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LETTER TO THE EDITOR

Southeast Asian ovalocytosis detected in a critical patient with COVID-19 pneumonia

Dear Editors,

Southeast Asian ovalocytosis (SAO) is an autosomal dominant disorder that occurs in Melanesians in Papua and New Guinea, the Solomon and Torres Strait Islands, in Malaysian aboriginals and the populations of Indonesia and the Philippines, being as many as 25%–30% of the individuals of the affected ethnic groups.[1] Heterozygotes for SAO are usually asymptomatic, being significant only in the neonatal period when clinically significant hemolysis and anemia may be detected.[2,3] In contrast, the homozygous state for this mutation is incompatible with fetal survival.[4] We report the case of a 73-year-old woman from Philippines admitted to the Hospital Clinic of Barcelona (HCB) Respiratory Intensive Care Unit for bilateral pneumonia for SARS-CoV-2 with need for mechanical ventilation. She had previous pathological history of hypertension and type 2 diabetes, both treated with oral drugs. There was no evidence of a previous history of anemia or hyperbilirubinemia. An automatic blood cell count showed normal values of leukocytes, hemoglobin and platelets. High levels of C-reactive protein (7.96 mg/dl; normal values [NV]: <1 mg/dl), lactate dehydrogenase (843 U/L; NV: <234 U/L), procalcitonin (1.98 ng/ml; NV: <0.5), ferritin (2934 ng/ml; NV: 15–200) and

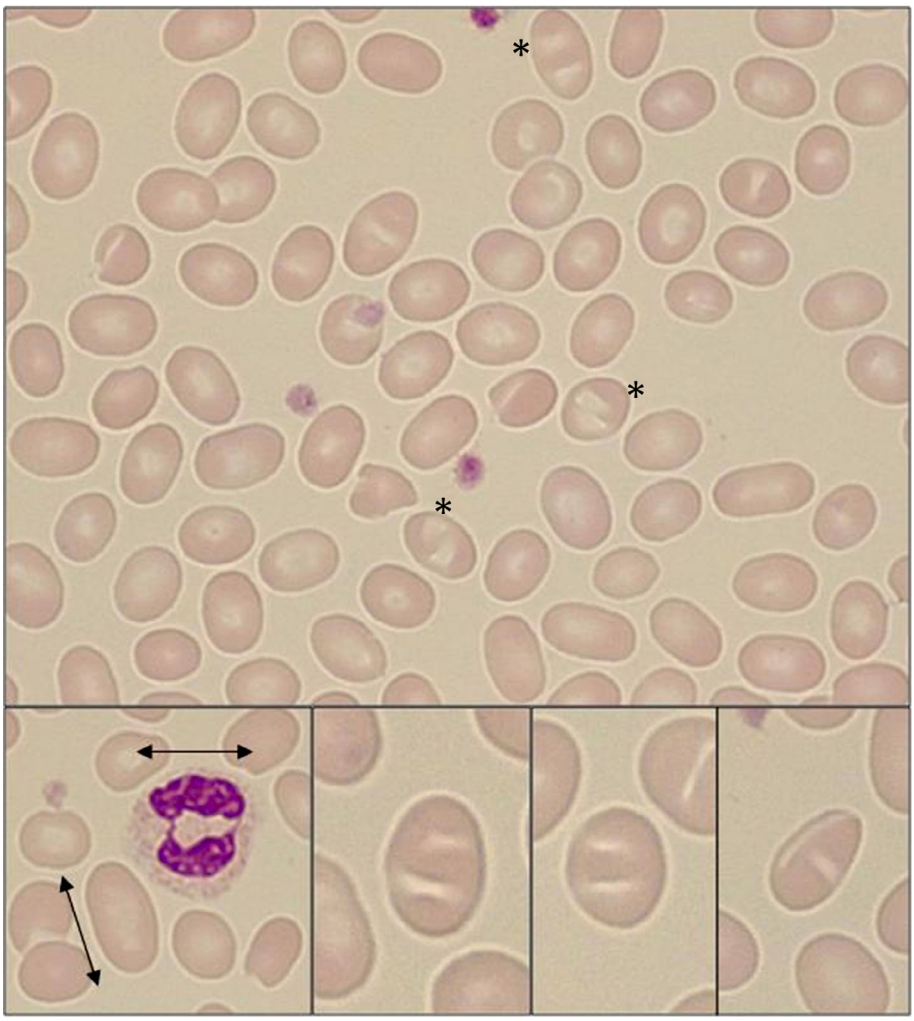

FIGURE 1 Peripheral blood film images of the patient with Southeast Asian ovalocytosis showing macro-ovalocytes, stomato-ovalocytes and stomatocytes. Stomas were longitudinal or transverse and some of the red blood cells showed more than two stomas (*). Note that the length of the arrows is the same, which shows that the longitudinal axis of the macro-ovalocyte and the neutrophil diameter are similar.

