Original Research Article

Evaluation of automated hematology analyser: Mindray BC-6800 plus as a screening tool for diagnosing malaria

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ABSTRACT

Background: Malaria is one of the major health problems of developing and tropical countries like India, causing huge financial burden also. Smear examination for diagnosis is a routine and cost-effective method for diagnosis. CBC is one the most commonly ordered test in clinical practice. The Mindray BC-6800 plus haematology analyser provide dedicated flag of “Infected RBC?” for suspected malaria while analysing CBC. Present study attempts to evaluate whether CBC done on BC-6800 plus can serve as an aid for malaria screening and diagnosis.

Methods: Prospective study of 30077 cases was carried out where thick and thin smear examination done by experienced microscopists and CBC was analysed on BC-6800 plus analyser.

Results: Total 323 cases were found to be positive for malaria by smear examination, out of which 285 were of P. vivax and 38 were of P. falciparum malaria. Sensitivity of 88.24%, specificity of 99.21%, PPV of 54.70%, NPV of 99.87% for Infected RBC flag for all cases of malaria. For P. vivax malaria cases analyser provided highest sensitivity of 99.65% with NPV of nearly 100%, however P. Falciparum malaria cases analyser produced sensitivity of 2.63% only. In P. vivax cases present study found very good correlation between InR count and parasite load under microscope. Present study also evaluated interference of parasitized red cells in differential count causing falsely elevated neutrophils and/or eosinophils.

Conclusions: By lowering threshold for “Infected RBC?” flag to 0, BC-6800 plus can serve as an excellent screening tool especially for P. vivax malaria.

Keywords: Abnormal scattergram, Infected RBC, Malaria, Mindray BC-6800 plus analyser

INTRODUCTION

According to world malaria report 2019 by WHO, an estimated 228 million cases of malaria occurred worldwide in the year 2018 and of which 85% of the global malaria burden was borne by nineteen African countries and India. The report states that while falciparum malaria is most prevalent in African Region, India carries staggering 47% Global P. vivax burden. India and African Region together accounted for 85% of global malarial death in 2018.1 According to some estimation, India bears malaria related total economic burden around US$ 1940 million, of which, major burden comes from lost earnings (75%), while 24% comes from treatment costs.2 So, Indian Government launched National Framework for Malaria Elimination (NFME) in 2014, with the aim of eliminating malaria from India by 2030. This requires INR 20 billion annually.3

Gujarat is the state of India situated at the mid-western part of India and due to its varied eco-types, the state is prone to malaria outbreaks.4 Surat, one of the fastest growing cities of India and world also bears burden of vector borne diseases as it receives high numbers of immigrants from other parts of country. According to Vector Borne Disease Control (VBDC) department,
Annual Report 2018, though cases of malaria have decreased to third from year 2010, still during monsoon malaria poses big health challenge. India has by far the greatest estimated P. vivax burden of any country, and in Surat P. vivax accounts for almost 90% of cases. Though P. vivax is found round the year, it definitely shows seasonal peak during monsoon between June and October.

It is easy to suspect malaria when presented with classic clinical features, however they are present in 50% to 70% cases only. In endemic regions, malaria can present with unusual features due to development of immunity, increasing resistance to anti-malarial drugs, and the indiscriminate use of anti-malarial drugs, making difficult to suspect malaria for a clinician. Microscopic detection of malarial parasite in thick and thin smears has continued to be a reliable tool for early diagnosis, species identification and assessment of parasitic load for malaria. Its cost effectiveness makes it a widely accepted method for malaria diagnosis and follow up for developing country like India. The shortcomings of microscopic detection are its subjectivity, many variables involved in thick and thin smear preparation and staining, it is time consuming as before labeling a case as negative, one must examine at least 100 fields and well-trained personals for microscopy are required.

