WBC histogram pattern contributes to diagnostic screening of malaria infestation

Dr. Parag Dharap¹, Shubham Rastogi², Aaruni Agarwal², Sébastien Raimbault³, Manuela Pastore³, Esther Tournier^{3,}

¹ Dr. Dharap's Diagnostic Centre, Mumbai, 2 Horiba Medical India, New Delhi, ³ Horiba Medical Montpellier, France

INTRODUCTION

With arrival of monsoons, each and every public hospitals as well as private clinical laboratories in India start getting flooded with requests for investigations for fever of unknown origin and specifically for malaria. As the workload is high, there is a need to develop methodologies to effectively screen & filter these samples, so as to help the microscopist. Most of these centers are equipped with three part differential hematology analysers. We would like to present our experience of malaria detection in a private clinical laboratory set up where a large number of complete blood count requests are received specifically to investigate for malaria or as a part of profile for Pyrexia of unknown origin.

AIM

Our study was conducted to assess whether flaggings generated in WBC histograms in three part differential hematology cell counter, can help as an important adjuvant diagnostic tool for malaria screening. The parasitic index was determined and its correlation with the abnormalities found on the Hematology analyzer was also studied.

MATERIALS AND METHODS

Blood samples were collected from patients who presented with requests for investigating specifically for malaria or acute febrile illness during July 2014 and November 2014. All those samples which were found to be Positive for Malaria on a peripheral blood smear stained by Field's staining method, were further analysed & scored for parasitic index, type & species of Malarial parasites per 2000 RBCs. These Malaria Positive blood samples were then processed on Horiba Microsemi CRP hematology analyser, which is a three part differential blood cell counter, within 6 hours of blood collection.

RESULTS

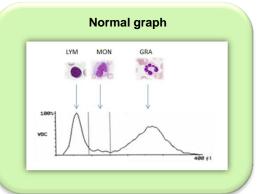
Of the 242 patients who were diagnosed to have Malaria infestation on Field stained blood smears, 91 were found to show L1 flagging on the WBC histogram. A clear & separate peak (\bowtie) was observed between 30 to 100 fl region placed on the ascending limb of a small cell peak (Lymphocyte peak). Other commonest abnormal parameters found were higher MCV & thrombocytopenia.

DISCUSSION

In a three part differential blood cell counter, quantification of WBCs is carried out by Impedance method with the help of dilution of whole blood by a diluents & a lyser. In addition to Red blood cell lysis, action of the lyser causes either stripping of the WBC cytoplasmic membrane as in Lymphocytes or causes extracellular leakage of cytoplasm resulting in collapse of the membrane around the nucleus & the cytoplasmic granules, if present. When the cells pass through the aperture the resultant pulse generated is proportional to the volume of the cell. These generated data are then converted & segregated into small cell volume regions allowing the leucocyte classification: (30 to 100 fl) occupied by lymphocytes, mid cell region (100 - 150 fl) occupied by monocytes & activated lymphocytes and large cell region (150 - 400 fl) occupied by granulocyte. After lysis of RBCs, bared intracellular malarial parasitic forms, being resistant to lytic action generate small pulses while passing through the aperture. These pulses being smaller than the average lymphocytes are then plotted as a separate peak in the small cell region. Other elements such as platelet aggregates & nucleated RBCs have been reported to activate this flag, presence or absence of which can be ruled out on peripheral smear examination.

We believe that in future with improved sensitivity, data characterization & use of differential lyser in a three part differential hematology analyser, a possibility exists of having a flag for Malaria suspicion which can be used as an important adjuvant diagnostic tool by laboratory personnel.

Graphical results



Examples of pathological cases

RESULT	RESULT	RESULT
ND. : 177/200 VALID 10/09/2014 19:58 70.13699-1	NG. : 049/200 VALID 30/09/2014 11:58 ID.16043-1	ND. : 173/200 VALID 10/09/2014 20:41 ID.13905-1
NBC: 9.2 10 ¹ /μ. RBC: 4.11 10 ⁵ /μ. RBC: 15.5 δ/d. RCN: 34.3 L% 5.74 RCN: 83.5 L M ³ 6.7 RCN: 83.6 L M ³ 6.7 RCN: 83.6 L M ³ 7.8 RCN: 83.6 L M ³ 7.8 RCN: 8.6 L M ³ 7.8 RCN: 6.7 μm ³ 7.8 RCN: 11.1 % 7.0 RCN: 16.3 % 6.7 RCN: 28.3 H Mg/L 7.8	NBC: 4.6 10 ¹ /μ_L NBC: 4.41 10 ¹ /μ_L NBC: 34.6 L NCV: 78.4 L m ¹ NCV: 70.4 L m ¹ NCV: 70.7 X ¹ POI: 91.1 10 ¹ /μ_L NV: 7.7 μ ² POI: 91.1 10 ¹ /μ_L POI: 18.5 H % POI: 18.5 H %	₩80: 5.3 10 ¹ /µL Hg0: 14.2 g/dL Hg0: 14.2 g/dL Hc1: 42.2 1 KON: 101.1 H m ² KON: 34.0 H pg KON: 36.6 g/dL HOW: 15.2 H FLT: 140 L 10 ¹ /µL HV1: 6.9 m ² FCT: 0.097 L 1 FOW: 14.4 1 GP: 14.4 1 GP: 5.1 H Hg/L
₩8C Flags L1 DIFF : LIM%T: 21.8 \$ MOM\$T: 21.3 L \$ GRAT: 75.9 \$ LYM#F: 2.0 10 ⁻⁷ /μL MON#F: 0.2 L 10 ⁻⁷ /μL GRAF: 7.0 H 10 ⁻⁷ /μL	WBC Flags L1 DIFF : LIMT: 53.9 H t MONT: 8.3 t DIMA: 37.8 L t LIMT: 2.5 10 ¹ /µL MONA: 0.4 10 ¹ /µL DRAE: 1.7 10 ¹ /µL	WBC Flags L1 DIFF: LYMN1: 13.2 L % MONA1: 4.8 % GRA1: 82.0 H % LYM4: 0.7 L 10 ³ /μL MON4: 4.3 % GRA1: 82.0 H % LYM4: 0.7 L 10 ³ /μL GRA8: 4.3 10 ³ /μL
	VBC 5 100 201 201 201 201	VBC
RBC	RBC	RBC
PLT	PLT	PLT