D-dimer (3800 ng/ml; NV: <500) were found. The absolute number of lymphocytes was low (0.60 x 10^9/L; NV: 0.90–4.50) showing the neutrophil/lymphocyte ratio increased values (6.12).

The morphological analysis of the peripheral blood (PB) smear using the CellaVision DM96 revealed round or oval red blood cells (RBC) with several stomatocytes. Macro-ovalocytes, some of them stomatocytic, were present. Stomas were longitudinal or transverse and some of the RBC showed more than two stomas (Figure 1). These morphological findings were distinctive of SAO.

Other complementary tests were performed, with the following results: decreased RBC osmotic fragility, normal acidified glycerol lysis test and positive eosin-5′-maleimide (EMA) binding test. Semi-quantification by densitometry of RBC-membrane proteins (Bands 3, 4.1, and 4.2), on SDS-PAGE gel, showed values comparable to the control sample.

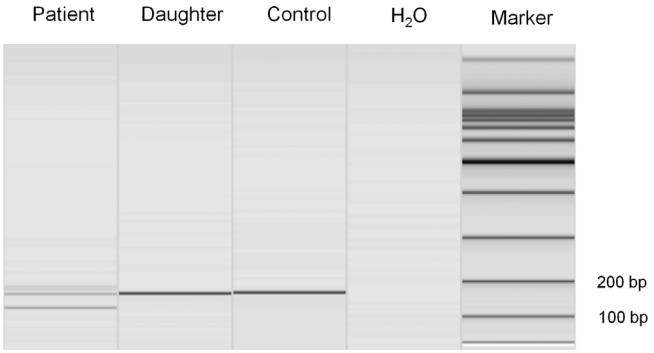

FIGURE 2 Molecular confirmation with PCR-GAP. From left to right: in the patient's sample lane, we observe a double band, the upper one corresponding to the 175 bp allele (normal) and, below, another corresponding to the 148 bp allele (the one with the 27 bp deletion). Lanes corresponding to daughter's and healthy control's samples show a single bland corresponding to the 175 bp normal allele in homozygosity. H_2O lane is used to assure lack of contamination in the technique.

SAO is caused by a 27 bp deletion in exon 11 of *SLC4A1* gene in chromosome 17, affecting aminoacids from 400 to 408. For the molecular demonstration of the responsible deletion, we performed a PCR-GAP.[5] Two amplification bands of 175 (normal) and 148 bp (amplicon with the deletion) typical of the heterozygote pattern were demonstrated in the capillary-electrophoretic analysis (Figure 2). To validate the PCR-GAP for diagnosis, we performed a second analysis with PCR-Sanger, which showed a peak-overlap in the sequence delimited to the genomic zone of the heterozygous deletion.

As previously described,[6] osmotic gradient ektacytometry analysis performed in patients with SAO show severely decreased maximal deformability (EImax) and decreased hypotonic osmolarity (Omin). The osmotic gradient ektacytometry analysis performed to the patient presented herein, using the Osmoscan module on the LoRRca MaxSis, confirmed a decreased deformability of the RBCs when compared with the control sample (Figure 3). Moreover, patient's RBC showed an increased surface to volume ratio and a decreased osmotic fragility. Interestingly, we observed a higher decrease in the patient's RBC deformability during the COVID-19 infection than when this infection was resolved (see Figure 3). Similar results were obtained previously by Kubánková et al,[7] which reported that patients with COVID-19 showed less deformable erythrocytes when compared to healthy controls.

The same tests were performed in the patient's daughter and their normal results (including those from the molecular analysis) allowed us to rule out her as a carrier of SAO.

The patient suffered various complications: (1) debut of atrial fibrillation at admission and initiating treatment with enoxaparin; (2) left radial artery thrombosis (day +4) leading to ischemia of the radial fingers of the left hand, for which a thrombectomy was performed (day +4); (3) re-thrombosis with distal necrosis that required transfhalangeal amputation of the first and second finger; (4) septic shock of probable respiratory origin (day +13); (5) *Clostridium difficile* enterocolitis (day +24) and (6) *Pseudomonas aeruginosa* pneumonia (day +34). After 62 days in the hospital, she was discharged (the average recovery time in COVID-19 patients admitted to the HCB is 20 days).

