Case Report: detection of Plasmodium falciparum gametozytes by Sysmex XN analyzers Teresa Villalba Hernandez¹, Jorge Medina Ugarelli¹, Marta Garcia Bernal², Nerea Ramos Gonzalez¹ ¹Catlab, Terrassa, Spain/²HU Mutua Terrassa, Terrassa, Spain



INTRODUCTION: Malaria is a disease caused by parasites transmitted to humans by the bite of infected mosquitoes. It is life-threatening but preventable and curable. It shows geographical predominance in tropical areas with low resources. In 2013 about 200 million cases occurred that caused around 580,000 deaths. An accurate and rapid diagnosis is necessary to begin effective treatment to minimize these risks. Within the diagnostic test, microscopic observation of the parasite in blood smear is very relevant. New analyzers can provide useful parameters that help us to suspect the presence of malaria, it is of high importance to use any available information to enable fast diagnosis and treatment.

METHODS: Case Report: After a 4-months travel to Gambia and a week after her return, a 13-years-old girl presented to her doctor with a 3 days history of mild abdominal pain, fever and dark urines. Initially nor the patient neither the family informed about their recent travel to Africa. Physical examination was unremarkable and diagnostic testing revealed anemia (Hemoglobin 78 g/L, MCV 95 fL) high reticulocyte count (306 x10⁹/L), high LDH and undetectable haptoglobin suggesting hemolysis.

RESULTS: HPLC analysis was performed to exclude haemoglobinopathies giving normal HbA1c value and a normal chromatogram, and, because abnormal lymphocytes flag by the Sysmex XN analyzer, a blood film was reviewed. Some ring forms of plasmodium were observed inside the erythrocytes, sometimes two or three in one. Also banana shaped gametocytes were seen outside the red blood cells (Fig 1) thus a Plasmodium falciparum infection was diagnosed. Questioning the family about international travels they admitted a recent stage in their hometown in Gambia.

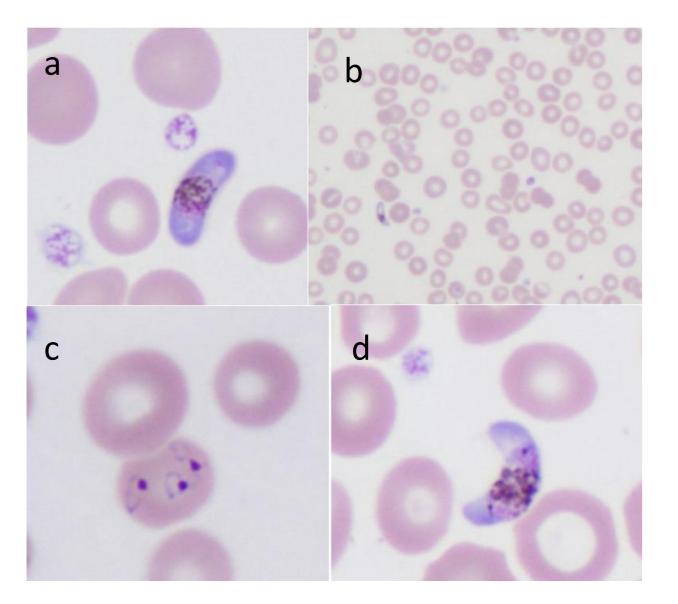


Fig 1: images of parasites in the blood film. MGG stain. Panels a and d: gametozytes of plasmodium falciparum, Panel b: overview, 200x, gametozyte of plasmodium falciparum. Panel c: multiple trophozoites into an erythrocyte.

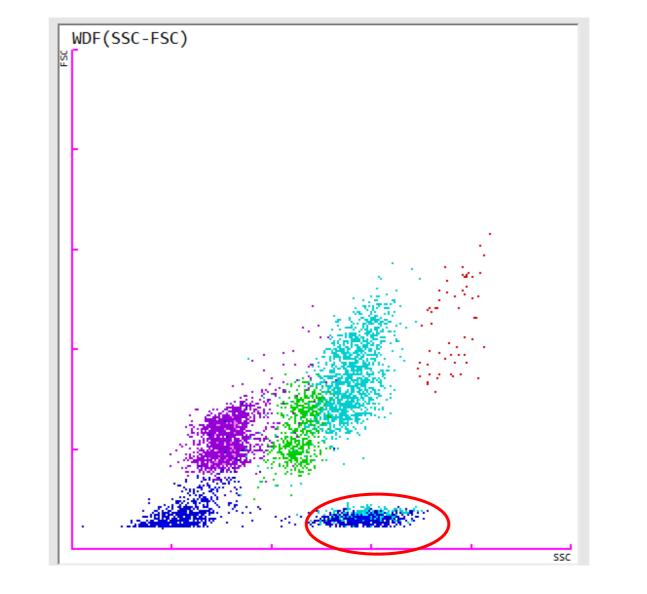


Fig 2: WDF scattergram of the patient, atypical cluster



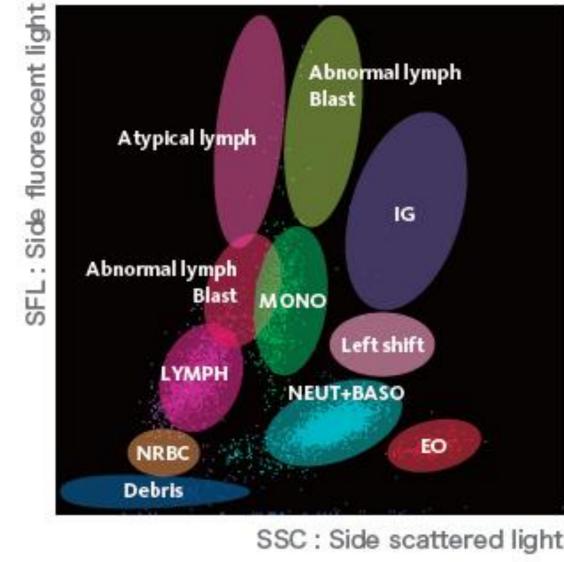


Fig 3: Sysmex XN, WDF scattergram, Leucocyte subpopulations placement.

