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Pseudo Eosinophilia: A Role for Automation in Plasmodium Vivax Detection

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Background: A 52-year-old male, Sudanese, diagnosed to have vivax malaria while being investigated for anemia and fever of 3 months duration. Automated eosinophil count turned to be erroneously elevated discordant with manual count which attributed to presence of Plasmodia and its byproducts in circulation suggesting a role for automated complete blood count (CBC) in malaria diagnosis. Because general screening tests like CBC are always undertaken for patients who present with fever. Hematologists should be aware that samples containing malaria may give erroneously high eosinophil counts, thus blood films should be reviewed carefully and the White Blood Cell (WBC) differential count should be confirmed microscopically.

Clinical History and Laboratory Work:

A 52 years old Sudanese male, diabetic, complained of fever, fatigability, productive cough and dyspnea of 3 months duration. On clinical examination, pallor and mild hepatomegaly were detected.

Laboratory investigations included CBC were as follows: white blood cells (WBCs): 4.46 × 109/L (4.0–10.0 × 109/L), with an absolute lymphopenia and eosinophilia; hemoglobin, 8.7 g per dL (14.0–17.0 g/dL) with Hematocrit 27.8 % (36-52), mean corpuscle-cell volume of 78.3 fL (80.0–98.0fL), mean corpuscular hemoglobin of 24.5 pg (27.0–34.0 pg), and mean corpuscular hemoglobin concentration of 31.3% (31.5%–36%); and platelets, 319 × 109/L (150–400 × 109/L). Chemistry revealed normal liver and kidney functions. Iron profile showed decreased serum iron, decreased TIBC and markedly elevated ferritin level 3067 (30-300 ng/mL).

Upon reviewing peripheral blood film with manual differential count, different stages of plasmodia (trophozoite, schizont and gametocyte) were detected (Figure 1: Panels A-H: original magnification×1000) with discordance between automated and manual differential eosinophil counts i.e. automated eosinophil count is higher than manual count by 10% (pseudo eosinophilia). Pseudo-eosinophilia or spurious eosinophilia means a gap of more than 5% in eosinophil counts between automated analyzer and microscopic examination. 1

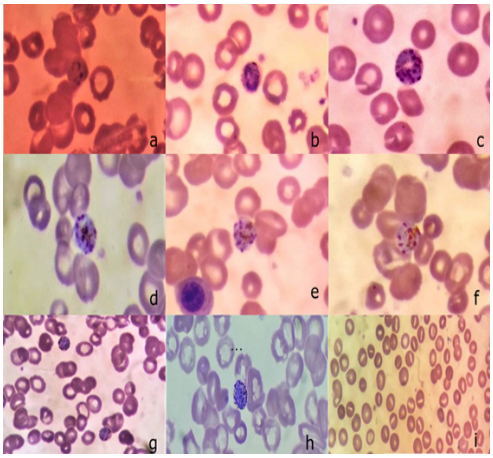

Figure (1): a. ring forms, b. amoeboid trophozoite, c-f. schizont forms, g&h. gametocytes, i. stomatocyte like RBCs

So, malarial infection was diagnosed but what about the species?

First it is not P. Falciparum as the predominant stage in falciparum is ring form and falciparum gametocyte is banana shaped which is not the case in our patient. So, our case is non falciparum malaria but which one? Most features fit to P. Vivax Why?

Because all stages appear with few ring forms, amoeboid trophozoite, large schizont with countable merozoites around a loose cluster of brown pigment, round/oval gametocyte with scattered brown pigment almost fill the RBCs, eroded forms with no cytoplasm, only chromatin and pigment. 2

Plasmodium Vivax, formerly known to cause mild malaria, was later proven to cause severe malaria, although malaria in Sudan is still largely attributed to P. falciparum, P. Vivax has been rising with worrying proportions in Eastern Sudan and spreading to new areas. 3-6

Discussion: Anemia of P. Vivax infection is multifactorial with hemoglobin levels comparable to P. falciparum despite lower parasitemia mainly due to Proportionately greater removal of uninfected RBCs, strong predilection for juvenile RBCs in particular reticulocytes i.e. choking the supply of mature RBCs, bone marrow insufficiency and dyserythropoiesis with marked nuclear abnormalities in erythroblasts. 7 RBCs with stoma like central pallor were observed which suggested autoimmune response against red cell membrane proteins namely Band 3, the major integral RBC membrane which maintain shape and deformability, either due to molecular mimicry with plasmodium vivax or neoantigen formation (Figure 1: Panel I: original magnification ×1000)

Lymphopenia is due to redistribution of lymphocytes from the circulating pool to the marginated pool and the destruction of lymphocytes due to Fas-induced apoptosis. 8

Acute malaria in adults, with no or limited previous exposure to Plasmodium infection, is usually associated with low eosinophil counts, followed by persistent eosinophilia in a proportion of patients after cure. 9

So, what is the cause of spurious eosinophilia?

