Transfusion Medicine and Hemotherapy

Case Report

Transfus Med Hemother 2023;50:360–364
DOI: 10.1159/000529500

Received: August 7, 2022
Accepted: January 29, 2023
Published online: March 7, 2023

# Diagnosis of Bone Marrow Necrosis following Severe Vaso-Occlusive Crisis in Patient with Compound Heterozygous Sickle Cell Disease

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**Keywords**

Bone marrow necrosis · Sickle cell disease · Leucoerythroblastic reaction · Fat embolism syndrome · Atypical hemolytic uremic syndrome · Microangiopathic anemia

**Abstract**

***Introduction:*** Bone marrow necrosis is a rare entity that can develop in context of a sickle cell disease vaso-occlusive crisis. Its physiopathology is related to an endothelial dysfunction taking place in bone marrow microvasculature. ***Case Presentation:*** A 30-year-old patient with history of compound heterozygous sickle cell disease was admitted following SARS-CoV-2 infection with fever and diarrhea. After initial favorable evolution, he developed a severe vaso-occlusive crisis with intense hemolysis and multi-organ ischemic complications. Patient then developed high fever and hypoxemia. With the suspicion of acute thoracic syndrome, a red blood cell exchange was performed. Respiratory symptoms ceased but patient persisted febrile with very high levels of acute phase reactants, persistent pancytopenia, and leucoerythroblastic reaction. An infectious cause was ruled out. Afterward, bone marrow aspiration and bone marrow biopsy showed a picture of bone marrow necrosis, which is an extremely rare complication of vaso-occlusive crisis but, paradoxically, more frequent in milder heterozygote cases of sickle cell disease. Ultimately, large deposits of complement membrane attack complex (particles C5b-9) were demonstrated after incubation of laboratory endothelial cells with activated plasma from the patient. ***Discussion:*** The clinical presentation and findings are consistent with a case of bone marrow necrosis. In this setting, the demonstration of complement as a potential cause of the endothelial dysfunction mimics the pattern of atypical hemolytic uremic syndrome and other microangiopathic anemias. This dysregulation may be a potential therapeutic target for new complement activation blockers.



## Introduction

Reported cases of bone marrow necrosis (BMN) in literature correspond mostly to acute hematological malignancies with massive infiltration of bone marrow stroma, affecting myeloid precursors, and preserving bone trabeculae structure. Other causes are autoimmune syndromes (e.g., systemic lupus erythematosus and antiphospholipid syndrome) and hemoglobinopathies. The common aspect of these varied pathologies is a dysfunction and lesion of sinusoidal endothelium, leading to a diffuse failure of bone marrow microcirculation [1]. As a result, an edema and infarction of the bone marrow initiate an inflammatory cascade which leads to liberation of fat, marrow cells, and bony spicules to the bloodstream.

