

Case Report

Myositis-specific autoantibodies in a non-traveler, patient from a non-endemic country, with *Plasmodium vivax* malaria

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Abstract

Introduction: Autoantibodies (AAb) are a hallmark of immune-mediated inflammatory diseases. Malaria is a parasitic disease caused by *Plasmodium* protozoa. Individuals with malaria may present with a wide range of symptoms. It is frequently linked to the development of different AAb.

Case description: A 35-year-old male presented with repeated episodes of fever, malaise, myalgia, dark urine, and yellowish sclera. Initial diagnostic workup revealed severe Coombs-positive anemia, increased C-reactive protein, and procalcitonin, pathological liver tests, high concentration of serum IgE, IgG, IgM, IgA, positive antinuclear antibodies (ANA), and positive antineutrophil cytoplasmic antibodies (ANCA). In addition, myositis-specific antibodies directed to polymyositis-scleroderma 75 protein (PmScl75), threonyl-tRNA synthetase (PL-7), alanyl-tRNA synthetase (PL-12), Mi-2 antigen (Mi-2), Ku DNA helicase complex (Ku), signal recognition particle (SRP), and anti-aminoacyl tRNA synthetase (EJ) were detected. The patient was suspected of having systemic lupus erythematosus and sent to the Clinic of Allergy and Immunology for further evaluation and treatment. A peripheral blood film examined by the hematologist during an episode of fever revealed intra-erythrocytic parasitic forms of *Plasmodium vivax* (*P. vivax*). After being diagnosed with *P. vivax* malaria, he was transferred to the Clinic for Infective and Tropical Diseases. The therapy consisted of artesunate/mefloquine and prednisone led to a complete clinical recovery and autoantibodies gradually disappeared.

Conclusions: Malaria would not normally be considered during the initial diagnostic workup in a non-traveler and a patient from a non-endemic country. However, a thorough parasitic evaluation in patients presenting with a broad range of autoantibodies might be of particular importance.

Key words: malaria; autoantibody; myositis; *Plasmodium vivax*.

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Introduction

Autoantibodies (AAb) are a hallmark of immune-mediated inflammatory diseases. Moreover, the presence of more specific AAb may enable the diagnosis of certain autoimmune diseases [1]. Malaria is a parasitic disease frequently linked to the development of different AAb. Whether these autoimmune-like responses are pathogenic or have a protective role has not yet been well understood [2]. However, malaria would not be suspected and considered during an early diagnostic assessment in a non-traveler patient from a non-endemic country presenting with AAb of broad-range specificity.

Case presentation

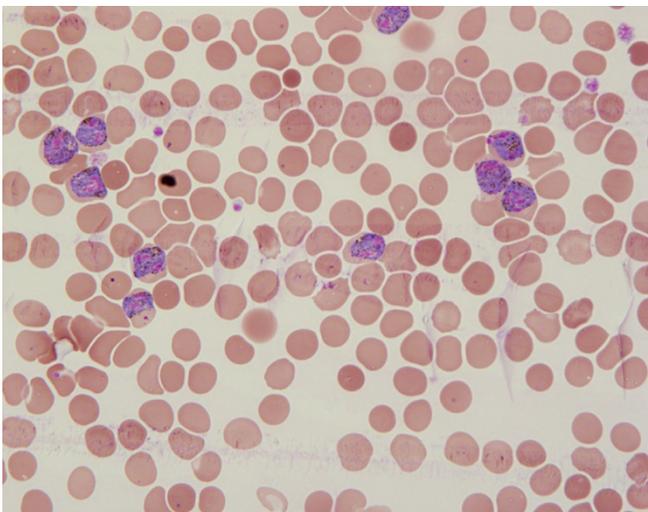
We report a 35-year-old male suspected of developing systemic lupus erythematosus (SLE), who was sent to the Clinic of Allergy and Immunology, University Clinical Center of Serbia for further diagnostic evaluation and treatment. He presented with repeated episodes of fever up to 40 °C, malaise, myalgia, dark urine, and yellowish sclera over several months.