Rapid Diagnostic Test (RDT) is a cheaper and objective alternative which also allows species identification. The drawback is, it is only ordered upon clinical suspicion and does not give information about parasite load. The sensitivity also varies greatly from one manufacturer to other. Other alternative methods include QBC, PCR, Loop Mediated Isothermal Amplification (LAMP), Microarray, Mass Spectrometry (MS) and Flow Cytometry (FCM). Most of which requires sophisticated setup and are costly making them difficult to apply as a mass screening test.

Hemogram/ Complete Blood Count (CBC), performed on hematology analyzers, is one the most commonly ordered blood test in clinical practice especially in patients presenting with fever. Ever since Wallace H. Coulter discovered method for counting particles in fluid and patented in 1953, technical up gradations of automated Hematology Analyzers have made them extremely sophisticated, fast and intelligent. Many articles have been published showing very wide sensitivity and specificity depending upon methodology in various hematology analyzers.

Authors attempted to assess one such sophisticated modern hematology analyzer, Mindray BC-6800 Plus as screening tool for malaria detection.

METHODS

A prospective study was carried out at the department of pathology of Sterling Accuris Wellness laboratory from 15th of March 2019 to 31st December 2019. Total 30,077 CBC samples were included. Samples were collected K2 EDTA vacutainer (Becton Dickinson, USA). Samples with unacceptable preanalytical variables like hemolyzed samples, low volume samples, delay of more than 6 hours between collection and analysis were excluded from study.

One thin smear and two thick smears were prepared immediately at the time of sample collection. Thin smears were stained with Leishman’s stain (Qualigens, Thermo Fisher Scientific, US) while all thick smears were stained with Giemsa stain (Qualigens, Thermo Fisher Scientific, US) using Sorenson’s phosphate buffer (pH 7.2). All smears were thoroughly examined under microscopes CX21 (Olympus, Tokyo, Japan) and Primo Star Zeiss (Carl Zeiss AG, Germany) and were recorded as positive only when any of four stages; early trophozoite (ring form), late trophozoite (amoeboid form), schizonts or gametocytes were detected. All positive smears are graded in to groups according to ‘+’ scale system; (+) for 1-10 parasites/100 oif, (++) for 10-100 parasites/100 oif, (+++) for 1-10 parasites/oif and (++++) for >10 parasites/oif. Based on ‘+’ scale parasite densities were also estimated as 10 to 99 parasites/µl for (+), 100 to 999 parasites/µl for (++), 1,000 to 9,999 parasites/µl for (+++) and more than 10,000 parasites/µl for (+++++). Species and morphology were identified in thin smear examination. Smears were reported negative only if parasites were not found after examining 100 oif.

The samples were analyzed on Mindray, BC-6800 plus using Count + Differential (CD) mode.

The analyzer performs differential count in WBC-DIFF channel where normal red cells are lysed and white cells are differentiated into different subpopulation based on size; measured by forward scatter (FS; z-axis), internal complexity; measured by side scatter (SS; x-axis) and nucleic acid content (measure of immaturity); measured by fluorescence (FL; y-axis). So, a 3-dimension cube is generated (Figure 1), differentiating WBCs in to Lymphocytes, Monocytes, Neutrophils (with Basophils) and Eosinophils along with immature cells with higher fluorescence if they are present. All lysed red cells occupy a region called GHOSTS. If analyzer algorithm is not been able to differentiate between various types, then they are colored white and labeled as NOTYPE cells. Total events recorded in WBC sensitive area in WBC-DIFF scattergram is expressed as total WBC-D count in research parameter.

Red cells infected with malarial parasites resist complete lysis and they occupy a separate zone as clustered dots/events in 3 D cube (Figure 2); an area marked as “Infected RBC” sensitive zone. All events recorded in this area are considered due to Infected RBC and absolute Infected Red Cells (InR #) and % Infected Red Cells
Figure 1: SF cube (scatters; side and forward and fluorescence) with differential WBC zones.

Figure 2: SF cube (SS vs. FS scattergram). Infected RBC zone (circled area) with dense clustered dots/events is best appreciated on rotating cube.