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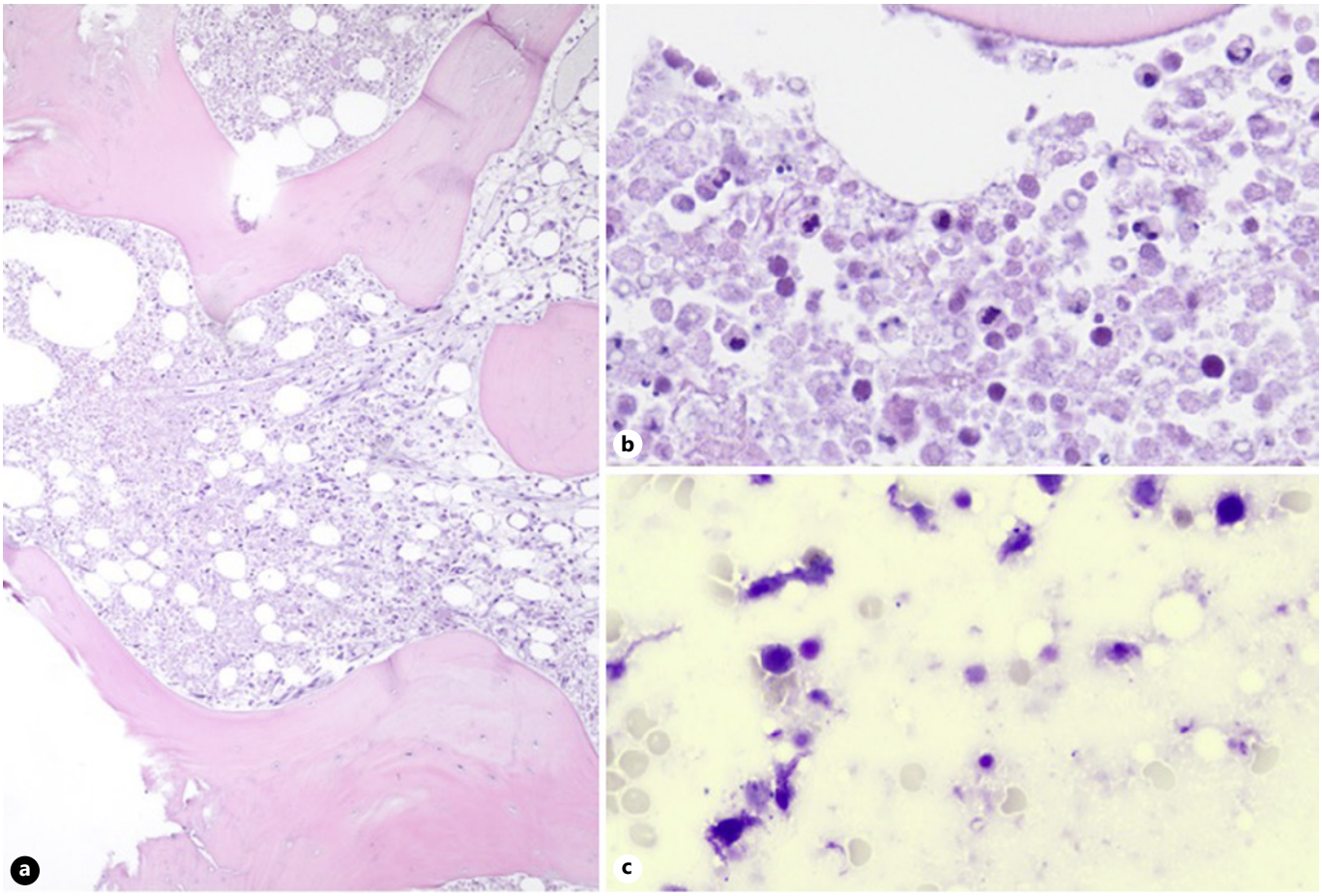


**Fig. 1.** Bone marrow aspirate and biopsy. **a** (H&E staining, × 100): micrography of bone marrow showing necrotic occupation of medullary spaces with conserved bone structure (no signs of osteonecrosis). **b** (H&E staining. , × 400): micrography of bone marrow showing large area of ghost cells. **c** (May-Grünwald Giemsa staining, × 400): bone marrow aspirate showing scarce viable content with predominance of necrotic material.

These lesions promote a pro-inflammatory state, dysregulation of adhesion molecules, and imbalance between vasodilator and vasoconstrictor agents in endothelium [2], resulting in severe multi-organ complications.

Sickle cell disease (SCD) is a group of inherited hemoglobinopathies affecting â-globin gene alleles. Regarding literature reports of BMN in SCD, it seems that cases of BMN tend to be delayed or confused with other microangiopathic anemias [2, 3], not deserving bone marrow evaluation in most cases. Regarding literature reports of BMN in SCD, it is paradoxically more frequent in those patients affected by less severe heterozygote genotypes. Perhaps because in those cases the total red blood cell (RBC) count tends to be higher and, therefore, blood viscosity increases [4]. The present case exemplifies the clinical presentation and diagnostic challenge of a patient with BMN in context of SCD vaso-occlusive crisis (VOC), stressing the need of better understanding of this entity and its implications toward endothelial dysfunction and interaction with the alternative complement pathway.

