A Case of Hyper-reactive Malarial Splenomegaly. The Role of Rapid Antigen-detecting and PCR-based Tests

B. Mothe, J. Lopez-Contreras, O.H. Torres, C. Muñoz, P. Domingo, M. Gurgui

Abstract

Hyper-reactive malarial splenomegaly (HMS) – originally referred to as tropical splenomegaly syndrome – is characterized by a massive splenomegaly, high titres of anti-malarial antibodies and polyclonal IgM hypergammaglobulinemia. It is believed to be a consequence of an aberrant immunological response to prolonged exposure to malarial parasites. Although it is a frequent disease in the tropics, it is infrequent in western countries and is only seen in long-term residents from endemic areas. We describe the case of a 67-year-old Spanish man, a missionary in Cameroon for 30 years, who presented with a clinical history that fulfilled the diagnosis of HMS. We discuss the role and importance of PCR-based techniques in demonstrating low-grade malarial parasitemia and the usefulness of new rapid antigen-detecting dipstick tests.

Infection 2008; 36: 167–169
DOI 10.1007/s15010-007-6025-z

Case Report

A 67-year-old male, originally from Barcelona, had worked as a missionary in a rural area of Cameroon for 30 years. He was referred to our hospital with a 5- to 6-month history of asthenia, left hyponchondrial pain and progressive exercise-induced shortness of breath. In reference to his past medical history he remembered having been diagnosed of filariasis at 42 years of age and three prior episodes of fever and chills attributed to malaria in Cameroon. The last of these episodes had occurred 8 months prior to the present illness, and was treated with intravenous quinine. On arrival to Barcelona and on admission to our hospital, he was pale, afebrile and haemodynamically stable. Hepatomegaly and massive splenomegaly of 17 cm below the costal margin were noted on palpation without tenderness or guarding. No peripheral lymphadenopathy was found and the rest of the physical exam was unremarkable.

Laboratory tests revealed hemoglobin of 75 g/l (135–170), with a mean cell volume of 90 fl, leukocyte count of 1.8×10^9/l (3.8–11) with 45% lymphocytes and 40% neutrophils, platelet count of 61×10^9/l (140–350), sedimentation rate of 138 mm/h (1–10), thromboplastin time of 1.57 ratio (0.75–1.30). Aspartate aminotransferase was 22 U/l (< 38), alkaline phosphatase 118 U/L (40–130), gamma-glutamyl transpeptidase 25 U/l (< 53), lactate dehydrogenase 520 U/l (240–480), C-reactive protein 11.9 mg/l (< 5). Serum protein electrophoresis showed polyclonal gamma-globulins of 30.8 g/l (6.3–16.2) with an immunoglobulin dosage of 3,120 mg/dl of IgM (40–320) and normal IgG (749 mg/dl, normal range 723–1,685) and IgA determinations (64 mg/dl, normal range 69–382). Glucose-6-phosphate dehydrogenase (G-6-PDH) deficiency screening test was negative.

The following work-up was added to rule out other causes of splenic enlargement and pancytopenia. Differentials included lymphoproliferative disorders, HIV infection, hepatic cirrhosis with portal hypertension, brucellosis, leishmaniasis, subacute forms of non-falciparum malaria, salmonellosis, schistosomiasis and tuberculosis. Direct and indirect Coomb's tests were negative. Lymphocyte subsets counts were normal (32% CD4+ –259/mm^3, and 13% CD8+ –105/mm^3), with no detection of monoclonal B cell populations using clusters of differentiation marker assays. Bone marrow biospsy showed unspecific erythroblastic hyperplasia. Immunological tests showed speckled ANA of > 1/320, anti-smooth muscle 1/160, reumathoid factor 528 IU (< 20), cryoglobulins 456 mg/100 ml (< 8), total complement CH50 of 15 mg/ml (34–71) with C3 fraction determination of 40 mg/dl (85–193), and C4 fraction < 5 mg/dl (12–36). Anti ds-DNA antibodies were negative.

