The utility of a three part differential hematology analyzer with CRP for assessing malaria

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The utility of a three part differential hematology analyzer with CRP for assessing malaria

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Abstract

Background

Hematology analyzers display abnormal hematology parameters during malaria infection providing insightful information for suspecting and assessing malaria infection. The goal of this study is to demonstrate the potential of a three part differential hematology analyzer to assess malaria, provide information about the parasitemia, and discuss the importance of combining CRP with hematology parameters to obtain further information about the malaria infection.

Methods

The present study shows the results of a retrospective study involving comparison of raw instrument data from a three part differential hematology analyzer with CRP measurement and corresponding microscopic findings of samples obtained during the monsoon season of years 2018 and 2019 in Mumbai, India. Mann-Whitney U and Krustal-Wallis tests were applied for obtaining statistical significance of hematology parameters and CRP among P. vivax, P. falciparum, dengue and negative samples.

Results

The study considers 1008 non-malaria febrile cases, 209 P. vivax, 31 P. falciparum positive malaria samples, five cases of mixed species malaria infection, and three co-infection of malaria and dengue. Median values of WBCs, PLTs, PCT and %LYM were lower in malaria and dengue samples compared to negative ones with statistical difference ($p < 0.05$). Contrary, medians of MCHC, MPV, PDW, and %MON were higher than negative samples.

The greatest difference in the analyzer results of malaria and dengue cases was observed in between their medians of CRP levels, which is evidenced by the values of the first quartiles of P. vivax and P. falciparum cases, and the third quartile of dengue cases being 8.6 and 10.4 mg/L, respectively.

The parameters with a statistical difference for different levels of parasitemia were WBC, PLT, %MON and CRP. An interfering abnormal peak was observed in the white blood cell histogram, below 37 fl, in malaria infected samples, especially in P. vivax cases, where the height of that peak showed a strong correlation with red blood cells infected predominantly with larger parasitic forms.

Conclusions

A three differential part hematology analyzer has the potential to not only trigger malaria diagnosis confirmation but also assess the severity of the infection when CRP is considered.

Keywords

Malaria; hematology analyzer; WBC histogram; CRP

Background

According to the WHO an estimated 228 million cases of malaria occurred in 2018 worldwide and 85% of this burden was located in 19 sub-Saharan Africa countries and

India. For a rapid disease management and surveillance of malaria, the WHO recommended two main strategies to eradicate malaria: closing the gaps of healthcare access between urban and rural areas, and diagnosis before antimalarial treatment by microscopy or malaria rapid diagnostic test (RDT) in all patients with malaria suspicion. Fever is the main manifestation for suspicion of malaria infection. In malaria endemic areas, it acts as a trigger for parasitology testing [1].

RDTs are currently replacing microscopy, the golden standard for diagnosing malaria, especially in endemic countries. Thus, complementary information should be considered to ensure correct interpretation and correct disease management particularly in surveillance programs [2]. The two main limitations of RDTs are the impossibility to obtain parasitemia quantification and, particularly in antibody based RDT, the persistent positivity after treatment [2]. Microscopy can overcome both limitations by quantifying the parasite density, however the activity is highly time-consuming.

Complete blood count (CBC) is one of the most frequently requested laboratory tests in patients with acute febrile illness symptoms to confirm infection status and/or severity of disease to initiate proper treatment. For endemic and non-endemic countries, abnormal hematology parameters might provide insightful information for managing malaria infection.

Malaria detection in standard automated cell counters (ACCs)

The potential to diagnose clinically unsuspected cases of malaria for patients subjected to CBC is one of the main benefits of flagging malaria in hematology analyzers [3]. A comprehensive summary of studies for diagnosing malaria by ACCs without a specific malaria-detection design is well described by Campuzano-Zuluaga [3], in which many of the referred studies provide performances to detect malaria related to abnormalities

in the white blood cell (WBC) differentiation channel. ACCs with cytometry capable of differentiating five WBC populations show a clear clustering for malaria population due to specific scattering of infected red blood cells (iRBCs) that are resistant to lyse and/or produce scatters due to residuals of the parasite infection. Pseudoeosinophilia resulting from neutrophils containing haemozoin have been reported as an abnormality caused by malaria infection [4 - 6]. Fourcade et al., demonstrated that large activated monocytes are present on malaria infections by measuring the standard deviation of the volume of LYM and MON calculating a malaria factor with sensitivity of 96.9% and specificity of 82.5% [7]. Many studies have identified a particular cluster by inspecting different angles of light after moderate lysing of RBC without using extra reagents. However the majority of these studies have a lower performance to detect small or immature forms of parasites due to their low lysing resistance and small size. For example, a recent evaluation on a malaria parameter to evaluate the percentage of iRBCs in the BC-6800 (Mindray) showed a lower sensitivity to detect P. falciparum in comparison with detecting P. vivax (24.1% and 88.3%, respectively), and related to it the difficulty to detect small size parasites and their low nucleic acid content [8], which are prevalent in P. falciparum infection where trophozoites and schizonts are barely seen in peripheral blood.

In spite of all promising results from ACCs to identify malaria, fewer studies have been conducted for three differential ACCs. One study combined HGB, TLC, platelets and RDW data in a KX-21 (Sysmex) achieving a 26.1% sensitivity and 96.3% of specificity in a study mainly consisting of P. vivax infected samples [9]. The majority of small ACCs utilize the Coulter-principle to classify WBCs in populations based on size and obtain an RBC distribution using limited reagents. Identifying intracellular presence of malaria

parasite without inspecting the internal content of blood cells based only on abnormal WBC histograms' distribution caused by the infection poses a difficult challenge.

C-reactive protein (CRP) to distinguish malaria

CRP is a member of the pentraxin family of proteins, an acute-phase reactant and a biomarker for inflammation. Although CRP is not a specific biomarker for detecting malaria infection, several studies have identified CRP to be a potential marker to assess the severity of malaria infections [10-13]. A study in the south-western part of the Brazilian Amazon has shown evidence in the association of P. vivax malaria infection with inflammatory activation, including CRP, and cytokine imbalance [14].