## Case Presentation

Patient was a 30-year-old male born in Ghana. He had been living in Spain for 10 years. Last trip to his country of origin was 2 years before. In February 2021, was diagnosed with SCD (hemoglobin SC genotype) with a hemoglobin level of 117 g/L. He had never had any complications and he was not taking any medication.

He was admitted with acute diarrhea and fever, oral intolerance, and mild dehydration. Blood tests at admission showed elevated acute phase reactants without anemia. Screening for SARS-CoV-2 resulted positive. Empiric antibiotic and standard COVID-19 treatment were initiated. During the first 5 days of hospitalization, digestive symptoms evolved favorably but patient developed an acute severe VOC. Despite treatment with abundant hydration and rigorous pain control, patient did not improve.

On day +6, he was febrile, tachypneic, and mildly tachycardic, blood pressure was 154/90 mm Hg, oxygen saturation was 89% on room air (improving to 97% with $O_2$ 3 L/min). On physical exam, he appeared uneasy. Cardiopulmonary auscultation was normal and abdomen exploration was soft and non-tender, without hepatosplenomegaly. He seemed somnolent but oriented and without focal neurological deficits. Arterial blood gas analysis at room air confirmed hypoxemia ($pO_2$ 67 mm Hg). Electrocardiogram

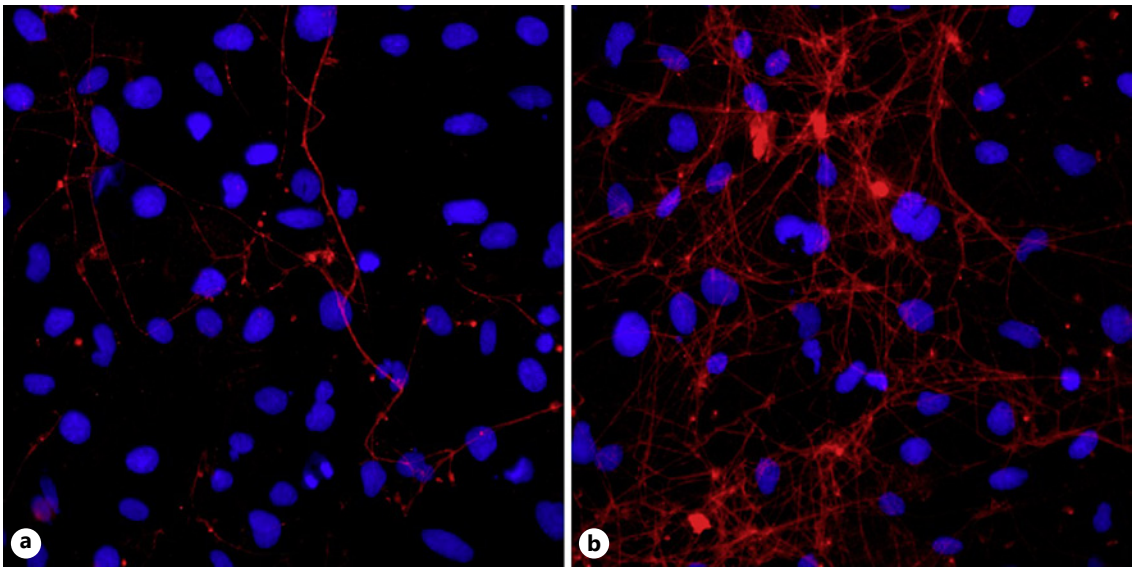


**Fig. 2.** C5b-9 fluorescence immunostaining imaging showing complement deposit (red) in laboratory endothelial cells (blue, DAPI staining). The human dermal microvascular endothelial cell line (American Type Culture Collection) was seeded on glass coverslip. Cells were washed with test medium (HBSS without calcium or magnesium, 0.5% BSA; Life Technologies) and activated or not with 10 μM ADP (Sigma-Aldrich) (10 min, 37°C). Cells were then incubated (4 h) with activated plasma (citrated plasma mixed with a control sera pool 1:1) diluted with test medium in proportion 1:2. Control sample was obtained by mixing healthy plasma from donors. Cultures were then washed and fixed. For C5b-9 immunostaining, cells were treated with 2% BSA (1 h) and incubated with a rabbit anti-human complement C5b-9 complex (Calbiochem), followed by Alexa594-conjugated goat anti-rabbit secondary antibody (Life Technologies). Micrographs were captured by fluorescent microscopy (Leica DM4000 B) through a video camera (Leica DFC310FX) and analyzed using Fiji (ImageJ). **a** Plasma control. **b** Plasma from patient.

showed sinus tachycardia and a chest X-ray did not show alterations.