Following the previous medical history, the patient was likely to have sepsis and disseminated intravascular coagulation when he presented with fever, severe anemia, thrombocytopenia, and markedly elevated inflammatory parameters and was initially hospitalized at the hematology unit of the local hospital. Abdominal

ultrasound revealed moderately enlarged liver and spleen. A further diagnostic evaluation by computed tomography of the abdomen revealed a round mass with lobulated border in the spleen (7.0 x 7.8 cm). Bone marrow biopsy trephine revealed reactive changes. Hematological neoplasm was excluded. Broad action antibiotics and multiple transfusions of packed red blood cells led him to a favorable outcome. The fever disappeared and all hematological parameters improved within the next few days.

Seven days later, the patient was admitted to the same hospital again due to fever, anemia, elevated C-reactive protein (CRP) and procalcitonin. This time, the patient was considered as having sepsis due to a splenic abscess. A causative pathogen could not be identified according to the negative tests for Epstein-Barr, herpes simplex, cytomegalovirus, hepatitis B and C virus, human immunodeficiency virus, multiple negative blood cultures, and urine culture analysis. In the presence of a treatment-resistant, direct Coombs test positive anemia, thrombocytopenia, and suspected splenic abscess, splenectomy was performed. Histopathological examination revealed a sclerosing angiomatoid nodular transformation of the spleen, while the histopathological evaluation of the liver showed hydropic degeneration. Immunological tests revealed positive ANA of a homogenous pattern on immunofluorescence, directed to the nucleoplasm (titer of 1/320), positive staining to the nucleolus (1/640), and cytoplasm (1/160). Additional tests showed low positive myositis specific AAb (MSA): anti-PL-7, anti-PL-12.

Figure 1. Malarial trophozoites within erythrocytes, not expected in Coombs positive hemolysis (blood film, 1000x, May Grünwald Giemsa stain).



After this initial diagnostic evaluation, infective and hematological etiology was temporarily excluded; the patient was referred to our clinic suspected of having SLE. Laboratory evaluation revealed elevated erythrocyte sedimentation rate (74 mm/h), CRP (16.8 mg/dL), procalcitonin level (5.75 ng/mL), D-dimer (3.86 mg/L), severe anemia (hemoglobin 8 g/dL) with a normal mean corpuscular volume (MCV) (90 fL), leukocytosis (20.600/mm³), lymphocytosis (9.690/mm³), elevated serum lactic dehydrogenase level (573 U/L), and indirect bilirubin (6.5 µmol/L). Liver tests also showed high aspartate (48 U/L) and alanine amino-transferase (152 U/L), gamma-glutamyl transferase (275 U/L), and alkaline phosphatase (167 U/L). Both the direct and indirect antiglobulin tests were positive. ANCA directed to elastase (59.5 U/mL) and lysozyme (29.7 U/mL) were detected. Additionally, MSA directed to PmScl75 (+++), PL-7 (+), PL-12 (+++), Mi-2 (+), Ku (+), SRP (+), EJ (+) were also found. Gamma globulin fraction accounted for 27.3% of total serum proteins, but without a peak of paraprotein. In addition, a high serum total IgE (1600 IU/mL), IgG (18.8 g/L), IgM (13.26 g/L), and IgA (4.96 g/L) were measured.

During the in-patient follow up, he experienced an episode of fever up to 39.9 °C. A peripheral blood film examined by the hematologist revealed intra-erythrocytic parasitic ring forms of *Plasmodium vivax* (*P. vivax*) (Figure 1).

The patient was diagnosed with *P. vivax* malaria and transferred to the Clinic for Infective and Tropical Diseases, where he was treated with artesunate/mefloquine and prednisone. The therapy led to a complete clinical recovery.

One month after completion of antimalarial treatment, he remained low positive for ANCA and MSA. Complete immune-serological tests, starting eight months since recovery, until the last follow up, two years later, remained negative (Figure 2).

