**The usefulness of Cell Population Data of leucocytes in accurately predicting the etiology of acute febrile illness**

Dr T. Bhuvaneshwari¹, Ms. Malini. S², Dr R. Vijayashree³*

¹ Assistant Professor, Department of Pathology, Chettinad Hospital and Research Institute (CHRI), Chettinad Academy of Research and Education (CARE), Kelambakkam, Chennai.

² II MBBS student, Chettinad Hospital and Research Institute (CHRI), Chettinad Academy of Research and Education (CARE), Kelambakkam, Chennai.

³ Professor/HOD, Department of Pathology, Chettinad Hospital and Research Institute (CHRI), Chettinad Academy of Research and Education (CARE), Kelambakkam, Chennai.

**ABSTRACT**

**TITLE:** The usefulness of Cell Population Data of leucocytes in accurately predicting the etiology of acute febrile illness.

**BACKGROUND & AIM:** A single diagnostic test for acute undifferentiated febrile illnesses is elusive and this study is undertaken on the premise that leucocytes undergo quantitative, structural and functional changes in acute fever, in differentiating the common etiologies of acute fever due to dengue, malaria and enteric fever, a major reason for the population in this part of the country to seek medical care. This study utilizes the cell population data (CPD) generated through VCS technology to identify the abnormal leucocytes, which help in screening and detection of hematologic and non-hematologic diseases. To retrospectively analyse and compare the Cell Population Data and lymph index in 100 seropositive cases for either dengue, malaria or enteric fever and to establish criteria for predicting these based on VCS parameter obtained from the automated analyzer LH 780 for analysis by comparing with age and gender matched 100 control samples. **RESULTS:** Lymph index (LV * LV-SD/ LC) was calculated for both cases and controls. The lymph index of cases were increased in cases of dengue and malaria when compared to controls. The Mean neutrophil volume, neutrophil conductance SD, mean lymphocyte volume and lymphocyte volume SD were increased significantly in malaria cases. Monocyte conductance SD were increased both in malaria and dengue cases. Lymphocyte scatter SD and monocyte scatter SD showed no difference in both dengue and malaria. These findings will be useful for physicians as the parameters are easily obtained from hematologic auto analyzer with no additional cost. **CONCLUSION:** CPD data are more accurate in evaluating morphological changes in leucocytes in acute febrile illness than a peripheral blood examination which involves time and experience.

Key words: Volume conductance and scatter VCS, Cell population data

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INTRODUCTION: In the developing world, the differential diagnosis for Acute onset fever (>38°C) of less than 2 weeks duration without an apparent cause despite a meticulous history and clinical examination includes significant illnesses such as bacterial sepsis, dengue fever, malaria, enteric fever, rickettsiosis, leptospirosis and Japanese encephalitis both in terms of mortality and morbidity.[1] The clinical signs and symptoms overlap significantly; therefore, diagnosing the specific etiology of Acute Febrile illness (AFI) can be difficult, incorrect and time consuming at times, precluding the appropriate patient management.

Until few years back, the major diagnostic test performed was the complete blood count (CBC) apart from serology. The semi automated and some of the fully automated haematology analyzers reported the differential counts only and the slides had to be screened for cell morphology to gather any further evidence of underlying disease condition. Recently, newer hematology analyzers utilize the cell population data (CPD) generated through VCS technology to flag abnormal cells, which help in screening and detection of hematologic and non hematologic diseases. A single diagnostic test for acute undifferentiated febrile illnesses is elusive and this study is undertaken on the premise that leucocytes undergo quantitative, structural and functional changes in acute fever.

The potential of VCS parameters of leucocytes, and lymph index generated with the haemogram report by the auto-analyzer, in differentiating the common aetiologies of acute fever due to dengue, malaria and enteric fever, a major reason for the population in this part of the country to seek medical care are evaluated in our study. In this study the CPD consisting of volumes of neutrophils, lymphocytes and monocytes (MNeV, MLyV, MMoV,) and their standard deviations (SD), also known as neutrophil volume distribution width; conductance of neutrophils, lymphocytes and monocytes (MNeC, MLyC, MMoC,) and their standard deviations (SD) and scatter of neutrophils, lymphocytes and monocytes (MNe S, MLyS, MMoS,) and their standard deviations (SD) along with lymph index, which is MLyV x Ly-SD / MLyC are analysed.

