

the 2 components was considered as an indicator of prothrombotic tendency. We found values in patients with CC similar to those of Tripodi *et al.* [7], but patients with ACLF had significantly higher ratios (Table 2). The ratio in the patients with CC had a direct and significant correlation with the MELD score compared to the ACLF group in which the coagulopathic defects were more serious. In patients with ACLF, other causes of hemostatic defects except for CLD, making more complex and heterogeneous coagulopathies, might interrupt correlation with MELD scores compared to those with CC [4, 8].

To conclude, the patients with ACLF have higher factor VIII and lower protein C than those with CC. The factor VIII levels and the ratio of factor VIII to protein C may be used as a predictable marker for the severity of liver disease in patients with CC.

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The first case of paroxysmal nocturnal hemoglobinuria and Budd-Chiari syndrome treated with complement inhibitor eculizumab in Korea

TO THE EDITOR: Budd-Chiari syndrome (BCS) is a rare and potentially life-threatening disorder characterized by hepatic venous outflow obstruction [1]. BCS is associated with thrombogenic conditions such as myeloproliferative neoplasms or inherited deficiencies in protein C, protein S, and antithrombin in at least 75% of patients [2]. However, paroxysmal nocturnal hemoglobinuria (PNH) is another well-recognized cause of BCS [3]. PNH is an acquired disorder of hematopoietic stem cells, characterized by chronic intravascular hemolysis, thromboembolic episodes, and varying degrees of bone marrow failure caused by uncontrolled complement activation [4]. Patients with BCS, in whom no other etiological factor has been identified after a thorough clinical and laboratory investigation, are required to be tested by routine flow cytometry screening for PNH in Western countries [5].

Eculizumab, a humanized monoclonal antibody that blocks the activation of terminal complement C5 components, is currently used in the treatment of PNH. Treatment with eculizumab reduces transfusion requirements, ameliorates anemia, decreases the risk of thrombosis, and improves quality of life by resolving the constitutional symptoms associated with chronic intravascular hemolysis [6, 7]. Long-term treatment with eculizumab in patients with concomitant BCS and PNH has shown a favorable safety profile [8-10]. To the best of our knowledge, this is the first report of eculizumab treatment in a patient with BCS and PNH in Korea.

CASE

A 39-year-old man was admitted to our hospital with newly developed abdominal pain, fatigue, pancytopenia, abdominal distension, and jaundice. He had a history of liver cirrhosis secondary to BCS and undergone splenectomy and inferior vena cava (IVC) stent insertion 15 years ago. The laboratory results on admission were as follows: white blood cell count, $3.2 \times 10^9/L$; hemoglobin, 5.3 g/dL; platelets, $41 \times 10^9/L$; reticulocyte count, 10.3%; haptoglobin, <100 mg/L (lower limit of reference range, 300 mg/L); lactate dehydrogenase (LDH), 5,005 IU/L (upper limit of reference

range, 378 IU/L); and total bilirubin, 55.7 mg/dL (reference range, 0.3–1.2 mg/dL). The findings of an abdominal computed tomography scan and ultrasonography were compatible with BCS, showing underlying liver cirrhosis with the presence of thrombus in IVC stent and hepatic venous congestion (Fig. 1). Despite the continuous treatment of BCS, his clinical condition progressively deteriorated and liver transplantation (LT) was planned. To investigate the cause of the severe hemolytic anemia and pancytopenia, a bone marrow examination and flow cytometric analysis for PNH were performed during preparation for an LT. The bone marrow aspirations showed hypercellular marrow with erythroid hyperplasia and no definite dysplasia. The flow cytometric analysis was performed using antibodies directed against glycosylphosphatidylinositol-anchored membrane proteins (GPI-APs), including CD59, CD24, and fluorescent proaerolysin (FLAER) on granulocytes, and CD59 on erythrocytes. The analysis revealed that 99.4% of granulocytes had FLAER deficiency (Fig. 2), and 5.7% of erythrocytes had a CD59 deficiency. The patient was newly diagnosed with classic PNH and concomitant BCS, and the LT was canceled. He was treated for PNH with intravenous

methylprednisolone (62.5 mg/day for 2 days), oral prednisolone (10 mg, 3 times/day), and warfarin (5 mg/day). Following treatment, the patient showed favorable complete blood count results (white blood cells, $3.83 \times 10^9/L$; hemoglobin, 8.4 g/dL; platelets, $120 \times 10^9/L$) and total bilirubin levels (5.1 mg/dL). However, his reticulocyte count (15.6%) and LDH level (5,995 IU/L) were still elevated. Despite steroid therapy, he experienced persistent hemolytic episodes, and eculizumab treatment was initiated. At 3 months of eculizumab treatment, the flow cytometric analysis revealed that 99.9% of granulocytes had a FLAER deficiency, and 50.4% of erythrocytes had a CD59 deficiency. Five months later, improvements in the hemoglobin levels (9.9 g/dL), reticulocytes count (3.0%), and LDH levels (691 IU/L), as well as complete cessation of hemolytic episodes, were observed (Table 1).

