Case Report: Hyperreactive Malarial Splenomegaly Syndrome Diagnosed with Loop-Mediated Isothermal Amplification and Treated with Artemisinin-Based Combination Therapy

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Abstract. Hyperreactive malarial splenomegaly syndrome (HMSS) is a rare cause of splenomegaly in the Western world. Hyperreactive malarial splenomegaly syndrome is caused by an aberrant immunological response to chronic malaria exposure in endemic areas. Revised Fakunle’s criteria may be helpful for diagnosis: persistent splenomegaly (> 10 cm below the costal margin), increased anti-*Plasmodium* antibodies, increased IgM levels, exclusion of other causes of splenomegaly or malignancy, and a favorable response to antimalarial treatment. We describe the case of a 16-year-old patient, who recently arrived in Belgium from Guinea with a history of splenomegaly and B symptoms in whom HMSS diagnosis was achieved, thanks to the loop-mediated isothermal amplification method. To our knowledge, this is also the first described case treated by dihydroartemisinin/piperaquine.

CASE REPORT

A 16-year-old boy, who recently arrived in Belgium from Guinea, was referred to our tropical diseases outpatient clinic in Brussels for splenomegaly. He stated recent B symptoms, such as weakness, hypersomnia, itching after shower, and evening febrile sensation. His past medical history was notable for multiple malarial episodes and HbS heterozygosity. The physical examination revealed a soft palpable splenomegaly 6 cm below the costal margin and two infracentimetric cervical lymph nodes, without hepatomegaly. The rest of the physical examination was unremarkable.

The first laboratory test revealed normal red blood cells and white blood cells (WBC), including lymphocyte and platelet counts. There was no inflammation, or kidney or liver dysfunction. Polyclonal hypergammaglobulinemia (18 g/L, N 6–13) was observed with increased IgM (2.8 g/L, N 0.5–1.9) and IgG levels (17.6 g/L, N 7.3–15).

Serological tests were performed (*Trypanosoma brucei*, *Leishmania donovani*, *Filaria* spp., *Schistosoma*, *Strongyloides*, HIV, Hepatitis B, and Hepatitis C) and were all negative. An interferon gamma release assay was negative. The patient showed positive IgG for cytomegalovirus. Epstein-Barr virus showed the following serological markers: Virus capsid antigen (VCA) IgM at 3.8 U/mL (N < 1.1), VCA IgG at 184 U/mL (N < 22) with a negative Paul-Bunnell test and positive Epstein-Barr nuclear antigen (EBNA) IgG. A first panel of malaria tests was performed with a rapid diagnostic test (SD Bioline Malaria Ag P.f./Pan; Abbott, Wavre, Belgium) and thick and thin blood smears. All tests were negative. A screening for autoimmune conditions (antinuclear Ab, rheumatoid factor, and complements) was also negative and hematological investigations were normal.

Three months later, the patient presented to emergency department because of the worsening of weakness associated with fever and cough. Blood test revealed thrombocytopenia (99,000/ μ L) and leucopenia (total WBC 3,000/ μ L with 29% of atypical lymphocytes) with normal C-reactive protein.

A second blood smear for malaria was performed and was negative. Hyperreactive malarial splenomegaly syndrome (HMSS) was suspected and the patient was hospitalized in infectious diseases department. Loop-mediated isothermal amplification (LAMP) method (illumigene Malaria LAMP assay by Meridian Biosciences, Cincinnati, OH) was performed as well as IgG anti-*Plasmodium falciparum*: LAMP was positive for the presence of *P. falciparum* and serology showed extremely high IgG levels (index 5,520, N < 0.8), both being in favor of HMSS diagnosis. Thick blood smear was read again by an experienced hematologist with a positive result < 0.1% parasitemia. Treatment with dihydroartemisinin/piperaquine (320/40 mg three tablets/day for 3 days) was associated with doxycycline 200 mg once a day for 6 weeks. A bone marrow biopsy and a fluorodeoxyglucose-positron emission Tomography/computed tomography (FDG-PET/CT) were performed to exclude a malignancy, showing a few *P. falciparum* in the bone marrow and pneumonia of the right inferior lobe on PET/CT. A treatment with amoxicillin/clavulanate was associated to antimalarial therapy. Clinical and biological evolution was rapidly favorable and patient was discharged after a few days.

One month later, spleen was clinically unpalpable; itching and B symptoms had disappeared. Two months later, hypergammaglobulinemia, including increased IgG and IgM, was completely resolved.