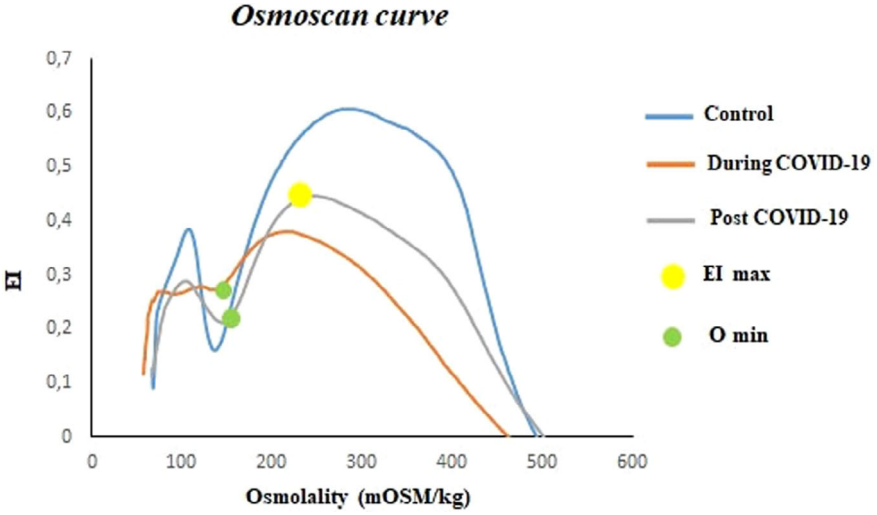

FIGURE 3 Osmotic gradient ektacytometry analysis. RBCs deformability curves in the patient with SAO during COVID-19 infection and after their resolution, compared to that of a normal individual, using osmotic gradient ektacytometry analysis. EImax (yellow point), that represents maximal deformability of RBCs, was found decreased in SAO. Additionally, Omin (green point), that reflects surface to volume ratio of the cells, was slightly shifted to the left suggesting increase in surface to volume ratio and decreased osmotic fragility. RBCs, red blood cells; SAO, Southeast Asian ovalocytosis.

The RBC Osmoscan analysis was performed on the sample obtained at discharge, 1 month apart from the previous sample and showing a marked improvement in RBC deformability.

Heterozygosity for SAO state has been described as a protective factor in front of severe forms of malaria.[8] The unique deletion causing the disease is responsible for a qualitative defect on the RBC-membrane protein band 3. This qualitative defect in the present case justifies the positivity of the EMA binding test, since the binding of EMA to the band 3 Lys430 residue is impaired, being the quantity of the protein strictly normal.[9]

Although the abnormality of RBC deformability in COVID-19 infection is an acquired defect, the result causes a similar defect in the RBC membrane conditioned by various pathophysiological mechanisms such as the degradation of the main structural proteins, the alteration of lipid metabolism and the deregulation of oxidative stress balance, among others.[10] In the mentioned study, the RBC structural protein damaged in the COVID-19 setting was detected by proteomics and no significant changes in the total levels of the structural proteins studied were observed. Moreover, COVID-19 has been associated with a deleterious impact in RBC rheology that might affect the microvascular blood flow.[11]

In summary, SAO is a congenital condition that causes a decreased RBC deformability, although without clinical repercussions in adult carriers. The impact of COVID-19 in RBC membrane proteins and in their rheology has also been described but, to the best of our knowledge, the functional effect of the coexistence of SAO and COVID-19 in the RBC has not been explored. Although our data is limited to one single case, we can conclude that SAO and COVID-19 have a synergistic effect in terms of analytical RBC deformability. It is possible that the coexistence of SAO and COVID-19 may increase the probability of suffering thrombotic events. Nevertheless, a higher number of patients are needed to confirm this affirmation.

AUTHOR CONTRIBUTIONS

Ana Belén Moreno-Castaño and Anna Merino wrote the manuscript. Angel Molina and Joana Faneca collected the data and images. María del Mar Mañú-Pereira and Amira Idrizovic performed the osmotic gradient ektacytometry analysis. Maribel Diaz-Ricart and Ginés Escolar analysed data and advised on the discussion of the laboratory findings. Estefanía García performed the molecular laboratory tests. Mónica Matute performed the clinical evaluation and patient management. All authors read and approved the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable.

Ana Belén Moreno-Castaño[1,2]
Maribel Diaz-Ricart[1,2]
Ginés Escolar[1,2]
Estefanía García[1]
María del Mar Mañú-Pereira[3]
Amira Idrizovic[3]
Mónica Matute[4]
Angel Molina[5]
Joana Faneca[5]
Anna Merino[5]

[1]*Pathology Department, Centre Diagnòstic Biomèdic (CDB), Hospital Clinic, Barcelona, Spain*
[2]*Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain*
[3]*Vall d'Hebron Institut de Recerca (VHIR), Hospital Universitari Vall d'Hebrón, Barcelona, Spain*
[4]*Pneumology Department, Clinic Respiratory Institute (ICR), Hospital Clinic, Barcelona, Spain*
[5]*Biochemistry and Molecular Genetics Department, Core Laboratory, Biomedical Diagnostic Centre, Hospital Clinic, Barcelona, Spain*

Correspondence
Anna Merino, Biochemistry and Molecular Genetics Department, Core Laboratory, Biomedical Diagnostic Centre, Hospital Clinic, Barcelona, Spain.
Email: amerino@clinic.cat

ORCID

Angel Molina https://orcid.org/0000-0002-9584-3646
Anna Merino https://orcid.org/0000-0002-1889-8889

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