Analyzer provides 14 adjustable flags with threshold between 0 and 100, expressed as i-Message count for each flag, for abnormal blood cell morphology. All flags come with a default threshold value of 40 except “Infected RBC?” flag which has default threshold value of 100. In present study, threshold value of “Infected RBC?” flag is kept at 0.

NRBCs, if present, are separated from WBCs and basophils are separated from neutrophils in WNB channel along with red cell ghosts using, separate reagents. A WNB 3 D cube is generated where FL is on x-axis, FS is on y-axis and SS is on z-axis. Analyzer reports total WBC count (also expressed as WBC-N in research parameter) from this channel correcting for presence of NRBCs. Under normal circumstances there is fair degree of agreement of total WBC count from both these channels, but in presence of parasite infected red cells, WBC count from WNB channel is less than that of WBC DIFF channel. Authors also analyzed this difference in WBC count from both channel and labelled as Delta WBC. Data from analyzer software were exported in Microsoft Excel-2019. Data were sorted/filtered and grouped and then Mean, Standard Deviation, Frequency distribution, Chart and relevant tables were prepared in MS Excel. OpenEpi 2.3 was used to analyze whether difference in observation was statistically significant or not.

RESULTS

All 30,077 samples were examined under microscope for presence or absence of malaria by experienced persons and total 323 cases (1.07%) were positive on smear.

Table 1: Incidence of malaria and distribution amongst male and female.

|        | P. vivax | P. falciparum |
|--------|----------|---------------|
| Males  | 198 (69.5%) | 26 (68.4%)    |
| Females| 87 (30.5%) | 12 (31.6%)    |
| Total  | 285 (88.2%)| 38 (11.8%)    |

P. vivax was present in 285 cases (Age range between 6 months and 90 years) and P. falciparum was present in 38 patients (Age range between 1½ years and 85 years) (Table 1). The cases of malaria showed sharp peak in monsoon season being highest in period between August and October. While P. falciparum cases were found predominantly during monsoon season, P. vivax cases were present round the year, though much less during non-rainy period (Figure 3).
Out of 30,077 samples, InR flag was generated in 531 (1.73%) samples. Based on microscopy for malarial parasites and presence or absence of “Infected RBC?” flag, authors separated them into 6 groups as shown in Table 2. Microscopy examination was considered as reference method for diagnosis. Smear negative cases without “Infected RBC?” flag were considered as false positive (Group 6). Smear positive cases with “Infected RBC?” flag were categorized as true positive for P. falciparum (Group 2) and P. falciparum (Group 3) malaria while smear positive without “Infected RBC?” flag were grouped into false negative P. vivax (Group 4) and P. falciparum (Group 5) malaria cases.

Few parameters were compared amongst smear positive malaria cases (True Positive), smear negative cases where “Infected RBC?” flag was not present (Control Cases) and smear negative cases where “Infected RBC?” flag was present (False Positive) these groups (Table 3).

Group 1 consists of all true negative samples for malaria constitute for 98.13% of all cases and were used for comparison as a control group.

Group 2 consists of True positive (P. vivax) cases which constitutes 284 of all 285 P. vivax positive cases. All these cases generated “Infected RBC?” flag and P. vivax malaria detected on smear.

Table 2: Group wise number of cases.

| Groups | Description | No. |
|--------|-------------|-----|
| 1      | MP Neg. and InR Neg. [Control] | Control Group 29517 |
| 2      | MP Pos. and InR | P. vivax and InR Pos. | True Positive (P. Vivax) 284 |
| 3      | Pos. [TP] | P. falciparum and InR Pos. | True Positive (P. falc) 1 |
| 4      | MP Pos. and InR | P. vivax and InR Neg. | False Negative (P. Vivax) 1 |
| 5      | Neg. [FN] | P. falciparum and InR Neg. | False Negative (P. falc) 37 |
| 6      | MP Neg. and InR Pos. [FP] | False Positive 237 |

Table 3: Statistical comparisons of some important parameters amongst different groups.