Discussion

Malaria is a potentially life-threatening parasitic disease caused by the protozoa *Plasmodium* which are transmitted to human population by bites of an infected female *Anopheles* mosquito [3]. Individuals with malaria may present with a wide range of symptoms. Fever and anemia appear after lysis of infected erythrocytes containing schizonts, subsequently releasing merozoites [4]. *P. vivax* is the most geographically widespread human malaria parasite. It may emerge weeks to months after the initial infection. In addition, it may have a hypnozoite form, during

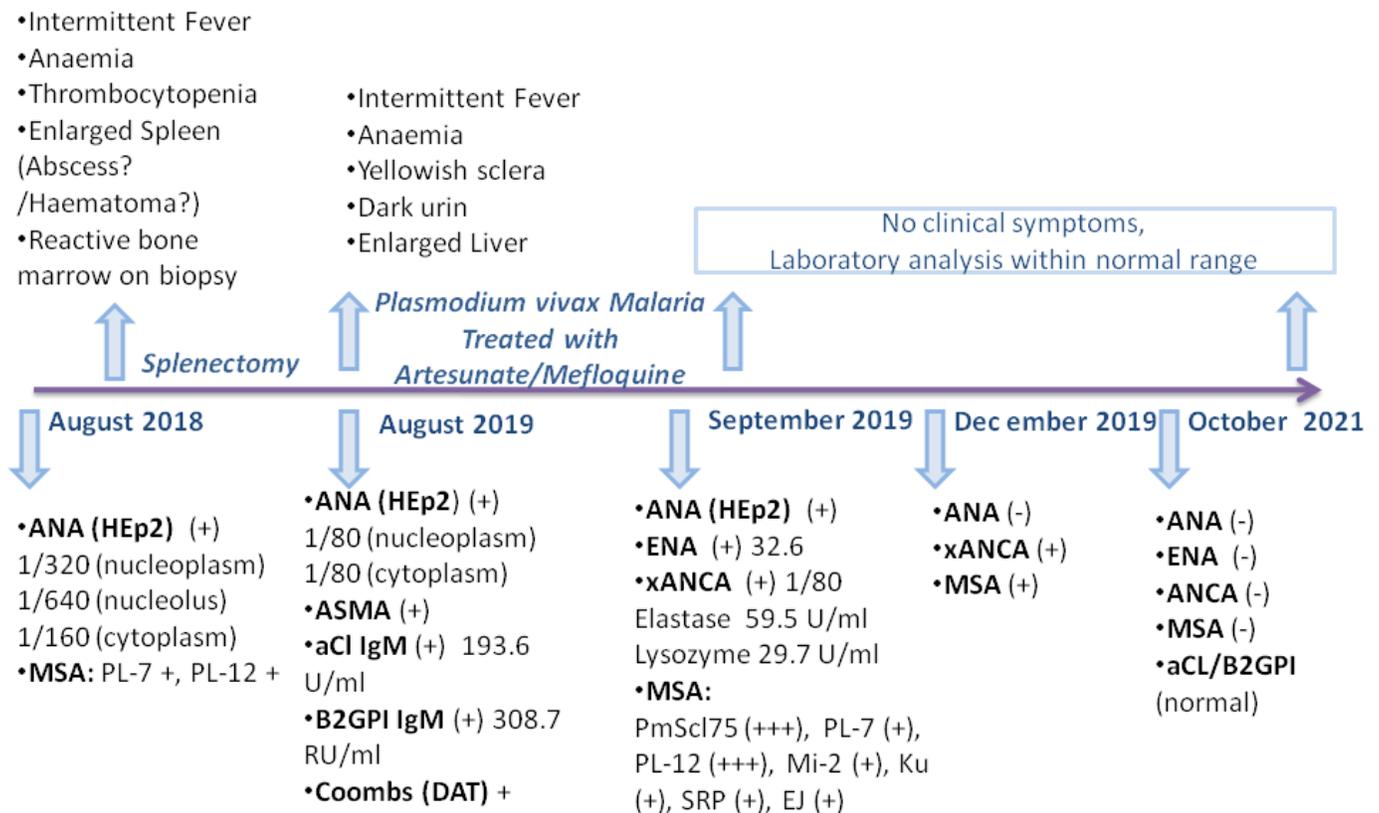
which the parasite exists in the liver for a long time, before recurrence after the initial infection [5]. Recent works have also demonstrated that *P. vivax* preferentially invades reticulocytes in the bone marrow while a substantial proportion of asexual *P. vivax* trophozoites and schizonts may occur in the extravascular spaces such as spleen and liver [6,7]. Consequently, the low density of *P. vivax* in the blood can make them undetectable, creating significant challenges for the diagnosis of infected individuals [5]. The specific lifecycle of *P. vivax* allows sporozoites to develop faster in the mosquito and across a wider range of temperatures in different geographic regions [8]. Interestingly, sporadic cases of locally acquired vivax malaria transmitted by mosquitoes continue to occur. It is considered that the introduction of *P. vivax* into those areas might be caused by travelers, immigrants, or soldiers from malaria-endemic countries [9,10]. Our patient denied travelling to endemic countries; he had received no recent transfusions except those during the

