Short Report: HMS-Related Hemolysis after Acute Attacks of *Plasmodium vivax* Malaria

Oriol Mitjà,* Russell Hays, James Malken, Anthony Ipai, Samson Kangapu, Jenny Robson, and Quique Bassat

Department of Medicine, Lihir Medical Centre - International SOS, Lihir Island, Papua New Guinea; Department of Microbiology, Lihir Medical Centre - International SOS, Lihir Island, Papua New Guinea; Department of Microbiology, Sullivan Nicolaides Pathology, Brisbane, Australia; Barcelona Centre for International Health Research/Hospital Clínic/University of Barcelona, Spain

Abstract. Among a cohort of 1,213 cases treated for *Plasmodium vivax* malaria from an isolated Papua New Guinean population, seven adults with severe and sustained hemolytic anemia after clearance of the peripheral parasitemia were prospectively investigated. All the patients fulfilled the criteria for hyper-reactive malarial splenomegaly and in 2 of 7 cases an IgG warm antibody was identified. Hereditary hemolytic anemia was excluded in 5 of 5 patients. All treated cases improved after an initial high dose of prednisone and antimalarial chemoprophylaxis. The persistence of marked anemia in a patient with splenomegaly after a *P. vivax* attack should raise the suspicion of hyper-reactive malarial splenomegaly.

The anemia that accompanies infection with *Plasmodium vivax* (Pv) in adults is sometimes persistent after appropriate antimalarial treatment. The enhanced inflammatory response, of greater magnitude than that to *Plasmodium falciparum* (Pf) may account for Pv having a comparably high potential to cause anemia.^{1,2} Conversely, a well-recognized chronic complication that increases susceptibility to anemia after repeated malaria infections is hyper-reactive malarial splenomegaly (HMS). Its defining features are chronic massive splenomegaly, elevated serum immunoglobulin M (IgM), high malarial antibody titers, and clinical and immunological response to long-term antimalarials.³ Most patients with HMS experience a chronic anemia attributable to pooling in the spleen and low-grade hemolysis.^{4,5} Occasionally sudden episodes of acute hemolytic anemia may become superimposed on this chronic status. Factors involved in the pathogenesis of HMS-related hemolytic crisis remain unclear and treatment recommendations are not well established. In many cases it seems to be caused by pregnancy, and could have an immune basis, because it has been reported to respond to treatment with steroids.⁶ In this series, we describe patients treated at our institution with a diagnosis of HMS-related hemolytic crisis.

THE STUDY

From July through December 2010, all patients presenting with Pv malaria and moderate anemia at Lihir Medical Center (LMC; Lihir Island; Papua New Guinea) were treated using artemether-lumefantrine without primaquine and followed up. The diagnosis of moderate anemia was based on laboratory parameters with a cut-off value of 8 g/dL. All patients who presented with sustained anemia 1 month after clearance of peripheral parasitemia by microscopy underwent further diagnostic investigation. Prolonged hemolysis was defined as a hemoglobin value below the pre-defined threshold in addition to total bilirubin > 1.4 mg/dL, lactate dehydrogenase (LDH) > 200 U/L, and haptoglobin < 0.41 g/L 1 month after elimination of peripheral parasitemia. Immunological tests, including direct anti-globulin test (DAT, direct Coombs' test), protein electrophoresis, total IgM and IgG quantitative determination, and serum concentrations of antibodies, IgM and IgG against *Plasmodium* were performed. For the purpose of this study an

individual who had splenomegaly of at least 10 cm below the costal margin, polyclonal increase in immunoglobulins, a serum concentration of IgM above 3.1 g/L, and a malaria antibody titer above 640, using the indirect fluorescent antibody test (IFA), was considered a case of hyper-reactive malarial splenomegaly (HMS). Following recommendations for primary autoimmune hemolytic anemia, we started treatment immediately with an initial dose of 1 mg/kg/d prednisone (PDN) orally for 1 week.^{6,7} Thereafter, the PDN dose was tapered slowly (by 10 mg/d/week) under careful monitoring of hemoglobin over a 3-week period. The patients were also prescribed chloroquine base (300 mg weekly) for 6 months. Clinical outcome was measured at a follow-up visit 21 days after initiation of steroid treatment. A successful response was defined as a complete normalization of bilirubin and LDH levels together with a hemoglobin level > 8 g/dL and an initial decrease of the palpable splenomegaly. All patients gave oral consent to participate in the study, and laboratory determinations were performed as part of their routine clinical management. The protocol of the study was approved by the Papua New Guinea Ministry of Health Medical Research Advisory Committee.