DISCUSSION

We present a case of a 16-year-old Guinean boy who recently arrived in Belgium and presented with splenomegaly and B symptoms. This case highlights several aspects of HMSS: the strengths and limitations of revised Fakunle’s criteria in HMSS diagnosis; the diagnostic value in HMSS of current molecular techniques such as LAMP; and the lack of guidelines for HMSS treatment in non-endemic areas.

Hyperreactive malarial splenomegaly syndrome is caused by an aberrant immunological response to chronic malaria exposure. A genetic predisposition such as human leukocyte antigen (HLA)-DR2 haplotype¹ is associated with repeated exposure to malarial antigens, leading to an excessive production of polyclonal hyperimmunoglobulinemia, especially macroglobulinemia formed by IgM and/or immune complexes. The persistent clearance of those by the reticuloendothelial

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TABLE 1
Review of published cases of HMSS in adults in malaria non-endemic countries

Study	Country (study period)	Patients (n), (age), country of origin	Diagnosis of HMSS and other special conditions	Treatment	Outcome
Van den Ende et al. ⁴	Belgium (1986–1997)	49 (10–76 years), sub-Saharan Africa	Fakunle 17/49 B symptoms 42/49 Positive smear 27/49	Different regimens anti- <i>Plasmodium falciparum</i> from 1 to 7 days according to the drug (no ACT)	9/49 relapse, all following reexposure
Puente et al. ²⁰	Spain (1988–2000)	5 (55–70 years), Equatorial Guinea and West-Central Africa	Fakunle Hepatic biopsy 3/5 (all with lympho sinusoidal infiltration) Negative OMB 5/5 Positive smear 0/5	Quinine 7 days (1) Halofantrine 4 days (4) All (5/5) + chloroquine 6–12 m	5/5 cured
De Franceschi et al. ¹⁸	Italy (not specified)	2 (6 years), Angola; (17 years), Nigeria	Fakunle Positive smear 0/2 2/2 SCD	Halofantrine + splenectomy for SCD (1) Artemeter 5 days + pyrimethamine sulfametopyrazine single dose (1)	2/2 cured
De Iaco et al. ¹⁹	Italy (2004)	1 (57 years), Cameroon	Fakunle Negative smear Positive PCR New diagnosis of HIV	Mefloquin single dose (renal impairment)	Cured
Mothe et al. ⁶	Spain (not specified)	1 (67 years), Cameroon	Fakunle Negative OMB Negative smear Positive PCR	Chloroquine 3 days + primaquine 2 weeks relapse: Quinine + doxycycline 1 week	Cured
Camara et al. ¹⁵	France (2001–2006)	3 (15–71 years), Cameroon	Fakunle Negative smear 3/4	Mefloquine + doxycycline for 30 days followed by chloroquine + doxycycline for other 30 days (1) Atovaquone/proguanil + doxycycline for 30 days (1) Atovaquone/proguanil (1)	3/3 cured
Masfrancx et al. ¹⁶	Belgium (2009)	1 (75 years), Democratic Rep. Congo	Fakunle Positive OMB Negative smear Positive rapid test (Pf HRP-2)	Atovaquone/proguanil	Cured
McGregor et al. ⁵	United Kingdom (2003–2010)	7 but only four confirmed (16–61 years), sub-Saharan Africa (four from Sierra Leone)	Fakunle Positive smear 1/7 PCR 1/4 (three not done, three negative) Schistosomiasis 3/7 and Hepatitis B 1/7, including two with portal hypertension	Different long regimens from 2 months to 3 years Chloroquine ± quinine and proguanil Mefloquine + chloroquine and proguanil Atovaquone/proguanil for 3 years	4/7 cured three with negative PCR not improved
Bisoffi et al. ³	Italy (1990–2015)	44 (56 years median), from Africa (not specified)	Fakunle Positive smear 22/38 Positive quantitative buffy coat malaria 9/38	Quinine 3 days + pyrimethamin/ sulfametopyrazine one dose, after 2002: ACT but precise regimen not specified	20/24 cured or improved others lost to follow-up
Genderini et al. 2018 ^[current]	Belgium (2017)	1 (16 years), Guinea Conakry	Fakunle Positive smear Positive LAMP	Dihydroartemisinin/ piperazine + doxycycline 6 weeks	Cured

ACT = artemisinin-based combination therapy; HMSS = hyperreactive malarial splenomegaly syndrome; LAMP = loop-mediated isothermal amplification; OMB = osteomedullary biopsy; PCR = polymerase chain reaction; SCD = sickle cell disease.

system causes eventually persistent and often massive splenomegaly.² Complications are increased susceptibility to infections, pancytopenia, and splenic rupture. Mortality of the syndrome may exceed 50% if left untreated.³

