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High erythropoietin may be associated with vascular complications in patients with secondary erythrocytosis caused by high oxygen affinity variant hemoglobin Coimbra

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To the Editor:

Mutations affecting globin regions comprising the oxygen binding pocket can generate variant high oxygen affinity hemoglobins (HOAHs), impairing oxygen delivery to the tissues, triggering a response to hypoxia that leads to polycythemia. One such variant, Hb Coimbra, was described in 1991 (*HBB* c.300T > A, p.Asp100Glu) [1], but there is limited published data of this condition. Despite being regarded as benign disorders, HOAHs have been associated with vascular complications. Here we describe laboratory findings and clinical features of a cohort of Hb Coimbra carriers with unexpectedly more severe clinical presentations in association with increased erythropoietin (EPO) levels. Our observation suggests that supranormal endogenous EPO production may be associated with complications in patients with HOAHs.

Clinical data and peripheral blood samples were collected upon signed informed consent from patients diagnosed with Hb Coimbra-associated polycythemia (local ethics committee approval number 41684915.8.0000.5404). Genotypes and β-globin cluster haplotypes were confirmed by direct sequencing of the *HBB* gene and by TaqMan SNP Genotyping Assay (rs7482144, rs968857, rs16911905) and other polymorphisms in the β-globin gene (rs713040, rs10768683, rs7480526, rs7946748).

Oxygen affinity was evaluated by determining the p50 (at pH 7.34, 37 °C) of oxygen dissociation and saturation curves to calculate $p50_{deoxy}$ and $p50_{oxy}$ in whole blood samples without Hb isolation in a Hemox Analyzer system (TCS Scientific Corporation, Southampton, PA, USA). Heme-heme cooperativity was calculated by Hill coefficients (n). Functional tests were performed in sample triplicates and compared to three control samples.

Table 1 summarizes demographic and clinical data from 10 patients identified from four unrelated families at the Hematology and Hemotherapy Center, Hemocentro Campinas, University of Campinas, in Campinas, Brazil, for an average of 6.5 years (range 1–15 years).

All patients bear heterozygosity for *HBB* c.300T > A, p.Asp100Glu and the same haplotype for the variant allele, which strongly supports a common origin for this mutation in Brazil.

Abnormalities in the α1β2 interface, such as the Asp100Glu substitution in Hb Coimbra, promote conformational changes favoring the "relaxed" (R) state of the hemoglobin molecule, favoring oxygen binding and reducing oxygen delivery to the peripheral tissues, which results in compensatory polycythemia [2]. The diagnosis of HOAHs should be considered in cases of familial erythrocytosis or in patients in whom more frequent causes of acquired polycythemia, such as chronic obstructive pulmonary disease and polycythemia vera, have been excluded [3].

We confirmed that samples containing Hb Coimbra presented higher affinity for oxygen ($p50_{deoxy}$ 8.22 ± 0.64 mm Hg and $p50_{oxy}$ 7.94 ± 0.98 mm Hg) than wild type samples ($p50_{deoxy}$ 15.56 ± 0.50 mm Hg, $p50_{oxy}$ 15.56 ± 0.77 mm Hg). Heme-heme cooperativity was also significantly reduced in Hb Coimbra samples, with a trend towards a non-cooperative event, with a Hill coefficient of 1.51 in the Hb-oxygen dissociation curve and 1.48 in the Hb-oxygen saturation curve, while control Hb A samples had Hill coefficients of 2.52 and 2.41, respectively.

Clinically, three of the patients had complications. Patient 1, family A, male, 64 years old, presented with pulmonary embolism in July 2012, diagnosed by computerized angiography after an episode of sudden dyspnea. He was managed with oral anticoagulation. Patient 1, family D, male, 61 years old, had a complicated left leg deep venous thrombosis with amputation in 2010 before he was diagnosed with Hb Coimbra and, five years later, he was also diagnosed with right pulmonary artery thromboembolism. He is currently also on oral anticoagulation. Patient 2, family B, male, 72 years old, presented with left hemiparesis in 2016 due to an intraparenchymal hemorrhage in the right basal ganglia, with no need for surgical intervention.

Treatment varied widely, ranging from watchful waiting and aspirin alone to phlebotomy with or without aspirin or warfarin. Age-related bias might explain certain treatment choices, since all patients above 60 years received phlebotomy, and patients on watchful waiting or aspirin alone were under 50. This may stem from the referral of elderly patients with elevated hematocrits to be investigated for polycythemia vera. They are commonly managed with phlebotomy and aspirin as recommended for low-risk polycythemia vera patients until this diagnosis is ruled out.

