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Evaluation of verification criteria for platelet scan for automated platelet counts generated by beckman Coulter LH-780 hematology analyser

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Abstract
Aims & Objectives: To evaluate verification criteria for reflex ordering of platelet scans for automated platelet counts generated by Beckman Coulter LH-780 Hematology Analyzer.
Materials & Methods: The study uses automated platelet counts <100x103/µL generated by the two Beckman Coulter LH-780 analyzers as part of evaluation and monitoring of thrombocytopenia.
The cases are grouped into 4 based on platelet flags generated by the analyzer as (1) positive for giant platelets, (2) positive for platelet clumps (CLP), (3) positive for both giant platelets & clumps (GP+CLP) and (4) without platelet flags. Corresponding smears were reviewed to determine if the automated platelet counts were acceptable and to note down the positive findings such as presence of giant platelets, clumps, fibrin strands, microclots. Positive and Negative predictive values (PPV and NPV) were calculated.
The automated platelet count was accepted and the same reported if the manual platelet count was within 10% of the automated count for counts >/=40x103/µL and within 20% for counts <40x103/µL.
Results: Of the total 1004 smears studied for thrombocytopenia, the category CLP had the least PPV of 0.27, followed by categories GP with 0.74 and GP+CLP with 0.92. The group with no platelet flags comprised of 645 samples. In these 645 samples, 13 samples had unacceptable counts - 12 smears showed giant platelets of which 8 were cases with initial presentation, 2 with a platelet count <20x103/µL and 2 cases with a positive smear finding noted on previous smear examination. One sample had no smear findings, but a repeat sample was requested in view of delta check failure which had unacceptable platelet count.
Conclusion: Based on the findings of (a) PPV of 0.74 and 0.92 respectively for the groups with GP and GP+CLP (b) NPV of 0.98 for the group with no flags, one could also understandably exclude all the platelet count <100 × 103/µL as a criteria for a reflex order of a platelet scan and instead limit it to only those with platelet flags; and for those negative for flags, reflex smears may be done for the initial presentation, counts with <20x103/µL, a delta check failure or a positive smear finding on a previous smear examination.
With the revised policy, we expect a significant reduction in the number of platelet scans performed daily in our laboratory.

Keywords: Thrombocytopenia, Beckman Coulter, Giant platelets, Platelet clumps

Introduction
Automated hematology analyzers used in the clinical laboratories for complete blood counts (CBCs) and differential leukocyte counts generate reliable results on essentially all blood specimens containing normal cellular elements and no interfering substance(s). Whereas, the results generated by these analyzers on blood specimens containing abnormal cellular elements and/or potentially interfering substances may not be always reliable and are consequently flagged for verification by alternate methods(1). Verification of platelet count below 100×109/L is important because pseudo- thrombocytopenia of this magnitude may unnecessarily trigger a hematology consult, additional laboratory work-up, postponement of surgery/special procedure, and may warrant a platelet transfusion(2). Manual peripheral smear examination is the most commonly employed alternate method for verification of the automated counts. For verification of the platelet count, the entire blood smear, including the feather edge, lateral edges, readable area and thick area, should be examined first under low power (10X) looking for platelet clumps, especially large clumps which are easily

discernible under this magnification. Smaller clumps, giant platelets need to be looked under high power (40X) or oil immersion (100X). Under higher magnification, it is important to note if red cell fragments, organisms (bacteria and fungi), and/or giant platelets are present in significant number which may lead to erroneous platelet counts by automated analysers. While clumps and giant platelets cause pseudothrombocytopenia, red cell fragments and organisms can lead to falsely higher counts [3, 4].

However manual method is a labor-intensive and time-consuming procedure that impacts overall laboratory efficiency. In order to maintain a reasonable degree of efficiency and hence the turn- around-time in the laboratory, there is a need to minimize the number of blood smear examinations performed daily. The Department of Laboratory Medicine at SAKRA World Hospital processes approximately 300-350 blood specimens per day for CBCs and/or platelet counts. Peripheral smears are prepared, stained and examined microscopically for nearly 30% samples. Platelet scans performed to verify only the platelet counts account for about 50% of blood smear examinations. The criteria for reflex order of a platelet scan in our laboratory routinely included (a) an automated platelet count <100 × 103/μL, irrespective of whether it is an initial or a follow-up count and irrespective of the platelet flags and (b) presence of 1 or more of the analyzer-generated flags (platelet clumps (CLP), and giant platelets (GP) for platelet counts between 100 – 150 × 103/μL.

