Rheologic Predictors of the Severity of the Painful Sickle Cell Crisis

By Samir K. Ballas, James Larner, Eugene D. Smith, Saul Surrey, Elias Schwartz, and Eric F. Rappaport

Deformable sickle erythrocytes have been reported by Mohandas and Evans to be more adherent to vascular endothelium than rigid irreversibly sickled cells (ISC). To define the clinical implications of this finding we have determined genetic, hematological, clinical, and rheological characteristics of sickle erythrocytes obtained from 65 patients with sickle cell anemia and fetal hemoglobin (Hb F) levels <15%. The α-globin gene number had a significant effect on the hematological parameters, the percentage of dense cells, ISC number, and Hb A2 levels. The presence or absence of α thalassemia, however, had no effect on the frequency and severity of the sickle cell painful crisis (r = 0.06, P > .05). RBC deformability, determined by an ektacytometer, showed great heterogeneity among patients with three or four α-globin genes. Linear regression analyses of the data showed significant positive correlation of the frequency and severity of the painful crisis with RBC deformability (r = 0.49, P < .001), and negative correlations with the percentage of dense cells (r = -0.37, P = .002), and the percentage of ISC (r = -0.46, P < .001). We propose that the more deformable the sickle RBC are, the greater their adherence to vascular endothelium, and the more they cause vaso-occlusive crises. RBC deformability and the percentage of dense cells (or ISC) seem to have a predictive value of the frequency and severity of painful crises in sickle cell anemia. In this study we have determined a number of disease parameters in patients with sickle cell anemia with and without co-existent α thalassemia in whom Hb F levels were <15% of total hemoglobin. We now report that rheological properties of sickle erythrocytes show significant correlation with clinical severity.

THE CLINICAL PICTURE of sickle cell anemia is highly heterogenous and varies from very mild to very severe. There is no simple relationship between clinical presentation and the degree of anemia in sickle cell disease. There has been a great interest in defining factors that modulate the clinical severity of this disease. The only such parameter on which there is reasonable consensus of opinion is the very high concentration and the pancellular distribution of fetal hemoglobin (Hb F). The high level of Hb F may decrease the severity of certain sickle cell syndromes because of its ability to inhibit polymerization of Hb S during the sickling process. In vitro polymer studies of Hb S and F demonstrated that FS hybrids are excluded from nuclei formation thereby interfering with polymerization in vitro and possibly with the sickling process in vivo. In order to have its salutary effect on the painful crisis, Hb F level must be very high, usually above 20% of total hemoglobin. Nevertheless, there are patients with sickle cell anemia and low Hb F level (<10%) who have mild disease indicating that there must be other parameters of clinical severity. The co-existence of α thalassemia with sickle cell anemia has a beneficial effect on the anemia itself and may also influence the incidence of other clinical features of the disease.

Patients. All patients were examined and diagnosed at the Sickle Cell Center of the Cardea Foundation at the Thomas Jefferson University Hospital (Philadelphia). The procedures described in this study were performed on blood obtained from 65 individuals with sickle cell anemia on whom the α genotype could be determined. Patients with sickle-β thalassemia, patients with other sickle combinations (SC, SD, etc), and patients with sickle cell anemia and unknown α genotype or whose Hb F levels were >15% were not included in this study. Sixty-four patients were American blacks, the remaining one was a white female (age 58 years) of Mediterranean origin. Only three patients in this study were pediatric subjects, a 10-year-old boy, a 12-year-old girl, and a 14-year-old girl; all others were adults whose ages ranged from 19 to 63 years.

Hematological and rheological data were obtained from patients in clinic who were in their usual asymptomatic steady state and who were free of crisis pain at least 1 week before their office visit. During the period of the study numerous hematological and rheological data points were determined on most patients. These were averaged and the values obtained for each patient were used in the statistical analyses described in the Results section. Hematological data on transfused patients were determined at least 4 months after the last transfusion.