Koening and Quillen demonstrated higher neutrophil volume distribution width (NVDW) in bacterial infections and higher lymphocyte volume distribution width (LVDW) in viral infections in childhood.[2] Celik et al. studied the levels of neutrophil VCS parameters in neonatal sepsis and observed significant increases in mean neutrophil volume (NV), neutrophil volume distribution width (NVDW), neutrophil conductivity distribution width (NCDW), and significant decreases in mean neutrophil conductivity (NC) and mean neutrophil scatter (NS).[3] Lee and Kim evaluated mean cell volumes of neutrophils and monocytes in elderly patients with sepsis. They observed that the mean NV and monocytes volume (MV) were higher in the sepsis group than in the localized infection and control groups (p<0.001 for both). A mean NV of 156.5 fL or higher was suggested as a predictor of sepsis with a high sensitivity (83.3%) and specificity (78%).[4] In a study conducted by Briggs et al., response to malarial infection was shown to cause an increased monocyte count and volume.[5] Similarly, the lymph index (LI), was observed to be significantly increased in viral infections when compared to acute bacterial infections and controls. However, literature for other causes of fever, like rickettsial diseases, enteric fever and TB, are not available.
AIMS AND OBJECTIVES: To retrospectively analyse the Cell Population Data and lymph index (retrieved from automated analyzer with VCS technology) from 100 febrile patients visited the hospital who are proven seropositive for either dengue, malaria or enteric fever. To establish a criteria for predicting dengue, malaria and enteric fever based on VCS parameter changes and lymph index.

MATERIAL AND METHODS: This is the Retrospective case control study with 100 cases and 100 controls has a two groups. The demographic, clinical and laboratory data of all those included are compiled from the hospital records and hematology analyzer. The VCS data obtained from blood samples drawn at the time of presentation to the hospital and analysed by the haematology analyzer LH780 are retrieved for analysis. The 100 adult patients with an established diagnosis of acute malaria, dengue fever, or enteric fever are included as cases and the 100 apparently healthy individuals are used as controls. Subjects with acute fever of less than 15 days duration, who are seropositive for dengue, malaria or enteric fever, are included in the study. Malaria is diagnosed by smear positivity, dengue infection by positive IgM, NS1 serology (rapid card test & ELISA) while Enteric fever was diagnosed on the basis of culture positivity for Salmonella sp., and/or positive Widal test (defined as somatic O and flagellar H titre >160 at baseline or a fourfold rise in titre over 2 weeks). Subjects with co-infections and/or with underlying chronic diseases like arthritis; malignancy; hypothyroidism, Diabetes, Megaloblastic anemia, leukemia and chronic kidney disease are excluded.

Experimental details: The VCS parameters of leucocytes and lymph index (LV X LV-SD / LC) calculated using Cell Population Data of the cases and controls are retrieved from the automated haematology analyzer LH 780. The obtained values are analysed for dengue, malaria and enteric fever independently and compared to each other subsequently. Also lymph index calculated is correlated with the specific diagnosis of febrile illness.

Statistical methods: Mean is used as the measure of central tendency and standard deviation as the measure of dispersion for descriptive statistics. Analysis of variance (ANOVA) with Bonferroni post-hoc analysis is used to compare the means of various etiological groups using the statistical software SPSS version 19 (IBM, Armonk, NY, USA). A p-value of <0.05 is considered as significant.

