INTRODUCTION

Congenital analbuminemia (CA) is a rare autosomal recessive inherited disorder characterized by strongly decreased concentration, or complete absence, of serum albumin (SA). Several lines of evidence indicate that SA has anti-thrombotic effect. In vivo platelet function and the role of oxidative stress (OS) in platelet aggregation promotion have never been studied in analbuminaemic patients.

Patients and Methods: We report two cases of congenital analbuminemia in a 38-year-old male and in a 67-year-old woman. We analyzed platelet activation (PA) and OS at baseline and 2 h after 40 g human albumin infusion. PA was evaluated as platelet aggregation, sCD40L and surface αIIbβ3 integrin and P-selectin expression. OS was evaluated measuring serum sNOX2dp, and 8-iso-PGF2α.

Findings: Analbuminemic patients displayed higher platelet aggregation, markers of PA and of OS. Albumin infusion reduced platelet activation by reducing oxidative stress.

KEYWORDS
albumin, congenital analbuminemia, oxidative stress, platelet function, platelets
deficiency of anticoagulant proteins, might predispose patients with analbuminemia to thrombotic events. Moreover, albumin exerts extracellular antioxidant and anticoagulant activity. Several studies suggest that albumin itself exerts an anticoagulant effect by binding antithrombin, which enhances the neutralization of coagulation factor Xa, and by inhibiting platelet aggregation. Finally, a recent metaanalysis showed that SA levels are associated with an increased risk of cardiovascular events in both patients in primary and secondary prevention for cardiovascular disease.

Despite this evidence, in vivo platelet activation (PA) and oxidative stress (OS) and their role in inducing platelet hyperreactivity have never been studied in analbuminemic patients. The aim of the present study was to investigate if in patients with CA, biomarkers of PA and OS are altered compared to healthy subjects and if albumin infusion could modulate platelet function.

2 | MATERIALS AND METHODS

2.1 | Study subjects

2.1.1 | Patient 1

A 38-year-old man was referred for the management of a severe hypercholesterolemia. His past medical history was unremarkable, except for sporadic lipothympic events and the presence of mild ankle edema during warm seasons. His family history was negative for hypercholesterolemia and premature cardiovascular or cerebrovascular events. Physical examination revealed no evidence of edema or pathological cardiovascular findings. Corneal inspection resulted in a corneal arcus. All laboratory tests were negative except for hypercholesterolemia and the absence of albumin peak at electrophoresis, with a concentration of SA of 1.0 g/dL. Genetic analysis found two new mutations, one in exon 10 and one in exon 11, respectively, named Rome2 and Fondo mutations, and we diagnosed, for the first time, a double compound heterozygosity accountable for CA.

2.1.2 | Patient 2

A 67-year-old woman with CA (homozygosity for one mutation in the HSA gene) was referred for severe hypercholesterolemia. She had a clinical history of severe premature multiorgan cardiovascular disease (non-ST-segment elevation myocardial infarction, ischemic stroke, bilateral internal carotid thromboembolectomy and peripheral artery disease). Physical examination revealed no signs of peripheral oedema. She died for heart failure 4 months after the study.

Healthy subjects

Two healthy subjects (34-year-old man and 64-year-old woman) were recruited in the study for comparison of baseline data. They had no history of cardiovascular disease and dyslipidemia and were not taking antiplatelet drugs.

2.2 | Albumin infusion

Patients were infused with human albumin (40 g) over a period of 30 min. Blood was collected immediately before infusion (T0) and 2 h after the end of it (T2h). A small aliquot of blood anticoagulated with sodium citrate was used to study PA by flow cytometry. The rest was centrifuged for platelet aggregation analysis in platelet-rich plasma. Biomarkers of PA, OS and NETs formation namely, TxB₂, sCD40L, serum NOX2-derived peptide (sNOX2-dp), H₂O₂, urinary 8-iso-PGF2α, NO bioavailability and citrullinated histone H3 (CitH3) were analyzed. Details on biomarker dosage were reported in Data S1.

Only in patient 1, a second albumin infusion (40 g) was performed after 6 months on regular albumin treatment (two 20 g infusions during the first week and once per week thereafter).

Statistical methods were reported in the Data S1.

3 | RESULTS

3.1 | Baseline characteristics

Baseline clinical and biochemical characteristics of analbuminemic patients and healthy subjects are summarized in Table S1. Laboratory examination revealed in both patients remarkably reduced serum total proteins and almost undetectable albumin levels (<0.5 g/L). Both patients had severe untreated hyperlipidemia with remarkable elevation of total and LDL cholesterol.

Analbuminemic patients displayed higher collagen-induced platelet aggregation (88.5 ± 2.6% vs. 76.0 ± 1.4%; *p < .05), elevated levels of TxB₂ (833.5 ± 71.4 vs. 376.0 ± 79.20 pg/mL; *p < .05) and sCD40L (6.7 ± 0.3 vs. 2.6 ± 0.6 ng/mL; *p < .05), compared to healthy subjects (Figure S1A–C). Moreover, patients displayed increased levels of OS markers such as sNox2dp (36.5 ± 2.1 vs. 13.5 ± 2.1 pg/mL; **p < .01), H₂O₂ production (52.0 ± 5.6 μM vs. 19.0 ± 2.8 μM; *p < .05) and urinary 8-iso-PGF2α (775.0 ± 35.4 pg/mL vs. 412.5 ± 53.0 pg/mL; *p < .05) (Figure S1D–F). Finally,
compared to controls, analbuminemic patients show a significant decrease of NO bioavailability (14.5 ± 4.9 vs. 47.5 ± 9.2 μM; *p < .05), and an increase of citrullinated histone H3 (CtH3), a marker of NETS formation (3.2 ± 0.6 vs. 0.7 ± 0.1 ng/mL; *p < .05). (Figure S1G,H).