Abdominal echography and abdominal CT scan demonstrated homogeneous hepatomegaly and massive splenomegaly (length of 27 cm on CT scan) without significant lymphadenopathy. Chest CT scan showed calcified nodules of < 1 cm diameter at right and left lower lobes of the lungs, suggestive of granuloma, and a non-significant cicatricial infiltrate at the right upper lobe was also observed.

Serological tests by enzyme immunoassay – EIA – were positive for cytomegalovirus (IgG+ and IgM+) and toxoplasmosis (IgG– and IgM+), and by indirect immunofluorescence – IFI – for Epstein Barr virus (IgM+), Human Herpes 6 (IgM+),

B. Mothe, J. Lopez-Contreras *(corresponding author)*, **O.H. Torres, P. Domingo, M. Gurgui**
Infectious Diseases Unit, Internal Medicine Department, Hospital de la Santa Creu i Sant Pau, Universitat de Barcelona, Barcelona, Spain;
e-mail: jlcontreras@santpau.es
C. Muñoz
Microbiology Department, Hospital de la Santa Creu i Sant Pau, Universitat de Barcelona, Barcelona, Spain

Received: January 16, 2006 · Revision accepted: March 27, 2007
Published online: September 28, 2007

parvovirus B19 (IgM+) and schistosomiasis (IgG+). Hepatitis B virus, hepatitis A virus, hepatitis C virus by EIA and Leishmania infection by IFI were all negative. Rose Bengal test for brucellosis was negative, as well as serology of Typhoid Fever (Widal TO and TH). Rapid plasma reagin test for *Treponema pallidum* and EIA and Western Blot for HIV 1 and 2 were also negative. Blood and bone marrow cultures were negative for bacteria and mycobacteria. No parasites were detected in bone marrow biopsy, and granulomas were neither identified. Bacteriological and parasitological tests of stool samples were negative. Schistosoma detection on 24-h urine collection was also negative. The titre of specific IgG anti-malarial antibodies by indirect immunofluorescence was high 1/1,280. Giemsa-stained thick and thin smears (×3 times) did not detect malarial parasites. The antigen-detecting dipstick test (Now® ICT Malaria test; Binax, Inc., Portland, ME, USA) was positive for non-*Plasmodium falciparum* species and negative for *Plasmodium falciparum* malaria. It was repeated three times with the same results.

Hyper-reactive malarial splenomegaly (HMS) was diagnosed on the basis of standard criteria. The patient was initially treated with chloroquine (total dose of 2,500 mg salt in 3 days) plus primaquine 15 mg PO qd for 2 weeks, in a non-*P. falciparum* treatment plan. No significant improvement was achieved and 15 days after discharge, the patient referred persistent dyspnea and appearance of cough with sputum. Pancytopenia had also worsened and the patient was readmitted. *P. falciparum* DNA in the blood was checked by a PCR-based method (as described [6]) and was positive. However, blood smears remained negative for parasites. A new treatment plan was then started with quinine 600 mg PO q8h and doxycycline 100 mg PO bid for 1 week. Chest X-ray and CT scans performed due to the cough were normal. However, sputum samples subjected to Ziehl-Neelsen, PCR and Lowenstein-Jensen culture turned positive for *Mycobacterium tuberculosis*. Tuberculostatic therapy was then started with isoniazid 300 mg PD qd, rifampicin 600 mg PD qd, ethambutol 1.2 g PO qd and pyrazinamide 1.5 g PO qd. Following discharge, the patient showed a slow but favorable improvement.

At present, 24 months later, splenomegaly is undetectable by palpation and laboratory data are returning to normal limits (150 g/l Hb, 3.15×10^9/l leukocyte count, 94×10^9/l platelet count, gamma globulin of 13.1 g/l with IgM dosage of 489 mg/dl, cryoglobulins 18 mg/100 ml – previous 456, and rheumatoid factor of 49 IU – previous 528). Serological tests for cytomegalovirus, toxoplasmosis, Epstein Barr virus and Human Herpes type 6 have been repeated and are now all negative for IgM. By CT scan, spleen length is now of 12 cm.

