Impedance platelet count in severe microcytosis-study of 161 patients

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Abstract

Introduction- Platelet count estimation is an important element of the diagnostic and treatment process in many disorders. The present study aims to determine the incidence of improper impedance platelet count in severe microcytosis and to state the sensitivity and specificity of various parameters like histogram, red cell distribution width (RDW), mean platelet volume (MPV), platelet flags

Methods-Total 161 patients with mean corpuscular volume less than 60 femtoliter and impedance platelet count more than 150 x 10^9/L were included. The samples were analyzed on CellDyn 3700 cell counter. Manual platelet count and stained peripheral smear review was done in each case. ROC (Receiver Operating Characteristics) analysis was used for MPV, RDW and histogram.

Results-18.01 % (29/161) patients did not show correlation between impedance and manual platelet count. For “Do not correlate” group mean platelet volume (>12.7 femtoliter) showed 79.55% specificity and 55.17% sensitivity, Abnormal Platelet/RBC histogram (>40%) showed 76.51% specificity and 51.72% sensitivity and RDW > 27% showed 87.12% sensitivity and 37.93% specificity.

Conclusion- Impedance platelet count is not always reliable in cases of severe microcytosis. Abnormal MPV and RBC/platelet histogram gives good hint for the same.

Key wards - Automated cell counter, Microcytosis, Platelet count

Introduction

Platelet count estimation is an important element of the diagnostic and treatment process in many disorders. In patients with thrombocytopenia, especially in the case of platelet transfusion, the reliability of the platelet estimation is highly desired and necessary to provide appropriate treatment.

The assessment of platelet count is essential in clinical haematology. Any numerical deficiency or defect in their function may lead to bleeding. The normal platelet count at all ages is widely quoted as 150-400 x 10^9/L of whole blood. At present manual counting using phase contrast microscopy, impedance analysis, optical light scatter or fluorescence analysis and immunoplatelet by flow cytometry are various available methods for platelet count. Early methods to enumerate platelets in blood were usually inaccurate and irreproducible until the mid-20th century. In 1953, the manual phase contrast microscopy method was developed, enabling platelets to be easily discriminated from lysed red cells within a counting chamber or haemocytometer. The manual method is time-consuming, subjective and tedious and results in high levels of imprecision with typical interobserver coefficient of variations (CV) in the range of 10–25%.

At low platelet numbers, because fewer cells are counted, observed CVs increase proportionally. Although relatively imprecise, the manual method still offers a relatively inexpensive, simple and viable means to enumerate platelets in the nonspecialized laboratory.

Although the development of the Coulter Principle revolutionized blood counting, platelet counts were only added to the automated full blood count in the late 1970s. Majority of cell counter uses impedance method to enumerate platelet and RBC. The particles analyzed are suspended in an electrolyte solution and dilution is passed through an aperture that links two chambers one containing a positive and the other a negative electrode. As cells pass through the orifice they cause a momentary increase in electrical resistance, which is registered as a pulse. One pulse represents a cell and the size of the pulse is proportional to size of cell. Platelet and RBC analyzed in same chamber(s) are discriminated according to their volume. The introduction of automated blood cell counters using impedance technology resulted in a dramatic improvement in precision, with typical coefficient of variation of <3% because much higher total numbers of platelets are counted.

A major disadvantage of the electrical impedance method for counting platelets is the difficulty in distinguishing large platelets from extremely microcytic or fragmented red cells, even with the use of hydrodynamic focusing methods. False increase in the platelet count will occur when red cell or white cell fragments, microcytic red cells, immune complexes, bacteria or cell debris are included in the reported platelet count. False decreases in the impedance platelet count will occur in the presence of large platelets and if there is platelet clumping as seen with pseudo-thrombocytopenia by ethylene diamine tetracetic acid (EDTA) dependent agglutinins.