Dengue and malaria infections, which frequently co-exist in endemic areas, present with similar symptomatology and produce similar abnormalities in CBC parameters. CRP has been observed to be a useful biomarker to discriminate malaria and dengue, Epelboin et al., using CRP > 5 mg/L as cut off to differentiate between malaria from dengue obtained sensitivity of 99% and specificity of 35% [15]. In addition, effective management and surveillance of malaria require a screening method facilitating quick transfer of patients to receive diagnostic confirmation and proper treatment. POCT hematology analyzers providing information about malaria and other co-existing infections such as dengue could play a major role to support this line of action.

The goal of this study is to demonstrate the potential of a three part differential hematology analyzer to assess malaria, provide information about the parasitemia, and discuss the importance of combining CRP with hematology parameters to obtain further information about the malaria infection.

Methods

The present study shows the results of a retrospective study involving comparison of raw instrument data from a three part differential hematology analyzer with CRP

measurement (LC-667G CRP, HORIBA) and corresponding microscopic findings of the patients visiting at Dr. Dharap's Diagnostic Centre, Mumbai, India from samples obtained in the monsoon season of years 2018 and 2019.

During monsoon season, the laboratory routinely caters to patients for diagnostic testing in case of acute febrile illness which comprises a CBC, peripheral examination of blood smear for malarial parasite and rapid screening tests for malaria and dengue NS1 being an endemic area with co-existent infections. Documented historical data of known malaria, dengue positive and negative cases was used for the study. Persons for routine health check-up without history of any specific ailments visiting the laboratory during the corresponding periods were considered for normal or control group.

For the current study, the historical documented data was further anonymized in coded fashion in order to protect patient confidentiality in accordance with local ethical or IRB guidelines, according to national and international standards for the conduct of clinical studies including 21 CFR Parts 50 and 56 and International Conference on Harmonization (ICH) E6-Good Clinical Practice Consolidated Guideline. Ethical clearance was not obtained as study was carried as a retrospective review of the available raw instrument and historical patient data, without any collection of extra specimen or monetary charges to patient and did not involve any communication of results of the study to the clinician so as to affect the diagnostic and therapeutic management of the patients.

Laboratory techniques

Testing for 'Acute Febrile Illness' in the laboratory comprises of a full blood count, peripheral examination of blood smear for malarial parasite and rapid screening tests for malaria and dengue NS1 being an endemic area with co-existent infections.

Full blood count is performed using a three part differential hematology analyzer (LC-667G CRP, HORIBA) which additionally provides CRP level estimation. For peripheral blood smear examination the following technique is used. Anticoagulated (K2-EDTA) blood of patient is used to prepare thick & thin blood smear for peripheral blood smear examination (PBSE). Both types of smear preparation are air dried. Thin preparation is fixed with methanol whereas thick blood smear are subjected to 'process of de-hemoglobinisation' with deionized water. Both smears are then stained by 'Field staining technique' using automated slide stainer. Thin smears are examined for presence of malarial parasite and species identification, presence of platelet aggregates, macroplatelets and nucleated red blood cells, if any.

Malaria cases are further assessed for parasite density by counting parasite life cycle forms against 100 white blood cells by an experienced pathologist. The life cycle forms are grouped as small forms being ring and early ameboid, and large forms including late ameboid with ample parasitic material, schizonts and gametocytes.

For additional screening and confirmation of malaria cases, Rapid Malaria Antigen detection test kits manufactured by SD Biosensor Healthcare Pvt. Ltd., Gurugram, India using monoclonal anti-P. falciparum HRP-II (0.75+/- 0.15 µg) with monoclonal anti-P. vivax pLDH (0.75+/- 0.15 µg) respectively to detect presence of P. falciparum & P. vivax related antigen is used.

For screening & diagnosis of dengue, RecombiLISA NS1 Antigen test utilizing pairs of specific polyclonal & monoclonal anti-dengue antibodies of all four serotypes (DEN1, 2, 3, 4) and analytical sensitivity of 0.3 ng/ml for type 2 NS1 antigen, manufactured by CTK Biotech, Inc., United States of America is used.

Statistical analysis

To determine the significance of the differences between the hematology parameters extracted from LC-667G CRP of positive & negative samples of P. vivax & P. falciparum samples respectively, as well as malaria & dengue positive samples, Mann-Whitney U and Krustal-Wallis tests were applied due to their non-parametric distribution.
All the statistical calculations were done using Minitab 19.1.1. and histogram graphs were created using R software 3.5.1.

Results

From the monsoon seasons of 2018 and 2019, based on selection criteria and availability of corresponding data, we selected 209 (144 males, 65 females) P. vivax positive cases, 31 (22 males, 9 females) P. falciparum samples, five cases of mixed species malaria infection (4 males, 1 female), and three co-infection of malaria and dengue (2 males, 1 female). In addition, 1008 non-malaria febrile cases were examined including 197 (124 males, 73 females) dengue positive cases. The median and range of age were 35 years (5-87) and 36 years (15-77) for only P. vivax and P. falciparum cases, respectively.
Using Krustal-Wallis test to compare the four groups (see table 1), median values of WBCs, PLTs, PCT and %LYM were lower in malaria and dengue samples compared to negative ones with a statistical difference ($p < 0.05$). Contrary, the median values of MCHC, MPV, PDW, and %MON were higher than negative samples. RBC, HGB, HCT, MCV, MCH and %GRA showed no significant difference in median between the four groups.
The medians of the parameters that had a significant difference between P. vivax infected and negative samples were WBCs, MCH, MCHC, RDW, PLTs, MPV, PCT, PDW and CRP. Similarly P. falciparum cases had the same parameters with significant difference excepting MCH, MCHC and RDW.

Comparing both malaria infected cases with dengue, only PLT, PCT and CRP medians had a significant difference, which makes difficult to differentiate between both diseases when observing solely the hematology parameters affection. The greatest difference of median between malaria infected patients and dengue was observed with CRP. Figure 1 shows the first quartiles of P. vivax and P. falciparum to have a difference with the third quartile of dengue patients of 8.6 and 10.4 mg/L, respectively.

In the current set of data only %LYM ($p = 0.029$) showed a significant difference between P. Vivax and P. Falciparum samples.

