CONCISE REPORT

The Incidence of Painful Crisis in Homozygous Sickle Cell Disease: Correlation With Red Cell Deformability

By William M. Lande, Deborah L. Andrews, Margaret R. Clark, Natalie V. Braham, Dennis M. Black, Stephen H. Embury, and William C. Mentzer

To determine whether the vasoocclusive severity of homozygous sickle cell (SS) disease is influenced by cellular dehydration, we correlated the incidence of painful crisis with steady-state measurements of red cell hydration. Sixteen children with SS disease were followed for 3.3 to 8 years (mean, 6.8 years), and a single crisis rate was calculated for each patient. At the time of well visits, cellular hydration was assessed by measuring cell deformability, the percentage of red cells with a density ≥1.1056 g/mL, and the percentage of irreversibly sickled cells (ISCs). The incidence of painful crisis showed a strong positive correlation with \( O_{\text{max}} \), a deformability measurement reflecting cellular hydration \((r = .84, P < .002)\), and with hemoglobin concentration \((r = .59, P = .04)\). That is, higher crisis rates were observed in patients with less dehydrated, more deformable red cells and also in patients with higher hemoglobin concentrations. Furthermore, cell deformability and hemoglobin concentration were independent predictors of the incidence of painful crisis, which is consistent with separate effects of these two red cell parameters on vasoocclusive severity.

I N HOMOZYGOUS sickle cell (SS) disease, the frequency of vasoocclusive events varies markedly between patients. A better understanding of the pathophysiologic basis of this variability would enhance our ability to devise new therapeutic strategies. To date, investigators have identified a number of possible modifying factors. At the molecular level, the \( \alpha \)-globin genotype\(^1\) and the \( \beta \)-globin haplotype\(^3\) may correlate with the frequency of vasoocclusive complications. At the cellular level, an increase in the percentage of fetal hemoglobin (HbF) above a threshold level of 10% lessens the incidence of major organ failure, while an increase greater than 20% reduces the incidence of recurrent events such as painful crisis.\(^4\) Finally, at the level of cell-cell interaction, the tendency of sickle cells to adhere to vascular endothelial cells correlates with an overall index of vasoocclusive severity and with specific complications such as painful crisis, leg ulcers and bone lesions.\(^5\)

Dehydration is a prominent feature of sickle cells.\(^6\) It may be an important determinant of vasoocclusive severity due to the attendant increase in intracellular hemoglobin concentration. The latter effect reduces the deformability of oxygenated sickle cells\(^7\) and increases the rate of hemoglobin polymerization when oxygen tension is reduced.\(^8\) In this study, we test the hypothesis that cellular dehydration is a predisposing factor for vasoocclusion. When patients were clinically well, we assessed cellular hydration by measuring cell deformability, the percentage of high-density red cells, and the percentage of irreversibly sickled cells (ISCs). Then, we correlated these laboratory measurements with the incidence of painful crisis documented over a long period of continuous observation. Since we did not collect study samples during painful crisis, we did not observe acute changes such as a decrease in the percentage of dense cells\(^9\) and an increase in the sedimentation rate.\(^10\)

PATIENTS AND METHODS

Determination of the incidence of painful crisis. Sixteen children with SS disease were studied. The observation period for each patient was the length of time that we were the sole providers of medical care for the patient, from January 1980 onward. This period ranged from 3.3 to 8 years, with a mean of 6.8 years (Fig 1). A single incidence of painful crisis was calculated for each patient for the entire period of observation. Painful crisis was defined as an acute event characterized by musculoskeletal and/or visceral pain not otherwise explained and, in the older patients, consistent with the presentation of previous crises. All painful crises that prompted the patient to come to the clinic were counted regardless of whether the patient was hospitalized or received narcotic analgesics. Acute chest syndrome and splenic sequestration crisis were counted separately and were not included in the calculation of incidence of painful crisis.

Collection of blood samples. Informed consent was obtained from the patient or the parent, as appropriate. Blood samples were collected at the time of well visits and analyzed the same day. The length of time separating blood collection from the most proximate documented illness of any kind was at least a month. Blood samples were anticoagulated with EDTA, except for the samples used to measure cell deformability and percentage of dense cells and ISCs, which were anticoagulated with acid-citrate-dextrose (solution A).