The trophozoite feeds on Hemoglobin; heme is toxic to the parasite and is therefore aggregated to the insoluble dark-brown crystal called hemozoin. Hemozoin is released by the rupture of parasitized red cells, reaches high concentrations in the circulation, and is actively phagocytized by circulating monocytes and neutrophils. The hemozoin-containing neutrophils show considerable side light scattering because of the birefringent character of hemozoin, subsequently, misclassified as eosinophils. 1,10,11 Figure 2

Another mechanism explaining spurious eosinophilia is that schizonts and gametocytes, resistant to lysis, have a significantly increased amount of nucleic acid higher than that of non-infested RBCs but still be much smaller than that from any nucleated cell. Accordingly, red blood cells or reticulocytes, whether infected or not, will be found in the WDF scattergram in the ghost area, however, infected forms will interfere with separation between the ghost and the eosinophils

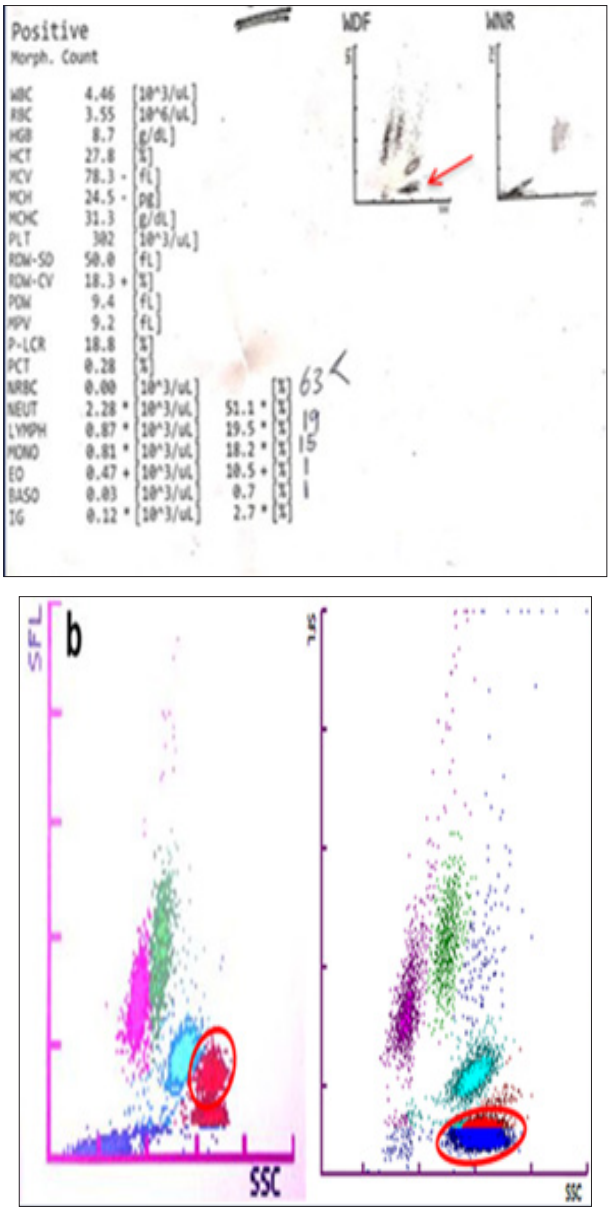

Figure (2): Left: CBC of the patient, the arrow refers to eosinophil area, Right: two possibilities of interference with eosinophil area.11

Then, how automated CBC contributes to malaria diagnosis?

Vivax malaria may show one or more of the following flags:

WBC Abn Scattergram: no clear separation between the ghost and the eosinophils area.

Eosinophilia is a customizable flag and can be helpful in these cases to detect the pseudo-eosinophilia.

Atypical Lympho? is also frequently found, caused by reactive lymphocytes, which might occur during the infection.11

Automation could detect not only presence of plasmodia but also help in determining spp.

Infections with P. falciparum and P. vivax generate different patterns in scattergrams:

- P. falciparum causes abnormal pattern in the RET scattergram.
- P. vivax causes abnormal pattern in the WDF scattergram.
- Automation also offers a new way to assess disease severity and response to therapy:
- Hemozoin containing WBCs seem to be a sensitive indicator of prognosis due to short life span of WBCs.
- Reticulocytes % and low fluorescence reticulocytes % (LFR%) should decrease significantly if the infested RBCs is declining.

Hyperferritinemia indicates Severe inflammatory state. Hemozoin activates the release of various proinflammatory mediators by monocytes/macrophages, including TNF-α, IL-1β and the chemokines MIP-1α and MIP-1β. 12

In conclusion, because it is commonly that CBC is always requested for feverish patients, noticing such abnormalities could help early detection of malaria.

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