This report describes the favorable response to eculizumab in the patient with progressive BCS and PNH. Given the various clinical manifestations and low frequency of PNH, it is inappropriate that every patient with anemia or thrombosis is screened for this disease. However, some clinical presentations, such as BCS or cerebral thrombosis, should

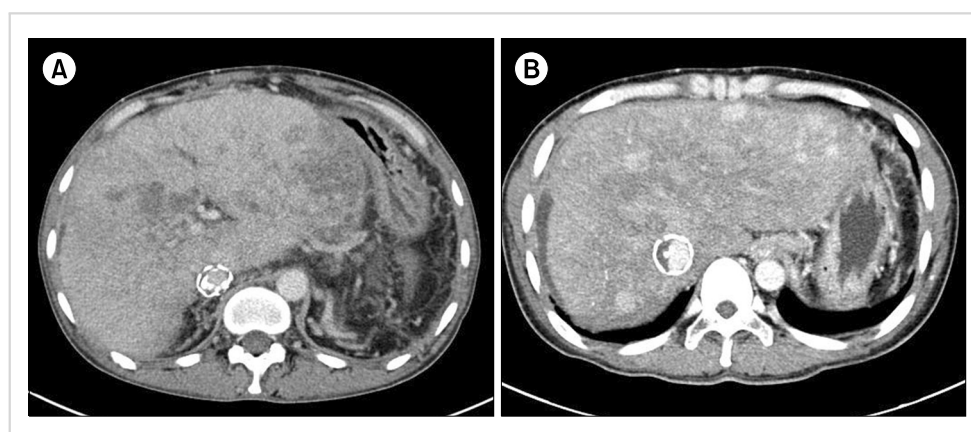


Fig. 1. Abdominal computed tomography evidence of Budd-Chiari Syndrome. (A) The liver shows heterogeneous enhancement and multiple ill-defined hypodense lesions with venous congestion. (B) Non-obstructive thrombus in inferior vena cava stent and multiple enhancing nodules at the liver are seen.

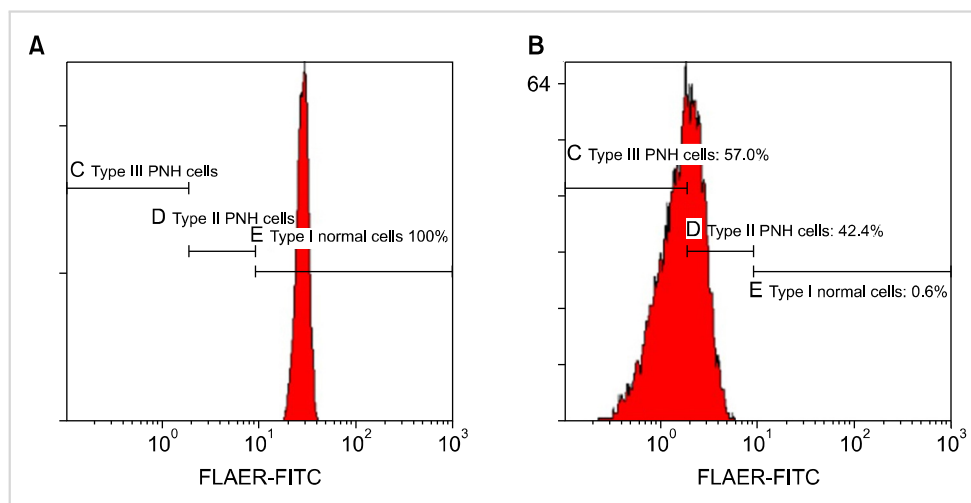


Fig. 2. Flow cytometric analysis of granulocytes using FLAER. (A) As a control setting, there was no evidence of decreased (type II PNH cells) or absent (type III PNH cells) expression of FLAER. (B) In this patient, decreased (42.4%) and absent (57.0%) FLAER fractions were detected, suggesting that 99.4% of PNH granulocyte clones had a FLAER deficiency, which is consistent with classic PNH. Abbreviations: FITC, fluorescein isothiocyanate; FLAER, fluorescein-labeled proaerolysin; PNH, paroxysmal nocturnal hemoglobinuria.

Table 1. Laboratory findings and transfusion events pre- and post-eculizumab treatment.

