

Concurrent juvenile myelomonocytic leukemia with thalassemia in a case with *Plasmodium knowlesi* infection from Sabah, Malaysian Borneo

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Abstract

A 3-year-old male child was presented with worsening abdominal pain, abdominal distension, lethargy, pallor and hepatosplenomegaly. The patient had multiple outpatient visits in the past and was treated with oral antibiotics, oral anthelmintic agents, albeit with minimal benefit. The patient also had non-neutropenic pyrexia spikes and oral ulcers. The patient was an adopted child; hence details about his biological parents' previous history were unclear. Differential diagnosis of Chronic Myelomonocytic Leukemia (CMML), Juvenile Myelomonocytic Leukemia (JMML), Gaucher's disease, Thalassemia and discrete pancreatic pathology was considered. Hemoglobin electrophoresis was indicative of thalassemia. Also, molecular detection method by polymerase chain reaction confirms a concurrent infection with *Plasmodium knowlesi* malaria. The BCR-ABL fusion gene was found to be negative. Correlating with peripheral monocytosis, bone marrow aspiration and trephine biopsy with blasts only 3-4% and hepatosplenomegaly, a diagnosis of JMML was established. We present a rare phenomenon with an overlap of signs and symptoms between JMML, underlying thalassemia, and *Plasmodium knowlesi*, posing a diagnostic challenge to physicians.

Introduction

Juvenile myelomonocytic leukemia (JMML) is a lethal myeloproliferative disease (MPD) of young childhood and is characterized clinically by the overproduction of myelomonocytic cells and by the *in vitro* phenotype of hematopoietic progenitor with hypersensitivity to granulocyte-macrophage-colony-stimulating factor (GM-CSF).^{1,2} In contrast to healthy subjects, the morphological composition of progenitor colonies from JMML patients is predominantly macrophages and monocytes.^{3,4} It is notable, however, that progenitor colonies from JMML patients contain monocytic cells along the full spectrum of differentiation, including blast forms, promonocytes, monocytes, and macrophages. In addition to monocytic cell overproduction, patients are often present with anemia and thrombocytopenia. About 50% of patients also show the presence of elevated fetal hemoglobin, hemoglobin F (HbF). JMML patients can progress to blast crisis, usually with French-American-British (FAB) M4 or M5 morphology, but more frequently succumb to the disease due to tissue infiltration of myeloid cells.^{5,6}

This case represents a rare diagnostic dilemma, as there is a considerable overlap of signs and symptoms between JMML with a hematologic phenotype of thalassemia and *Plasmodium knowlesi* infection. In this report, we describe the findings from a child with the JMML syndrome with thalassemia with concurrent *Plasmodium knowlesi* infection.

Case Report

Clinical findings

A 3-year-old male child presented with complaints of loss of appetite and loss of weight, lethargy, abdominal pain, abdominal distension for six months. The abdominal pain was colicky, with an on and off occurrence. The patient had multiple outpatient visits in the past and was treated with oral antibiotics, oral anthelmintic agents. However, the patient was hospitalized in a district hospital in Sabah, Malaysia due to worsening abdominal pain and gradually increasing abdominal distension. On examination, the patient was lethargic, pale with hepatosplenomegaly and matted cervical, axillary and inguinal lymph nodes. The hemoglobin was low, and stool for occult blood was suspected of being positive. There was no history of blood transfusion.

The patient was brought again to a pri-

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vate medical center in Sabah 3 months later for similar symptoms. Ultrasonography (abdomen) revealed hepatosplenomegaly (spleen 12.6 cm, liver 12.2cm), and focal area at the pancreatic head region with duct at its center. The patient was referred to Sabah Women and Children Hospital (SWACH) for further work up. Differential diagnosis of CMML/JMML, Gaucher's disease, Thalassemia and discrete pancreatic pathology was considered. The patient was an adopted child; hence biological parents' previous history was unclear.