Since the platelet scans contributed significantly to the total workload and adversely impacted the turnaround time of platelet count results, we decided to examine the outcome in terms of percent positive yield of the individual platelet flags and to find ways, if possible, to improve efficiency by reducing the number of daily platelet scans without an adverse impact on patient care.

Materials and Methods

The study uses automated platelet counts <100x103/μL generated by the two Beckman Coulter LH-780 analyzers as part of evaluation and monitoring of thrombocytopenia.

We use 2 Beckman Coulter LH780 analyzers for performing CBCs and Diffs. Both analyzers were calibrated and quality controlled according to manufacturer's recommendations. The LH780 employs electrical impedance technology as the primary method to generate platelet counts. The platelet-associated flags routinely generated by the LH780 are GP and CLP. The threshold for the CLP flag was adjusted by the manufacturer's representative to an optimal setting prior to the start of the study.

Inclusion criteria: Automated platelet counts <100x103/μL irrespective of flags generated by the hematology analyzer.

Exclusion criteria: None

The cases are grouped into 4 categories based on platelet flags generated by the analyzer as

[1] positive for giant platelets, [2] positive for platelet clumps (CLP), [3] positive for both giant platelets & clumps (GP+CLP) and [4] without platelet flags. Corresponding smears were reviewed to determine if the automated platelet counts were acceptable and to note down the positive findings such as presence of giant platelets, clumps, fibrin strands and microclots. A smear was said to have positive findings if it revealed any 1 or more of the following findings: 1 or more platelet clumps, giant platelets, red cell fragments, any fibrin strands, microclots. The grading of morphologic findings was based on published guidelines [5]. Positive and Negative predictive values (PPV and NPV) were calculated.

The automated platelet count was accepted and the same reported if the manual platelet count was within 10% of the automated count for counts >/=40x103/μL and within 20% for counts <40x103/μL [6].

Results

A total of 1004 samples were reviewed for thrombocytopenia. Of the total 1004 smears studied, 73 samples belonged to category 1 (CLP), 141 in category 2 (GP), 145 in category 3 (GP+CLP) and 645 in category 4 (no flags).

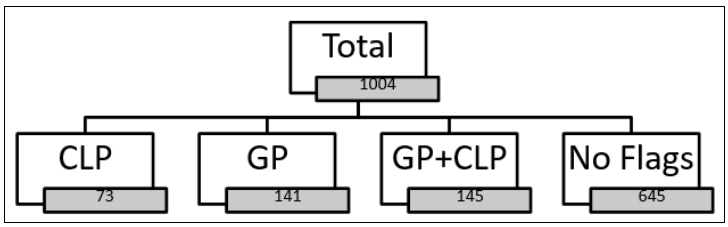

Fig 1: Total no. of smears studied in the 4 categories

In the category 1, out of the 73 samples having CLP flag, only 20 of them actually had a positive smear finding, with a PPV of 0.27. In the category 2, out of 141 samples showing GP flag, 104 of them showed giant platelets on smear, with a PPV of 0.74. The category 3 includes 145 samples, of which 134 had positive smear findings, with a PPV of 0.92.

The last category, comprising 645 samples without an analyser-generated platelet flags, 16 smears showed a positive smear finding, either GP or CLP, however 4 of these still had acceptable platelet count. The remaining 12 smears with unacceptable platelet count include 8 were cases with initial presentation, 2 with a platelet count <20x103/μL and 2 cases with a positive smear finding noted on previous smear examination. One sample had no smear findings, but a repeat sample was requested in view of delta check failure which had unacceptable platelet count. The category 4 therefore includes a total of 13 samples with unacceptable platelet count, with a NPV of 0.98.

