A New Method for Studying Splenic Reticuloendothelial Dysfunction in Sickle Cell Disease Patients and Its Clinical Application: A Brief Report

By James T. Casper, Susan Koethe, Glenn E. Rodey, and L. Gilbert Thatcher

Differential interference contrast (DIC) microscopy (Nomarsky optics) readily demonstrates the formation of "pits" or crater-like depressions in red cell membranes of splenectomized individuals. Splenic reticuloendothelial dysfunction characteristic of many patients with sickle cell disease (SCD) can be demonstrated by technetium spleen scans, but this technique is expensive, requires injection of radioactive material into children, and is cumbersome to perform at regular intervals. However, pit formation in red cells, which also appears to reflect splenic dysfunction, can readily be quantitated in a finger-stick blood sample using DIC microscopy. In this study, the degree of red cell pitting was compared with results of technetium spleen scans and measurements of Howell-Jolly bodies in individuals with sickle cell disease. The average pitted cell percentage in the control population was 0.5% ± 0.5 (range 0.0–2.6) and 30.5% ± 13.9 in the SCD population (range 2.4–71.1) (<0.001). Of the individuals studied with SCD, 12 also had technetium (99mTc) sulfur colloid scans and measurements of Howell-Jolly bodies. The percentage of Howell-Jolly bodies was low and did not correlate well with the degree of splenic visualization. However, there was an excellent correlation between pit count and splenic dysfunction as measured by spleen scan. Determination of red cell pitting, therefore, appears to offer a simple means for clinical evaluation of splenic reticuloendothelial function in patients with SCD.

The normal spleen appears to play a significant role in defense against infection. A property of the spleen which contributes to safeguarding the host is phagocytosis of foreign or damaged particles, also known as the reticuloendothelial (RE) function. One measurable RE function of the spleen is its ability to remove particulate matter from the red blood cell (RBC), or to remove the entire cell if it is markedly abnormal. Splenectomized individuals lack this capacity and consequently RBCs with altered morphology appear in their peripheral blood. The presence of Howell-Jolly (H-J) bodies is such an example. A number of investigators have used differential interference contrast (DIC) microscopy (Nomarsky optics) to evaluate RBC morphology more critically. Red cells examined with Nomarsky optics assume a three-dimensional appearance, permitting more detailed examination of the RBC surface. Red cells from a splenectomized individual possess a high percentage of cells with pits or crater-like depressions in the red cell membrane when viewed with this technique. There is still some disagreement as to the...
nature of the pits. Holroyde and Gardner\textsuperscript{5} have suggested they are "optical illusions" of vacuoles of low optical density lying beneath the plasma membrane. Schnitzer and colleagues\textsuperscript{6} using the electron microscope have demonstrated true surface indentations. Nathan and Dunn\textsuperscript{7} have suggested that pits may be surface indentations, vacuole formations, precipitated hemoglobin, or degenerated mitochondria. Whatever their nature, it is agreed that pitted RBCs are found in increased numbers in splenectomized individuals.

Pearson and colleagues\textsuperscript{7} have examined splenic RE function in sickle cell disease (SCD) patients using $^{99}$Tc spleen scans. The $^{99}$Tc-labeled sulfur colloid is phagocytized by the RE system and the radioactive uptake is measured in the splenic area. The absence of a splenic shadow in 11 of 12 Hb SS patients they studied indicated a lack of splenic RE function.

The purpose of this study was to determine if examination of the peripheral blood for "pitted RBCs" in SCD patients using Nomarsky optics might be more convenient and as quantitative as splenic scans as a measure of splenic RE function. If splenic RE function could be assessed in this manner (pitted cells), the technique might be utilized for early identification of SCD patients at risk of developing severe infection.