	P. vivax (n = 209) Median (IQR)	P. falciparum (31) Median (IQR)	Dengue (n=197) Median (IQR)	Patients with fever but Non-malaria non-dengue (n=811) Median (IQR)	p_{KW}	p_{VF}	p_{VD}	p_{FD}	p_{VN}	p_{FN}
WBC ($10^3/\mu L$)	5.4 (4.1 - 6.6)	4.7 (3.8 - 5.5)	4.4 (3.4 - 6)	6.6 (5.1 - 8.5)	< 0.001	0.056	< 0.001	0.404	< 0.001	< 0.001
RBC ($10^6/\mu L$)	4.67 (4.3 - 5.15)	4.5 (4.09 - 4.89)	4.74 (4.4 - 5.18)	4.73 (4.36 - 5.09)	0.19	0.255	0.437	0.199	0.509	0.119
HGB (g/dL)	13.5 (12.32 - 14.67)	12.7 (11.8 - 14.4)	13.4 (12.5 - 14.6)	13.3 (11.95 - 14.8)	0.24	0.082	0.894	0.114	0.203	0.206
HCT (%)	40.05 (36.32 - 43.4)	37.7 (35.8 - 42.4)	40.3 (37.3 - 43.5)	39.7 (35.72 - 43.87)	0.318	0.136	0.436	0.053	0.601	0.187
MCV (μm^3)	86.05 (82 - 89.37)	84.2 (74.6 - 89.9)	84.8 (81.1 - 89)	85.4 (80.6 - 89.8)	0.437	0.148	0.667	0.309	0.358	0.335
MCH (pg)	29.15 (27.5 - 30.47)	28.7 (25.9 - 30.9)	28.6 (27.1 - 30.1)	28.6 (26.75 - 30.3)	0.123	0.185	0.139	0.669	0.04	0.593
MCHC (g/dL)	33.80 (33.2 - 34.75)	33.80 (33 - 34.4)	33.35 (33.1 - 33.9)	33.50 (32.9 - 33.9)	< 0.001	0.461	<0.001	0.115	< 0.001	0.238
RDW (%)	14.45 (13.8 - 15.1)	15 (13.6 - 15.6)	14.5 (14.1 - 15.2)	14.7 (14.2 - 15.4)	< 0.001	0.333	0.152	0.09	< 0.001	0.698
PLT ($10^3/\mu L$)	102.5 (66.25 - 149)	109 (79 - 140)	174 (136 - 219)	223 (174 - 274)	< 0.001	0.991	<0.001	<0.001	< 0.001	< 0.001
MPV (μm^3)	8.4 (7.9 - 9)	8.6 (7.8 - 9.3)	8.2 (7.7 - 8.8)	8.1 (7.6 - 8.6)	< 0.001	0.168	0.732	0.14	< 0.001	0.001
PCT (%)	0.0865 (0.056 - 0.119)	0.088 (0.063 - 0.113)	0.144 (0.118 - 0.175)	0.179 (0.143 - 0.217)	< 0.001	0.769	<0.001	<0.001	< 0.001	< 0.001
PDW (%)	15.35 (13.9 - 17.3)	16.9 (13.8 - 19.4)	15 (14 - 16.4)	14.6 (13.7 - 15.9)	< 0.001	0.274	0.122	0.102	< 0.001	0.023
%LYM	25.9 (20.4 - 34.2)	22.8 (18.5 - 27)	23.4 (16.75 - 31.22)	26.1 (18 - 35.3)	0.016	0.029	0.015	0.919	0.406	0.117
%MON	5.6 (4 - 7.3)	5.8 (4.4 - 7.2)	5.5 (4.2 - 7.2)	5.2 (4 - 6.8)	0.04	0.658	0.217	0.636	0.055	0.156
%GRA	68 (59.1 - 73.8)	71.2 (66.3 - 77.2)	70.35 (61.55 - 79.25)	68.4 (58.32 - 77.6)	0.071	0.061	0.053	0.876	0.257	0.236
CRP (mg/L)	67.4 (35.7 - 111.2)	81.4 (37.5 - 122.3)	9.7 (3.3 - 27.1)	10.4 (2.9 - 27.9)	< 0.001	0.455	< 0.001	< 0.001	< 0.001	< 0.001

Table 1. Hematology parameters statistics of the LC-667G of data sets 2018 and 2019.

p-value of less than 0.05 was considered statistically significant

* p_{KW}, p_{VF}, p_{VD}, p_{FD}, p_{VN}, and p_{FN} *corresponds to the p-value of Kruskal-Wallis test, PV vs PF, PV vs dengue, PF vs dengue, PV vs negative, and PF vs negative, respectively*

Co-infections of malaria and dengue or PV and PF were not contemplated

Insert here Figure 1

Parasitemia association with different hematology parameters

To inspect the affection of the hematology parameters regarding the parasite density observed in peripheral blood, only the data-set of P. vivax samples in the current study has enough data variation to provide interesting results. The distribution of parasitemia in P. falciparum samples of the present study is limited, more than 60% of the cases have less than 0.2% of parasitemia, thus the analysis of associating parasitemia with the impact on hematology parameters was performed solely for the P. vivax set of data. The parasitemia percentage establishes the relation of the number of parasites found in thin smear against the total count of RBCs and it was calculated with the following formula:

$$\%\text{Parasitemia} = \frac{\frac{\#Parasites}{100WBC}\left(\frac{\#WBC}{\mu L}\right)}{\frac{\#RBC}{\mu L}} \text{x}100 \ldots \text{Eq. } 1$$

The total counts of WBCs and RBCs were retrieved from the hematology analyzer.

The parasitemia ranges from 0.00005% to 2.15% and no cases of high parasitemia were found (>5% in low intensity transmission areas). Table 2 summarizes the medians of different hematology parameters provided by the LC-667G in relation with three divisions of parasitemia (< 0.5%, 0.5% < parasitemia < 1%, > 1%). The parameters with a statistical difference in the median were WBC ($p = 0.001$), PLT ($p = 0.022$), %MON ($p < 0.001$) and CRP ($p < 0.001$). A negative slope in the significant different medians was found in WBC, PLT, PCT and %MON, whereas CRP increases with its parasite load.

