IN-VITRO LEUCOAGGLUTINATION:
A STUDY OF 11 CASES

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ABSTRACT

Objective: Cold agglutination of red blood cells and platelets are well recognized and extensively studied in literature. However aggregation of leukocytes in vitro is rare and may result in spuriously low leucocyte count. We present a case series of 11 patients with spurious leukopenia due to leucoagglutination in-vitro.

Methods: A retrospective study was conducted at Clinical laboratory & Haematology division, Kasturba Hospital, compiling 11 cases which showed WBC clumping on the peripheral smear in the last 2 years.

Results: The aetiologies ranged from immune mediated (4 cases), liver diseases (2 cases) and infections/drug induced (5 cases). The incubation of samples at 37°C for 30 minutes caused disaggregation of leucocytes; thus denoting the role of temperature dependent antibody.

Conclusion: Leucoagglutination in-vitro can be linked to an immunological phenomenon, possibly due to a temperature-dependent antibody

Key words: Leucoaggregates, EDTA (Ethylenediaminetetraacetic acid), antibody, leucocytes

INTRODUCTION

The increasing application of automated analyzers for routine hematologic procedures has several advantages. Several of them are known for their unbeaten service excellence in terms of data reproducibility and accuracy. However possibility of spurious cannot be overseen. They may be due to the interaction of blood cells with components of serum such as antibodies, drugs and their derivatives or even something trivial as additives in reagents. Among these, platelet aggregation, neutrophil-platelet clumping (satellitosis), and aggregation of platelets, resulting in pseudothrombocytopenia or pseudoleukocytoysis have been repeatedly described.¹-³ In-vitro aggregation is a well-known laboratory phenomenon for platelets and erythrocytes due to EDTA (Ethylenediaminetetraacetic acid) & cold agglutinins respectively. But aggregation of neutrophils (also known as leuco-aggregation, neutrophil agglutination and leucocyte clumping) in peripheral smear is a very rare phenomenon and seldom described.⁴,⁵

We present a case series of 11 patients with spurious leukopenia due to leucoagglutination in-vitro. The objective of our study was to assess the clinico-pathological profile of leucoagglutination in-vitro and also to determine the potential usefulness of the Research Population Data (RPD) such as Mean Neutrophil Volume (MNV) in predicting this phenomenon.

Only few case reports of leucoagglutination leading to spurious leukopenia have been published in literature. We believe it is not a very uncommon phenomenon but it is basically underrated or overlooked due to unawareness. This anomalous low WBC count can be clinically perplexing and lead to additional patient testing & unnecessary treatment, thus necessitating the recognition of this entity. A morphological evaluation along with new
automated analyser parameters could possibly predict this phenomenon.

**MATERIALS AND METHODS**

A retrospective study conducted at Clinical laboratory & Haematology division, Kasturba Hospital, Manipal. A total of 11 cases of WBC clumping in the peripheral smear in the last two years (2011 – 2013); were included in the study. WBC counts in these cases were flagged by Beckman Coulter LH 750™ series analyzer as “uncorrected” WBC count and were lower than the normal reference range in most cases. Thus the corresponding peripheral smears before and after incubating (37°C for 30 minutes) the sample were also studied. The Volume conductivity scatter (VCS) parameters and research population data (RPD) were noted for all cases. A correlation with the clinical profile was done using the patient case files from medical records department. An Institutional ethical clearance was obtained for the study.

**RESULTS**

In our study the age ranged from 35-85 years with an equal sex predilection (table 1). The aetiologies ranged from immune mediated (4 cases), liver diseases (2 cases) and infections/drug induced (5 cases).

2 out of 11 cases showed serologic positivity for HIV. The WBC counts in all the 11 cases increased after incubating blood samples at 37°C for 30 minutes; suggesting that

<table>
<thead>
<tr>
<th>Age yrs</th>
<th>Sex</th>
<th>Clinical features</th>
<th>HIV status</th>
<th>WBC count before warming/μl</th>
<th>WBC count after warming/μl</th>
<th>Neutrophil%</th>
<th>MNV +/- SD(f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>F</td>
<td>Fever with LRTI</td>
<td>Neg</td>
<td>2.3x10^3</td>
<td>5.1x10^3</td>
<td>73%</td>
<td>163 +/- 42</td>
</tr>
<tr>
<td>76</td>
<td>M</td>
<td>Rheumatoid arthritis on treatment</td>
<td>Neg</td>
<td>1.4x10^3</td>
<td>4.6x10^3</td>
<td>50%</td>
<td>174 +/- 41</td>
</tr>
<tr>
<td>61</td>
<td>M</td>
<td>Viral hepatitis</td>
<td>NA</td>
<td>5.7x10^3</td>
<td>8.6x10^3</td>
<td>91%</td>
<td>184 +/- 49</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>Rhabdomyosarcoma on chemotherapy</td>
<td>Neg</td>
<td>1.1x10^3</td>
<td>2.6x10^3</td>
<td>28%</td>
<td>176 +/- 38</td>
</tr>
<tr>
<td>50</td>
<td>M</td>
<td>Tuberculosis</td>
<td>Pos</td>
<td>2.9x10^3</td>
<td>4.2x10^3</td>
<td>55%</td>
<td>155 +/- 36</td>
</tr>
<tr>
<td>84</td>
<td>F</td>
<td>Adenocarcinoma lung on treatment</td>
<td>Neg</td>
<td>15.6x10^3</td>
<td>25.0x10^3</td>
<td>89%</td>
<td>156 +/- 32</td>
</tr>
<tr>
<td>40</td>
<td>M</td>
<td>Alcoholic liver disease</td>
<td>Neg</td>
<td>3.2x10^3</td>
<td>7.9x10^3</td>
<td>69%</td>
<td>161 +/- 22</td>
</tr>
<tr>
<td>80</td>
<td>F</td>
<td>Bronchiectasis</td>
<td>NA</td>
<td>3.8x10^3</td>
<td>5.4x10^3</td>
<td>59%</td>
<td>164 +/- 38</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>PUO with arthralgia</td>
<td>Neg</td>
<td>17.2x10^3</td>
<td>23.5x10^3</td>
<td>88%</td>
<td>138 +/- 30</td>
</tr>
<tr>
<td>50</td>
<td>M</td>
<td>Disseminated Tuberculosis</td>
<td>Pos</td>
<td>2.5x10^3</td>
<td>6.1x10^3</td>
<td>77%</td>
<td>159 +/- 28</td>
</tr>
<tr>
<td>40</td>
<td>M</td>
<td>PUO</td>
<td>NA</td>
<td>7.5x10^3</td>
<td>9.0x10^3</td>
<td>63%</td>
<td>146 +/- 21</td>
</tr>
</tbody>
</table>