previous hospitalization. Since no cases of imported malaria had been reported in Serbia ever, we can speculate that current infection might have been transmitted from an infected person in the immigrant camp located two kilometers away from the patient's home. However, additional similar cases have not been reported so far.

There is accumulating evidence of autoantibody generation during malaria and other infectious diseases, while their potential pathological role remains insufficiently known [11]. Autoimmune hemolytic anemia, antiphospholipid antibodies, new fluorescence type of ANA, and different ANCA patterns had previously been described in patients suffering from malaria [12–15].

However, to the best of our knowledge, our case is the first report of positivity to multiple MSA during the one-year-long course of unrecognized malaria. This case emphasizes the importance of thorough parasitic workup in patients with a broad range of AAb. The

Figure 2. The patient's disease course and immuno-serological analysis (August 2018- October 2021).



ANA: anti-nuclear antibody (indirect immunofluorescence, HEp2 cell line substrate); AAb: autoantibodies; MSA: myositis specific antibodies; PL-7: anti-threonyl-tRNA synthetase antibodies, PL-12: anti-alanyl-tRNA synthetase antibodies, ASMA: anti smooth muscle antibodies; DAT: direct antiglobulin test; ENA: extractable nuclear antigen antibody; ANCA: anti-neutrophil cytoplasmic antibodies; PmScl75: anti-polymyositis-scleroderma 75 protein, Mi-2: anti Mi-2 antibodies, Ku: anti-Ku DNA helicase complex antibody, SRP: anti-signal recognition particle antibodies, EJ: anti-aminoacyl tRNA synthetase antibodies, aCL: Anticardiolipin IgM; B2GPI: Anti-beta 2 Glycoprotein antibodies.

origin and role of these AAb remain to be clarified. The complete disappearance of AAb after recovery, as seen in our patient, may indicate their putative protective role rather than a contribution to the induction of autoimmunity.

Authors' contributions

MS, ABa and ABo contributed to the conception and design of this report. The case was diagnosed and followed up by MS, ABa, JB, GS, SJ and ABo. MS, ABa, RM, JB, DJ, JL and ABo participated in data collection. MS, ABa and ABo wrote the article. All authors contributed to data interpretation, critically reviewed the article and approved the final draft for submission.

Ethics statement

The work offers a retrospective analysis of diagnostic evaluation and patient's treatment, and does not describe a medical experiment. Additionally, it brings a description of a single clinical case and the opinion of the Institutional Review Board was not required, as per local guidance for publishing case reports.

Informed consent

The patient has given a written informed consent for the publication.