Frequency and severity of the painful crisis. A painful crisis in this study was defined as one for which a patient with sickle cell anemia sought treatment for pain with parenteral narcotic analgesics in the emergency room after failure of self-treatment at home or for which hospitalization was required. Because the use of narcotic analgesics varies greatly among patients and because one worries that factors other than pain may determine use, we have taken the following precautionary measures to ensure that the patients did have authentic painful crises before they came to the Emergency Room. First, patients were instructed and encouraged to treat themselves at home with oral analgesics in an effort to break the crisis. Second, patients were encouraged to call the clinic if they developed crises to determine if treatment with oral analgesics would suffice. In such cases the patients were seen on the same day and were given prescriptions for oral medication if needed. Third, the frequency and severity of painful crises were analyzed by boxplots.
PREDICTORS OF SEVERE PAINFUL SICKLE CRISIS

which identify those patients (outliers) who had more crises than the group average. The data was then analyzed with and without these outliers. When the patients came to the Emergency Room they were examined, evaluated, and treated with parenteral narcotic analgesics every two hours for three doses. If they still had severe pain or significant objective signs they were admitted to the hospital. Otherwise they were sent home to be followed-up in the clinic. The number of painful crises and the number of days during which narcotic analgesics were given for pain were determined for each patient. For the purposes of this study we defined severity as the number of days per year for which the patient received parenteral narcotic analgesics. Each patient was followed-up a minimum of 3 years.

Some of our patients were occasionally admitted to other hospitals in the area because the local ambulance took them to the emergency room nearest to their residence. In order to be sure that such crises are included in our records the following approaches were taken to maximize the accuracy of our data. First, whenever seen in the clinic, patients were asked about crises, if any, treated in other hospitals and signed release forms were obtained in order to get the appropriate records. Patients were also encouraged to call our center whenever they were admitted to other hospitals. Second, on a quarterly basis, we contacted the Medical Records Department of the major hospitals in the area and obtained information about the dates, duration, and reason for admission of any of our patients to those hospitals and the dates they were treated in the emergency room there. Third, the Medical Assistance office was also contacted quarterly to give us dates and places of admission of our patients to other hospitals. Whenever we learned of an admission to other hospitals through these measures, the crisis data were included in our records for each patient. In general, the decision whether patients required hospital admission or not was made by the emergency department staff and medical house staff; decisions regarding discharge from the hospital were based on the discontinuation of the administration of parenteral narcotic analgesics.

Erythrocyte preparation. Erythrocytes from patients and controls were drawn into heparin or acid-citrate dextrose, cooled to 4°C, and processed as follows: hemoglobin, hematocrit, red cell indices, and reticulocyte count were performed on freshly drawn samples within a few hours of collection. Ektacytometric studies were performed within five days of collection in acid-citrate dextrose.

Other procedures were performed within 24 to 48 hours of collection or as indicated. Sickle red cells suspended in citrate, phosphate, and dextrose do not exhibit a density change for five days, and, therefore, maintain the pattern of their distribution on discontinuous Stractan density gradients during this period. In a series of initial studies we documented that both the ektacytometric profile and the number of dense cells remained constant for up to 2 weeks if RBC were collected in a dextrose-containing medium (ACD or CPD).

Collection of RBC in EDTA or heparin, however, altered these parameters after 24 hours of venipuncture. All procedures, unless otherwise noted, were performed at 4°C. Aliquots of cells were washed at least three times in buffers as specified for the various procedures described below, and theuffy coat was removed by aspiration after each wash.

Hematological data. Hemoglobin and reticulocyte determinations were made by routine methods and the microhematocrit was obtained by centrifugation. Red cells were counted on a Coulter Model F cell counter (Coulter Electronics, Hialeah, FL).

Hemoglobin electrophoresis on starch gel, starch block, or agar gel was performed on membrane-free hemolysates by established methods. Hb F was measured by the alkaline-resistance method and Hb A2 by column microchromatography. Hemoglobin solubility testing was conducted using a commercial assay (Sicklemed; Ortho Diagnostics, Raritan, NJ), and individual globin chains were differentiated by cellulose acetate electrophoresis of membrane-free hemolysate as described by Ueda and Schneider.

Determination of α-globin genotype. Alpha-globin genotypes (αα/αα, αα/αα, and αα/αα) were determined by Southern blot hybridization of genomic DNA extracted from peripheral blood leukocytes. The restriction endonucleases used were BamHI and BglII, and the probe was BamHI-linearized α-cDNA plasmid JW 1017 that was 32P-labeled with calf thymus DNA primers.