Recently arrived from a highly malaria-endemic country, our young patient presented with weakness and febrile sensation,

which are described as the most usual symptoms in patients with HMSS in non-endemic countries.^{3,4} Negative thick blood smear is often (42–100%) reported in series of HMSS,^{3,5,6} and when detected, parasite density is very low most of the time.³ Thick blood smear, read by an experienced hematologist, is able to detect 50 parasites/ μ L; a rapid test about 100–500

parasites/ μ L; and LAMP two parasites/ μ L, as is by polymerase chain reaction (PCR).^{7,8} Loop-mediated isothermal amplification method is a molecular technique that uses unique DNA polymerases and specially designed primers to amplify nucleic acid without thermocyclers.⁷ As already described, LAMP is useful to detect malaria in asymptomatic patients with very low parasitemia (submicroscopic and subclinical parasitemia).⁹ The use of molecular techniques for malaria diagnosis increased the yield of *Plasmodium* detection in HMSS.¹⁰ Detection of submicroscopic parasitemia has been proposed as a good prognosis factor for a favorable outcome after treatment.⁵ To our knowledge, this is the first case in which LAMP was used to diagnose HMSS. All Fakunle's criteria¹¹ were present in our patient: a massive splenomegaly, an extremely increased anti-*P. falciparum* antibody level, raised IgM titer, and a favorable clinical-immunological response to a prolonged antimalarial therapy. The lymphocyte morphological heterogeneity, the absence of lymphocyte monoclonality demonstrated by cytometry, and the normal bone marrow and peripheral blood excluded hematological malignancy (revised criteria).¹² In our case, Epstein-Barr virus (EBV) infection could have explained symptoms as well as splenomegaly and polyclonal hypergammaglobulinemia, but serological markers (negative Paul-Bunnell and positive EBNA) were discrepant. Extremely increased anti-*P. falciparum* antibody level, normalization of the spleen and the IgM after treatment, long stay in a hyperendemic malaria area, and multiple malarial episodes in childhood added to the cluster of arguments for HMSS. Exaggerated polyclonal stimulation of B lymphocytes is one of the mechanisms initiating HMSS and led probably to falsely positive EBV VCA IgG and IgM as already reported.^{3,6} Anyway, given the risk of developing a severe disease with high mortality in case of reexposure, treatment would have been justified.³ Increased susceptibility to bacterial infections is a known complication of HMSS and a historically described leading cause of mortality.¹³ Pneumonia was incidentally detected on PET/CT: it is difficult to assess if fever was due to pneumonia or to HMSS, with pneumonia as an incidental phenomenon.

In non-endemic countries without reexposure to *P. falciparum*, a short antimalarial regimen may be an option to treat HMSS.^{3,14} We administrated dihydroartemisinin/piperazine, which is our first-line treatment for malaria. To our knowledge, this is the first case of HMSS treated with this combination. We continued antimalarial treatment with 6 weeks of doxycycline, as most case series report long treatments.^{5,15} Retrospectively, considering the low risk of reexposure, a short regimen would have been probably enough.^{3,14,16}

A potential link between HbS and HMSS has not been studied. High prevalence of HbS heterozygosity in endemic areas and its protective role against acute *P. falciparum* malaria (decreased rate of cerebral malaria, mortality, and hyperparasitemia) are well known.¹⁷ Hyperreactive malarial splenomegaly syndrome in patients with sickle cell disease has already been reported,¹⁸ but incidence in this particular group is not known. The presence of HbS probably does not prevent submicroscopic parasitemia and the development of HMSS, which is mostly secondary to immunological phenomena² and not to the presence of the parasite itself.

In Table 1, we present a summary of the publications since year 2000, reporting at least one case of HMSS in adults in non-endemic countries, which represents a total of nine

studies and 113 patients. Several patients were treated with ACT in one study, but precise regimen and duration were not specified.³ In nearly all studies, revised Fakunle's criteria were the cornerstones of HMSS diagnosis and in three reports, PCR was used to confirm submicroscopic infection.^{5,6,19}

In conclusion, hyperreactive malarial splenomegaly syndrome is a challenging diagnosis in non-endemic areas. Revised Fakunle's criteria may guide the diagnosis, and the role of molecular techniques such as LAMP or PCR is emerging to obtain a definite diagnosis in certain cases, a prognosis tool in others. We lack specific treatment guidelines for non-endemic areas: cure may possibly be achieved with an ACT-based short regimen even if this strategy should be validated in larger studies.

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