In optical light scattering method, platelets are counted and sized by a flow cytometry system in which the cells in a suitable diluent pass through a narrow beam of light (i.e., helium-neon laser). The illumination and light scatter by each cell is measured at a single angle (2° to 3°). This allows assessment of the number of electrical pulses generated in proportion to the number of cells and cell volume. To improve discrimination of platelets accurately from nonplatelet particles, two-dimensional laser light scatter was developed which measure two
angles of laser light scatter at 2°–3° and at 5°–15°. In optical fluorescent platelet count polymethine dye is used to stain the RNA/DNA of reticulated cells and platelet membrane and granules. The fluorescence intensity of each cell is analyzed, which allows the separation of platelets from red cells and reticulocytes. The fluorescent staining of the platelets not only allows the exclusion of nonplatelet particles from the count, but also allows the inclusion of large or giant platelets. The immunologic platelet count involves labeling EDTA-anticoagulated blood with a suitable antiplatelet monoclonal antibody, which has been fluorescently conjugated with, for example, fluorescein isothiocyanate. The method simply derives the platelet count from the ratio of fluorescent platelets to red cells within the sample. Immunological platelet counting is simple, rapid, reliable and easily transferable to any laboratory with a flow cytometer. There is good correlation with the existing recommended manual method at normal platelet counts. Superior precision is demonstrated compared with the manual method especially in thrombocytopenic samples.

More recently, the combination/convergence of flow cytometric principles (e.g., laser light and hydrodynamic focusing) with impedance technology has facilitated the simultaneous detection of multiple light scatter and fluorescent parameters of platelets and cells within a range of modern automated hematology analysers. Modern analysers can therefore offer a range of platelet counting methods including impedance counting, optical counting (light scatter or fluorescence), and an immunological platelet counting method. These advances have improved the ability of automated analysers to discriminate platelets and should theoretically increase the accuracy of platelet counting in many clinical conditions.

In India, particularly Gujarat state has high prevalence of iron deficiency and beta thalassemia trait (BTT) which are associated with severe microcytosis. As severe microcytosis can lead to false increase of impedance platelet count, it is very important to identify actual platelet count. The person with BTT and severe iron deficiency anaemia can have normal impedance platelet count even though actual platelet count is low or borderline low. In high volume laboratories and laboratories with less trained manpower, it is important to identify and correct impedance platelet count in severe microcytosis from cell counter data or by other available methods. The correct platelet count should be informed to the clinician as in common conditions like malaria and dengue fever, where actual platelet count is reduced but impedance platelet count is normal or near normal and clinician try to follow the platelet count report for response to the therapy or recovery.

Aims of study were (1) To determine the incidence of improper impedance platelet count in severe microcytosis. (2) To state sensitivity and specificity of various parameters like abnormal platelet/RBC histogram, abnormal MPV, platelet flags and RDW as indicators of improper impedance platelet count.

**Material and methods**

All blood samples were drawn in 3ml K$_3$ EDTA-collection tube. Total 191 samples were included in the study. 30 samples from normal individuals were analysed as control group. The 161 patients with mean corpuscular volume (MCV) value less than 60 femtoliter and impedance platelet count more than 150 x 10⁹/L were included. The patients who received any blood transfusion (whole blood or components) or haematinics within last 3 months were excluded.

The samples were analyzed in auto sampler mode on Cell Dyn 3700 (Abbott diagnostics, Santa Clara, CA) cell counter within 6 hours of phlebotomy. The Cell Dyn 3700 uses impedance principal for red blood cell (RBC) and platelet count. The software version 1.3 is used in that cell counter. The cell counter is maintained as per manufacturer’s instructions. Cell counter was calibrated according to manufacturers’ specifications by field service representatives. The manual platelet count was done by ammonium oxalate method. For each patient manual platelet count was performed by two trained persons. The manual platelet count is considered as gold standard. Because of high intra-individual variation and intermethodology variation cut off 30% is considered as significant. If the difference between impedance platelet and manual platelet count is more than 30%, it is stamped as “Do not correlate” group. If the difference is less than 30% it is stamped as “Correlate” group. Peripheral blood smears stained with Leishman stain were reviewed in all study samples for the presence of erythrocyte or leukocyte fragments, marked microcytosis, giant platelets, platelet clumps, fibrin strands and number of platelets. The persons who performed manual platelet count and smear review were unaware of cell counter data.

The MPV more than >12.7 femtoliter and MPV (>>> and “no value”) on cell counter were considered as abnormal MPV. In Cell Dyn 3700 URI (Upper Region Interference) and LURI (Lower and Upper Region Interference) are platelet flags which indicate presence of microcytic RBC, giant platelet, schistocytes, sickle cell and platelet clumps. The histogram of RBC and platelet is consider significant when platelet peak merge with RBC peak (“Valley”) at >40% where height platelet peak is considered as 100%. The height of platelet peak and valley is measured in centimetres from printout of histogram.
**Figure-1** Abnormal RBC/platelet histogram (>40%), where left side peak is of platelet and right side peak is of RBC. The platelet peak measure 1.5 centimetre and subsequent valley measures 0.97 centimetre.