Figure 2 shows the association of CRP value with a more extended parasitemia classification where the median of each population has a positive correlation with the parasite load, however all groups have a wide variation (CV>20% in all groups).

Parameter	paras < 0.5 (n = 156)	0.5 <= paras < 1 (n=28)	paras >= 1 (n=14)	p-value
WBC (10^3/µL)	5.10	6.55	6.40	0.001
RBC (10^6/µL)	4.66	4.75	4.53	0.52
HGB (g/dL)	13.60	13.05	12.85	0.262
HCT (%)	40.30	37.95	38.40	0.341
MCV (µm^3)	86.10	85.80	84.55	0.723
MCH (pg)	29.20	28.90	28.60	0.663
MCHC (g/dL)	33.75	33.75	33.75	0.757
RDW (%)	14.55	14.55	14.65	0.366
PLT (10^3/µL)	104.0	81.0	72.5	0.022
MPV (µm^3)	8.40	8.55	8.35	0.49
PCT (%)	0.0875	0.0765	0.0685	0.05
PDW (%)	15.20	15.75	15.85	0.172
%LYM	25.75	31.05	31.10	0.065
%MON	6.1	4.2	4.0	<0.001
%GRA	68.65	64.50	63.50	0.49
CRP (mg/L)	61.50	98.40	116.65	<0.001

Table 2. Medians and statistical significance of hematology parameters in association to parasitemia of P. vivax samples. *Data set from years 2018 and 2019. Kruskal – Wallis, p-value of less than 0.05 was considered statistically significant.*

**10 samples from the original data set were incomplete on parasites counting, therefore they were not contemplated for the calculation of table 2 results*

Insert figure 2

For correctly associating the CRP with the affection of malaria infection, a consideration on the age dependency has to be considered. Figure 3 shows the CRP association separated in different age groups of P. vivax samples with parasitemia less than 0.2%.

Insert figure 3

WBC histogram interference in the RBC ghosting area

In the LC-667G CRP HORIBA analyzer, an abnormal peak located approximately at the 37fL channel in the WBC histogram appears mainly in malaria P. vivax samples with high density of large forms of parasites (see figure 4). Using the parasite density information from datasets 2018 and 2019 of P. vivax samples, a statistically significant multiple linear regression model ($p < 0.001$) was calculated obtaining the following equation $y = 1229 + 7.771x10^{-3}\alpha_1 + 48.1x10^{-3}\alpha_2$ (see figure 5), where y represents the events per µL from 19.3fL to 43.9fL in the WBC histogram (eq. 2), α_1 the coefficient related to the small forms parasite density and α_2 to the parasite density of large forms. The contribution of large forms is approximately 6.2 times bigger than the contribution of small forms(α_2/α_1) and the percentage of variation of the model is 74.43%. Additionally, the small and large forms of parasitemia showed a small multicollinearity (VIF = 1.05). For the case of positive P. falciparum multiple regression model, we could not conclude a statistically significant association between the large forms parasite density and the events in the above mentioned range of the WBC histogram ($p = 0.142$).

Insert figure 4

Insert figure 5

$$Events\ per\ \mu l\ from\ 19.3\text{fL to } 43.9\text{fL} = \frac{Sum\ events\ 19.3\text{fL to } 43.9\text{fL}}{Total\ events\ of\ WBC\ hist} x(\#WBCx10^3) \quad [\frac{events}{\mu L}] \ldots \text{Eq. 2}$$

Large forms of P. falciparum samples are barely seen in peripheral blood due to sequestration in the spleen, thus interference in the RBC ghosting area of the WBC

histogram will be smaller than in P. vivax samples were large forms of parasites remains in peripheral blood, compare subplots of figure 4. In the analyzed set of data, different interferences non-related to parasite inclusion located in the 37-fL area are displayed in figure 4, these interferences include platelet aggregations, macro platelets, and nRBCs.

Discussion

The most common reported affectations on hematology parameters observed in malaria infection are thrombocytopenia, anemia and leucopenia [16, 17]. Thrombocytopenia in malaria infection occurs due to complex platelet roles and abnormalities, including enabling cytoadhesion of iRBCs to endothelial cells, clumping and killing iRBCs [18], coagulation disturbances, bone marrow alterations, antibody-mediated platelet destruction, systemic platelet activation, vascular pooling and oxidative stress [19]. Moreover, in hematological analyzers, PLTs complexed with RBCs are not identified, therefore complexed particles would lead to an apparent platelet loss [20]. In the present study, thrombocytopenia ($< 150\ 10^3/\mu L$) was predominantly observed in malaria P. falciparum cases (83.87%) than P. vivax (76.44%), which is similar to the observations reported in [21, 22]. Authors in [20] have concluded that in malaria cases, in addition to type of specie, the severity of thrombocytopenia is also affected by parasite density. In present study, similar association with parasitemia ($p = 0.022$) is seen in P. vivax infection with thrombocytopenia cases. While understanding thrombocytopenia dependency on malaria specie, it must be analyzed considering the parasite load and patient condition, as pointed out in a study where parasitemia was significantly higher in individuals with thrombocytopenia only in P. vivax and not in P. falciparum infections [23].

Anemia caused by malaria infection has a multi-factorial and complex pathogenesis that is quite pronounced in P. falciparum, contrary to P. vivax which target mainly young RBCs [24]. In the present analysis, although P. falciparum medians of RBC counting, HGB and HCT were lesser than the rest of the inspected groups (see table 1), no statistical significant difference was found in parameters concerning anemia affectation which might be related to the non-severe parasite invasion and small parasitemia variability of P. falciparum current data.

In study performed using a five differential part ACC in Thailand, Tak Province the neutrophil percentage, RBC count, MCV, MCH, MCHC and platelets count were found to be significantly different between P. falciparum and P. vivax species [25]. In contrast, in the current study, none of the above-mentioned parameters were significantly different between malaria species excepting the leukocyte count ($p = 0.029$), which most probably is caused by the interference of the WBC channel by the large forms of P. vivax infection compared to P. falciparum samples.

Among the hematology parameters with significant statistical difference in medians between malaria positive and negative samples, platelets related parameters, especially MPV, should be further analyzed due to the strong affection with the elapsed time between collection and measurement of sample. Unfortunately, due to the retrospective nature of the study, the exact amount of elapsed time from collection to measurement is not available.