**MATERIALS AND METHODS**

Forty-seven patients with SCD (38 Hb SS and 9 Hb SC) were evaluated for percentage of pitted red cells in their peripheral blood. The median age was 5 yr with a range of 6 mo to 39 yr. Twelve of these patients were further evaluated with H-J body counts and $^{99}$Tc-labeled sulfur colloid spleen scans. The patients whose clinical course is subsequently described in the Discussion...
Table 1. Pitted RBCs in Normals Versus Sickle Cell Patients

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>Age Range</th>
<th>% Pits*</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle cell disease</td>
<td>47</td>
<td>5 mo–39 yr</td>
<td>30.5 ± 13.9</td>
<td>2.4–71.1</td>
</tr>
<tr>
<td>Control group</td>
<td>88</td>
<td>2 mo–43 yr</td>
<td>0.5 ± 0.5</td>
<td>0.0–2.6</td>
</tr>
</tbody>
</table>

*Student’s t test (p < 0.001).

Pitted RBCs

A drop of blood obtained from a finger stick was mixed in a plastic tube containing 0.3–0.5 ml of isotonic saline. The contents of the tube were gently agitated for 5 sec and then carefully dispersed onto a clean glass slide. A cover slip was carefully applied and the preparation immediately examined with (DIC) microscopy (Zeiss Universal Research Microscope equipped with Nomarsky optics). Five hundred consecutive RBCs were examined on the slide.

“Pitted” RBCs were considered to be red cells having one or more discrete crater-like indentations on their surfaces, regardless of the sizes of the indentations or the number of indentations per cell (Fig. 1). The results are shown in Table 1.

Howell-Jolly (H-J) Bodies

A Wright’s stained peripheral blood smear from each patient was examined for the presence of H-J bodies. To quantitate the number of H-J bodies, three separate areas of 1000 RBCs were examined and the results expressed as a percentage of the RBCs counted.

Spleen Scan

Spleen scans were performed with $^{99m}$Tc-labeled sulfur colloid (Squibb & Sons), 45 μCi being injected intravenously. Patients were scanned with a Picker Magnascanner. The scans were interpreted by a pathologist who did not have prior knowledge of other splenic RE parameters being investigated. Scans were graded as normal, decreased, no uptake, or compatible with splenic infarct.

The above three parameters were carried out simultaneously, when possible, in the patients described in Table 2. The risks of spleen scans were explained to the patients, and a consent form approved by the Research and Education Committee of the Hospital was signed if the procedure was being done for research purposes.

Table 2. Splenic Function Parameters in Sickle Cell Disease Patients

<table>
<thead>
<tr>
<th>Pt. No.</th>
<th>Hb</th>
<th>Age (yr)</th>
<th>% Pits</th>
<th>% H-J</th>
<th>Spleen Scan</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>SS</td>
<td>3½</td>
<td>12</td>
<td>1.1</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>SS</td>
<td>16</td>
<td>23</td>
<td>1.0</td>
<td>No uptake</td>
</tr>
<tr>
<td>3</td>
<td>SS</td>
<td>10</td>
<td>23</td>
<td>0.3</td>
<td>No uptake</td>
</tr>
<tr>
<td>4</td>
<td>SS</td>
<td>2</td>
<td>25</td>
<td>0.1</td>
<td>Decreased</td>
</tr>
<tr>
<td>5</td>
<td>SS</td>
<td>25</td>
<td>30</td>
<td>0.1</td>
<td>No uptake</td>
</tr>
<tr>
<td>6</td>
<td>SS</td>
<td>5½</td>
<td>35</td>
<td>0.0</td>
<td>Decreased</td>
</tr>
<tr>
<td>7</td>
<td>SS</td>
<td>4</td>
<td>36</td>
<td>0.1</td>
<td>No uptake</td>
</tr>
<tr>
<td>8</td>
<td>SS</td>
<td>5</td>
<td>37</td>
<td>1.0</td>
<td>No uptake</td>
</tr>
<tr>
<td>9</td>
<td>SC</td>
<td>12</td>
<td>12</td>
<td>0.3</td>
<td>Splenic infarct</td>
</tr>
<tr>
<td>10</td>
<td>SC</td>
<td>16</td>
<td>14</td>
<td>0.1</td>
<td>Splenic infarct</td>
</tr>
<tr>
<td>11</td>
<td>SC</td>
<td>29</td>
<td>51</td>
<td>0.0</td>
<td>No uptake</td>
</tr>
<tr>
<td>12</td>
<td>SC</td>
<td>39</td>
<td>50</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>
RESULTS

Determination of Per Cent Pitted RBCs in a Normal Control Population and a Sickle Cell Disease (SCD) Population

The frequency of pitted RBCs in SCD patients (30.5% ± 13.9) was significantly greater than the control population (0.5% ± 0.5) (Table 1). In the SCD group a 6-mo-old Hb SS patient had the lowest pit count, 2.4%, while a Hb SC patient with pneumococcal meningitis exhibited the highest pit count, 71.1%.