**Figure-2** Correlation of the results of Platelet counts using the impedance method and the manual method in blood samples from patients with microcytosis.

X- axis size femtoliter, Y-axis numbers of events PLT-Platelets, RBC- Red blood cells

The data analysis was done on Microsoft office 2003 Excel. ROC analysis and other statistics were done on MedCalc software version 11.6.1.0. The beta thalassemia trait status was confirmed on D-10 VARIANT (Bio-Rad Laboratories, Hercules, CA).

**Results**

In control group total 30 normal individuals data were included. The mean MCV was 83.6 femtoliter (Standard deviation - 4.16), mean platelet count was $332.714 \times 10^9$ /L, mean MPV was 7.63 femtoliter (Standard deviation -1.02). None of them show abnormal MPV, platelet flags and abnormal RBC platelet histogram (>40%).

In study group, “correlate group” showed average MCV, RDW and MPV 56.18, 24.34 and 9.05 respectively with standard deviation 3.17, 3.52 and 1.36 respectively. “Do not correlate” group showed average MCV, RDW and MPV 55.60, 25.83 and 9.36 respectively with standard deviation 3.29, 3.98 and 3.10 respectively.

**Table-1** Distribution of various parameters in study group.

<table>
<thead>
<tr>
<th></th>
<th>Correlate (%)</th>
<th>Do not correlate (%)</th>
<th>Specificity (%)</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Participant (n=161)</td>
<td>132 /161 (81.98)</td>
<td>29/161 (18.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal MPV(n=42)</td>
<td>27/132 (20.45)</td>
<td>15/29 (51.72)</td>
<td>79.54</td>
<td>51.72</td>
</tr>
<tr>
<td>Platelet flags (URL/LURL)</td>
<td>11/132 (8.34)</td>
<td>27/29 (84.61)</td>
<td>18.24</td>
<td>84.61</td>
</tr>
<tr>
<td>Histogram(RBC-platelet &gt;40%) (n=46)</td>
<td>31/132 (23.48)</td>
<td>15/29 (51.72)</td>
<td>76.51</td>
<td>51.72</td>
</tr>
<tr>
<td>RDW&gt;27(N=29)</td>
<td>06/132 (4.54)</td>
<td>10/29 (34.48)</td>
<td>87.12</td>
<td>37.93</td>
</tr>
<tr>
<td>BTT (n=12)</td>
<td>3/132 (2.72)</td>
<td>9/29 (31.03)</td>
<td>97.72</td>
<td>31.03</td>
</tr>
<tr>
<td>MPV + Histogram (RBC-platelet &gt;40%) (n=35)</td>
<td>21/132 (15.90)</td>
<td>14/29 (48.29)</td>
<td>84.09</td>
<td>48.27</td>
</tr>
<tr>
<td>BTT+ Abnormal MPV (n=06)</td>
<td>2/132 (1.51)</td>
<td>4/29 (13.79)</td>
<td>98.48</td>
<td>13.79</td>
</tr>
<tr>
<td>BTT+ Histogram (RBC-platelet &gt;40%) (n=05)</td>
<td>1/132 (0.75)</td>
<td>4/29 (13.79)</td>
<td>99.24</td>
<td>13.79</td>
</tr>
<tr>
<td>BTT+ Histogram (RBC-platelet &gt;40%)+ Abnormal MPV (n=05)</td>
<td>1/132 (0.75)</td>
<td>4/29 (13.79)</td>
<td>99.24</td>
<td>13.79</td>
</tr>
</tbody>
</table>

MPV-Mean platelet volume, BTT- Beta thalassemia trait, RBC-Red blood cells, URI-upper region Interference, LURI- lower and upper region Interference
As depicted in table-1 81.98% (132/161) patients showed correlation, while 18.01% (29/161) patients did not show correlation with manual platelet count. Out of 29 patients, 2 patients showed false decreased and 27 patients showed false increased impedance platelet count. In “Correlate” group histogram (Platelet-RBC >40%) showed highest incidence of 23.48% (31/132). In “Do not correlate” group MPV and histogram (Platelet-RBC >40%) showed highest incidence of 51.72%(15/29).The cut-off of MPV, RDW and histogram(Platelet-RBC) are derived by ROC analysis. The correlation of coefficient between impedance and manual platelet count in severe microcytosis is 0.692, which show poor correlation.