OBSERVATIONS AND RESULTS: In this study of 100 patients with acute febrile illness, 80 are proven seropositive for dengue, 19 for malaria and 1 for enteric fever. The cell population data (VCS parameters) for these patients and 100 age & sex matched controls are retrieved from the automated analyzer LH 780. Although the total count and platelet count revealed significant differences between the patients and the controls, we analyzed the VCS parameters of leucocytes for cases and controls independently and then compared. Also lymph index (LV * LV-SD/ LC) is calculated for both cases and controls. Table:1
Table: 1 Cell population data of cases and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls n=100</th>
<th>Cases(patients with acute febrile illness) seropositive for Dengue n=80</th>
<th>Malaria n=19</th>
<th>Enteric fever n=1</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNV</td>
<td>146.07± 5.65</td>
<td>147.94± 6.93</td>
<td>159.22± 10.04</td>
<td>138.3</td>
</tr>
<tr>
<td>NVDW</td>
<td>21.13± 8.48</td>
<td>23.08± 3.96</td>
<td>25.17± 3.15</td>
<td>20.33</td>
</tr>
<tr>
<td>MNC</td>
<td>147.28± 4.43</td>
<td>144.91± 3.91</td>
<td>143.31± 4.55</td>
<td>150.7</td>
</tr>
<tr>
<td>NCDW</td>
<td>6.20± 1.10</td>
<td>6.73± 1.13</td>
<td>7.32± 1.29</td>
<td>5.58</td>
</tr>
<tr>
<td>MNS</td>
<td>141.21± 7.35</td>
<td>140.55± 5.45</td>
<td>136.94± 7.58</td>
<td>140.1</td>
</tr>
<tr>
<td>NSDW</td>
<td>10.95± 1.67</td>
<td>12.96± 3.60</td>
<td>12.70± 2.17</td>
<td>9.66</td>
</tr>
<tr>
<td>MLV</td>
<td>82.34± 2.70</td>
<td>86.57± 4.91</td>
<td>91.42± 6.05</td>
<td>90.5</td>
</tr>
<tr>
<td>LVDW</td>
<td>14.22± 1.57</td>
<td>18.99± 3.42</td>
<td>17.72± 2.05</td>
<td>14.36</td>
</tr>
<tr>
<td>MLC</td>
<td>116.70± 20.70</td>
<td>113.68± 12.08</td>
<td>113.02± 4.52</td>
<td>117.2</td>
</tr>
<tr>
<td>LCDW</td>
<td>11± 2.14</td>
<td>13.5± 2.73</td>
<td>12.18± 1.53</td>
<td>9.78</td>
</tr>
<tr>
<td>MLS</td>
<td>62.60± 5.62</td>
<td>61.3± 5.07</td>
<td>65.15± 6.30</td>
<td>63.2</td>
</tr>
<tr>
<td>LSDW</td>
<td>18.49± 1.50</td>
<td>18.1± 1.52</td>
<td>17.46± 1.63</td>
<td>15.23</td>
</tr>
<tr>
<td>MMoV</td>
<td>163.83± 6.10</td>
<td>177.21± 8.34</td>
<td>189.40± 11.78</td>
<td>168.3</td>
</tr>
<tr>
<td>MoVDW</td>
<td>18.12± 2.14</td>
<td>25.22± 3.22</td>
<td>26.33± 3.90</td>
<td>20.76</td>
</tr>
<tr>
<td>MMoC</td>
<td>122.07± 11.37</td>
<td>124.05± 3.98</td>
<td>121.88± 5.13</td>
<td>127.9</td>
</tr>
<tr>
<td>MoCDW</td>
<td>4.45± 0.53</td>
<td>5.81± 1.33</td>
<td>5.33± 1.24</td>
<td>4.6</td>
</tr>
<tr>
<td>MMoS</td>
<td>85.73± 5.35</td>
<td>86.51± 3.67</td>
<td>83.99± 6.29</td>
<td>90.2</td>
</tr>
<tr>
<td>MoSDW</td>
<td>11.51± 4.05</td>
<td>11.16± 1.26</td>
<td>10.56± 1.25</td>
<td>9.81</td>
</tr>
<tr>
<td>LYMPH INDEX</td>
<td>10.17± 1.44</td>
<td>15.54± 10.36</td>
<td>14.38± 2.16</td>
<td>11.08</td>
</tr>
</tbody>
</table>

MNV-mean neutrophil volume; NVDW- neutrophil volume distribution width; MNC-mean neutrophil conductance; NCDW- neutrophil conductance distribution width; MNS- mean neutrophil scatter; NSDW- neutrophil scatter distribution width; MLV-mean lymphocyte volume; LVDW- lymphocyte volume distribution width; MLC-mean lymphocyte conductance; LCDW- lymphocyte conductance distribution width; MLS- mean lymphocyte scatter; LSDW- lymphocyte scatter distribution width; MMoV-mean monocyte volume; MoVDW- monocyte volume distribution width; MMoC-mean monocyte conductance; MoCDW- monocyte conductance distribution width; MMoS- mean monocyte scatter; MoSDW- monocyte scatter distribution width.
Of the 100 patients included in this study, 89 were males and 11 were females. The mean age of the patients was 29.04±13.6 with male predominance. The Neutrophil volume SD, lymphocyte volume mean and SD, monocyte volume mean and SD are increased in cases of dengue significantly in our study. The Mean neutrophil volume, neutrophil conductance SD, mean lymphocyte volume and lymphocyte volume SD are increased significantly in malaria cases with p value of <0.05. However neutrophil conductance mean, scatter mean and monocyte scatter mean are reduced when compared to controls. Lymphocyte volume mean and SD; monocyte volume mean and SD; monocyte conductance SD are increased both in malaria and dengue cases. Lymphocyte scatter SD and monocyte scatter SD showed no difference in both dengue and malaria.

