Short Communication: Elevated Platelet Count and Megathrombocyte Number in Sickle Cell Anemia

By Michael L. Freedman and Simon Karpatkin

Eight adult patients with sickle cell anemia were followed over a 6-mo period. During this time all patients had elevated platelet counts, 1.7-fold (mean, 438,398 ± 86,223), and megathrombocyte numbers, 2.3-fold (mean, 79,535 ± 38,907), during asymptomatic periods. These data are interpreted as suggesting that the elevated platelets and megathrombocytes in asymptomatic sickle cell patients result from lack of splenic sequestration. During three crises in two patients, both megathrombocyte number and platelet count fell significantly. It is speculated that platelets may be associated with the pathology in this disease. Platelet function studies performed during crises, therefore, must take into account the number of megathrombocytes.

The vascular pathology in sickle cell anemia is located in the capillaries, terminal arterioles, and venules and is due primarily to occlusive aggregation of sickled erythrocytes. This phenomenon is associated with the deposition of fibrin and platelets. Several workers have reported elevated platelet counts in sickle cell anemia. Haut et al. showed that platelet counts were moderately elevated during asymptomatic periods, somewhat lower during crisis, and markedly elevated postcrisis. Platelet survival was normal or greater than normal, except during crisis, when it was reduced. These investigators also found normal platelet aggregation during asymptomatic periods or crisis and greater than normal platelet aggregation postcrisis. Stuart et al. have reported abnormalities of platelet aggregation during crisis. Taken together, these results suggest that platelets are involved either directly or indirectly in the vaso-occlusive crises and raise the possibility that there is an increased utilization of functionally active platelets during crises.

Megathrombocytes (large-heavy platelets) have been shown to be more functionally active than other platelets. Recent work in our laboratory has shown that the spleen preferentially sequesters megathrombocytes. Using two methods of bypassing splenic sequestration in rabbits (splenectomy and acute hemolytic anemia leading to splenic blockade), it was found that the average platelet count rose 1.5-fold, whereas the average megathrombocyte number rose 2.0-fold. We have calculated splenic platelet and megathrombocyte pools of 35% and 54%, respectively. The platelet pool in rabbits was very similar to that reported by Aster in humans. Since adult patients with sickle cell anemia have autofaercted their spleens and lost splenic platelet pool function, one
might expect that these patients would have an increased number of both circulating platelets and megathrombocytes. In this respect, it is of interest that a similar elevation in platelet count and megathrombocyte number was noted in animals subjected to splenic blockade via phenylhydrazine-induced hemolytic anemia.9

**MATERIALS AND METHODS**

Platelet count and megathrombocyte number were measured in five normals and eight sickle cell anemia patients on numerous occasions. The diagnosis of sickle cell anemia was made by hemoglobin electrophoresis on cellulose acetate and acid-agar gels, sickle cell preparation in sodium metabisulfite, and family studies.

All blood samples were collected into EDTA-vacutainer test tubes (Becton Dickinson & Co., Rutherford, N.J.). All determinations were performed at room temperature. Platelet counts were performed manually under phase microscopy, employing 3% procaine hydrochloride as diluent.12

Megathrombocyte number was obtained by multiplying the per cent megathrombocytes by the platelet count per cubic millimeter. Per cent megathrombocyte was measured with a Coulter Counter Model B from platelet-rich plasma, obtained by centrifugation in plastic 2-mm diameter tubes at 600 g for 30 sec. The specimen was diluted in isoton (Coulter Electronics, Hialeah, Fla.), with the aid of a 3.3-μl pipette, to a platelet count of 5–10,000 per 0.1 ml volume in order to reduce coincidence counting to less than 1%. A 70-μ aperture tube was employed and the equipment calibrated with latex particles of 3.35 cu μ so that each window was equal to 0.25 cu μ. Per cent megathrombocytes represented windows 52 through 100, divided by windows 8 through 100, multiplied by 100: (13–25 cu μ/2–25 cu μ x 100). Windows 1–8 were excluded because of electronic noise interference. In order to standardize conditions, the diluting fluid, time interval between blood removal and volume measurement (less than 2 hr), and concentration of platelets were held constant at all times. All measurements were made in duplicate. The aperture tube was kept clear by continual monitoring of the oscilloscope screen for "interference" patterns. Background counts, as determined on the model B, were kept below 50 prior to use.

**RESULTS AND DISCUSSION**

Eight adult patients (aged 24–38) with sickle cell anemia were followed over a 6-mo period. All patients had elevated platelet counts (1.7-fold) and megathrombocyte numbers (2.3-fold) during asymptomatic periods (p < 0.001). Table 1 shows the mean values for normal and sickle cell patients with respect to platelet counts and megathrombocytes numbers. The normal controls were matched for age and sex. Females were matched for time in their menstrual cycle. One patient was exchange transfused with normal blood in order to maintain his percentage endogenous sickle cells at 10%–20% of total. After the initial exchange, approximately 2–3 U of blood were removed and 3 U of normal packed buffy-coat-poor washed cells given every 6 wk. His hematocrit was kept between 40%–45%, and his per cent reticulocytes was kept at less than 3%. Maintenance transfusion did not reduce his platelet count or megathrombocyte number.