Discussion

Hyper-reactive malarial splenomegaly is believed to be a consequence of an immunological dysfunction due to recurrent episodes of malaria. It is generally considered that prolonged exposure to malarial parasites (5–10 years) is needed to develop HMS [4]. This response is based on an overproduction of polyclonal IgM antibodies, leading to accumulation of high molecular-weight immunocomplexes and complement consumption, as seen in the patient presented herein [3, 7, 9]. Long-term purification of immunocomplexes induces progressive hyperplasia of the spleen. Patients usually present hypersplenism, and may have anaemia, leukopenia, thrombopenia and even haemolytic crises, depending on the severity. Increased susceptibility to infections conferred by the immunosuppressive state has been reported during HMS [2]. Reactivation of pulmonary tuberculosis could be related to this state in our patient.

In 1981, *Fakunle* established major and minor diagnostic criteria for HMS, attempting to distinguish this entity from other tropical splenomegaly syndromes [5]. Gross splenomegaly, elevated antimalarial antibodies, serum IgM > 2 SD above the mean of a given population, and favorable response to antimalarials are mandatory criteria. Minor diagnostic criteria include hepatic sinusoidal lymphocytosis, normal cellular and humoral immune responses to antigenic challenge, hypersplenism, lymphocyte proliferation and occurrence within families. However, some of the minor criteria have now fallen into disuse; a liver biopsy is rarely necessary today for the diagnosis, and epidemiological criteria such as familial and tribal occurrence are of little relevance outside tropical areas.

In relation to lymphocytic proliferation, it has recently been described that chronic exposure to paludism may also lead to B-cell splenic lymphoma [11]. Therefore, differential diagnosis in these patients must always include lymphoproliferative disorders. Absence of both villous lymphocytes and monoclonal lymphoid populations, as well as a significant response to antimalarials, ruled out a lymphoproliferative origin in our patient.

Our patient had positive serological tests for CMV, toxoplasmosis, Epstein Barr virus, Human Herpes 6, parvovirus B19 and schistosomiasis, as well as elevated speckled ANA, anti-smoth muscle and cryoglobulines titers. Probably due to unspecific B-cell activation, false-positive tests for serologies and autoantibodies can be present, leading to difficulties in their interpretation. Today, 24 months after antimalarials, the patient has negative serological tests and the autoantibodies titers are almost normal. To our knowledge, this cross-reactivity phenomenon in HMS has not been previously described in literature.

With PCR-based methods, malarial DNA can be demonstrated in peripheral blood from patients with previously negative smears [7]. This suggests an association between HMS and active malarial infection, and implies a subacute but persistent low-grade parasitaemia, which may be important in clinical practice in terms of management. Classical lifelong-antimalarial prophylaxis is now being substituted with short treatment plans when the patient does not return to the endemic area. Furthermore, multiplex PCR can identify the *Plasmodium* species involved [8].

The ICT Malaria Test is based on the detection of circulating *P. falciparum*-specific antigen histidine-rich protein-2 (HRP2) and a panmalarial antigen (PMA: aldolase) found in *P. falciparum*, *P. vivax* and *P. ovale* but possibly not in *P. malariae*. The main benefit of rapid antigen-detecting dipstick tests is that they are quick to perform and easy to interpret, without the need for spe-

cialized laboratory personnel. They are almost as sensitive as blood smear microscopy in the detection of malarial crises but they cannot determine parasite load, and sensitivity is poor in cases of low parasitaemias as in HMS. Furthermore, false negative results for *P. falciparum* may also occur occasionally even at high parasitaemias. Potential explanations include a prozone effect (a high concentration of antibodies may mask the antigen, making it undetectable with this assay) and the presence of a mutation or deletion within the *hrpii* gene [6, 10]. Due to permanent gametocytemia, panmalarial antigens may persist in patients in endemic areas despite correct treatment, accounting for a considerable number of false positive results. This is a potential problem in semi-immune residents of these areas, where chronically infected states can result in high rates of gametocytemias, especially in countries where chloroquine is widely used. Moreover, cross-reactivity with rheumatoid factor – especially high in cases of hyperimmune reactions – often gives false positives for non-*falciparum* species [1], as occurred in our patient, leading us to erroneously diagnose a non-*falciparum* infection.