In the 6-month study period, among 1,213 cases of Pv malaria evaluated, 232 patients received a diagnosis of moderate anemia. Mean age (standard deviation) of patients with anemia was 7.6 (9.8) years. Out of 159 patients for whom follow-up data were available, 29 (18.2%) cases presented with persistent anemia 1 month after elimination of the parasite. Seventy-five percent (22 of 29) of these cases had a non-inflammatory plausible explanation for their persisting anemia. There were 5 cases of *P. vivax* malaria recurrence, 3 cases of new infection with Pf, 7 cases with iron deficiency anemia caused by menstrual blood loss, 3 cases of gastrointestinal bleeding caused by hookworm infestation, and 4 cases of megaloblastic anemia caused by folic acid deficiency. The remaining 7 patients (25%) met the diagnostic criteria of acute hemolytic crisis and all of them fulfilled the criteria for HMS. The demographic characteristics, clinical presentation, diagnostic test results, and outcome for these 7 cases from LMC are shown in Table 1. The cases were all adults resident in malaria holoendemic areas. Patients presented with fatigue and other constitutional symptoms (7 [100%] of 7), gross splenomegaly (7 [100%] of 7), and jaundice (5 [71.4%] of 7). One patient presented with dyspnoea on minimal activity and signs of congestive heart failure. Laboratory findings included hemoglobin levels ranging from 4.1 to 7.5 g/dL, with normocytic or macrocytic indices. In addition 4 (57.1%) cases had severe thrombocytopenia (defined as $\leq 50 \times 10^3$ cells/mm³),

*Address correspondence to Oriol Mitjà, Department of Medicine, Lihir Medical Centre, PO Box 34, Lihir Island, New Ireland Province, Papua New Guinea. E-mail: oriolmitja@hotmail.com

TABLE 1

Demographic characteristics, clinical presentation, and laboratory results for seven patients with prolonged hemolysis and *Plasmodium vivax* malaria

Patient	Sex, age (in years)	Clinical features	Spleen, cm*	First determined Hb (Day 0)	Day 28 Hb, g/dL	Platelets $\times 10^3$ cells/mm ³	Lymphocytes (%)	LDH†, U/L	Haptoglobin, g/L	Bilirubin, mg/dL	Hb on Day 21 post initiation of steroids treatment	Spleen on Day 21 post initiation of steroids treatment
1	M, 34	Jaundice, fatigue	17	6.8	4.6	27	36	ND	0.16	3.90	8.1	10
2	F, 25	Jaundice, dyspnoea	11	5.0	4.1	70	31	3,694	0.08	2.33	8.9	4
3	M, 28	Microscopic hemoglobinuria	14	6.9	7.5	130	ND	1,601	0.40	1.16	9.6	8
4	M, 26	Jaundice, fatigue	10	6.0	5.6	28	22	ND	ND	2.26	10.4	6
5	M, 16	Jaundice	22	4.3	6.6	70	30	2,460	0.08	2.10	8.4	14
6	F, 21	Fatigue, fever	13	7.0	5.2	42	43	391	0.31	3.23	9.2	7
7	M, 46	Jaundice, fatigue	11	6.5	6.9	50	ND	ND	ND	3.05	9.5	3

M = Male; F = female; Hb = hemoglobin, LDH = lactate dehydrogenase; ND = not determined; Pos = positive; Neg = negative.

*Spleen measured below the costal margin by palpation.

†Normal range, < 470 U/L.

‡Normal range, 0.41–1.65 g/L.

§Normal range 0.1–1.4 mg/dL.

however none of them presented with purpura or any other bleeding manifestations. Laboratory evidence of ongoing hemolysis was present in all the cases. Microscopical examination of blood smears remained negative for malaria parasites in all 7 cases, however two cases were checked by a polymerase chain reaction (PCR)-based method and both were positive for Pv DNA. The investigations for hereditary hemolytic anemia performed in five cases were all negative. The main immunological laboratory results are shown in Table 2. The Direct Coombs' test with mono-specific antibodies to IgG was positive in two cases (patient nos. 1 and 6), but C3d components were not detected. The administration of PDN in combination with antimalarial chemoprophylaxis, in all treated patients, resulted in prompt and dramatic improvement of all parameters of hemolysis, a gradual rise in hemoglobin and, improvement of the clinical condition. The mean duration of follow-up was 6 months at the time of writing and only one case (patient no. 1) presented a relapse of hemolysis with a drop of hemoglobin to 5 mg/dL after cessation of immunosuppressive therapy.

