To the editor:

Long-term efficacy of the complement inhibitor eculizumab in cold agglutinin disease

Cold agglutinin disease (CAD) is characterized by immunoglobulin M (IgM)–mediated hemagglutination and robust complement activation leading to intravascular hemolysis and hemolysis-related symptoms including anemia, fatigue, dyspnea, hemoglobinuria, and acrocyanosis.[1-6] Although CAD can have a mild course, it may be life-threatening, and its chronic nature leads to transfusion dependence in approximately 50% of cases.[7] Conventional approaches are largely ineffective in controlling hemolysis in patients with CAD.[2,4,7]

Eculizumab (Soliris; Alexion Pharmaceuticals, Cheshire, CT) is a monoclonal antibody that targets complement protein C5 and prevents the cytolytic and proinflammatory effects of complement activation.[8,9] Eculizumab dramatically reduces intravascular hemolysis thereby improving fatigue, anemia, dyspnea, and quality of life and reducing thrombotic events and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria (PNH), a rare form of hemolytic anemia.[6,10,11] Here we report the efficacy of an off-label use of eculizumab in a transfusion-dependent patient with CAD.

A 66-year-old male was diagnosed with CAD in 2001 after experiencing progressive dyspnea and fatigue. Laboratory tests revealed anemia (hemoglobin 10.8 g/dL), increased hemolysis (lactate dehydrogenase [LDH] 438 U/L, bilirubin 4.0 mg/dL) and a monoclonal IgM κ antibody with a cold agglutinin titer of >1:2000. Bone marrow biopsy showed increased erythropoiesis and a minor infiltration by a light chain restricted B-cell population without evidence of lymphoma. The patient was unresponsive to prednisone (1 mg/kg with consecutive reductions over 3 months) and subsequently received 5 courses of rituximab (375 mg/m^2 weekly for 4 cycles). After an initial response to rituximab, the patient became refractory during additional administrations between 2004 and 2006, and failed treatment with intravenous immunoglobulins in early 2007. Eculizumab therapy was initiated in June 2007 after hemolysis and transfusion requirements increased with frequent episodes of angina pectoris due to concomitant coronary artery disease. As instructed in the prescribing information, the patient was vaccinated against *Neisseria meningitidis* 14 days before receiving the first dose of eculizumab.

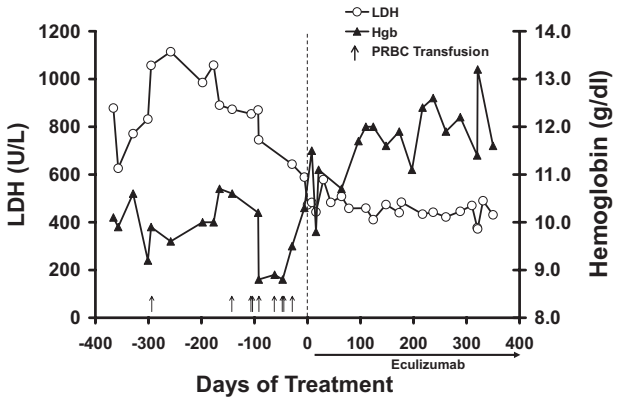

Figure 1. Effect of eculizumab in a patient with cold agglutinin disease. Levels of hemolysis (as measured by LDH, ○) and anemia (hemoglobin, ▲) were documented in the year before and after initiation of eculizumab treatment. Transfusion episodes (↑) are also shown.

Eculizumab was dosed at 600 mg intravenously every 7 days for 4 weeks, at 900 mg 7 days later, and then chronically at 900 mg every 14 days.[12] Although detectable cold agglutinins persisted during treatment, hemolysis was significantly reduced as demonstrated by a reduction in levels of serum LDH from a mean of 850 (± 159) U/L in the year before treatment to 456 (± 44) U/L in the year after initiation of eculizumab (normal range, 100-247 U/L; Figure 1). Reduction in chronic hemolysis resulted in an improvement of anemia from a mean hemoglobin level of 9.8 g/dL and transfusion dependence (18 units) before treatment to a hemoglobin of 11.7 g/dL and transfusion independence (0 units) in the year after initiation of eculizumab treatment. Ferritin levels decreased from 666 (± 71) μg/L to 311 (± 26) μg/L (normal range, 20-290 μg/L). The patient reported having "a new active life" with a major improvement in fatigue and quality of life; tolerance to cold weather and temperatures has improved dramatically with no further hemolytic crises or episodes of angina pectoris. Thus far, eculizumab treatment has been continued for 18 months and has been well tolerated.