References

- Agmon-Levin N, Damoiseaux J, Kallenberg C, Sack U, Witte T, Herold M, Bossuyt X, Musset L, Cervera R, Plaza-Lopez A, Dias C, Sousa MJ, Radice A, Eriksson C, Hultgren O, Viander M, Khamashta M, Regenass S, Andrade LE, Wiik A, Tincani A, Rönnelid J, Bloch DB, Fritzler MJ, Chan EK, Garcia-De La Torre I, Konstantinov KN, Lahita R, Wilson M, Vainio O, Fabien N, Sinico RA, Meroni P, Shoenfeld Y (2014) International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *Ann Rheum Dis* 73: 17–23. doi: 10.1136/annrheumdis-2013-203863.
- Rivera-Correa J, Rodriguez A (2018) Divergent roles of antiself antibodies during infection. *Trends Immunol* 39: 515–522. doi: 10.1016/j.it.2018.04.003.
- Tijani MK, Lugaajju A, Persson KEM (2021) Naturally acquired antibodies against *Plasmodium falciparum*: friend or foe? *Pathogens* 10: 832. doi: 10.3390/pathogens10070832.
- Siciliano G, Alano P (2015) Enlightening the malaria parasite life cycle: bioluminescent *Plasmodium* in fundamental and applied research. *Front Microbiol* 6: 391. doi: 10.3389/fmicb.2015.00391.
- Angrisano F, Robinson LJ (2022) *Plasmodium vivax* – how hidden reservoirs hinder global malaria elimination. *Parasitol Int* 87: 102526. doi: 10.1016/j.parint.2021.102526.
- Obaldia N, Meibalan E, Sa JM, Ma S, Clark MA, Mejia P, Moraes Barros RR, Otero W, Ferreira MU, Mitchell JR, Milner DA, Huttenhower C, Wirth DF, Duraisingh MT, Wellems TE, Marti M (2018) Bone marrow is a major parasite reservoir in *Plasmodium vivax* infection. *mBio* 9: e00625. doi: 10.1128/mBio.00625-18.
- Kho S, Qotrunnada L, Leonardo L, Andries B, Wardani PAI, Fricot A, Henry B, Hardy D, Margyaningsih NI, Apriyanti D, Puspitasari AM, Prayoga P, Trianty L, Kenangalem E, Chretien F, Brousse V, Safeukui I, Del Portillo HA, Fernandez-Becerra C, Meibalan E, Marti M, Price RN, Woodberry T, Ndour PA, Russell BM, Yeo TW, Minigo G, Noviyanti R, Poespoprodjo JR, Siregar NC, Buffet PA, Anstey NM (2021) Evaluation of splenic accumulation and colocalization of immature reticulocytes and *Plasmodium vivax* in asymptomatic malaria: a prospective human splenectomy study. *PLoS Medicine* 18: e1003632. doi: 10.1371/journal.pmed.1003632.
- Sattabongkot J, Tsuboi T, Zollner GE, Sirichaisinthop J, Cui L (2004) *Plasmodium vivax* transmission: chances for control? *Trends Parasitol* 20:192–198. doi: 10.1016/j.pt.2004.02.001.
- Pastor A, Neely J, Goodfriend D, Leesburg JM, Jenkins S, Woolard D, Pettit D, Gaines D, Sockwell D, Garvey C, Jordan C, Lacey C, DuVernoy T, Roberts D, Robert L, Santos P, Wirtz R, MacArthur J, O'Brien M, Causer L (2002) From the Centers for Disease Control and Prevention. Local transmission of *Plasmodium vivax* malaria –Virginia. *JAMA* 288: 2113–2114.
- Kim MB (2001) Epidemiologic characteristics of malaria in non-malarious area, Jeollabuk-do, Korea in 2000. *Korean J Parasitol* 39: 223–226. doi: 10.3347/kjp.2001.39.3.223.
- Rivera-Correa J, Rodriguez A (2022) Autoantibodies during infectious diseases: lessons from malaria applied to COVID-19 and other infections. *Front Immunol* 13: 938011. doi: 10.3389/fimmu.2022.938011.
- Rivera-Correa J, Rodriguez A (2020) Autoimmune anemia in malaria. *Trends Parasitol* 36: 91–97. doi: 10.1016/j.pt.2019.12.002.
- Gomes LR, Martins YC, Ferreira-da-Cruz MF, Daniel.Ribeiro CT (2014) Autoimmunity, phospholipid-reacting antibodies and malaria immunity. *Lupus* 23: 1295. doi: 10.1177/0961203314546021.
- Hommel B, Charuel JL, Jaureguierry S, Arnaud L, Courtin R, Kassab P, Prendki V, Paris L, Ghillani-Dablin P, Thellier M, Caumes E, Amoura Z, Mazier D, Musset L, Buffet P, Miyara M (2014) Chronic malaria revealed by a new fluorescence pattern on the antinuclear autoantibodies. *PloS One* 9: e8854815. doi: 10.1371/journal.pone.0088548.
- Pradhan V, Badakere SS, Shankarkumar U, Iyer YS, Ghosh K, Karnad D (2002) Anti- neutrophil cytoplasmic antibodies (ANCA) in malaria. *Indian J Malariol* 39: 51-59.

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