| No. of cases | True positive cases | Control cases | False positive cases | True positive vs. control | True positive vs. false positive |
|--------------|---------------------|---------------|----------------------|---------------------------|---------------------------------|
| WBC Mean     | 5.96                | 8.04          | 8.94                 | <0.001*                   | <0.001*                         |
| x10^3/μl SD  | 3.09                | 2.24          | 4.93                 |                           |                                 |
| NLR Mean     | 6.11                | 2.76          | 4.49                 | <0.001*                   | <0.001*                         |
| Ratio SD     | 5.44                | 1.66          | 4.87                 |                           |                                 |
| PLR Mean     | 163.56              | 142.28        | 165.22               |                           |                                 |
| Ratio SD     | 146.23              | 58.60         | 139.55               |                           |                                 |
| Haemoglobin  Mean | 12.59            | 13.21         | 12.85               | <0.001*                   | 0.178                           |
| gm/dl SD     | 1.99                | 1.67          | 2.03                 |                           |                                 |
| RBC Mean     | 4.37                | 4.52          | 4.42                 | <0.001*                   | 0.355                           |
| x10^3/μl SD  | 0.66                | 0.50          | 0.64                 |                           |                                 |
| PLT Mean     | 115.22              | 282.51        | 243.84               | <0.001*                   | <0.001*                         |
| x10^3/μl SD  | 62.44               | 76.38         | 117.67               | <0.001*                   | <0.001*                         |
| Delta WBC Mean | 0.80               | -0.01         | 0.06                 | <0.001*                   | <0.001*                         |
| x10^3/μl SD  | 1.87                | 0.24          | 0.28                 |                           |                                 |
| InR # Mean   | 0.88                | 0.00          | 0.03                 | <0.001*                   | <0.001*                         |
| x10^3/L SD   | 1.83                | 0.00          | 0.03                 |                           |                                 |
| InR % Mean   | 0.20                | 0.00          | 0.01                 | <0.001*                   | <0.001*                         |
| % SD         | 0.43                | 0.00          | 0.01                 |                           |                                 |

NLR=Neutrophil:Lymphocyte ratio, PLR=Platelet:Lymphocyte ratio, Delta WBC=WBC-DIFF count minus WBC-WNB count, InR# = Infected red cell count, InR%= Infected red cell percentage. (* indicates statistically highly significant)

Density of clustered events/dots in Infected RBC sensitive zone was directly proportional to lyse resistant malaria infected red cells (Figures 4 to 7). Partially lysed infected red cells generated enough fluorescence so that they were included in total WBC-D count and then differentiated either in neutrophils or eosinophils based on their SS, FS and software algorithm. Authors found interference of these events/dots in differential count of...
either neutrophil or eosinophils or both, as shown in Table 4 as well as Figures 4 to 7.

Authors also tried to establish correlation between thick smear parasite grade and InR count. As shown in Table 5 and Figure 8 microscopy grade was well correlated with InR count. However, as shown in Figure 8, there were few cases in each group where InR count was found to be lower than microscopy. Likely reason behind that is when early trophozoite (small ring) forms are more in number, many of them would show low fluorescence and would not qualify to be included in Infected RBC sensitive zone. They occupy ghost population but slightly on right side than usual and under such circumstance, InR count would be falsely low (Figure 9).

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**Figure 4:** SF cube, SS vs. FS scattergram. Extremely low density of clustered dots/events in Infected RBC sensitive region in a case where peripheral parasitemia of *P. vivax* was very low.

**Figure 5:** SF cube, SS vs. FS scattergram. Low density of clustered dots/events in Infected RBC sensitive region where dots were included in neutrophils and eosinophils falsely.

**Figure 6:** SF cube, SS vs. FS scattergram. High density of clustered dots/events in Infected RBC sensitive region where all dots were included in neutrophils falsely.