Checking graphics provided by the Sysmex XN analyzer, a cluster of dots in the WDF graphic was found, below the neutrophils (Fig 2). Fig 3 shows the placement of normal and pathological cell populations on the WDF scattergram. No other sample showed the same pattern. After collection of csv files from analyzers and checking the values of multiple routine and investigational parameters provided (table 1) we found an abnormal low value for the NE-FSC (also named WDF-Z, on the Z axis of the WDF channel), an investigational parameter. For this analysis, data were collected from over 3000 negative samples (without morphology flags or abnormal counts) and 3000 positive and this was the only parameter outside lower and higher limits of this series (Table 2).

Q-Flag(Blasts/Abn Lympho?)	Q-Flag(Blasts?)	Q-Flag(Abn Lympho?)	Q-Flag(Left Shift?)	Q-Flag(Atypical Lympho?)
Q-Flag(RBC	Q-Flag(Turbidity/HGB	Q-Flag(Iron	Q-Flag(HGB	O Elag(Eragmonts2)
Agglutination?)	Interf?)	Deficiency?)	Defect?)	Q-Flag(Fragments?)
Q-Flag(PLT Clumps?)	WBC(10^9/L)	RBC(10^12/L)	HGB(g/dL)	HCT(%)
MCV(fL)	MCH(pg)	MCHC(g/dL)	PLT(10^9/L)	RDW-SD(fL)
RDW-CV(%)	PDW(fL)	MPV(fL)	P-LCR(%)	PCT(%)
NRBC#(10^9/L)	NRBC%(%)	NEUT#(10^9/L)	LYMPH#(10^9/L)	MONO#(10^9/L)
EO#(10^9/L)	BASO#(10^9/L)	NEUT%(%)	LYMPH%(%)	MONO%(%)
EO%(%)	BASO%(%)	IG#(10^9/L)	IG%(%)	[PLT-I(10^9/L)]
[MicroR(%)]	[MacroR(%)]	[TNC(10^9/L)]	[WBC-N(10^9/L)]	[TNC-N(10^9/L)]
[BA-N#(10^9/L)]	[BA-N%(%)]	[WBC-D(10^9/L)]	[TNC-D(10^9/L)]	[NEUT#&(10^9/L)]
[NEUT%&(%)]	[LYMP#&(10^9/L)]	[LYMP%&(%)]	[HFLC#(10^9/L)]	[HFLC%(%)]
[BA-D#(10^9/L)]	[BA-D%(%)]	[NE-SSC(ch)]	[NE-SFL(ch)]	[NE-FSC(ch)]
[LY-X(ch)]	[LY-Y(ch)]	[LY-Z(ch)]	[MO-X(ch)]	[MO-Y(ch)]
[MO-Z(ch)]	[NE-WX]	[NE-WY]	[NE-WZ]	[LY-WX]
[LY-WY]	[LY-WZ]	[LY-WZ/M]	[MO-WX]	[MO-WY]
[MO-WZ]				

Table 1, Sysmex XN,	, analyzed	parameters
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		[NE-FSC(ch)]
total	MIN	43,00
N=6948	MAX	109,50
	MEAN	94,73
	DS	5,39
	CV	5,69
negative	MIN	91,00
N=3338	MAX	109,50
	MEAN	94,73
	DS	2,81
	CV	2,97
positive	MIN	43,00
n=3610	MAX	91,00
	MEAN	86,55
	DS	2,50
	CV	2,89
		[NE-FSC(ch)]
Patient		18,10
Patient, same sample, analyzed +20h		24,00

Table 2, NE-FSC values for controls and patient

For this patient, the NE-FSC value was 18.1 (24 we reanalyzed the same sample 20h later) while the lower limit of this series was 43 (mean value 94.73).

Values under 70 for this parameter were seen in patients (25 in a series of 3610) with severe neutropenia, or "abnormal lymph-blast" and "Basts?" flags.

No other abnormalities as pseudohyperesosinophilia or false reticulocytosis were found. Similar WDF graphics have been published without identification as P. falciparum gametozytes and without analysis of WDF signal values. It could be useful to set a flag related to the NE-FSC low values to help to diagnose P. falciparum malaria.

The patient received Proguanil Atovaquone with good evolution. Samples obtained after 5 and 12 days showed no parasites at the slide review, no clusters at the graphics, and normalized (85) NE-FSC value.

CONCLUSIONS:

- Gametozytes of P. falciparum can be located at a Sysmex XN WDF graphic
- the parameter NE-FSC (WDF-Z) at low values can trigger an alarm to inform suspicion of P. falciparum infection
- -Further studies are required to confirm these findings.

REFERENCES:

- 1. Jain M, Gupta S, Grover RK. Cautions before interpreting scattergram abnormalities on XE-2100 in malaria detection. Int J Lab Hematol. 2015 Aug;37(4):e62-3
- 2.-Campuzano-Zuluaga G, Hänscheid T, Grobusch MP. Automated haematology analysis to diagnose malaria. Malar J. 2010 Nov 30;9:346.

3.- Dubreuil P, Pihet M, Cau S, Croquefer S, Deguigne PA, Godon A, Genevieve F, De Gentile L, Zandecki M. Use of Sysmex XE-2100 and XE-5000 hematology analyzers for the diagnosis of malaria in a nonendemic country (France). Int J Lab Hematol. 2014 Apr;36(2):124-34.