In conclusion, although HMS is still infrequent in our population, it can become more common as a result of migration. Fakunle's diagnostic criteria remain valid but there are several limitations. New concepts related to HMS pathogenesis and its relationships with lymphoproliferative disorders are being proposed and novel approaches with short therapy alone are proving effective in expatriates returning from endemic areas. Rapid malaria tests are of immediate benefit for diagnostic management in several malarial situations, but their sensitivity and specificity are limited in HMS as compared to PCR-based methods. New tests are presently under development and will hopefully overcome these shortcomings in the near future. In terms of diagnostic criteria, when HMS is suspected in patients returning from endemic areas and smears are negative, we suggest that PCR could be a useful alternative on the diagnostic work-up for this entity.

References

1. Craig MH, Bredenkamp BL, Williams CH, Rossouw EJ, Kelly VJ, Kleinschmidt I, Martineau A, Henry GF: Field and laboratory comparative evaluation of ten rapid malaria diagnostic tests. Trans R Soc Trop Med Hyg 2002; 96: 258–265.
2. Crane GG: Hyperreactive malarious splenomegaly (tropical splenomegaly syndrome). Parasitol Today 1986; 2: 4–9.
3. Corachan M, Cerralbo M: Síndrome de esplenomagalia tropical o esplenomegalia malárica hiperreactiva. Med Clin (Barc) 1986; 87: 76–81.
4. Van den Ende J, Van Gompel A, Van den Enden E, Taelman H: Hyperreactive malaria in expatriates returning from sub-saharan Africa. Trop Med Int Health 2000; 5: 607–611.
5. Fakunle YM: Tropical splenomegaly. Part 1: tropical Africa. Clin Hematol 1981; 10: 963–975.
6. Farcas GA, Zhong KJ, Lovegrove FE, Graham CM, Kain KC: Evaluation of the Binax NOW ICT test versus polymerase chain reaction and microscopy for the detection of malaria in returned travelers. Am J Trop Med Hyg 2003; 69: 589–592.
7. Moraes MF, Soares M, Arroz MJ, Do Rosario VE, Da Graca JP, Abecasis P: New concepts in hyperreactive malarial splenomegaly. Acta Med Port 2003; 16: 41–46.
8. Puente S, Subirats M, Benito A, Rubio JM, Gonzalez-Lahoz JM: Hyperreactive malarial splenomegaly in Europeans: report of five cases. J Travel Med 2001; 8: 322–324.
9. Puente S, Rubio JM, Subirats M, Lago M, Gonzalez-Lahoz J, Benito A: The use of PCR in the diagnosis of hyper-reactive malarial splenomegaly (HMS). Ann Trop Med Parasitol 2000; 94: 559–563.
10. Tjitra E, Suprianto S, McBroom J, Currie BJ, Anstey NM: Persistent ICT malaria P.f/P.v panmalarial and HRP2 antigen reactivity after treatment of *Plasmodium falciparum* malaria is associated with gametocytemia and results in false-positive diagnoses of *Plasmodium vivax* in convalescence. J Clin Microbiol 2001; 39: 1025–1031.
11. Wallace S, Bedu-Addo G, Rutherford TR, Bates I: Serological similarities between hyperreactive malarial splenomegaly and splenic lymphoma in West Africa. Trans R Soc Trop Med Hyg 1998; 92: 463–467.