**Figure 7:** SF cube, SS vs. FS scattergram. High density of clustered dots/events in Infected RBC sensitive region where most of the dots were included falsely in eosinophils while few included in neutrophils.
Table 4: Interference in differential counts of neutrophil and/or eosinophils due to dots/events recorded in Infected RBC sensitive area.

| Interference in differential count of | No. cases | Percentage |
|--------------------------------------|-----------|------------|
| Neutrophils                          | 185       | 65.14%     |
| Eosinophils                          | 44        | 15.49%     |
| Both                                 | 55        | 19.37%     |

Table 5: Correlation between microscopy grades with InR count.

| Microscopy Grade | Cases | InR# (x10^9/L) | Analysis of Variance (ANOVA) |
|------------------|-------|----------------|-----------------------------|
|                  |       | Mean | SD | Range |                                  |
| 1 +              | 51    | 0.033 | 0.021 | (0.00-0.1) |                                |
| 2 +              | 147   | 0.377 | 0.24 | (0.05-1.03) | <0.001*                        |
| 3 +              | 81    | 2.236 | 1.783 | (0.04-9.92) |                                |
| 4 +              | 6     | 12.775 | 5.234 | (4.56-21.87) |                                |

(* indicates statistically highly significant)

In few cases, the scattering due to infected red cells went haywire due to marked overlapping, so analyzer algorithm could not differentiate between various cells, typing all of them as NOTYPE cells (Figure 10 and 11).

The analyzer’s default threshold of “Infected RBC?” flag is 100. As shown in Table 6, default threshold of 100 can give 100% specificity and 100% PPV, but sensitivity reduces to 48.07% leaving 148 out of 285 smear positive P. vivax cases without flag.

Primary objective of “Infected RBC?” flag is to alert about presence of malarial parasites, so that further action is taken. In order to use it as screening tool, authors suggest that threshold should be kept at 0. By keeping threshold at 0 authors could achieve sensitivity of 99.65% and NPV of nearly 100% with only 237 (0.79%) false positive “Infected RBC?” flagged cases out of total 30077 cases.

Authors also evaluated one case of smear positive P. Vivax case which did not generate “Infected RBC?” flag.

Figure 8: Correlation between microscopy grade with infected red cell count.

Figure 9: SF cube, SS vs. FS scattergram. Showing a case of P. vivax where predominant form of parasite was early trophozoite (small ring form), so InR count was falsely low.
(Group 4) where peripheral parasitemia was extremely low generating only two clustered events/dots in Infected RBC sensitive zone (Figure 12). Even in such cases where parasitemia is very low, close examination of SS vs FS in 3D cube raises suspicion.

Delta WBC is the difference of WBC count of DIFF and WNB channel. WBC count in DIFF channel is positively affected by presence of infected red cells which are included in total WBC-DIFF. Generally, InR count is well correlated with delta WBC as shown in Figure 15, however authors found 8 out of 285 P. vivax positive cases where delta WBC was not correlating with InR count and delta WBC count was low. When authors analyzed WNB scattergram, authors found that red cells with parasites in such cases, could not be lysed completely, so they were included in total WBC count elevating total WBC count falsely (Figure 13 and 14). Authors recommend that in such cases, total WBC count should be done on different analyzer or done manually.

Group 3 consists of true Falciparum positive cases which constitute only 1 out of 38 cases of all P. falciparum malaria detected on smear making sensitivity of 2.63% only and positive predictive value of 0.19% only in present study. Rest of smear positive P. falciparum cases (37/38) did not produce “Infected RBC?” flag (Group 5). The likely reason is the early ring forms of P. falciparum generate low SS, FS and FL signals making them difficult to differentiate from RBC ghosts. When late trophozoites, or schizonts are present in significant numbers, which are larger and complex structures, can be detected.

