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CASE REPORT

Oxidative stress mediated platelet activation in patients with congenital analbuminemia: Effect of albumin infusion

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Abstract

Background and Aims: Congenital analbuminemia 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 antithrombotic 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

1 | INTRODUCTION

Congenital analbuminemia (CA) is a rare autosomal recessive inherited disorder characterized by a strongly decreased concentration, or complete absence, of serum albumin (SA) and severe compensatory hypercholesterolemia, which may increase cardiovascular risk.[1–3] CA is conventionally defined by SA levels lower than 1 g/dL associated with normal liver function and absence of proteinuria. Patients are paucisymptomatic exept for fatigue, minimal ankle oedema and hypotension. CA is a Mendelian recessive trait caused by a variety of mutations in the gene located on chromosome 4 q13.3, coding for albumin.[4,5] Only 78 cases were reported, and the estimated disease prevalence is less than one in a million.

Albumin is the most important protein contributing to the regulation of plasma osmotic pressure. Individuals with analbuminemia have elevated levels of low-density lipoproteins and other plasma proteins, including coagulation factors, as a compensatory mechanism.[6–8] The increase of some blood coagulation factors and the

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deficiency of anticoagulant proteins, might predispose patients with analbuminemia to thrombotic events. Moreover, albumin exerts extracellular antioxidant and anticoagulant activity.[9] Several studies suggest that albumin itself exerts an anticoagulant effect by binding antithrombin, which enhances the neutralization of coagulation factor Xa,[10] and by inhibiting platelet aggregation.[11] Finally, a recent metanalysis showed that SA levels are associated with an increased risk of cardiovascular events in both patients in primary and secondary prevention for cardiovascular disease.[12]

Despite this evidence, in vivo platelet activation (PA) and oxidative stress (OS) and their role in inducing platelet hyperreactivity have never been studied in analbuminaemic 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.

Essential

- Several studies suggest that serum albumin exerts antioxidant, antithrombotic and anticoagulant activity.
- Congenital analbuminemia, an inherited disorder, characterized by a strong albumin concentration decrease is an interesting clinical setting to evaluate albumin antioxidant and antithrombotic effect.
- Patients with congenital analbuminemia showed a prothrombotic state and impaired oxidative stress compared to control subjects.
- In these patients, a reduction in platelet activation and oxidative stress was observed 2 h after album infusion.

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.[8] His past medical history was unremarkable, except for sporadic lipothymic 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 Fondi mutations, and we diagnosed, for the first time, a double compound heterozygosity accountable for CA.[5]

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, TxB_2, sCD40L, serum NOX2-derived peptide (sNOX2-dp), H_2O_2, 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.% vs. 76.0 ± 1.4%; $^*p < .05$), elevate levels of TxB_2 (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; $^{**}p < .01$), H_2O_2 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,