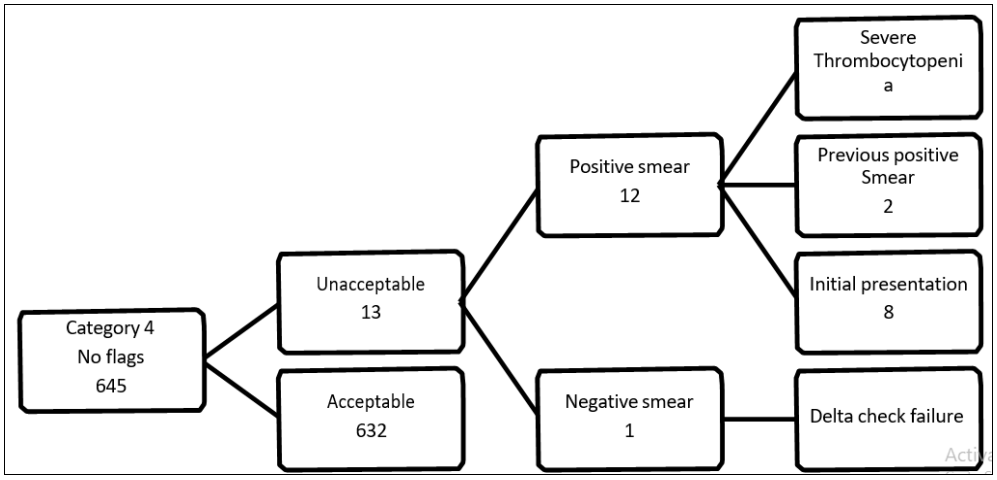

Fig 2: Distribution of samples in category 4

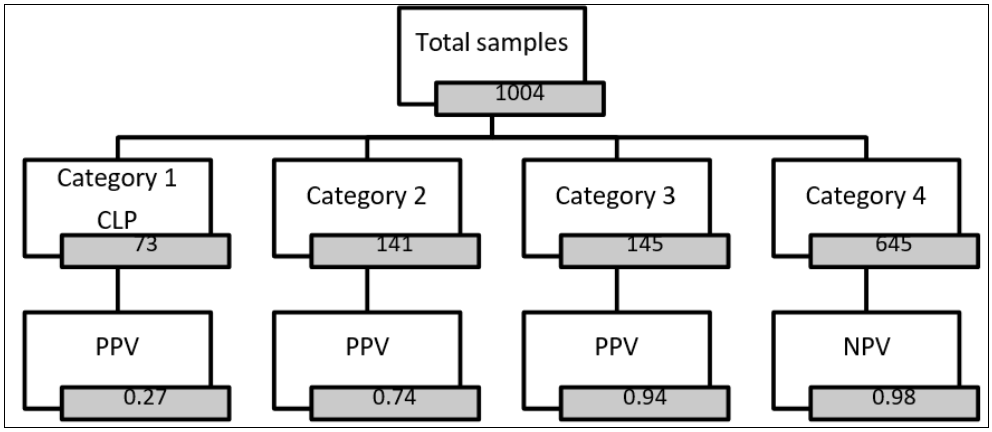

Fig 3: Distribution of samples in the 4 categories and their PPV and NPV

Discussion

Among the 3 categories of platelet-associated flags, CLP had the least PPV while the category

GP had a PPV of 0.74 and GP+CLP had a PPV of 0.94. The least positive yield for CLP flag is in agreement with the study by Gulati GL *et al*., which showed the CLP flag sensitivity as low as 25%, the possible reason could be platelet clumping that may have occurred in the interval between specimen processing through the analyzer and blood smear preparation [2].

The category 4, with no platelet associated flags, had a NPV of 0.98 which was in agreement with study by Gulati *et al*. [6]. In this category, 13 samples had unacceptable platelet count by manual method. 12 of these samples had positive smear findings (GP or CLP), but were not flagged by the analyser-8 of these were first presentation of thrombocytopenia, 2 samples had a positive smear finding in the previous examination and 2 samples were of severe thrombocytopenia (<20x103/µL). One sample with a platelet count of 83x103/µL, had no platelet-associated flags or positive smear findings, but a repeat sample was requested and processed in view of delta check failure (platelet count reduced by >50% in 24 hours) and found to have normal platelet count, which was unacceptable.

Conclusion

The above study intends to evaluate our criteria for ordering reflex platelet scans for automated platelet counts and verifying the predictive values of individual platelet flags generated by the analyser. Our current criteria for ordering reflex platelet scan includes all the samples showing platelet count <100 × 103/µL irrespective of the platelet-associated flags generated by the analyser.

Based on the findings of (a) PPV of 0.27, 0.74 and 0.92 respectively for the categories CLP, GP and GP+CLP (b) NPV of 0.98 for the group with no flags, one could understandably exclude all the platelet count <100 × 103/µL as a criteria for a reflex order of a platelet scan and instead limit it to only those with any of the platelet-associated flags; And for those negative for platelet flags, reflex smears may be done for the initial presentation, counts with <20x103/µL, a positive smear finding on a previous smear examination or a delta check failure. So a reflex smear may not need to be done for follow up counts within the delta check limits, and when there is no previous positive smear.

With the revised policy, we have been able to reduce the number of reflex smears by more than 60%, which has significantly reduced the turn-around time and in turn, improved the overall efficiency of the lab.

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