A significant association was found between platelet aggregation and urinary 8-iso-PGF2α (p = .01, r² = 0.972) and sNOX2dp (p = .02, r² = 0.953) and between sCD40L and urinary 8-iso-PGF2α (p = .02, r² = 0.919), sCD40L and sNOX2dp (p = .03, r² = 0.939).

3.2 | Response to albumin infusions

There was a significant reduction of platelet aggregation (88.5 ± 2.1–58.5 ± 2.1%; **p < .001), TxB2 excretion (833.5 ± 71.4–443.6 ± 15.6 pg/mL; *p < .05), sCD40L (6.7 ± 0.2–3.1 ± 0.4 ng/mL; *p < .05), sNOX2dp (36.5 ± 2.1–18.5 ± 2.1 pg/mL; *p < .05), H₂O₂ production (52.0 ± 5.6–25.2 ± 6.4 μM; *p < .05), urinary 8-iso-PGF2α (775.0 ± 35.4–416.0 ± 70.0 pg/mL; *p < .05) and an increase of NO bioavailability (14.5 ± 4.9–39.5 ± 3.5 μM; *p < .05) (Figure 1A–G).

In line with the results obtained by standard aggregometry, we found higher levels of active αIIbβ3 integrin in both basal and stimulated conditions (Figure 1H–I). Interestingly, surface P-selectin levels (Figure 1J), were increased in basal but not in stimulated conditions.

Before the infusion, increased platelet reactivity was most evident in basal conditions as we detected in unstimulated whole blood (Figure 1J, T0), platelets with active αIIbβ3, exposed P-selectin and increased platelet-leukocyte aggregates (Figure 1K). Infusion of albumin immediately normalized PA in both basal and stimulated conditions.

Moreover, at T2h we observed a decrease of CtH3 (3.2 ± 0.6 vs. 1.1 ± 0.1 ng/mL; *p < .05), a marker of NETS formation. (Figure 1L).

When patient 1 returned for a second study after 6 months on regular 20 g/week albumin treatment, we performed the assay with the same experimental conditions. Basal level of SA had increased from 0.3 g/dL to 2.0 g/dL. Cholesterol variation induced by weekly infusion of 20 g albumin is reported in Figure S2. After 40 g albumin infusion, SA levels rose from 20 to 26 g/dL while serum cholesterol decreased (total cholesterol from 269 to 235 mg/dL; LDL-C from 207 to 178 mg/dL). This time we observed no difference in platelet integrin activation (Figure 1M), platelet α-granule secretion (Figure 1N) before and after the infusion of albumin.

4 | DISCUSSION

Analbuminemic patients showed a pro-thrombotic state compared to control subjects, as detected by increased basal levels of circulating activated platelets, elevated plasmatic concentrations of sCD40L, an indirect marker of PA, and augmented platelet response to agonists as measured by standard aggregometry and by flow cytometry. Moreover, they also presented increased OS, as detected by increased serum levels of sNox2dp, a marker of NOX2 activation by blood cells which plays an important role in ROS generation and of urinary 8-iso-PGF2α, which is widely accepted as a reliable indicator of OS and platelet PA in vivo. Of note, also in patient 2, who took aspirin because of her clinical history PA (90%) and sCD40L (6.9 ng/mL) were higher than those observed in healthy subjects. Patient 2 did not take statins since they were the cause of worsening of edema, due to the reduction of the blood oncotic pressure after LDL decrease. Conversely, smoking habits of both patients could partially affect oxidative stress parameters.

In both patients, a reduction in PA indices and, at the same time, in OS markers was observed after a single 40 g albumin infusion. The albumin inhibitory effect on PA could in part be due to its ability to bind arachidonic acid and interfere with the release of this fatty acid from phospholipids, thus preventing its subsequent metabolism by cyclooxygenases into the platelet agonist thromboxane A2. Moreover, it has also been reported that in the presence of increasing levels of albumin, platelet-activating factor (PAF)-induced PA is suppressed in a concentration-dependent manner as results of albumin binding to PAF with high affinity. Another mechanism through which albumin could exert its anti-aggregatory action might be its ability to induce the expression of inducible NO synthase in macrophages.

Flow cytometry experiments showed higher levels of αIIbβ3 integrin activation in both basal and stimulated conditions and of surface P-selectin levels in basal conditions only. These findings suggest that SA may have a greater effect on the affinity regulation of the integrins, than on α-granule secretion. Albumin infusion immediately normalized PA in both basal and stimulated conditions.

Overall, our findings demonstrate that patients with analbuminemia have a thrombogenic profile, increased OS, and severe hypercholesterolemia that revert after exogenous albumin infusion.
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CONFLICT OF INTEREST
None.

AUTHOR CONTRIBUTION
FB, MDB, FA defined study concept and design, draft of the manuscript and critical revision; FB, SB, RC, LS: analysis and interpretation of data; SB, RC, LS: acquisition of data and critical revision of the manuscript; MDB is the guarantor of the study.

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SUPPORTING INFORMATION
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