No significant differences in oxygen affinity tests were observed between patients with and without clinical complications. Nevertheless, those three patients presented with elevated levels of serum EPO (81.1 mUI/mL, 423.5 mUI/mL, and 35.4 mUI/mL, respectively, reference range 2.6–18.5 mUI/mL), while EPO levels were within normal limits in the remaining patients. In patients with a high oxygen affinity Hb, EPO production may be expected to be normal at the equilibrium hematocrit [4]. While treatment with erythropoiesis stimulating agents has been shown to increase the risk for thrombosis, it is unclear whether high endogenous EPO levels could predispose to vascular events. A previous report showed that two patients with Hb Pierre-Benite and

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Table 1
Demographic, clinical, and laboratory data of the population studied.

Family/patient	Gender	Age at diagnosis, years	Hb, g/dL	Ht, %	EPO, mUI/mL	Comorbidities	Treatment	Complications
A/patient 1	M	65	16.7	56.3	**81.1**	AF, metabolic syndrome	Phlebotomy, warfarin	PE
B/patient 1	F	64	18.2	54.0	8.2	–	Phlebotomy, aspirin	None
B/patient 2	M	72	18.3	58.4	**35.4**	HTN	Phlebotomy	Hemorrhagic stroke
B/patient 3	F	38	16.5	52.7	14.1	–	Watchful waiting	None
B/patient 4	M	63	15.7	46.9	17.4	Pharynx carcinoma, former smoker	Phlebotomy, aspirin, hydroxyurea	None
B/patient 5	F	41	17.2	55.4	17.4	–	Watchful waiting	None
C/patient 1	F	65	18.2	60.0	13.9	HTN, DM, liver steatosis	Phlebotomy, aspirin	None
C/patient 2	F	42	17.4	55.1	9.2	–	Aspirin	None
D/patient 1	M	61	24.9	78.1	**423.5**	HTN, AF, former smoker	Phlebotomy, warfarin	DVT, PE
D/patient 2	F	32	20.4	59.5	5.7	–	Aspirin	None

Values in **bold** are outside the EPO reference range 2.6–18.5 mUI/mL. Hb, hemoglobin; Ht, hematocrit; EPO, serum erythropoietin; M, male gender; F, female gender; AF, atrial fibrillation; PE, pulmonary embolism; HTN, systemic arterial hypertension; DM, diabetes mellitus; DVT, deep vein thrombosis.

thrombotic events had elevated EPO, patients with Hb Santa Clara and Hb Olympia and normal EPO levels did not, and patients with Hb Heathrow and mild elevation of EPO also did not have complications but may have been protected by phlebotomies [5]. High EPO could be secondary to the treatment with phlebotomies in patients A-1 and B-2, but it does not explain the DVT in patient D-1, which happened prior to his diagnosis. Common risk factors for thrombosis, such as male gender and age, along with comorbidities, such as atrial fibrillation and hypertension, that could explain the vascular events observed are important confounders. Another limitation of this report is that we did not exclude concurrent diagnoses of hereditary thrombophilias.

This study shows a relatively long follow up of a cohort of patients with the same exact genotypic background for the beta globin cluster, but it is limited by the small number of patients and lack of serial measurements of EPO, which precludes the generalization of our findings to all patients with Hb Coimbra or with other HOAHs.

Taken together, our data show that not all patients with erythrocytosis secondary to HOAHs will follow a benign course, most noticeably Hb Coimbra carriers with high erythropoietin levels. This underscores the need for larger, multicenter, prospective studies of the clinical complications secondary to high oxygen affinity hemoglobins that could help identify prognostic markers that impact the management of this rare type of erythrocytosis.

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Bruna Cunha Santos[a], Susan Elisabeth Jorge[b], Dulcinéia Martins de Albuquerque[a], Simone Cristina Olenscki Gilli[a], Maria de Fatima Sonati[b], Kleber Yotsumoto Fertrin[a,c], Fernando Ferreira Costa[a,*]

[a] *Hematology and Hemotherapy Center, University of Campinas - UNICAMP, Campinas, Brazil*

[b] *School of Medical Sciences, Department of Clinical Pathology, University of Campinas - UNICAMP, Campinas, Brazil*

[c] *Division of Hematology, University of Washington, Seattle, United States*

E-mail address: ferreira@unicamp.br (F.F. Costa).

* Corresponding author at: Hemocentro - UNICAMP, Rua Carlos Chagas, 480, Cidade Universitária Zeferino Vaz, Campinas, SP 13083-878, Brazil.