Determination of “dense” erythrocytes. The proportion of dense erythrocytes was determined by zymohemoglobin determination24 of the fraction of hemoglobin found in a dense (d = 1.110 g/mL) cushion of Stractan II in a 100-μL microhematocrit tube (Corning Glass Works, Corning, NY) after a 20-minute centrifugation in a microhematocrit centrifuge as previously described.26

ISC counting. Counts of irreversibly sickled cells (ISC) were performed on peripheral smears after the cells were oxygenated in room air by a modification of a previously described technique.27 The ISC number was determined as the percentage of elongated (axial-to-width ratio of greater than 2:1) erythrocytes among 500 RBC examined by light microscopy.28 Ovalocytes, tear drop cells, poikilocytes, and spiculated RBC were excluded from analysis. To minimize subjective variations in this determination, all counts were performed by the same observer (SKB).

Ektacytometric studies. The ektacytometer, a visco-diffactometer, designed and previously described29 was used to measure whole cell deformability of erythrocytes as a continuous function of the suspending medium osmolality at a constant applied shear stress of 170 dynes/cm² (osmotic gradient ektacytometry). For these studies the deformability index (DI) of red cells was continuously recorded as the suspending medium osmolality was increased from 50 to 700 mosm/kg as previously described.30

Statistical methods. The data generated by this study were analyzed by a Systat software package32 using an IBM PC-AT computer. The mean, standard deviation, two-tail t test, P value, boxplots, graphs, correlations, multiple linear regression, and the analysis of variance were all determined by the various modules of the Systat program. The advantage of the boxplot analysis is that it identifies outliers and thus readily allows the determination of statistical correlations with and without them.

RESULTS

α-Globin genotypes. We have studied 65 patients with sickle cell anemia. Six of these (9.2%) had two genes (−α/−α genotype), 20 (30.8%) had three (−α/αα genotype), and the remaining 39 (60.0%) had four (αα/αα genotype). All these patients had Hb F values <15.0%.

Clinical and hematological characteristics. Table 1 depicts the clinical and hematological characteristics of the patients studied. The mean age of all patients was 29.5 ± 9.6 years and 33 of the 65 patients were females. The α-gene number showed no significant correlation with age, sex, or the Hb F level. There was significant negative correlation of the α-gene number with the Hb (r = −0.37, P < .003), Ht (r = −0.52, P < .001), and Hb A2 (r = −0.44, P < .001) values. These findings are in agreement with previous reports about the effect of α-gene number on these parameters.46,17

Red cell indices showed significant positive correlation with the α-gene number (for MCV: r = 0.57, P < .001; for MCHC: r = 0.62, P < .001). The severity of hemolysis was directly related to the α-gene number as indicated by significant positive correlation with the reticulocyte count (r = 0.48, P < .001) and the serum level of total bilirubin (r =
1218

BALLAS ET AL

Table 1. Summary of Genetic, Clinical, and Hematological Characteristics of the Patients Studied

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SS + 2α</th>
<th>SS + 3α</th>
<th>SS + 4α</th>
<th>All SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>6</td>
<td>20</td>
<td>39</td>
<td>65</td>
</tr>
<tr>
<td>Female (%)</td>
<td>33.3</td>
<td>55.0</td>
<td>51.1</td>
<td>50.8</td>
</tr>
<tr>
<td>Age</td>
<td>30.7 ± 4.8</td>
<td>30.3 ± 11.2</td>
<td>28.9 ± 9.3</td>
<td>29.5 ± 9.6</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>9.2 ± 1.2</td>
<td>8.8 ± 1.2</td>
<td>7.9 ± 1.3</td>
<td>8.3 ± 1.3</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>28.5 ± 4.0</td>
<td>26.4 ± 3.7</td>
<td>22.4 ± 3.7</td>
<td>24.2 ± 4.3</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>74.0 ± 3.6</td>
<td>86.5 ± 5.9</td>
<td>91.5 ± 7.8</td>
<td>88.4 ± 8.6</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>32.4 ± 0.94</td>
<td>33.4 ± 1.3</td>
<td>35.5 ± 1.64</td>
<td>34.6 ± 1.90</td>
</tr>
<tr>
<td>Reticulocyte (%)</td>
<td>8.8 ± 2.6</td>
<td>10.6 ± 3.2</td>
<td>15.2 ± 5.3</td>
<td>13.2 ± 5.2</td>
</tr>
<tr>
<td>Dense cells (%)</td>
<td>4.6 ± 2.7</td>
<td>9.5 ± 5.9</td>
<td>17.2 ± 7.9</td>
<td>13.7 ± 8.3</td>
</tr>
<tr>
<td>ISC (%)</td>
<td>3.5 ± 3.4</td>
<td>7.6 ± 5.2</td>
<td>14.0 ± 6.8</td>
<td>11.1 ± 7.2</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>1.3 ± 0.2</td>
<td>2.5 ± 1.2</td>
<td>3.6 ± 1.8</td>
<td>3.1 ± 1.7</td>
</tr>
<tr>
<td>Hb F (%)</td>
<td>5.5 ± 2.2</td>
<td>6.2 ± 3.8</td>
<td>5.4 ± 3.3</td>
<td>5.7 ± 3.4</td>
</tr>
<tr>
<td>Hb A₂ (%)</td>
<td>4.0 ± 0.7</td>
<td>3.7 ± 0.5</td>
<td>3.5 ± 0.8</td>
<td>3.7 ± 0.8</td>
</tr>
</tbody>
</table>

