AML and protein C deficiency were only reported in one AML-M3 patient in the form of myocardial infarction at day 15 of induction. In that study, the significance of their observation in terms of predisposition to thrombosis or DIC was not established [10]. Protein C deficiency has been reported in several AML cases in the absence of DIC, but without significant thrombotic events. Rodeghiero *et al.* [11] reported slightly lower levels of protein C and antithrombin III in acute leukemia patients. Protein C levels were not lower in patients with DIC when compared to patients without DIC. A close correlation was noted between the levels of these inhibitors and the synthetic function of the liver as expressed by indices such as serum albumin and pseudocholinesterase levels. As such, liver dysfunction rather than DIC was considered to be the cause of decreased anticoagulants. Troy *et al.* [12] reported low levels of protein C antigen and protein C activity in 50 patients with acute leukemia (34 acute myelogenous leukemia and 16 acute lymphoblastic leukemia). In that study, no correlation was made between protein C levels and liver dysfunction. There were no thrombotic events due to protein C deficiency; thrombotic events occurred only in three patients who had developed DIC.

In the present case, there was no evidence of clinical DIC, infection, or shock, and the chemotherapy agents used for treatment of this case of AML-M3 are not known to induce a hypercoagulable state. As it seems, the patient had developed protein C deficiency along the course of his AML. Consequently, the patient developed splenic, mesenteric, and renal infarction. The cause of protein C deficiency remains unclear. When compared with previous literature, one can postulate that deficiency may have been caused by liver dysfunction as evidenced by fatty infiltrate of the liver seen on CT scan of the abdomen, demonstrating possible inflammatory reactions and a mild decrease in albumin levels at the time of protein C deficiency, although liver enzymes remained normal throughout the course of the disease.

The development of protein C deficiency in an AML patient resulting in significant intra-abdominal organ ischemia has rarely been reported. Physicians should be aware of this potential complication. Early diagnosis and management in order to avoid further complications is extremely important. Considering the possibility of treatment with APC concentrate should be investigated in the future.

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