The child started to have multiple fever spikes (37.8-38.5°C, non-neutropenic fever) and was treated with IV Amoxicillin/Clavulanic acid and subsequently with Piperacillin/tazobactam. The total WBC count was 17.6×10^9 cells per liter, and the Absolute Neutrophil Count was 9.9

cells/ μL . Cytomegalovirus IgM/IgG was found to be reactive. However, there was no ocular infection. Chest X-ray showed patchy haziness over the right perihilar region. Simultaneously the patient also suffered from an oral ulcer and was treated with Gengigel (0.2% hyaluronic acid) topical application.

Hematological findings

Peripheral Blood Smear (PBS) examination exhibited mild anemia, moderate microcytic hypochromic, mild anisopoikilocytosis with few polychromes, and few teardrop cells; white cells show moderate leucocytosis, monocytosis and eosinophilia with a presence of 4% Blast cells. Moderate thrombocytopenia was seen. The polymerase chain reaction was positive for *Plasmodium knowlesi*. Iron studies revealed Iron - 3.6 $\mu\text{mol/L}$, Unsaturated Iron Binding Capacity - 61.3 $\mu\text{mol/L}$, Total Iron Binding Capacity - 64.9 $\mu\text{mol/L}$ and serum ferritin - 38.1 ng/ml . LDH was 417 U/L, Coombs' test was found to be negative.

Hemoglobin findings

Hb analysis was conducted to exclude thalassemia/hemoglobinopathy as it is high prevalence in Sabah. Capillary Electrophoresis (CE) of the patient's hemoglobin demonstrated a high percentage of Hb F, and a low percentage of Hb A. High-Performance Liquid Chromatography (HPLC) report revealed a moderate increase in Hb A2 10.2%. Based on the clinical history, CE and HPLC a differential diagnosis of Compound Hb E/B⁺ (or Hb E/B^o) and Heterozygous Hb E with concurrent alpha thalassemia was considered. (Table 1 and Figure 1).

Bone marrow aspiration and trephine biopsy findings

A leukemia-lymphoma immunophenotyping analysis was done to rule out acute leukemia (Figure 2). It revealed a scant population of cells positive for CD34, CD117, and HLA-DR and negative for CD19, CD79a, cyCD3, MPO & other markers tested. Bone Marrow Aspiration and Trephine Biopsy was suggested. BCR - ABL (Breakpoint Cluster Region - Abelson murine leukemia viral oncogene homolog) fusion transcript was not detected in the clinical, hematological analysis.

Peripheral blood smear and bone marrow aspiration (BMA) revealed a leucoerythroblastic picture with bicytopenia and presence of 4% blasts with some dysplastic features. Because of the presence of dysplastic features with monocytosis and clinical hepatosplenomegaly, Myelodysplasia/Myeloproliferative neoplasm (MDS/MPN

– possibly Juvenile Myelomonocytic Leukemia) was considered.

Trephine biopsy depicted markedly reduced megakaryocytes and erythrocytes (E-cadherin and CD61 reduced). There was a presence of granulocytic precursors and mature neutrophils in the intertrabecular spaces – increased mononuclear cells which were myeloperoxidase positive and co-expressing CD68 (active), suggestive of monocytic components. Thus, correlating clinical history with findings of peripheral monocytosis, BMA blasts only 3-4% and hepatosplenomegaly, the features were consistent with Juvenile Myelomonocytic Leukemia.

High-Risk Screening for Inborn Errors of Metabolism performed by derivatization with butanol chloride/LC-MS/MS method revealed low levels of few amino acids probably due to prolonged illness or poor feeding. CT scan of thorax, abdomen-pelvis showed multiple matted cervicals, hilar and mediastinal lymph nodes with hepatosplenomegaly. The skeletal survey did not show any skeletal abnormality.