**DISCUSSION:** Activated white blood cells in acute infection undergo changes both in terms of relative numbers as well as their morphological properties proving that these are the main cells responsible for the body’s defense. Jung et al. evaluated CPD of Unicel DxH800 Coulter system to screen for viral infection in children using a combination of CBC and CPD parameters. They achieved a sensitivity of 96.1% & specificity of 93.7% for detecting viral infection. Koening and Quillen demonstrated higher NVDW in bacterial infections and higher lymphocyte volume distribution width (LVDW) in viral infections in childhood. Simon observed that the monocytes anisocytosis was a new hematological marker for detection of dengue fever, similar to our results.

Lymph index was significantly increased in malaria and dengue cases similar to Ranjana et al., who used lymph index as a marker of dengue infection and found that a lymph index cut off of >13.6 achieved a sensitivity & specificity of 71.17% and 78.05% respectively in predicting dengue infection as compared to controls. Zhu et al. developed lymph index, a potential hematological parameter for viral infection. They observed that the lymph index was significantly increased in viral infections & very minimal increase was observed in bacterial infections. Using a cut off of lymph index >12.92, they achieved a sensitivity of 91.67% and specificity of 97.2% for diagnosis viral infections. Two recent abstract from Puerto Rico have described a high area-under-curve for a dengue factor calculated by combining quantitative and morphologic monocyte information in an equation (% monocyte + SD of monocyte volume). This factor was superior to the conventionally used platelet and leucocyte counts in differentiating dengue positive from dengue negative controls.

Briggs et al. developed an automated malaria discrimination factor using VCS technology. In this study response to malarial infection was shown to cause an increased monocyte count and production of large activated monocytes detectable by the VCS technology. By using a calculation derived from the SD of the volume of the lymphocytes and monocytes, a malaria factor was put forth for the detection of malaria with a very high sensitivity (98%) and specificity (94%). Compared to the controls, the mean NV, LV, MV and monocyte scatter (MS) were increased, and NS and MC were reduced in malaria in the study by Briggs et al. Our study didn't show difference in monocyte and neutrophil scatter.

Fourcade et al. conducted a study to detect malaria by means of hematology analyzer. This was probably the first study for malaria. Subsequently many studies have been conducted on the usefulness of CPD in detection of malaria.
Earlier, it was contested that there is a significant variation in the VCS parameters of the leucocytes even in the healthy individuals. Tang et al. in 2012 demonstrated little fluctuation in biological variations for cell population data in healthy individuals at homeostatic set point. Thus, this makes them reasonably reliable parameters clinically.[11]

In 2005, Chaves Fernando et al conducted a study for the quantitative determination of neutrophils VCS parameters as indicators of acute bacterial infection. They observed that the elevation of MNeV was associated with a higher WBC count and at a cut off value of 150 for the MNeV, there was 91% specificity and 70% sensitivity. Chaves Fernando et al in 2006 conducted a study on neutrophils volume distribution width in acute bacterial infection. They observed a significant increase in NeVDW in bacteremic patients. With a cut off of 23 for NeVDW, they achieved 100% specificity and 69% sensitivity. [12,13] Raimondi et al., Mardi et al. and Park et al. conducted similar studies with neutrophils volumes in pediatric patients, non systemic bacterial infections respectively.[14,15,16,17,18,19,20,21,22]

CONCLUSION: Our study showed that there are significant changes in some of the VCS parameters of Dengue and malaria cases when compared to controls. Our findings will be useful for physicians as these parameters are easily obtained from hematology auto analyzer. There is no additional cost involved and CPD data are more accurate in evaluating morphological changes in leucocytes in acute febrile illness than a peripheral blood examination which involves time and experience. An accurate, single, reliable and rapid hematology analyzer-based diagnostic test for the acute febrile illness can be developed for routine screening at no extra cost by studying the trend of VCS parameter changes in acute fever.

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