Complete blood count showed marked hemolysis (hemoglobin had dropped to 72 g/L with a reticulocyte count of 177 × $10^9$/L) with an extremely high LDH (39,000 IU/L), and thrombocytopenia (41 × $10^9$/L). Peripheral blood smear reported 3 schistocytes per field, immature granulocytes, and nucleated RBC. No *Plasmodium* spp. was reported on peripheral smear review. Bilirubin level was 2.9 mg/dL, ferritin was 27,469 ng/mL, D-dimer was >35,000 ng/mL (DDU) with normal basic coagulation tests. Patient associated acute kidney injury (GFR 56 mL/min) and the remainder of the tests showed analytic signs of ischemic damage of different organs (pancreas, liver, muscle, and myocardium). Blood, stool, and urine cultures were negative. ADAMTS-13 activity was 57%. A CTA scan excluded acute thromboembolic embolism, COVID-19 signs of lung injury or organizing pneumonia, showing normal pattern of pulmonary parenchyma.

In the light of rapidly progressive anemia and thrombocytopenia, the most likely scenario was a severe VOC drepanocytic crisis triggered by the viral infection, with subsequent bone and multiorgan microinfarctions. Although the respiratory failure did not accomplish diagnostic criteria for acute chest syndrome (ACS), a RBC exchange was performed on day +8. Patient hematocrit at that time was 21% and his total blood volume based on Nadler method was 5,933 mL, corresponding to 1,245 mL of RBC volume. A total of 2.5 RBC volumes of the patient were exchanged in 66 min with the goal of achieving a hematocrit of 30%. RBC exchange was performed with Spectra Optia v12.0 (TerumoBCT, Lakewood, CO) and 10 RBC units (accounting for 2,772 mL of RBC fluid), with an estimated 30% fraction of patient cells remaining after the procedure. Overall, after the procedure, hemoglobin was 115 g/L and levels of S/C hemoglobin dropped from 41%/45% to 9%/8%.

Following RBC exchange, pain improved rapidly and the hypoxemia ceased. Levels of hemoglobin stabilized around 80–100 g/L and a progressive improvement of ischemic alterations was observed. Despite this apparent improvement, from day +9, the patient continued with daily high fever (up to 39°C), progressive thrombocytopenia with a nadir of 29 × $10^9$/L, persistent elevation of acute phase reactants (C-reactive protein 17 mg/dL, ferritin 24,667 ng/mL), and a leucoerythroblastic reaction in peripheral blood. On the first hand, the combination of signs and symptoms could be consistent with a hemophagocytic syndrome considering the fever, pancytopenia, and ferritin elevation. Nevertheless, it would not be expected to find a leucoerythroblastic reaction in peripheral blood. On the other hand, a possible concealed infection was considered and multiple serologies (including human immunodeficiency virus, Epstein-Barr virus, Cytomegalovirus, Parvovirus-B19, atypical bacteria, *Toxoplasma*, and *Leishmania*) were performed, obtaining either passed infections or negative results.