This is the first report of a clinical response to the complement inhibitor eculizumab in a patient with CAD. Continuous therapy with eculizumab resulted in sustained reduction in hemolysis, elimination of transfusion requirements, and improvement in anemia, fatigue, and overall quality of life. Importantly, since the initiation of eculizumab treatment, hemolysis has been controlled with no exacerbations of the disease. These results provide a rationale for a prospective study of eculizumab in patients with CAD.

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To the editor:

Homozygous deletion of *HFE*: the Sardinian hemochromatosis?

Type 1 hemochromatosis is generally due to homozygous p.C282Y mutation in *HFE*.[1,2] We report the case of a young woman with a classical hemochromatosis phenotype due to a homozygous deletion in the 6p chromosome region containing *HFE*.

The proband is a 29-year-old woman of Sardinian (Italian) origin. At the age of 27, serum iron was 33.2 μmol/L (186 μg/dL), serum ferritin 3146 pmol/L (1400 μg/L), and serum alanine aminotransferase (s-ALT) 70 U/L. Diagnosis of hemochromatosis was confirmed at subsequent analyses including magnetic resonance. A test for *HFE* mutations (NLM, Settala, Italy) gave no signal. There were no causes of secondary iron overload. She began phlebotomies every 2 weeks and after 3.5 g of iron removal serum ferritin decreased to 422 pmol/L (188 μg/L), s-ALT normalized (23 U/L), whereas serum iron did not change (36 μmol/L [201 μg/dL]), indicating she was not yet iron-depleted. Nevertheless, therapy was stopped, and after 3 months serum ferritin raised to 1229 pmol/L (547 μg/L). She came to our attention in June 2008. She denied family consanguinity up to the fifth generation. Her grandparents originated from different regions of Sardinia.

After obtaining informed consent, we analyzed her DNA sample for pC282Y and pH63D by real-time polymerase chain reaction (PCR; Celbio, Milano, Italy), confirming the absence of signal and suggesting a deletion in *HFE*-containing region. By analyzing several polymorphic markers centromeric and telomeric to *HFE*, we roughly define the upstream and downstream limits at 26 181 749 and 26 225 235, respectively. Meanwhile, Le Gac et al[3] reported a very similar rearrangement in a different woman from Sardinia. By using their primers (gap-F and gap-R) and those for *HFE* exon 5,[4] we confirmed that the proband was homozygous and her parents heterozygous for a gross deletion (Figure 1). To better mark deletion boundaries, we designed an internal forward primer and confirmed, by sequencing, that the proband and her parents had the same deletion [Chr6 g.(26 175 442)_g.(26 208 186)del] reported by Le Gac et al.[3]

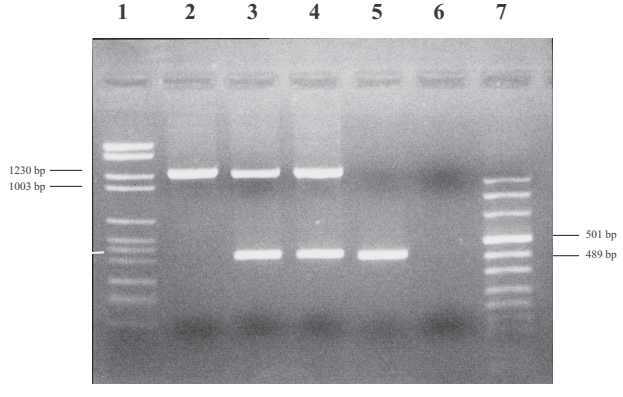

Figure 1. Electrophoresis of PCR products of proband, parents and control. Upper bands correspond to PCR products obtained by using gap-F and gap-R primers designed by Le Gac et al.[3] Lower bands correspond to *HFE* exon 5. Lane 1: DNA molecular marker II; lane 2: proband; lane 3: father; lane 4: mother; lane 5: control; lane 6: negative; and lane 7: DNA molecular marker VIII (Roche Diagnostics, Mannheim, Germany).

The patient we describe in the present report showed a more marked iron overload than that described by the French group, as indicated by the higher serum ferritin and iron removed despite her younger age, supporting the role of modifier factors in modulating phenotype expression in HFE-related hemochromatosis.[5] The absence of close consanguinity within the present family and, as far as we could assess, with the case reported by LeGac et al,[3] suggest that this deletion is present at the population level in Sardinia and may derive from a common ancestor. Sardinian population is an ancient genetic isolate, and the few data available indicate that p.C282Y mutation is very rare or even absent.[6] Thus, it is possible that the deletion might be the most common cause of hemochromatosis in this geographic area. However, considering the mechanism of deletion, it cannot be excluded it can result from multiple mutational events and explain other hemochromatosis cases.

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