CONCLUSIONS

This study describes the infrequent (0.6%) but life-threatening occurrence of HMS-related hemolysis, after acute

uncomplicated attacks of Pv malaria, which is mostly DAT negative. After the exclusion of other causes of hemolytic anemia, including hereditary red blood cell disorders, toxin exposure or vaccination, and neoplasia, we suspect it may have an immune basis. The dysregulated immune response to long-term carriage of parasites at low levels likely explains the occurrence of HMS and also promotes the development of complications such as marginal zone splenic lymphoma.⁸

Experimental studies have shown evidence that *Plasmodium* spp. invasion proteins bind to uninfected erythrocytes serving as targets for complement-mediated lysis or macrophage phagocytosis.^{9,10} However, the design of the study does not allow any firm conclusion on the potential triggering effect of acute Pv attack on hemolytic crisis, because the Pv infection was an inclusion criterion. On the other hand, mild hemolytic anemia in HMS patients occurred in 42 of 49 travelers in the absence of any recent malaria attack.⁵ The practical interest of our observations is that the persistence of marked anemia in a patient with splenomegaly after a Pv attack should raise the suspicion of HMS. Confirmation of the diagnosis (total IgM levels, specific IgG levels) will lead to a specific management. Our results also provide new data that support corticosteroid administration, combined with antimalarial prophylaxis,

TABLE 2

Immunology and other specific study results at the time of diagnosis of hemolytic anemia in seven cases

Patient	Serum gamma zone protein quantification*	Total IgM†	Total IgG‡	Malaria IFA, antibody titer		Coombs test	Platelet antibodies IgM and IgG	HHA§ investigation	Other studies
				IgM antibody	IgG antibody				
1	37	10.7	20.9	1,600	> 3,200	Pos	Neg	Neg	Cold agglutinin screen normal
2	23	3.9	25.9	800	> 3,200	Neg	Neg	Neg	<i>P. vivax</i> PCR positive
3	35	8.4	22.4	800	> 3,200	Neg	ND	ND	Occult blood stools positive and ova of hookworm
4	ND	4.6	20.4	3,200	> 3,200	Neg	ND	ND	—
5	25	15.4	20.8	3,200	> 3,200	Neg	Neg	Neg	Cold agglutinin screen normal
6	37	10.3	20.1	1,600	> 3,200	Pos	Neg	Neg	<i>P. vivax</i> PCR positive
7	34	ND	ND	1,600	> 3,200	Neg	ND	Neg	—

ND = not determined; Pos = positive; Neg = negative; IFA = indirect fluorescent antibody test; HHA = hereditary hemolytic anemia; PCR = polymerase chain reaction.

*Normal range, 6–15 g/L.

†Normal range, 0.48–3.1 g/L.

‡Normal range, 5.76–15.36 g/L.

§Hereditary hemolytic anemia investigation included: Sickle cell disease, Thalassemia, South East Asian Ovalocytosis, and G6PD deficiency.

which may be beneficial for the treatment of hemolytic anaemia related to HMS.

Papua New Guinea, where all four malaria parasites infecting humans coexist, is reported to have the highest prevalence of HMS in the world.¹¹ Whether HMS is due exclusively to Pf or not, is a complex, still open discussion. There are some strong indirect indications implicating Pf. HMS is more frequent in Pf holoendemic areas, acute attacks of Pf have been reported after splenectomy in HMS patients,^{12,13} and transfusion in sickle cell disease patients with HMS-like syndrome has lead to appearance of Pf trophozoites in the blood stream.¹⁴ There are results of positive PCR at diagnosis of HMS for both species, *P. falciparum* and *P. vivax*,^{15–17} but the interpretation of this finding is more complex. It is generally accepted that patients with HMS no longer have acute attacks of Pf. The coexistence, described in this work of acute Pv attacks in patients with HMS therefore suggests that Pf-induced HMS does not give protection against Pv malaria and that Pv-induced HMS is based on a different process or that Pv-induced HMS does not exist.

Received March 12, 2011. Accepted for publication June 24, 2011.

Acknowledgments: We thank the patients and their families, and all the clinical and laboratorial personnel of the Lihir Medical Centre.

Financial support: This work was supported by InternationalSOS (Australasia) Pty Ltd and Newcrest Mining Ltd. The sponsor had no role in the study design, data collection and analysis, data interpretation, or writing of the manuscript.

Authors' addresses: Oriol Mitjà, Russell Hays, James Malken, and Anthony Ipai, Department of Medicine, Lihir Medical Centre, Lihir Island, NIP, Papua New Guinea, E-mails: oriolmitja@hotmail.com, rhays@ozemail.com.au, James.Malken@newcrest.au.com, and Anthony.Ipai@newcrest.au.com. Samson Kangapu, Department of Microbiology, Lihir Medical Centre, Lihir Island, NIP, Papua New Guinea, E-mail: Samson.Kangapu@newcrest.com.au. Jenny Robson, Department of Microbiology, Sullivan Nicolaides Pathology, Brisbane, Australia, E-mail: jrobson@snp.com.au. Quique Bassat, Barcelona Centre for International Health Research (CRESIB), Hospital Clinic/University of Barcelona, Barcelona, Spain, E-mail: quique.bassat@cresib.cat.

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