Values (except for number of patients and percent female) are mean ± SD.

0.45, P < .001). Finally, both dense cells and ISC numbers showed significant correlation with the number of α genes (r = 0.53, P < .001). There was a significant correlation between the percentages of ISC and dense cells (r = 0.95, P < .001), a finding that is consistent with a previous study. The effect of the α-gene number on the proportion of dense sickle RBC is shown in Fig 1. Patients with four α genes had a significantly (P < .05) higher percentage of dense sickle RBC than either of the other two groups. The proportion of ISC was directly related to that of dense sickle RBC (r = 0.95, P < .001) and both parameters held the same relation to other characteristics of the patients. The above findings are in agreement with previous studies on the effect of α-gene number on the severity of the hemolytic component of sickle cell anemia, with the known association between ISC number and severity of the anemia, the association between the percentage of dense cells and the α-gene number, and with the close correlation between dense sickle RBC and the proportion of ISC.

Frequency and severity of the painful crises. Of all the painful crises that required treatment with parenteral narcotic analgesics 21.0% were associated only with fever defined as an oral temperature of 100°F (37.8°C) or higher, 8.7% presented with infection (± fever) such as upper respiratory tract infection, pneumonia, urinary tract infection, and 14.9% of the crises were associated with other objective signs such as swelling, tenderness, nausea, or vomiting. Thus, on the average, approximately 45% of painful crises were accompanied by objective signs, whereas the remaining 55% were subjective in nature.

Although the α-gene number had a bearing on the hematological characteristics of the patients studied, it had no effect on the frequency or severity of the sickle cell painful crises. Table 2 lists the mean number of crises per patient per year and the mean number of crisis days per patient per year for each group of subjects studied. There was no significant difference (P > .05) in these parameters among the three groups of patients. Moreover, the α-gene number showed no significant correlation with the severity of the crises (r = 0.06, P < .05). Figure 2 is a boxplot of the crisis days per patient per year as a function of the α-gene number. Noteworthy is that there were two outliers each for the group with three α and four α genes. Exclusion of these outliers did not

Table 2. Effect of α-Globin Gene Number on the Frequency and Severity of Sickle Painful Crisis

<table>
<thead>
<tr>
<th>α-Gene Number</th>
<th>No. of Crises/Year/Patient</th>
<th>Crisis Days/Year/Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS + 2α (6)</td>
<td>5.8 ± 3.6</td>
<td>31.8 ± 28.2</td>
</tr>
<tr>
<td>SS + 3α (20)</td>
<td>7.4 ± 6.3</td>
<td>42.2 ± 54.7</td>
</tr>
<tr>
<td>SS + 4α (39)</td>
<td>5.1 ± 5.2</td>
<td>32.0 ± 36.4</td>
</tr>
<tr>
<td>All SS (65)</td>
<td>5.8 ± 5.5</td>
<td>35.1 ± 41.8</td>
</tr>
</tbody>
</table>

Values shown are mean ± SD. Number in parentheses is number of patients tested.