Meanwhile, bone marrow aspiration was performed showing scarce material, with complete absence of viable hematopoietic cells and abundance of necrotic material. Consequently, a bone marrow biopsy was performed on day +15: revealing predominant necrotic occupation of medullary spaces (Fig. 1), without histologic signs of osteonecrosis, congruent with a case of BMN following the severe VOC. In view of this event, supportive treatment with transfusions was maintained until spontaneous recovering occurred, with normalization of complete blood count and analytic alterations by day +26. Patient was discharged after a month of hospitalization.

Retrospectively, complement membrane attack complex (C5b-9) deposition after incubation of laboratory endothelial cells with activated plasma from the patient was assessed (Fig. 2). This novel technique provides a semiquantitative estimation of plasmatic in vitro ability to induce complement activation [5], and in our patient, the endothelial reactivity to C5b-9 was reported as intensely positive.

## Conclusion

Several cases of SARS-CoV-2 infection and drepanocytic crisis have been reported [6]. In this particular case, respiratory failure was initially interpreted as an ACS. Diagnostic criteria for ACS are based in guiding symptoms plus lung infiltrates on chest imaging, in the context of VOC [7]. The patient had a congruent clinical presentation but CT-scan ruled out the diagnosis. Putting respiratory support aside, the treatment of ACS is the same of a severe VOC: thus, RBC exchange was guaranteed. RBC exchange terminated the VOC had little effect on BMN and hematopoietic function. In fact, platelet count kept dropping. High persistent fever, extremely elevated LDH (× 64 upper limits of normal), and ferritin levels (× 68 upper limits of normal) along with the leucoerythroblastic reaction were cornerstones to raise suspicion of this rare complication [2–4]. It is a genuine example of the frequent underdiagnosis of BMN following VOC. It also stresses the importance of a prompt actuation (e.g., RBC exchange) in case of severe VOC to prevent potential complications.

More precisely, the pathogenesis of the inflammation implicates the secretory form of phospholipase A2, which converts phospholipids of bone marrow fat into free fatty acids, which have a potent inflammatory activity and are prone to generate tissue injury (compared to neutral fats) when unleashed from bone marrow microinfarctions to different organs, notably with pulmonary involvement. This phenomenon is called fat embolism syndrome [8].

Notwithstanding the previous considerations, the novel aspect is the investigation of complement activation in the patient plasma. This finding is consistent with the endothelial dysfunction hypothesis and mimics the pattern of varied pathologies gathered under the "atypical hemolytic uremic syndrome" (aHUS). New evidence suggests that several thrombotic microangiopathies other than complement-mediated aHUS could also be associated with dysregulation of the alternative complement pathway [9]. Particularly, there is an increasing interest regarding VOC in the setting of SCD, which could involve both coagulation and complement pathways in its complex pathophysiology [10, 11]. Further research is needed to elucidate if these findings can act as the primary cause of the inflammatory cascade or whether are another consequence of the vicious circle it creates. Hence, recognition of patients with complement overactivation is a matter of interest because treatment with complement activation blockers (e.g., Eculizumab) may have a role in SCD [9]. Future prospective studies are needed to better understand the role of complement and testing new potential therapeutic targets for SCD.

## Statement of Ethics

The case report was written based on our center protocol concerning publication of patient-related information. The patient described in the text has given his written informed consent to publish his case (including publication of images). All data that could lead to identification of the patient has been omitted. Ethical approval was not required for this study in accordance with local/national guidelines.

## Conflict of Interest Statement

The authors have no conflicts of interest to declare.


## Funding Sources

Authors of the manuscript do not have any funding sources to declare.


## Author Contributions

Marco, Garrote, Seguí, and Bullich took part in the writing of the manuscript. Lozano and Cid supervised and corrected it before submission.

## Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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