Comparison of Pit Counts With Howell-Jolly (H-J) Bodies and Spleen Scans

Twelve patients with SCD (eight Hb SS and four Hb SC) were further evaluated with pit counts, H-J body determinations, and spleen scans. Seven of eight patients with Hb SS demonstrated elevated pit counts (23%-37%), with varying degrees of splenic RE dysfunction by technetium scan (Table 2). One patient had a normal scan with 12% pits. The H-J body percentages varied only slightly (0-1.1%) and, in one patient (No. 6 in Table 2) with 35% pits and a decreased uptake on scan, no H-J bodies were identified in the areas of the peripheral blood smear counted.

Two of the four patients with Hb SC had pit counts of 50% and spleen scans that exhibited no uptake. Although it is unusual for Hb SC patients to have splenic dysfunction, both of these men were older (29 and 39 yr) than patients studied by other investigators. The other two patients (aged 12 and 16 yr) had evidence of splenic infarct (left upper quadrant pain) with mild elevation of pit counts (14%) and, again, no good correlation with the percentage of H-J bodies.

DISCUSSION

Sickle cell disease patients develop progressive splenic dysfunction with age. A simple method to quantitate the splenic RE function appears to be the observation of pitted RBCs in the peripheral blood. Our data indicate a significant difference between the mean pit counts in SCD patients and our control population (p < 0.001). The presence of H-J bodies on examination of a Wright's stained peripheral blood smear is an indication that splenic RE function is abnormal. However, examination of 3000 RBCs on a peripheral blood smear for identification of H-J bodies is not a quantitative measure of splenic RE dysfunction. Counting 10,000 RBCs does not appreciably enhance the quantitation of splenic RE function by this technique.

Technetium-99 spleen scanning is helpful in quantifying splenic RE function, but has several disadvantages. It is expensive and time consuming, necessitates moving the patient to a special area for the procedure, and requires the injection of radioactive material. Our data suggest that determination of per cent pitted RBCs correlates well with spleen scans as a measure of splenic function and makes it possible to quantitate splenic RE function at frequent intervals.

Pit counts would appear to have clinical application in the longitudinal observations of young SCD patients with normal initial splenic RE function. Smith and other investigators have shown that most splenectomized individuals (> 80%) who develop severe infections postsplenectomy do so in the first 24 mo after splenectomy. Therefore, the SCD patient who manifests an
increase in pit count may be at greatest risk of developing an infection within the next 2 yr.

An example of such a correlation is shown in Fig. 2, which depicts the clinical course of a 4½-yr-old black girl with Hb SS. She complained of bone pain and exhibited a pit count of 5% when first examined but had not had serious infections. Following a mild respiratory illness, her pit count rose to 10%. Four months later, she entered the hospital with diarrhea and an Escherichia coli urinary tract infection. Her pit count had increased to 50%. One week later she developed Haemophilus influenza septicemia. Subsequent pit counts have all remained above 36%. The rising pit count in this child suggests progressively increasing splenic RE dysfunction and appeared to be associated with an increased susceptibility to infection.

We have now seen five SCD patients who developed sepsis and/or meningitis, all of whom had pit counts > 15% (range 16%-71%). One of these was a patient with Hb SC disease. More longitudinal studies are necessary before any generalizations can be drawn regarding the efficacy of pit counts in predicting increased susceptibility to infection. These preliminary observations suggest, however, that 15% or greater pitted RBCs is an indication of significant splenic RE dysfunction and may provide a warning signal in the young SCD patient that he is at risk to develop a severe infection.

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