Fig 1. Effect of α-globin gene number on the percentage of dense sickle RBC. The fraction of RBC with density >1.1055 g/mL is shown on the ordinate and the α-globin gene number characteristic of each group is shown on the abscissa. The number of patients studied is shown in Table 1. These data are depicted using boxplots. The boxes contain the middle 50% of the data (the interquartile range); the line within the box is the median value; and except for the outliers, the rest of the data points reside within the two crossbars. The asterisk represents an outlier that is further than one interquartile distance from the upper extremity (hinge) of the box. An outlier represented by an open circle is any data point (far out point) that is farther than 1.5 times the interquartile distance from the hinges of the box.

From www.bloodjournal.org by guest on September 14, 2017. For personal use only.
Effect of \( \alpha \)-globin gene number on the severity of sickle cell anemia. Crisis days per patient per year is shown on the ordinate and the \( \alpha \)-globin gene number on the abscissa. The number of patients studied is shown in Table 1. See Fig 1 regarding the boxplot method of displaying data. In the three \( \alpha \)-globin gene group the open circle represents an outlier that is 1.5 times the interquartile distance from the upper hinge of the box.

Fig 2. Effect of \( \alpha \)-globin gene number on the severity of sickle cell anemia. Crisis days per patient per year is shown on the ordinate and the \( \alpha \)-globin gene number on the abscissa. The number of patients studied is shown in Table 1.

Table 3 lists the DI values of sickle RBC in isotonic medium (290 mosm/kg) and in two hypotonic media (210 and 150 mosm/kg). Noteworthy is that sickle RBC had significantly lower DI than normal control RBC at 290 and 210 mosm/kg, and significantly higher DI than normal control RBC at 150 mosm/kg. These findings are compatible with a previous report of decreased deformability of sickle RBC in isotonic medium, which improves on exposure to hypotonic media. In this study, however, we found no significant difference in the DI at isotonicity among the three groups of patients studied. Moreover, the \( \alpha \)-gene number showed no significant correlation with the DI at 290 mosm/kg (\( r = 0.19, P > .05 \)). These differences may be due to the

<table>
<thead>
<tr>
<th>( \alpha )-Gene Number</th>
<th>DI 290</th>
<th>DI 210</th>
<th>DI 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS + 2(6)</td>
<td>0.297 + 0.063*</td>
<td>0.443 + 0.043†</td>
<td>0.408 + 0.030*</td>
</tr>
<tr>
<td>SS + 3α (20)</td>
<td>0.318 + 0.100*</td>
<td>0.446 + 0.093‡</td>
<td>0.350 + 0.078*</td>
</tr>
<tr>
<td>SS + 4α (39)</td>
<td>0.267 + 0.094*</td>
<td>0.410 + 0.084*</td>
<td>0.331 + 0.056*</td>
</tr>
<tr>
<td>All SS (65)</td>
<td>0.285 + 0.095*</td>
<td>0.424 + 0.085*</td>
<td>0.344 + 0.065*</td>
</tr>
<tr>
<td>Control (242)</td>
<td>0.528 ± 0.050</td>
<td>0.497 ± 0.050</td>
<td>0.154 ± 0.054</td>
</tr>
</tbody>
</table>

Values shown are mean ± SD. Number of individuals studied is indicated in parentheses. All \( P \) values were compared with their respective controls.

* \( P < .001 \).
† \( P < .005 \).
‡ \( P < .025 \).
fact that the number of patients studied by Embury et al. is smaller than that in the present study. There was, however, significant negative correlation between the α-gene number and the DI at 150 mosm/kg (r = −0.32, P = 0.009) indicating that the higher the α-gene number, the greater is the cellular dehydration of sickle erythrocytes, a finding that is in agreement with a previous report.37

Regression analysis of data. The wide range of variation in the deformability profile among patients with three and four α genes suggested that there may be intragroup, rather than intergroup, correlations with the severity of the painful crisis. To investigate this possibility we conducted regression analyses in which each characteristic was analyzed for every other parameter in the study. This was done for each group of patients and for all the patients combined together. The pertinent findings are summarized in Fig 4 and Table 4. It is important to note that age, sex, and Hb F level showed no significant correlation with the frequency or severity of the painful crises. Surprisingly, the frequency of crises and the crises days per patient per year showed significant positive correlation with the deformability index, the severity of the hemolytic anemia, and the MCHC of all patients combined and of the patients with four α genes. These findings were true for each group of patients and for all the patients combined together. The percentage of dense cells (as well as that of ISC), in turn, showed significant correlation with the deformability index, the severity of the hemolytic anemia, and the MCHC of all patients combined and of the patients with four α genes. Patients with three α genes were intermediate between the other two groups in that dense cells showed significant correlation with RBC deformability and severity of hemolysis (reticulocytes + bilirubin) but no significant correlation with the other parameters. These findings are in agreement with the known association between ISC numbers and the severity of anemia.27,31,34 It must be emphasized that this association does not seem to exist in patients with two or three α genes, perhaps because these patients have a relatively low number of ISC and dense cells and because the sample size in each of these two groups is relatively small.