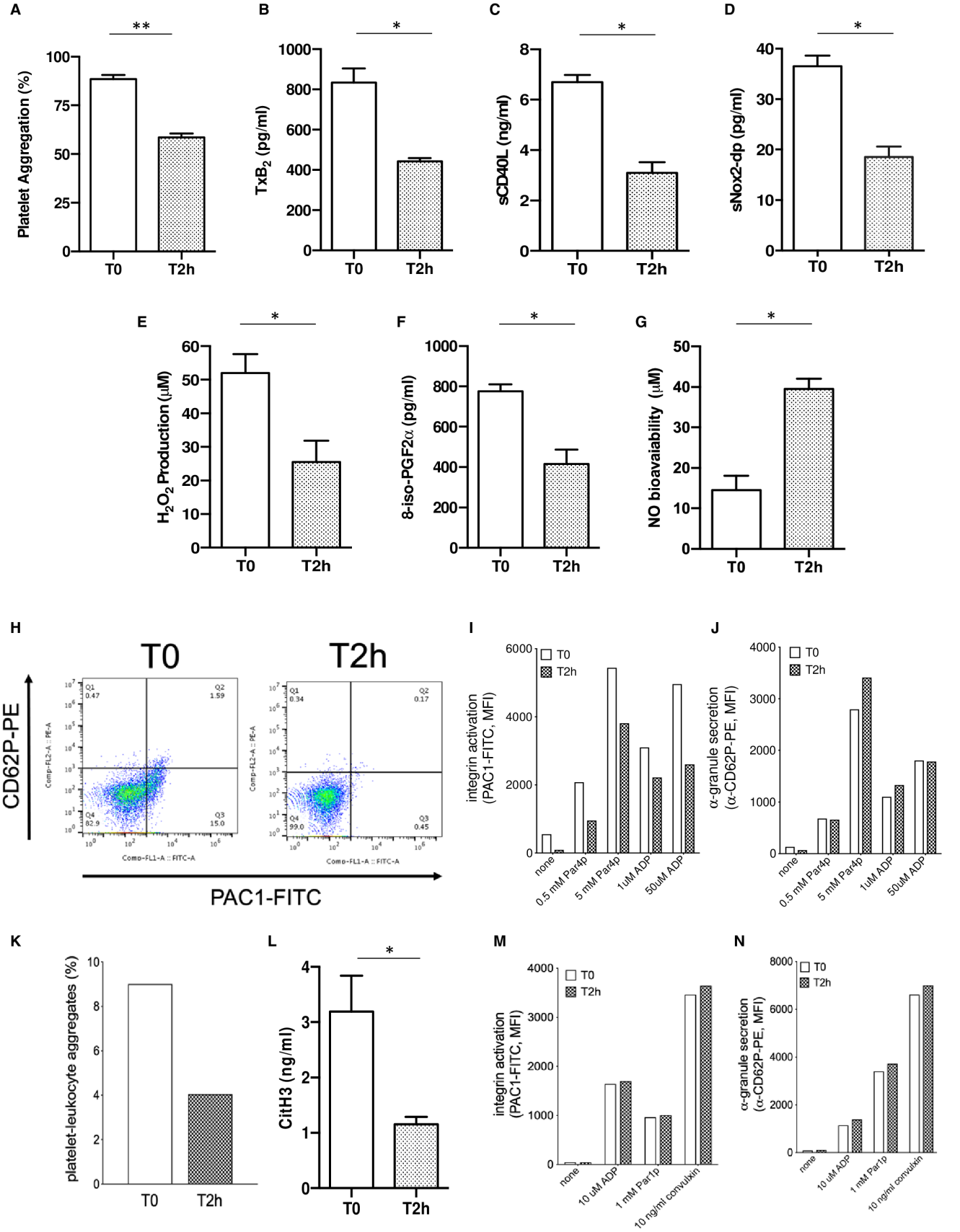
A
Platelet Aggregation (%)
T0
T2h
B
TxB2 (pg/ml)
C
sCD40L (ng/ml)
D
sNox2-dp (pg/ml)
E
H2O2 Production (μM)
F
8-iso-PGF2α (pg/ml)
G
NO bioavaiability (μM)
H
T0
T2h
CD62P-PE
PAC1-FITC
I
integrin activation (PAC1-FITC, MFI)
none
0.5 mM Par4p
5 mM Par4p
1uM ADP
50uM ADP
J
α-granule secretion (α-CD62P-PE, MFI)
K
platelet-leukocyte aggregates (%)
L
CitH3 (ng/ml)
M
integrin activation (PAC1-FITC, MFI)
none
10 uM ADP
1 mM Par1p
10 ng/ml convulxin
N
α-granule secretion (α-CD62P-PE, MFI)

FIGURE 1 (A) Platelet aggregation and (B) plasma concentration of TxB_2, (C) sCD40L, (D) sNOX2dp (E) H_2O_2 production, (F) 8-iso-PGF2α and (G) NO bioavailability in patients with analbuminemia before and 2 h after albumin infusion ($n = 2$; $^*p < .05$, $^{**}p < .01$). (H) Representative flow cytometry plots of platelets labelled directly in whole blood with PAC1-FITC, antibody that binds the active form of integrin αIIbβ3, and anti-CD62P-PE, that binds P-selectin exposed on the surface of platelets that have degranulated, before (left plot) and 2 h after (right plot) albumin infusion. (I) Integrin activation, (J) α-granule secretion, (K) platelet-leukocytes aggregate in platelets stimulated with or without PAR4p (0.5 mM and 5 mM) or ADP (1 μM and 50 μM) before and 2 h (T2h) after albumin infusion, in patient 1 with analbuminemia ($n = 1$). (L) CitH3 concentration in in patients with analbuminemia before and 2 h after albumin infusion ($n = 2$; $^*p < .05$, $^{**}p < .01$). (M) Integrin activation, (N) α-granule secretion, in platelets stimulated with or without PAR1p, or ADP or convulxin before and 2 h after albumin infusion, in analbuminemic patient 1 returned after 6 months with controlled levels of albumin ($n = 1$)

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 (CitH3), 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 ($p = .01$, $r^2 = 0.972$) and sNOX2dp ($p = .02$, $r^2 = 0.953$) and between sCD40L and urinary 8-iso-PGF2α ($p = .02$, $r^2 = 0.919$), sCD40L and sNOX2dp ($p = .03$, $r^2 = 0.939$).

3.2 | Response to albumin infusions

There was a significant reduction of platelet aggregation (88.5 ± 2.1–58.5 ± 2.1%; $^{**}p < .001$), TxB_2 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; $^*p < .05$), H_2O_2 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 CitH3 (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.[13] 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.[14,15] Additionally, albumin has been shown to directly bind and inactivate 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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