Discussion
As impedance platelet count is based only on the estimation of volume, it proves imperfect in the presence of nonplatelet elements similar in size to platelets. Severe microcytosis interfere with impedance platelet counts to various degrees. Excessive results in the 150–400 x 10^9/L area of the scope or slight thrombocytosis do not constitute clinical problems. False-negative results in the case of thrombocytopenia may jeopardize life.

In present study total 18.01% (29/161) patients showed improper impedance platelet count, while 16.66% (5/30) showed improper impedance platelet count in study done by R. Pinkowski. In R. Pinkowski study MCV range from 57-80 femtoliter, while in present study all 161 patients had MCV less than 60 femtoliter. Total 27 patients out 29 showed falsely increased impedance platelet count. This is due to severe microcytic RBC which comes in platelet region as the Cell dyn 3700 measure platelet between 1-35 femtoliter. 2 cases showed false decrease impedance platelet count. Both patients showed increased MPV and giant platelets on smear, this can be explained by the fact that giant platelet goes outside of upper limit of detection for impedance platelet (35 femtoliter in Cell Dyn 3700). The coefficient of correlation for impedance and manual platelet was 0.692 as compared to the coefficient of correlation of 0.98 from R. Pinkowski study. The difference might be because of different number of samples and MCV range. R. Pinkowski studied 30 patients’ data with iron deficiency and MCV ranges from 57-80 femtoliter, whereas present study 161 patients with MCV less than 60 femtoliter. The cell counter used in present study was Cell-Dyn 3700, R. Pinkowski study was done on Cell-Dyn 4000, both cell counter work on same impedance principal for platelet and RBC. Software version 1.3 is used in present study, the detail of software version was not provided in R. Pinkowski study.

In laboratory scenario of high workload, high incidence of severe microcytosis, non availability of high end cell counter, less trained manpower and less turnaround time, it is important to do correction with manual platelet count and peripheral smear. If we can derive a factor for correction by comparing impedance platelet count with immunoplatelet count it will be very useful but it will vary from cell counter to cell counter even with same manufacturer company. There is significant variation in the platelet count obtained on different analyser with the same sample. It might be because of difference in the method of analysis, linearity over the entire measuring range and number of events actually counted. The Beckman Coulter and Sysmex impedance platelet counts showed better correlation with the reference method than the optical platelet counts by the Advia and the Sysmex.

The differences between impedance platelet and optical platelet count had an inverse correlation with MCV patients with microcytosis. Therefore all the patients less than MCV 60 femtoliter are included in present study to increase the chances of finding improper impedance platelet count. The abnormal RBC/Platelet histogram more than 40 % was considered only giving more specificity to this indicator for correction of impedance platelet count. Lower cut off will increase the sensitivity but decrease specificity while increased cutoff will increase specificity and reduce sensitivity. Abnormal high MPV give hint about giant platelet and abnormal platelet/RBC histogram from cell counter gives idea regarding severe microcytic RBC entering the impedance platelet detection area or giant platelets are entering in the impedance RBC detection area. An abnormal MPV and platelet/RBC histogram both are appear it means microcytic RBC and/or giant platelets are cause for improper impedance platelet. An abnormal MPV without abnormal platelet/RBC histogram appears it suggests giant platelets are cause of concern for improper impedance platelet count. An abnormal platelet/RBC histogram without abnormal MPV suggests microcytic RBC to be a cause for improper impedance platelet count.

MPV, histogram analysis and RDW can be put in practice for knowing the reliability of impedance platelet count in severe microcytosis (MCV less than 60 femtoliter) particularly in near normal impedance platelet count. The authorised signatory of that report should make a remark on report that impedance platelet count is corrected so clinical or laboratory physician can take a note of the same.

Following conclusions derived from present study-
1: Impedance platelets count is not always reliable in cases of severe microcytosis.
2: Abnormal MPV, RDW and platelet/RBC histogram can provide very important hint for correction of impedance platelet count.
3: Peripheral smear review in severe microcytosis is very helpful.
References