Severe inflammatory and infectious conditions can elicit different reactions including pyogenic, granulomatous, lymphocytic and also non-inflammatory changes. The most common tissue response in acute inflammation is dominated by neutrophils. During inflammatory or infectious conditions, mature neutrophils can display toxic granulation (TG). TG is characterised as dark blue-black granules in the cytoplasm of mature neutrophils and is especially helpful in predicting acute bacterial infection. The appearance of TG is the result of an increase in acid mucosubstance in azurophilic granules, which stains more prominently than under normal circumstances.

These toxic granules are formed in the promyelocyte stage during neutrophil maturation, and contain antimicrobial substances. The acidic mucosubstance accumulated in the toxic granules also has the potential to acidify phagosomes. These toxic granules enhance bactericidal activity, since bacteria in phagosomes are killed more effectively at a pH of 5.5 than at a pH of 7. The formation of toxic granulated neutrophils (TGN) is induced by granulocyte colony-stimulating factor (G-CSF), which increases the number of granulocyte precursors in bone marrow that activate mature granulocytes. During inflammation, the serum concentrations of G-CSF and plasma interleukin-6 (IL-6) increase.

The correlation between C-reactive protein and toxic granulation of neutrophils can possibly be used as a surrogate marker to assess infection or inflammation and their response to treatment. It may be of particular use in cases where traditional infectious or inflammatory markers cannot be used, owing to inherent problems associated with the respective conditions.

**Background.** During inflammation, the serum concentrations of granulocyte colony-stimulating factor (G-CSF), plasma interleukin-6 (IL-6), and C-reactive protein (CRP) increase. A positive correlation between CRP and the percentages of neutrophils exhibiting toxic granulation during inflammation has been demonstrated, and that the fluctuations of CRP and toxic granulation of neutrophils were similar.

**Objectives.** We studied whether grading of toxic granulated neutrophils can be used as a surrogate marker for infection or inflammation, and also be an easier method than previously described methods.

**Materials and methods.** We graded 357 consecutive peripheral blood slides from patients on whom a full blood count with differential count and CRP level was performed, according to intensity of toxic granulation in the neutrophil population, according to a newly proposed grading system.

**Results.** The CRP range was between 1 and 530.3 mg/l. The results confirm the association between a rise in CRP and progressive intensity of toxic granulation in neutrophils in peripheral blood. Kruskal-Wallis equality of populations rank test showed a statistically significant difference between the graded categories ($p=0.0001$). The Trend test was also statistically significant ($p=0.000$).

**Conclusion.** The proposed system can be applied to patients with inflammatory or infectious conditions, where grading of toxic granulation of neutrophils can possibly be used as a surrogate marker to assess infection or inflammation and their response to treatment. It may be of particular use in cases where traditional infectious or inflammatory markers cannot be used, owing to inherent problems associated with the respective conditions.
a surrogate marker for infection or inflammation. This method is less labour-intensive and less time-consuming than the methods used in previous studies.

**Materials and methodology**

Ethical approval was obtained from the Faculty of Health Science Research Ethics Committee, University of Pretoria, on 29 April 2009, protocol number 61/2009.

From patients seen in Steve Biko Academic Hospital, Pretoria, we examined 357 consecutive peripheral blood slides, where the treating clinician had requested a full blood count, with a differential white cell count, as well as CRP. Neonates and patients with suspected or confirmed acute leukaemia were excluded. Peripheral blood smears were made from venous blood samples collected in vacutainer tubes, containing ethylene diamine tetra-acetic acid (EDTA), and stained with Wright Giemsa stain. Morphological examination of neutrophils was performed at magnifications of 500x and 1 000x.

We evaluated a possible correlation between the serum level of CRP and the graded intensity of observed TG in neutrophils, according to number and stain intensity. A minimum of 50 cells were evaluated on every slide, and an average impression was obtained and categorised. Neutrophils with TG were defined by the presence of dark blue to purple cytoplasmic granules in the neutrophils (Fig. 1).

The TG intensity was graded into 5 categories (0, 1+, 2+, 3+ and 4+), as set out in Table I. Serum levels of CRP were measured by a nephelometric method utilising CRP antibodies (Beckman Coulter DX1). The CRP distribution between the 5 TG categories was evaluated to assess a possible trend or relationship.

Statistical analysis was performed using STATA 9 statistical package (STATA Corporation). The results were expressed in box and whisker plots analysis to determine the mean, minimum and maximum CRP levels, in each graded category. Statistical difference between the categories was evaluated using Kruskal-Wallis equality of populations rank test. A trend test was also performed.

Table I. CRP characteristics in TG categories

<table>
<thead>
<tr>
<th>Group</th>
<th>CRP mean (mg/l)</th>
<th>Minimum CRP</th>
<th>Maximum CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.3</td>
<td>1</td>
<td>470.8</td>
</tr>
<tr>
<td>1</td>
<td>108.2</td>
<td>1</td>
<td>530.3</td>
</tr>
<tr>
<td>2</td>
<td>129.3</td>
<td>1</td>
<td>405.8</td>
</tr>
<tr>
<td>3</td>
<td>193.7</td>
<td>1</td>
<td>405.8</td>
</tr>
<tr>
<td>4</td>
<td>129.3</td>
<td>3</td>
<td>405.8</td>
</tr>
</tbody>
</table>

After the initial analysis into 5 categories of graded toxic granulation (0, 1+, 2+, 3+ and 4+), the categories were combined and all specimens were grouped as 0 (no TG observed), 1 (1+ and 2+ TG) and 2 (3+ and 4+ TG), to improve statistical significance.

**Results**

In total, 357 consecutive peripheral smears were evaluated. The CRP figures ranged between 1 and 530.3 mg/l. A statistically significant difference between the graded categories was demonstrated (p<0.001), as well as the trend between incremental TG categories (p<0.001) (Fig. 2, Table II). These results confirm a trend in the rise of CRP in proportion to the TG of neutrophils in peripheral blood. This could be applied to patients with inflammatory or infectious conditions, where the grading of TG of neutrophils can possibly be used as a marker of the severity of infection or inflammation.

**Discussion**

The relationship between TG in mature neutrophils and high CRP levels in infectious disease and inflammation has been...
diseases and HIV-1 infection. The degree of TG can be a useful adjunct in this setting, where most other markers have been confounded.

We found a statistically significant trend between grading the intensity of toxic granulation in neutrophils and a rise in CRP. Statistically significant differences in CRP were found between the graded TG categories. Morphological changes in neutrophils, especially TG, are therefore helpful in evaluating disease severity in patients with underlying inflammatory or infectious conditions.

Conclusion

The above results confirm a trend in the rise of CRP, as the intensity of TG of neutrophils in peripheral blood increases. This finding could be applied in patients with inflammatory or infectious conditions, where grading of TG of neutrophils can possibly be used as a marker to assess infection or inflammation severity. This method could be especially valuable in cases where the infective markers do not reflect the clinical picture.

References


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