*Neg-negative, Pos-Positive, NA-not done, MNV-Mean Neutrophil Volume, SD-Standard Deviation, WBC-White blood cells, PUO-Pyrexia of unknown origin, LRTI-Lower respiratory tract infection*
the WBC counts were spuriously low before incubation. So a peripheral smear examination was conducted for all the cases which showed neutrophil aggregates of 10-20 neutrophils near the tail end of the smears (fig. 1a). These aggregates disappeared following incubation of samples at 37°C for 30 minutes (fig. 1b); thus denoting the role of temperature-dependent antibody. The Mean Neutrophil Volume (MNV) was increased in most of the cases with a mean of 161.5 fl (normal range of MNV is 138.2 to 147.8 fl).

**DISCUSSION**

Leucoagglutination in-vitro is an elusive phenomenon. Most reports in literature are based on single case studies. This phenomenon has been linked to underlying malignancies, infections, hepatic disorders or autoimmune diseases. Interestingly, it has also been noted in apparently healthy subjects as well. One does come across these leucocyte aggregations in many smears; albeit infrequently. But in most of the cases it is just a spreading or smearing artefact. It should be distinguished from true leucocyte aggregation wherein additionally, the total WBC count will also be spuriously low. The explanation for this spurious low WBC count being that the size of the clumps which is larger than upper threshold set for WBC detection. So we presume that if the clump is large it not counted at all and if the clump is small consisting of 2-3 neutrophils, it may be counted as a single event thus decreasing the total WBC count even if the counts appear normal on corresponding peripheral smears. In our study only aggregates of neutrophils were seen unlike many other studies where aggregates of lymphocytes, mature neutrophils around immature neutrophils & platelets around neutrophils were also seen. The number of cells in aggregates varied from 10 to 100 cells.

The exact cause for this phenomenon is still not known. One of the proposed mechanisms incriminates the interaction of the anticoagulant EDTA with the leucocytes. Accordingly, EDTA unmasks antigens on the surface of WBCs, which creates the potential for any antibody in the serum to non-specifically bind and cross-link the leucocytes. In our study, EDTA was the anticoagulant used in all the smears. Secondly, it was noted that there was a reversal of clumping of the neutrophils after warming the sample to 37°C; suggesting that this reaction was also temperature-dependent. Was it the IgM antibodies then, which caused this phenomenon to manifest? The possible clues to this query may be in the medical condition of the patients. 4 cases had some underlying immune dysregulation, while 2 others had liver disorders. Still others had infectious aetiologies. These may explain the role of IgM antibodies, although the titre of antibodies was never assessed.

Another interesting aspect of this phenomenon is that leucocyte agglutination is also a time-dependent phenomenon. The number and the size of the clumps depend on the time elapsed. In our laboratory, analysis of blood sample is conducted within 20 minutes of phlebotomy. Peripheral smear is prepared & reviewed within next 30 min. Mixture of both small and large clumps of neutrophils were seen. Repeat smear from the sample showed a marginal increase in the size of the neutrophil aggregates; possibly denoting the time dependent nature of the event.
The Coulter LH-750 VCS technology determines the 5-part WBC differential using 3 measurements - individual cell volume, high-frequency conductivity, and laser-light scatter. We depend a lot on VCS parameters in our routine reporting to look for reactive lymphocytes, left shift, etc. However, not many studies have been done to identify this rare phenomenon of leucoagglutination using VCS technology. We had made an attempt to use Mean neutrophil volume (MNV) to suspect the presence of leucoagglutination. In our study all 11 cases showed an increase MNV, with the mean being 161.5 fl (normal range of MNV is 138.2 to 147.8 fl). But the necessary disclaimer to this finding is that the same MNV can increase in acute infections, malaria as well as leukaemia's. Thus, with an appropriate clinical perspective, the MNV can potentially prove to be a useful clue in identifying this phenomenon.

CONCLUSION

Leucoagglutination in-vitro can be linked to an immunological phenomenon. The role of a temperature-dependent antibody cannot be ruled out. An increased MNV is a useful predictor in this regard. Hematopathologist should be aware of this spurious leukopenia to avoid unnecessary diagnostic tests and inappropriate treatment. Further studies are necessary to shed light on this elusive phenomenon.

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REFERENCES