**Table 6: Sensitivity, specificity, PPV, NPV and accuracy at various “infected RBC?” flag thresholds.**

| Threshold | Sensitivity | Specificity | PPV   | NPV   | Accuracy |
|-----------|-------------|-------------|-------|-------|----------|
| 0         | 99.65%      | 99.20%      | 54.51%| 100.00%| 99.21%   |
| 1         | 99.30%      | 99.40%      | 61.39%| 99.99%| 99.40%   |
| 5         | 89.12%      | 99.82%      | 82.47%| 99.90%| 99.72%   |
| 10        | 85.61%      | 99.91%      | 89.71%| 99.86%| 99.77%   |
| 15        | 80.70%      | 99.95%      | 94.26%| 99.82%| 99.77%   |
| 20        | 78.60%      | 99.97%      | 96.14%| 99.80%| 99.77%   |
| 30        | 74.04%      | 99.99%      | 98.60%| 99.75%| 99.74%   |
| 40        | 69.47%      | 100.00%     | 100.00%| 99.71%| 99.71%   |
| 50        | 65.61%      | 100.00%     | 100.00%| 99.67%| 99.67%   |
| 100       | 48.07%      | 100.00%     | 100.00%| 99.51%| 99.51%   |

Figure 10: SF cube, SS vs. FS scattergram. Showing a case where all cells and InRs are classified as NOTYPE cells as instrument algorithm was not able to differentiate between them due to heavy overlapping.

Figure 11: SF cube, SS vs. FS scattergram. Showing same case after treatment of one day, shown in Figure 10, where instrument algorithm was able to give differential count along with InR.
Authors had a case where along with small ring forms, gametocytes of *P. Falciparum* were present which generated clustered events/dots in area which was different, again emphasizing close examination of SS vs. FS plot (Figure 16).

Group 6 Consists of 237 smear negative but “Infected RBC?” flagged cases. On examining 3 D cube of these cases authors found very scattered dots/events in these cases against the densely clustered dots/events in malaria positive cases.

When authors further analyzed these cases, authors found that out of 237 cases, 93 showed PLT clump? flag, 66 showed various other abnormal WBC flags, commonest are “Immature Gran?” in 60 cases, “NRBC present” in 48 cases, “Eosinophilia” in 24 cases and “Lipid Particles?” flag in 15 cases.

The mean i-Message count was 5.56 with SD of 5.89 and range was between 1 and >100. When authors compared with smear positive malaria cases, the mean i-Message count was 64.40 with SD of 38.52 and range was between 1 and >100.
The sensitivity and specificity of *P. vivax* and *P. falciparum* malaria were evaluated in the present study. The sensitivity and specificity of *P. vivax* were found to be 88.24% and 99.21%, respectively, while for *P. falciparum*, the sensitivity and specificity were 21.4% and 84.3%. Based upon the present study, authors derived some conclusions and authors have few suggestions for the users of Mindray BC-6800 plus hematology analyzer; if they want to use it as a screening tool of malaria.

- Upon evaluating Mindray BC-6800 plus hematology analyzer as screening tool for malaria, authors found overall sensitivity of 88.24%, specificity of 99.21%, PPV of 54.70%, NPV of 99.87% for all cases of *P. vivax* and *P. falciparum* malaria while keeping threshold for “Infected RBC?” flag at 0.
- While evaluating for *P. vivax* malaria, in countries like India where *P. vivax* burden is very high and atypical presentation of *P. vivax* is common, this instrument can be an excellent screening tool with sensitivity of 99.65% and NPV of nearly 100%.
- However present study shows that at present BC-6800 plus cannot be used as screening tool for *P. falciparum* as we estimated sensitivity of 2.63% only.
- Authors suggest that manufacturer should come up with modification in algorithm where smaller forms of parasite can also be detected raising sensitivity of *P. falciparum* detection.
- Whenever dots/events are recorded in Infected RBC zone, manual differential count must be performed as these parasitized red cells are included either in neutrophils or eosinophils.
- Authors suggest that manufacturer should come up with change in algorithm and type them as NOTYPE cells so that they do not interfere in reported differential count.
- In most of the cases of *P. vivax*, InR count is very well correlated with microscopic grade of parasite load. Sometimes when early ring forms are more in number, InR count may be lower than actual parasitemia.
- False total WBC count can be suspected by correlating InR value with delta WBC or by close observation of WBC-WNB 3 D cube. In such cases total WBC count should be rechecked by other alternative method.

Close examination of scattergram is mandatory. Very scanty parasitemia or early ring forms can only be suspected by close observation of WBC-DIFF 3 D cube.

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