Hemoglobin analyses. The diagnosis of SS disease was established by thin-layer isoelectric focusing, citrate agar electrophoresis, and solubility testing. Hemoglobin concentration was measured with a Coulter \( S + V \) cell counter (Coulter Electronics, Hialeah, FL), and percent HbF was determined by alkali denaturation.\(^11\) HbF ranged from 4% to 18%, with a mean of 10% (SD = 4%) (Fig 1). For one patient (O.B.), the only repeated laboratory measurements available were hemoglobin concentration and percent HbF.

DNA analyses. The \( \alpha \)-globin genotype of each patient was determined by restriction endonuclease mapping of genomic DNA extracted from peripheral blood leukocytes as previously described.\(^12,13\) Of the 16 SS patients, eight had a normal, nonthalas-
that were related to the fetal-to-adult hemoglobin switch. Since there were large changes during the study period, the data were omitted. The number of crises in that cluster. The number of crises per year was calculated for each patient. For the four youngest patients, percent HbF is shown next to each patient's initials. For the four youngest patients, percent HbF is shown next to each patient's initials. For the four youngest patients, percent HbF is shown next to each patient's initials.

Fig 1. Patient population. The length of each line represents the observation period, and the ticks indicate individual painful crises. When the tick marks are closely spaced, the number above the line indicates the number of crises in that cluster. The number of \( \alpha \)-globin genes and percentage of HbF are shown next to each patient's initials. For the four youngest patients, percent HbF is shown next to each patient's initials. For the four youngest patients, percent HbF is shown next to each patient's initials.

Fig 2. Osmotic gradient deformability profiles. Representative patient samples (R.E. and V.A.), normal range (shaded area), and method of measuring \( O_{\text{max}} \) and \( D_{\text{iso}} \) are shown. Red cell deformability was decreased in both patients relative to the normal range. In addition, red cells from R.E. were less dehydrated and more deformable in isotonic medium than were cells from V.A., as can be seen by comparing the two osmotic deformability profiles.

Fig 3. Correlation of the incidence of painful crisis with the steady-state measurement of cell hydration, \( O_{\text{max}} \), in SS patients followed beyond 8 years of age. Squares and triangles represent patients with four and three \( \alpha \)-globin genes, respectively. Increasing \( O_{\text{max}} \) indicates less severe cellular dehydration and is associated with improved deformability in isotonic medium.

was the osmolality yielding the maximum DI, and \( D_{\text{iso}} \) was the DI in isotonic medium. In SS disease, \( O_{\text{max}} \) is characteristically shifted to lower values than those observed in normal subjects because of cellular dehydration. Thus, when comparing different sickle blood samples, an increase in \( O_{\text{max}} \) indicates less severe dehydration and correspondingly less impairment of deformability in isotonic medium. The percentage of cells with a density \( \geq 1.1055 \, \text{g/mL} \) and the percent ISC were measured as previously described.

Statistical analysis of data. For repeated laboratory measurements, mean values over all visits were calculated for each patient and used in the analyses. Within-patient variability (SD/mean \times 100) of these measurements was calculated for each patient and then averaged over all patients. The youngest patients (R.L., T.H., M.A., and J.T.), unless otherwise specified, were analyzed separately from the remaining patients due to the marked developmental changes in the hematologic of SS disease that occur during infancy. The correlations reported are Pearson product-moment correlations. The significance of the adjusted associations between the incidence of painful crisis and the red cell parameters was assessed with a multiple linear regression model using crisis rate as the dependent variable and the red cell measurements as simultaneous independent variables. All analyses were performed on an IBM 4341 main frame computer with the Statistical Analysis System.

RESULTS

Assessment of red cell deformability in SS patients during the steady state. Although cell deformability was reduced in all patients relative to the normal range, the degree of impairment differed between patients (Fig 2). Cell deformability was measured on one to five separate occasions for each patient (mean, 3.3 times), and the overall within patient variability for \( O_{\text{max}} \) and \( D_{\text{iso}} \) was 7.5% and 43%, respectively. The large variation in \( D_{\text{iso}} \) was likely due to the difficulty of reproducibly measuring the DI from the steep portion of the osmotic deformability profile.

Predictors of the incidence of painful crisis. In patients followed beyond 8 years of age, there was a strong positive correlation between the incidence of painful crisis and the steady-state measurements of cell deformability. That is,
higher crisis rates were observed in patients with less dehydrated, more deformable red cells. Crisis rate correlated with both deformability measurements, $O_{\text{max}}$ (r = 0.84, P < 0.002) (Fig 3) and DI$_{190}$ (r = 0.79, P = 0.004). These correlations remained significant after adjustment for $\alpha$-globin gene number, hemoglobin concentration, percent HbF, percent dense cells, and percent ISC.