<table>
<thead>
<tr>
<th>Number of Observations</th>
<th>Category</th>
<th>Platelets/cu mm ± SD</th>
<th>Per Cent Megathrombocytes</th>
<th>Megathrombocytes/cu mm ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>Normals</td>
<td>262,576 ± 56,617</td>
<td>13.2</td>
<td>34,639 ± 12,131</td>
</tr>
<tr>
<td>85</td>
<td>Sickle cell anemia</td>
<td>438,398 ± 86,223</td>
<td>18.1</td>
<td>79,535 ± 38,907</td>
</tr>
</tbody>
</table>
SICKLE CELL ANEMIA

Table 2. Platelet Count and Megathrombocyte Number During Painful Crisis in Sickle Cell Anemia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Precrisis*</th>
<th>Platelets/cu mm Crisis†</th>
<th>Postcrisis</th>
<th>Megathrombocytes/cu mm Precrisis*</th>
<th>Crisis†</th>
<th>Postcrisis</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS</td>
<td>500,000</td>
<td>490,000</td>
<td>1,101,000</td>
<td>134,500</td>
<td>46,774</td>
<td>146,433</td>
</tr>
<tr>
<td>DS</td>
<td>330,000</td>
<td>770,000</td>
<td>51,705</td>
<td>83,098</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>530,000</td>
<td>190,000</td>
<td>460,000</td>
<td>84,800</td>
<td>15,200</td>
<td>78,200</td>
</tr>
</tbody>
</table>

*Determinations performed within 1 mo of the time of admission for painful crisis.
†Determinations performed within 24 hr of the time of admission for painful crisis. The crisis in DS began 4 days and 2 days prior to admission and in LP 2 days prior to admission.
‡Determinations for patient DS were performed 2 days postcrises for patient LP 1 mo postcrisis.

Two patients had three painful crises without detectable precipitating cause. These crises were defined as subjective pain and terminated when the patients had become asymptomatic. Their temperature was <100°F. No source of infection was detected. Patient DS has cor pulmonale and pulmonary fibrosis, while LP has no other medical problems at present. The changes in platelet count and megathrombocyte number during crises and postcrises are shown in Table 2. Of note is the decrease in megathrombocyte number at the height of crises. In addition, there appeared to be a moderate decrease in platelet count during crises as well as a marked thrombocytosis postcrises, as has been previously reported.³ It should be mentioned that Green et al.⁴ reported no change in platelet count during crises when comparing mean platelet counts in a group of patients before and during a crisis. However, it is not clear when in the course of the crisis the platelet counts were obtained. For example, it is conceivable that some of their samples may have been drawn in the early postcrisis period (see Table 2).

In order to evaluate the possibility that the elevated platelet count and megathrombocyte number in asymptomatic sickle cell anemia could be due to lack of splenic sequestration, we calculated the theoretical splenic pools. If one assumes that the difference in platelet counts between normal and sickle cell patients represents the splenic pool, this theoretical pool would be about 40%.

\[
\frac{(438,398 - 262,576)}{438,398} = 0.40
\]

When a similar calculation is performed for megathrombocytes,

\[
\frac{(79,535 - 34,639)}{79,535} = 0.56
\]

a megathrombocyte splenic pool of 56% is obtained. These pool sizes are strikingly similar to those we have found in experimental animals.¹⁰ These results suggest that the elevated platelets and megathrombocytes in asymptomatic sickle cell patients result from lack of splenic sequestration. This conclusion is supported by the failure of maintenance exchange transfusion to lower either platelet count or megathrombocyte number (in one patient).

The decreased platelet function described during crises⁶ could be explained by an increased utilization of megathrombocytes. Therefore, the platelet popu-
lation tested by Stuart et al.\(^6\) could have been deficient in functionally active megathrombocytes. Similarly, increased platelet aggregation postcrisis\(^3\) might be explained by an increased percentage of megathrombocytes. Studies during crises must, therefore, take into account the number of megathrombocytes. It seems likely that the increased consumption of platelets during crisis is a secondary phenomenon, although once a crisis is initiated, it is possible that an elevated platelet count and megathrombocyte number could aggravate the situation.

While these results are preliminary, it is hoped that other investigators will be stimulated to consider the role of platelets and megathrombocytes in the vaso-occlusive crises of sickle cell anemia. Future work should consider closer examination of platelet function and kinetics in this disorder.

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REFERENCES

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