DISCUSSION

In this study we have attempted to identify those red cell characteristics that correlate with the frequency and severity of the sickle cell painful crises. In order to achieve this we have developed comprehensive profiles on those patients with sickle cell anemia whose Hb F is <15% of total hemoglobin.

Table 4. Summary of Regression Analysis Data of Certain Parameters in Each Group of Patients Studied

<table>
<thead>
<tr>
<th></th>
<th>SS + 2α (n = 6)</th>
<th>SS + 3α (n = 20)</th>
<th>SS + 4α (n = 39)</th>
<th>All SS (n = 65)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r/P</td>
<td>r/P</td>
<td>r/P</td>
<td>r/P</td>
</tr>
<tr>
<td>Crisis days v</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI 290</td>
<td>0.92/0.11</td>
<td>0.52/0.028*</td>
<td>0.50/0.001</td>
<td>0.48/0.001</td>
</tr>
<tr>
<td>Dense cells</td>
<td>−0.85/0.032</td>
<td>−0.57/0.018*</td>
<td>0.42/0.007</td>
<td>0.37/0.002</td>
</tr>
<tr>
<td>ISC</td>
<td>−0.91/0.012</td>
<td>−0.59/0.012*</td>
<td>−0.57/0.001</td>
<td>−0.46/0.001</td>
</tr>
<tr>
<td>Dense cells v</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI 290</td>
<td>NS</td>
<td>−0.95/0.001</td>
<td>−0.78/0.001</td>
<td>−0.77/0.001</td>
</tr>
<tr>
<td>Hb + Hct</td>
<td>NS</td>
<td>NS</td>
<td>−0.61/0.001</td>
<td>−0.68/0.001</td>
</tr>
<tr>
<td>MCHC</td>
<td>NS</td>
<td>NS</td>
<td>0.43/0.006</td>
<td>0.57/0.001</td>
</tr>
<tr>
<td>Reticulocytes + bilirubin</td>
<td>NS</td>
<td>0.75/0.003</td>
<td>0.44/0.020</td>
<td>0.63/0.001</td>
</tr>
</tbody>
</table>

*These analyses do not include the two outliers shown in Fig 2.
Our data confirmed a number of findings previously reported by others. Most important among these is that the α-globin gene number has no predictive value of the frequency and severity of the painful crises.17,19 Billett et al.,28 however, indicated that patients with sickle cell anemia and the −α/αα genotype have a marginally increased number of hospital admissions for sickle cell painful crises. In this study, the frequency and severity of the painful crises in this group of patients were not significantly different from other patients. In both studies, however, the −α/αα sample size is relatively small and a much larger sample of patients defined by α-gene number will have to be investigated to clarify this issue.

The most important, and unexpected, finding in this study is that red cell deformability shows a significant positive correlation with the frequency and severity of the painful crisis. Thus, patients with a high RBC deformability index experienced more crises and crisis days than those with low RBC deformability. This finding was consistent in each group of patients studied and in all the patients combined together. Another important finding is the significant negative correlation between the percentage of dense cells (and of ISC) and the severity of the painful crisis. Thus, patients with a high percentage of dense cells had low Hb levels, low RBC deformability, and relatively mild disease. It must be emphasized that at first glance it may be a little bit surprising that there are correlations of α-gene number with the number of dense cells and ISC but no correlations with the severity of painful crises which, themselves, were correlated with the number of dense cells and ISC. The probable reason for this is that the correlations have r values around 0.5 and even though statistical validity of these correlations is high, the inherent correlation is not strong enough to demand the logical sequential correlations among these variables. Another point to be emphasized is that despite the statistical significance shown in Fig 4, there is a large amount of variability in the data as presented indicating that there may be other unknown factors that modulate the overall clinical picture of the patients studied.