In the same group of older children, the incidence of painful crisis also correlated with the steady-state measurement of hemoglobin concentration (r = 0.59, P = 0.04). Thus, higher crisis rates were noted in patients with higher hemoglobin concentrations, and this correlation remained significant after adjustment for $\alpha$-globin gene number, percent HbF, and cell deformability ($O_{\text{max}}$ and DI$_{190}$). In addition, we observed trends between crisis rate and both percent dense cells (r = -0.59, P = 0.06) and percent ISC (r = -0.47, P = 0.15). Although not statistically significant, these trends are consistent with the correlation of crisis rate with hemoglobin concentration since the latter is inversely related to both percent dense cells and percent ISC (see the next section).

Predictors of steady-state hemoglobin concentration. In all children studied, the baseline hemoglobin concentration correlated with both percent dense cells (r = -0.85, P = 0.0001) (Fig 4) and percent ISC (r = -0.65, P = 0.009). That is, more severe anemia was observed in patients with greater numbers of these cells. The correlation of hemoglobin concentration with both parameters was expected since the percentage of dense cells and ISC were closely correlated (r = 0.89, P = 0.0001). These correlations remained significant after adjustment for $\alpha$-globin gene number, percent HbF, and cell deformability ($O_{\text{max}}$ and DI$_{190}$).

DISCUSSION

In our study of painful crisis in children with SS disease, we observed that children with less dehydrated, more deformable red cells experienced more frequent crises. Although surprising, this result is supported by a similar finding in SS adults. To account for this correlation, we considered the possibility that severely dehydrated, undeformable cells might be sequestered or destroyed to a greater extent in the more ill patients. In this case, the measurement of cellular hydration would provide an index of disease severity but would not help to explain why some patients are more severely affected than are others.

A second possible explanation is that better hydrated, more deformable sickle cells initiate vasoocclusive events by virtue of their tendency to adhere to endothelial cells. Barabin et al reported that the adherence of sickle cells to endothelial cells is greatest in the top fraction of density-separated cells. Furthermore, Mohandas and Evans showed that deformable, irregular sickle cells are more adherent than nondeformable ISC. Last, Hebbel et al found that endothelial cell adherence correlates with a clinical severity index and, in particular, with the incidence of painful crisis. These observations prompt us to speculate that cellular hydration, by its effect on cell deformability, influences endothelial cell adherence and, ultimately, vasoocclusive severity. An adherent cell might reside in an area of low oxygen tension long enough to permit nucleation and the explosive growth of polymer, such that the cell becomes sickled and capable of obstructing flow and initiating vasocclusion.

We also noted that patients with higher steady-state hemoglobin concentrations have fewer dense cells and ISC but more frequent crises. Although Billett et al found no significant correlation between hemoglobin concentration and the percentage of dense cells, our observations are consistent with the dependence of hemolytic rate on percent ISC, and the strong correlation between percent dense cells and percent ISC. The influence of hemoglobin concentration on crisis rate was previously shown by Baum et al and was dramatically illustrated in a report describing compound heterozygotes for hemoglobins S and S Antilles (a hemoglobin containing the sickle mutation plus a second mutation that has a lower solubility than does HbS by itself). Although incapacitated by an extremely low hemoglobin concentration, these patients experienced few painful crises. These clinical correlations support the concept that anemia serves a protective role by minimizing the increase in whole blood viscosity that occurs when oxygen tension is reduced.

In conclusion, our observations suggest that the sickle cells that are responsible for initiating painful crises are the relatively deformable cells rather than the cells that are undeformable due to severe cellular dehydration. Further work is needed to understand how cell deformability might influence the initiation of vasoocclusive events. One possible mechanism that deserves further investigation is that cell deformability facilitates the adherence of sickle cells to endothelial cells. In any case, we do not expect a simple relationship to emerge since many factors, from the molecular to the tissue levels of organization, may modulate the initial stages of vasocclusion.
REFERENCES


The incidence of painful crisis in homozygous sickle cell disease: correlation with red cell deformability

WM Lande, DL Andrews, MR Clark, NV Braham, DM Black, SH Embury and WC Mentzer