Interference in the WBC channel and association with parasitemia

In both three and five part differential ACCs a peak around the RBC ghosting area in the WBC histogram has been identified as one of the main abnormalities to predict malaria infection [26 - 29]. Briggs et al., observed this peak before the 35-fL threshold at WBC histograms with malaria infection and used it for improving the specificity to detect

malaria in false-positives results coming from other infections [26]. In the present study, the number of events within this abnormal peak were correlated with the parasite density of P. vivax large forms, supporting the same observation of moderate correlation (r = 0.79) in [28]. We concluded the abnormal peak under malaria infection is caused mainly by the lysing-resistant large forms of parasites, regardless on the specie, and probably affected by the presence of PLT aggregation and/or macro PLTs. The abnormal peak is less often observed in P. falciparum infections as the larger forms are sequestered by the spleen. Mature trophozoites and schizonts in P. falciparum infection are barely present in peripheral blood samples, consequently according to [30] the presence of schizontaemia, especially in P. falciparum infections, can be used as a marker for disease severity. Therefore a semi-quantitative estimation of schizontaemia can be inferred from the events before the 37fL channel of the WBC histogram from the LC-667G and might help to assess the severity of malaria infections. The abnormal peak in WBC histogram is not exclusively due to malaria iRBCs. Common interferences include RBC lysed debris, PLT aggregations, macro-PLTs, nRBCs, and others. Researchers used this abnormal peak along with two other parameters of the LH780 (Beckman Coulter) to identify malaria infection having an 83.1% specificity but failed to discriminate from samples with nRBCs [28]. We feel that the distribution pattern of the abnormal peak might provide a way to differentiate the exact cause of it. We observed that PLT aggregations or macro PLTs show a bigger/wider distribution due to the random nature of formation, whereas gametocytes, schizonts and late stages of trophozoites show a narrower size distribution having a smaller dispersion close to the abnormal peak area.

A parameter to increase the specificity of detecting malaria and understand better the parasite density: CRP

In low cost ACCs where the Coulter principle is the only technology responsible of differentiating the WBC population into three parts based on volume, the identification of malaria or dengue infected samples is rather difficult due to the impossibility of differentiating granulocytes populations and to distinguishing interferences of lysing resistant particles in the WBC channel. Addition of CRP increases the specificity of detecting malaria due to the inflammatory response secondary to parasite infection. Some studies have positively correlated the parasite density of malaria infected samples with CRP levels [31-34], even in afebrile malaria patients [34]. CRP has been proposed for assessing the severity of malaria infection and for following-up response to treatments [35]. However in the present study, a direct correlation between the CRP value and the parasitemia was not found probably due to the non-specific CRP response and the debated age dependency [36, 37]. Only when grouping the parasitemia in different ranges, a positive association between parasitemia in P. vivax samples and the CRP median was observed (see figure 2). Similar to results of [11], in this study no significant difference of CRP value was observed between P. vivax and P. falciparum. Dengue and malaria in many countries are co-endemic diseases that present similar clinical presentation. The importance of distinguishing them has been reported by [38], who developed a tree decision model for discriminating malaria and dengue infection. Dengue samples collected in the present study had a lower counting of WBCs and MCHC in comparison to both malaria species supporting observations of [38], however only the difference in median with P. vivax samples was statistically significant. In contrast, PLT medians of both species of malaria infections were lower than dengue cases ($p<0.001$). Discrepancy of results with [38] might be related to the severity of each disease. For example, the hematology parameters affection in dengue infections is

greatly dependent on day of fever. Unfortunately current data has not any information about the fever progression of dengue infected individuals.

By far, the CRP parameter showed the most significant difference between malaria and dengue. Although CRP is not a specific biomarker for malaria, the response of CRP to virus infections is rather small making a good parameter to understand the etiology of the infection and help to differentiate malaria and dengue from similar hematology parameters. Nevertheless, dengue and other non-viral infections can co-exist in a single sample, thus relying only in CRP should not be an exclusive criteria to differentiate both diseases.

Conclusions

The LC-667G CRP, a three differential part hematology analyzer has the potential to not only trigger malaria diagnosis confirmation but also assess the severity of the infection due to simultaneous CRP estimation. The current study suggests that CRP might be helpful for distinguishing malaria from dengue, and for evaluating the severity of the parasite load. In addition to CRP, the abnormal peak located around the 37fL channel in the WBC histogram can provide a good estimation of the number of large parasites present during the infection, however the calculation might be interfered by other particles such as nRBCs and PLT aggregations. The combination of providing hematology parameters plus CRP value in a hematology analyzer makes a preferred method for screening malaria on routinely basis of any infection suspicion.

Current data-set is limited by the number of P. falciparum samples, the discussion and observations provided were focused on P. vivax positive cases. Further research following this study should be oriented to measure P. falciparum samples, evaluate the potential of using hematology analyzers to provide additional information to the RDTs, and monitor the anti-malaria drugs treatment.

Abbreviations

WHO: World Health Organization

RDT: rapid diagnostic test

ACC: automated cell counter

CBC: complete blood count

WBC: white blood cell

LYM: lymphocytes

MON: monocytes

RBC: red blood cell

iRBC: infected red blood cell

HGB: hemoglobin

TLC: total lymphocyte count

RDW: red cell distribution width

CRP: C-reactive protein

POCT: point of care testing

PLT: platelet

PCT: plateletcrit

MCHC: mean corpuscular hemoglobin concentration

MPV: mean platelet volume

PDW: platelet distribution width

HCT: hematocrit

MCV: mean corpuscular volume

MCH: mean corpuscular hemoglobin

GRA: granulocytes

CV: Coefficient of variation

VIF: variance inflation factor

nRBC: nucleic red blood cell

Declarations

Ethics approval and consent to participate

For the current study, the historical documented data was further anonymized in coded fashion in order to protect patient confidentiality in accordance with local ethical or IRB guidelines, according to national and international standards for the conduct of clinical studies including 21 CFR Parts 50 and 56 and International Conference on Harmonization (ICH) E6-Good Clinical Practice Consolidated Guideline. Ethical clearance was not obtained as study was carried as a retrospective review of the available raw instrument and historical patient data, without any collection of extra specimen or monetary charges to patient and did not involve any communication of results of the study to the clinician so as to affect the diagnostic and therapeutic management of the patients.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

JN and SR are employees of HORIBA Ltd. PD is an external consultant of HORIBA Ltd.