The reasons why decreased RBC deformability has a beneficial effect on the clinical picture of sickle cell anemia are unknown, but we wish to consider three possibilities. First, rigid erythrocytes may not be able to negotiate the microvasculature and enter the capillary and as a result may be trapped at the entrance of narrow capillaries. This is well supported by a number of studies on the rheology of sickle cells and the microcirculation. Studies of the microcirculation of animals receiving human sickle erythrocytes39 and of the retina of patients with sickle cell disease40 have shown that such cells tend to be trapped at the entrance of narrow capillaries. Using a noninvasive laser-Doppler technique to measure blood flow in the forearm skin of patients with sickle cell disease, Rodgers et al.41 found that the blood flow in the skin of all six patients studied exhibited large oscillations, with a periodicity of seven to ten seconds, which were not seen in normal subjects or in two patients with sickle-β-thalassemia. Chien indicated that, a relatively small fraction of rigid erythrocytes can reduce the microcirculatory blood flow, and hence change the local metabolic environment in patients with sickle cell anemia to initiate vasomotion in the less sensitive, larger arterioles upstream.42,43 The initial reduction in blood flow, which sets up the reactive compensation in this scenario, however, is not sufficient to induce painful crisis. Consequently, undeformable, rigid, sickle erythrocytes trapped at the entrance of capillaries may be destroyed in situ, may be dislodged by the vasomotion initiated by the oscillatory blood flow and channeled through functional or morphologic shunts,44 or may be re-oriented by the oscillatory motion in order to facilitate their passage into the capillary. Second, the ability of sickle RBC to adhere to the endothelium may be inversely related to their deformability. Sickle RBC are known to demonstrate increased adhesion to cultured vascular endothelium and to each other.45,46 Moreover, the number of oxygenated sickle RBC that adhere to endothelial monolayers correlates with the clinical severity among sickle cell syndromes47 and with the severity of vaso-occlusive crises in sickle cell anemia.48,49 Accordingly, rigid sickle RBC that enter the capillary may not adhere tenaciously to the endothelium in order to initiate the process of occlusion of the capillary and compromise of the blood supply of the organ in question. Deformable sickle erythrocytes, on the other hand, do enter the capillary, adhere to the endothelium, and compromise the blood flow. This possibility is in keeping with a recent report by Mohandas and Evans50 who found, by using micropipette detachment techniques, that irregularly shaped, deformable sickle cells, suspended in autologous citrated or heparinized plasma, were four- to fivefold more adherent than discoid sickle cells, whereas rigid ISC were least adherent, perhaps because these cells have a decreased ability to form multiple surface contacts with endothelial cells. Third, the severe anemia consequent to the high number of dense cells and ISC, in patients with four α genes, confers a dilution effect that will reduce the chances for cell-cell adherence at the capillary level and, thus, decrease the chances to compromise the blood flow by a plug of erythrocytes. The inverse relation between the percentage of ISC and Hb level has been reported before and seems to be due to the increased propensity of these cells to be destroyed rapidly in vivo and thus account for the reduced concentration. In vitro studies45,46 have shown that ISC adhere to and are phagocytized more rapidly by peripheral blood monocytes than normal cells. Whether the same mechanism of destruction of ISC occurs in vivo is not yet established.

Taken together, the data indicate that patients with sickle cell anemia and Hb F < 15% have a heterogeneous clinical picture. Patients who generate a relatively large number of dense cells and ISC, have decreased RBC deformability and have milder disease than those patients whose RBC are more deformable. RBC deformability, the percentage of dense cells, and the percentage of ISC seem to have a predictive value of the frequency and severity of the sickle cell painful crisis.

Finally, we wish to indicate that Lande et al have reported in abstract form54 similar studies that indicate that the sickle cell painful crisis is positively correlated with red cell deformability but negatively correlated with the number of dense cells and ISC.
REFERENCES

8. Singer K, Fisher B: Studies on abnormal hemoglobins V: The distribution of type S (sickle cell) and type F (alkali-resistant) hemoglobin within the red cell population in sickle cell anemia. Blood 7:1216, 1952
1988 72: 1216-1223

Rheologic predictors of the severity of the painful sickle cell crisis
SK Ballas, J Larner, ED Smith, S Surrey, E Schwartz and EF Rappaport

Updated information and services can be found at:
http://www.bloodjournal.org/content/72/4/1216.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml