Clinical Commentary: Granulocytic Fragments in Sepsis

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Abstract. Granulocytic fragments have been described in the peripheral blood of patients with sepsis and the systemic inflammatory response syndrome (SIRS). Although initially proposed as a morphologic clue for distinguishing the leukoerythroblastosis of sepsis from that of myelophthisis or marrow replacement by tumor, granulocyte-derived fragments may be part of a spectrum of cellular fragmentation associated with pathological inflammation and thrombosis, and thus play an important role in the pathophysiology of sepsis and SIRS. Pathologists, hematologists, and medical technologists should be aware of their existence, the morphologic features that distinguish them from macrothrombocytes and schistocytes, and their potential significance. (received 24 July 2001; accepted 23 August 2001)

Keywords: granulocyte, neutrophil, microparticle, sepsis, systemic inflammatory response syndrome

Early descriptions of granulocytic fragments

Granulocytic fragments in the peripheral blood were first described in leukemia and lymphoma [1,2]. When these were later identified in patients with fatal sepsis [3], it was suggested that granulocyte-derived fragments could be a morphological aid to distinguish the leukoerythroblastosis of sepsis from that of myelophthisis or marrow replacement by tumor and to identify patients at high risk of death [3,4]. Interestingly, granulocytic fragments were not detected when peripheral blood smears of patients with leukemoid reactions were examined [4].

The identification of granulocytic fragments in a recent case of fatal neonatal disseminated herpes simplex virus infection [5], coupled with a recent report of granulocyte-derived fragments (microparticles) in non-fatal meningococcal sepsis [6], prompted this review and summary of 10 cases of fatal sepsis in which granulocytic fragments were identified in antemortem blood smears. The authors present a re-appraisal of the potential significance of circulating granulocytic fragments in systemic inflammatory conditions.

Granulocytic fragments in peripheral blood smears

While granulocyte-derived fragments are not specifically identified by automated cell counters, they are readily recognized on Wright’s-stained peripheral blood smears. The photomicrograph in Fig. 1 illustrates a granulocytic fragment among erythrocytes in the peripheral blood smear of a 10-day-old infant who died from fatal disseminated herpesvirus infection [5]. As illustrated, granulocytic fragments are characteristically 5-10 µm in diameter with evenly distributed primary granules within faintly basophilic cytoplasm; they can readily be distinguished from macrothrombocytes (which have more basophilic cytoplasm and irregularly distributed granules) and schistocytes. It is probable that unfamiliarity of pathologists, hematologists, and medical technologists with the existence of granulocyte-derived fragments has resulted in their infrequent recognition and underreporting.

Origin of granulocytic fragments

Granulocytic fragments in the peripheral blood appear to be derived from circulating leukoerythroblastic precursors (as opposed to bone marrow), as evidenced by the lack of identifiable fragments in the marrow when thin sections of bone were...
examined [3,4]. Current models of neutrophil activation and adhesion, and of leukocyte-platelet and leukocyte-endothelial cell interactions provide a conceptual framework for understanding the potential mechanisms for fragmentation of circulating granulocytes in sepsis. After circulating leukocytes are activated by cytokines such as IL-1, TNF-alpha, and IL-6 [7,8], altered gene expression can be identified by a change in mRNA expression [9]. Neutrophil rolling, tethering, and adhesion to endothelial surfaces is mediated by tightly regulated expression of p-selectin, p-selectin ligand, and other cellular adhesion molecules [10]. Using high speed, high-resolution differential interface contrast videomicroscopy, membrane tethers have been directly observed when neutrophils are exposed to p-selectin coated surfaces [11]. Previously observed unusual projections of granulocyte fragments may be related to these tethers [4]. Altered biomechanical properties of neutrophils bound to activated endothelial cells may render them “stiffer” and more susceptible to fragmentation [12]. Little is presently known about the fate of these fragments, whether contents of cytoplasmic granules are released locally or systemically at the time of fragmentation, and what role the released contents may play in activating other granulocytes, endothelial cells, or platelets.

Granulocytic fragments in sepsis and SIRS

Ten patients with granulocytic fragments have been identified at our institution during a period of 10 years (Table 1). Clinical data and diagnostic laboratory findings from these patients with severe sepsis and/or systemic inflammatory response syndrome (SIRS) have been reported in detail [3-5]. In summary, Wright’s-stained peripheral blood smears from samples submitted for multichannel analysis and automated hemograms during routine clinical care were reviewed by a pathologist when the uncorrected WBC count was >50 x10⁹/L. Granulocyte-derived fragments were identified by morphology as described above and shown in Fig. 1.

Because these patients were identified during a period of 10 years, various laboratory instruments and techniques were used to analyze these samples [3,4]. In some patients, cytochemical stains, centrifugation and analysis of buffy coat smears, thin sections of bone, flow cytometry, and electron microscopy were performed [3,4]. These 10 patients are excellent examples of the diverse underlying conditions associated with sepsis and SIRS [8].

Cellular fragmentation in inflammation

Inflammatory and procoagulant mediators are now known to be intimately involved in the pathophysiology of sepsis and SIRS [13]. The importance of platelet-derived microparticles in disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, thrombosis of atherosclerosis, and other disorders where inappropriate coagulation occurs is well documented and seems related to the presence of tissue factor and platelet antigens [6]. Platelets are not, however, the sole source of microparticles; granulocytes, monocytes, and erythrocytes have recently been confirmed as sources of microparticles [6]. Perhaps due to their underrecognition, little attention has been directed to granulocytic fragments.

Recently described interactions between leukocytes and platelet-derived microparticles and the established links between inflammation and thrombosis support our contention that granulocyte-derived fragments may play an important role in the pathophysiology of severe sepsis. Platelet microparticles have been found to bind to and activate neutrophils [14]. Tissue factor has been localized to granulocyte fragments and has been shown to be incorporated into spontaneous human thrombi [15]. Leukocyte microparticles have also been demonstrated to stimulate endothelial cell cytokine release via a JNK-mediated pathway [16,17]. Finally, therapeutic approaches to severe sepsis and SIRS based on the removal of activated neutrophils and microparticles are under active investigation [18-20].

Granulocyte-derived fragments or microparticles are recognizable on Wright’s-stained peripheral blood smears and should be considered part of the spectrum of cellular fragmentation associated with sepsis and SIRS. Indirect evidence suggests that granulocytic fragments may play a role in the pathophysiology of sepsis and represent another link between inflammation and thrombosis.
Table 1. Clinical data and blood leukocyte counts for 10 patients with granulocytic fragments in Wright’s-stained smears of peripheral blood, who were observed at the Medical College of Georgia.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Gender</th>
<th>Underlying Disease</th>
<th>WBC (x10⁹/L)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55 yr</td>
<td>female</td>
<td>Crohn’s disease with perineal fistula</td>
<td>68</td>
</tr>
<tr>
<td>2</td>
<td>6 yr</td>
<td>male</td>
<td>Postoperative heart surgery</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>60 yr</td>
<td>male</td>
<td>Necrotizing pancreatitis</td>
<td>57</td>
</tr>
<tr>
<td>4</td>
<td>60 yr</td>
<td>female</td>
<td>Hepatic failure, gangrenous cholecystitis</td>
<td>34</td>
</tr>
<tr>
<td>5</td>
<td>37 yr</td>
<td>male</td>
<td>Ruptured berry aneurysm</td>
<td>57</td>
</tr>
<tr>
<td>6</td>
<td>33 yr</td>
<td>male</td>
<td>Multiple blunt trauma, Klebsiella pneumonia</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>1 mo</td>
<td>male</td>
<td>Inoperable necrotizing enterocolitis</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>31 yr</td>
<td>male</td>
<td>Multiple blunt traumatic injuries</td>
<td>31</td>
</tr>
<tr>
<td>9</td>
<td>42 yr</td>
<td>female</td>
<td>Abdominal gunshot wound, sepsis</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td>10 da</td>
<td>female</td>
<td>Disseminated neonatal herpes</td>
<td>19</td>
</tr>
</tbody>
</table>

*When nucleated red blood cells (nRBCs) were present, the corrected white blood cell count (cWBC) was calculated by dividing the automated total WBC x 100 by 100 plus the number of nRBCs.

Fig. 1. Granulocytic fragment in a patient with fatal disseminated neonatal herpesvirus infection. Granulocyte-derived fragments are recognizable by their size and the even distribution of granules (Wright’s stain, x1,000, oil immersion).
Although all of our patients had fatal sepsis or SIRS with granulocytic fragments that were identified hours or days prior to death, other workers have reported granulocyte-derived fragments (microparticles) earlier in the course of disease in patients who survived meningococcal sepsis, consistent with reversibility of this process [6]. We, therefore, believe that the recognition of granulocytic fragments has diagnostic value. Pathologists, hematologists, and medical technologists should remain aware of their existence when examining peripheral blood smears in patients with leukemoid reactions, leukoerythroblastosis, or suspected sepsis.

References