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The study was funded by HORIBA Ltd. The funder designed the study, provided all the consumables for the hematology analyzer and helped with the analysis and interpretation of the hematology analyzer results.

Authors' contributions

PD, SR and JN designed the study. JN drafted the manuscript and performed the data analysis. PD collected all samples, performed the smear inspections and clinical observations. SR and PD reviewed and corrected the manuscript. All authors read and approved the final manuscript.

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Authors' information (optional)

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Figure Legends

Figure 1. Box plot of CRP values on different groups. *Dengue (D), malaria and dengue (MD), non-malaria-dengue samples (N), P. falciparum (PF), P. vivax (PV) and the combination of both malaria species (PV + PF) distributions are represented with their corresponding median value and outliers.*

Figure 2. Box plot of CRP in association with parasitemia of P. vivax samples. *All individual points are displayed in the box plots*

Figure 3. Box plot of CRP in association with age of P. vivax samples with parasitemia less than 0.2%. *All individual points are displayed in the box plots*

Figure 4. WBC histograms of 2019 data-set with interferences around 37-fL. *Bold lines represent the median of the histograms.*

Figure 5. Multiple linear regression model between the events before 43.9fL and parasite density. *The vertical axis corresponds to the number of events from 19.3fL to 43.9fL in the WBC histogram and the horizontal axis to the parasite density of small and large forms of samples infected by malaria P. vivax. The regression model shows that the contribution of the linearity is mainly provided by large forms of P. vivax malaria infection.*

Figures

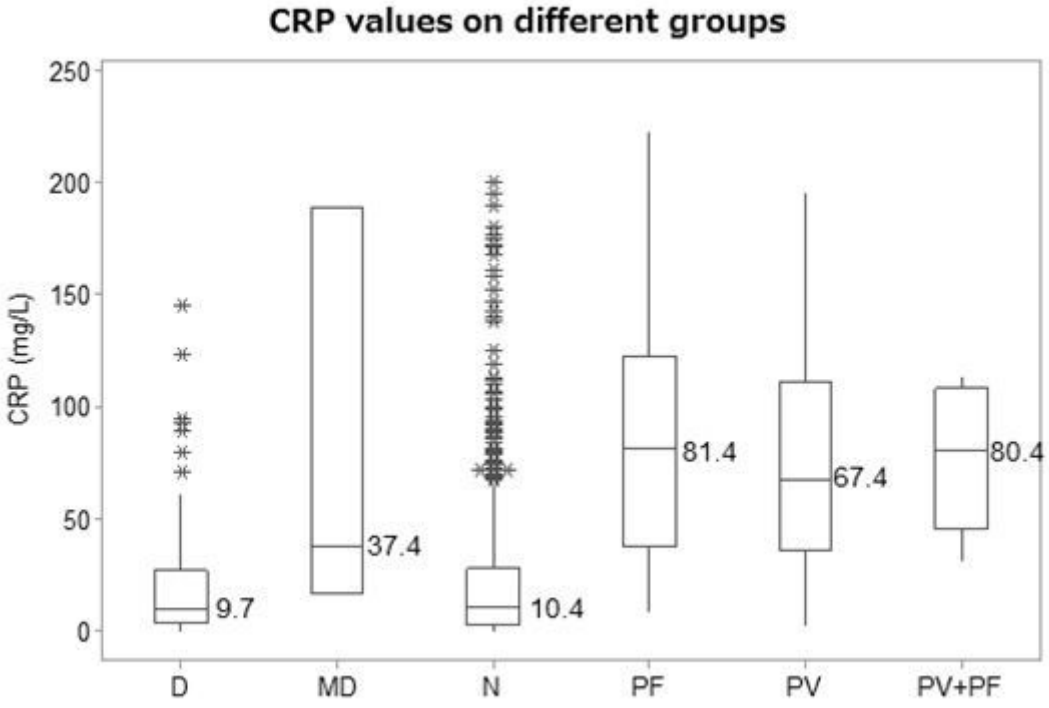


Figure 1

Box plot of CRP values on different groups. Dengue (D), malaria and dengue (MD), non-malaria-dengue samples (N), P. falciparum (PF), P. vivax (PV) and the combination of both malaria species (PV + PF) distributions are represented with their corresponding median value and outliers.

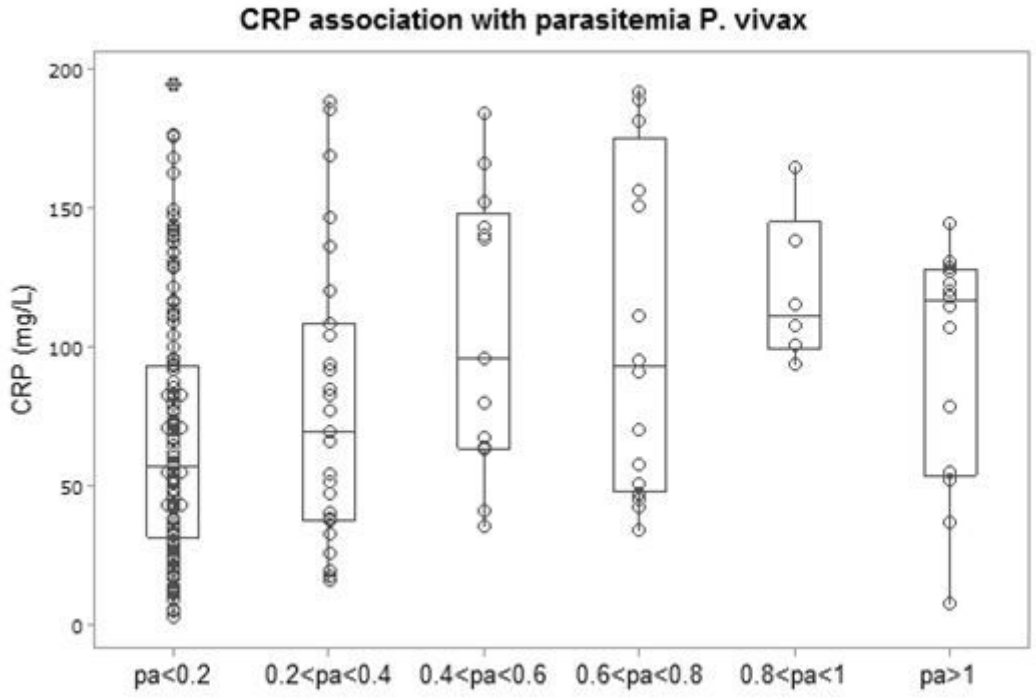


Figure 2

Box plot of CRP in association with parasitemia of P. vivax samples. All individual points are displayed in the box plots