Days	Laboratory value								Transfusion amounts of packed RBCs
	Hb (g/dL)	Platelets (× 10 ⁹ /L)	LDH (U/L)	Total bilirubin (mg/dL)	AST (IU/L)	ALT (IU/L)	Albumin (g/dL)	PNH clone in WBC (%)	
Pre-eculizumab treatment									
70	5.3	41	5,005	55.7	159	29	3.1	—	2
35	8.4	120	5,995	5.1	135	49	4.5	99.4	3
Post-eculizumab treatment									
0	7.7	130	3,981	3.8	139	51	4.3	100	0
7	7.9	130	1,465	2	44	41	4	—	0
14	7.2	121	858	2.5	53	46	3.8	—	0
70	9.4	94	600	2.8	27	20	4.2	99.9	0
140	9.8	89	650	3.9	39	24	4.1	99.9	0
235	10.5	89	636	3.8	34	23	4.2	99.9	0

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, hemoglobin; LDH, lactate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria; RBC, red blood cells; WBC, white blood cells.

warrant PNH testing [11]. According to the retrospective analysis of the South Korean National PNH Registry (N=301), 81 events of thromboembolism occurred in 54 patients (17.9%). Among 81 events, 7 events were identified at the hepatic portal vein (8.6% of events) [12]. Although 424 patients with BCS were identified in Korea during 2009–2013, there is no previous report of PNH with BCS [13]. Thus, routine screening for PNH will be necessary for Korean patients with BCS to examine the exact prevalence of PNH.

Flow cytometric analysis has emerged as the technique of choice for the screening and diagnosis of PNH. At the time of diagnosis, our patient showed 99.4% PNH granulocyte clones and 5.7% PNH erythrocyte clones. The discrepancy of two lineages might result from the complement-mediated destruction of PNH erythrocytes, as compared with other blood cell types resistant to destruction [14]. Additionally, red blood cell transfusions prior to eculizumab treatment might dilute the proportion of PNH erythrocytes, thereby reducing the accuracy of estimating the true proportion of PNH erythrocytes. The monitoring of PNH erythrocyte clones is useful for assessing the efficacy of the response to eculizumab treatment [15]. Since eculizumab inhibits complement-mediated lysis of GPI-AP-deficient cells, more PNH erythrocytes can survive and reflect the true size of PNH clone. In fact, 5.7% PNH erythrocytes at diagnosis had increased to 50.4% in our patient after 3 months of eculizumab treatment. In addition, the patient showed sustained improvements in hemoglobin levels and had not exhibited major episodes of intravascular hemolysis. Given the successful treatment of previous reports [8–10] and our case, eculizumab may be tried a primary therapeutic strategy in patients with concomitant BCS and PNH.

In conclusion, to our knowledge, this is the first Korean report of eculizumab treatment in a patient with PNH and

BCS. This case supports PNH screening in BCS patients, otherwise the patient could receive unnecessary and aggressive therapeutic options such as liver transplantation. In addition, we demonstrated the utility of eculizumab in the treatment of PNH patients with BCS.

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Ultrasound-guided sternal bone marrow aspiration

TO THE EDITOR: Bone marrow examination is a common procedure during the diagnosis of hematological disorders such as acute and chronic leukemia, non-Hodgkin lymphoma, and aplastic anemia. It is usually performed on the posterior iliac crest or the sternum. Although rare, a serious complication may occasionally occur when it is performed on the sternum due to the anatomical localization of the vasculature (ascending aortae) just beneath the sternum [1]. The injury mainly results from the use of an incorrect needle length according to the sternal bone width and is mostly life threatening.

Here we describe a novel sternal bone marrow aspiration technique, named “ultrasound-guided sternal bone marrow aspiration,” which enables the decrease of such risks to nearly zero. The high-resolution ultrasound imaging modality with a linear probe of a frequency higher than 8 MHz and a sterile probe cover (CIV-Flex Transducer Cover, CIVCO, Iowa, USA) were used (Fig. 1A). A linear high-resolution ultrasound probe with its frequency at 9 MHz (Vivid-E9, GE, San Jose, CA, USA) was applied perpendicular to the long axis of the sternum to obtain the image (Fig. 1B). The sternum is identified in the center of the figure as a hypoechoic structure surrounded by the hyperechoic periosteum (Fig. 1C). The image clearly reveals that the depth from the skin to the sternum is approximately 1 cm, and the width of the sternum is 1 cm. Considering the pressure that potentially would decrease the depth from the skin to the sternum during the puncture of the needle, the needle depth was adjusted to 1.3–1.5 cm. Just beneath the sternum, the blood flow of the ascending aortae is clearly visible using a B-mode ultrasonography with a color flow imaging (Fig. 1C). Since bony structures have high ultrasound absorption coefficients, we suggest that the probe be applied just beside the sternum, longitudinal to the body trunk for cases in which the blood flow and depth of the ascending aortae are not visible. The thoracic costae can be identified as a hypoechoic circle just in the center of the figure (Fig. 1D). The depth of the ascending aortae is measured as 2.5–3 cm from the skin (Fig. 1D). Finally, using an out-of-plane technique [2], an aspiration needle is inserted (Fig. 1B). In brief, the probe is applied perpendicular to the long axis of the sternum and adjusted to show the sternum just in the center of the screen. Next, the needle is positioned in the middle of the transducer and punctures the skin at the same distance from the transducer to the sternum at the depth estimated from the screen. By rotating the transducer probe, the needle tip is clearly visible during the aspiration procedure.

Recently, ultrasound guidance for central venous catheterization as well as for peripheral nerve block has gained popularity and been shown to reduce the rate of complica-