Discussion

Typical clinical features of JMML include leucocytosis with the presence of early myeloid and monocytic elements, thrombocytopenia, skin rash, and hepatosplenomegaly. Progression of the disease is often rapid, with infiltration of the bone marrow and other tissues with monocytic and myeloid cells.⁷

The nonspecific clinical findings early during this patient's illness made it difficult initially to arrive at a specific diagnosis due to the concurrent occurrence of thalassemia, and *Plasmodium knowlesi* infection. In our patient, as the disease progressed, with the appearance of blast cells (4% blast cell seen) in the bone marrow and characteristic

pattern of trephine biopsy, it became apparent that this syndrome represented JMML. Moreover, Hb analysis was diagnostic of concurrent thalassemia. Certain bacterial and viral infections can cause a response called leukemoid reaction that can resemble the clinical symptoms of JMML. However, during a leukemoid reaction, there is a temporarily elevated white blood cell count in response to an infection or similar trigger. Additional nonspecific findings including fever and splenomegaly may also be present. Since our patient had co-infection with *Plasmodium knowlesi* an initial speculation of a leukemoid reaction stands valid. Niemeyer *et al.* have defined 6 clinical variables (white blood cell count, platelet count, hematopoietic precursors and blasts in peripheral blood, bone marrow blast per-

Table 1. Capillary-electrophoresis findings.

Hb Name	Percentage
Hb A	30.5
Hb F	60.3
Hb E	8.3
Hb A2	0.9

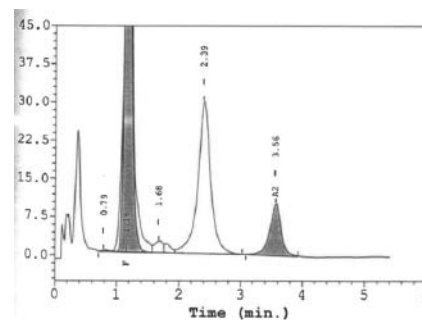
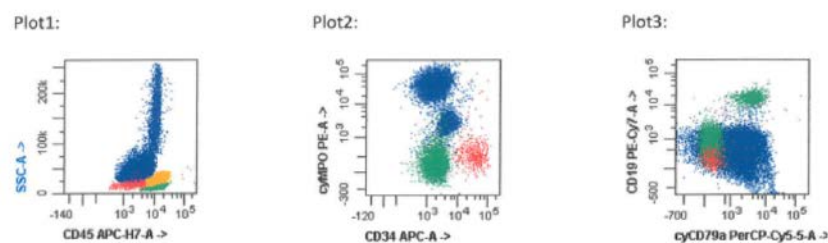


Figure 1. High-performance liquid chromatography (HPLC).



Findings: 2.5-2.7% cell population with dim CD45 and low SSC were gated and analysed. These cells were positive for CD34, CD117, HLA-DR and negative for CD19, CD79a, cyCD3, MPO and other markers tested.

Figure 2. Leukemia-lymphoma immunophenotyping analysis.

centage, spleen size and extramedullary disease) and 3 genetic variables (cytogenetic, molecular and chimerism response) which serve to describe the heterogeneous picture of response to therapy in each individual case, and further evaluation of non-transplant therapy.⁸

Honig *et al.* have described a case of elevated level of Hb F in a child with JMML that appeared to be part of an acquired Cooley's anemia-like hematologic phenotype. However, the diagnosis of JMML was a hematologic phenotype of severe beta-thalassemia.⁹

JMML is categorized as an overlap myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) by the World Health Organization and shares some clinical and molecular features with chronic myelomonocytic leukemia, a similar disease in adults. Insights from cancer predisposition syndromes have led to the discovery of nearly 90% of driver mutations in JMML, all of which thus far converge on the Ras signaling pathway. This has improved the ability of accurate diagnoses, development of molecular markers to measure disease burden and choose therapeutic agents to test in clinical trials.¹⁰

Conclusions

Our case presents a rare phenomenon,

with concurrent co-infection with *Plasmodium knowlesi* in a patient with JMML with thalassemia, which posed a diagnostic dilemma. Clinicians need to be vigilant, as patients of JMML are prone for infections, and should keep in mind the differential diagnosis as there is considerable overlap of signs and symptoms between JMML, and concurrent thalassemia as well as *Plasmodium knowlesi* infection.

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