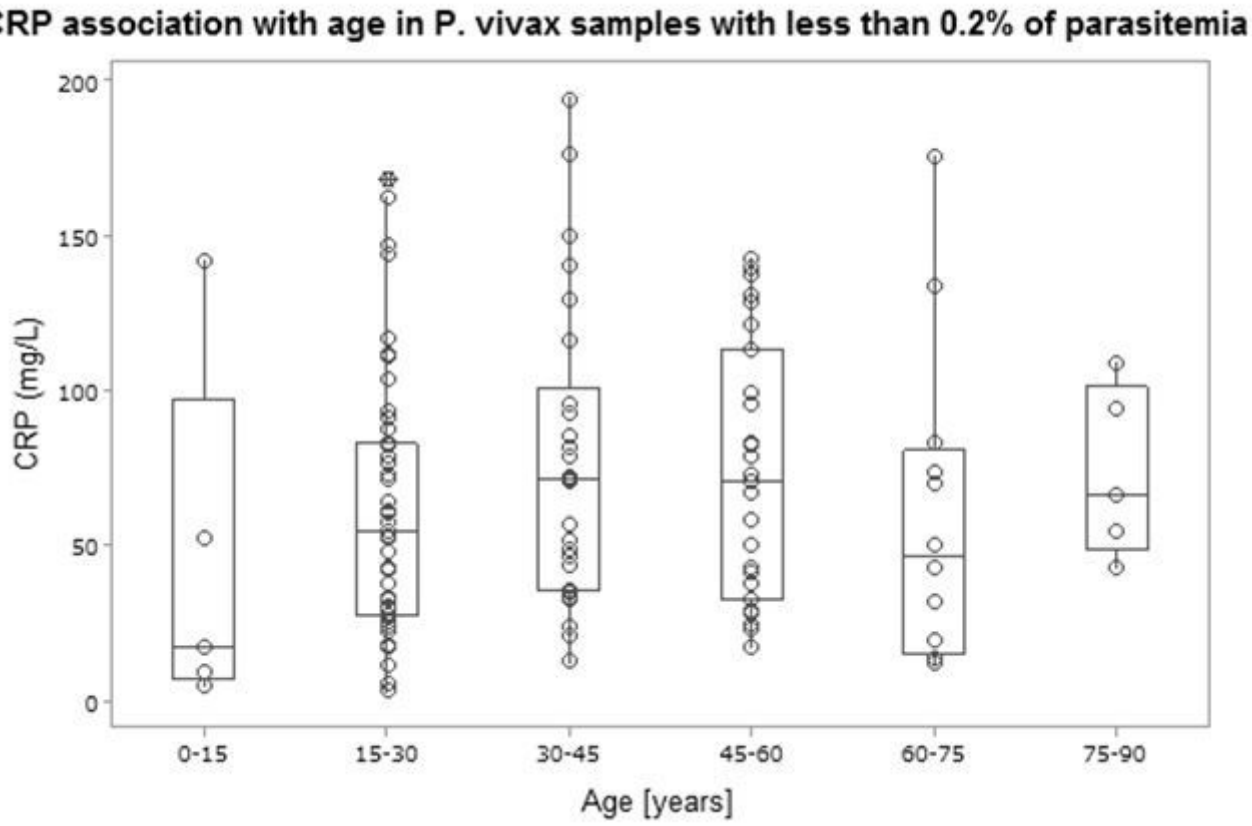


Figure 3

Box plot of CRP in association with age of P. vivax samples with parasitemia less than 0.2%. All individual points are displayed in the box plots

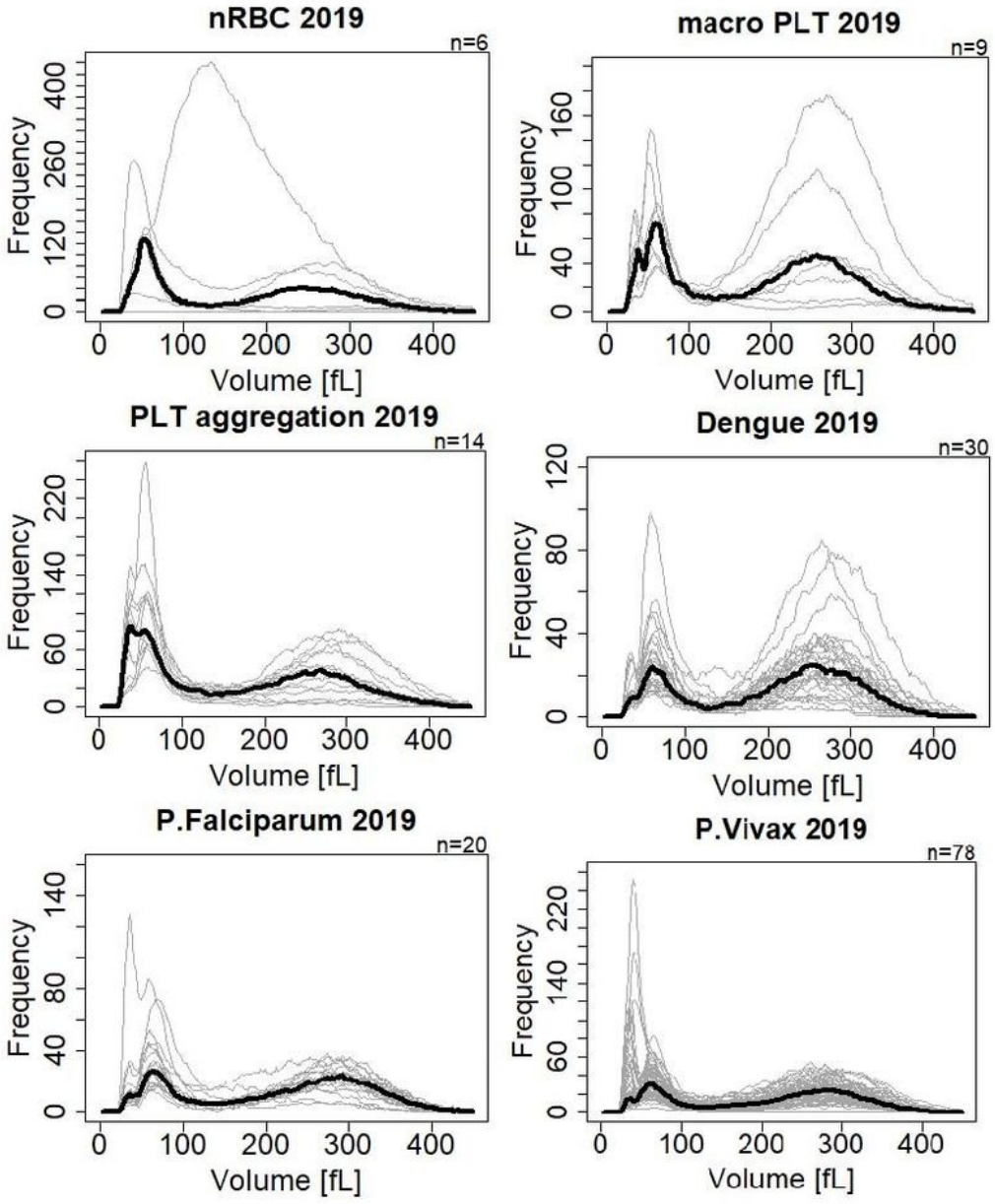
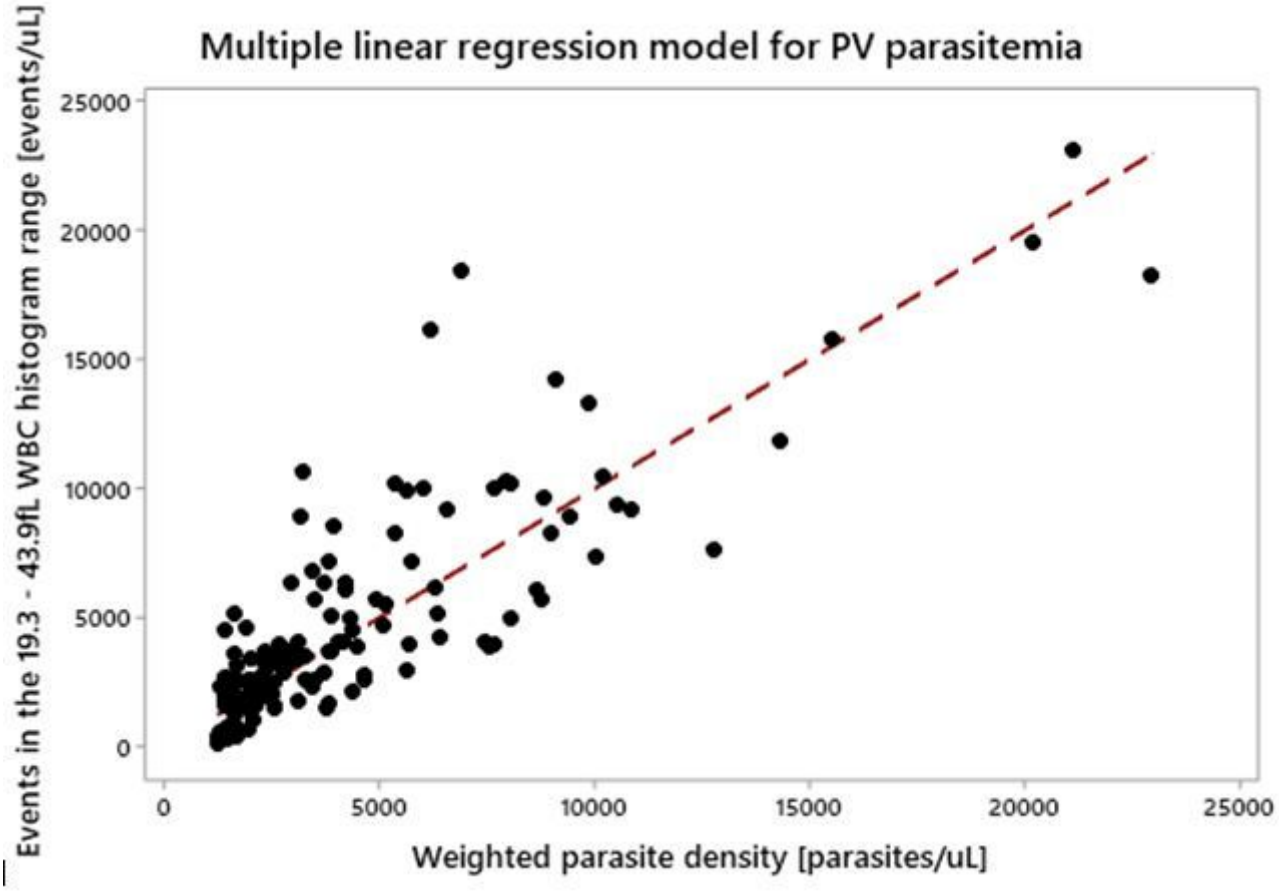


Figure 4

WBC histograms of 2019 data-set with interferences around 37-fL. Bold lines represent the median of the histograms.



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Multiple linear regression model between the events before 43.9fL and parasite density. The vertical axis corresponds to the number of events from 19.3fL to 43.9fL in the WBC histogram and the horizontal axis to the parasite density of small and large forms of samples infected by malaria P. vivax. The regression model shows that the contribution of the linearity is mainly provided by large forms of P. vivax malaria infection.