Is There a Threshold Level of Fetal Hemoglobin That Ameliorates Morbidity in Sickle Cell Anemia?

By Darleen R. Powars, Joyce N. Weiss, Linda S. Chan, and W. A. Schroeder

When the clinical manifestations of 272 patients with sickle cell anemia are compared with their level of fetal hemoglobin (HbF), the results suggest that there may be a threshold above which HbF is effective in ameliorating the morbidity of this disease. The age of entry of these SS patients into the study ranged from birth to 56 yr; the average length of follow-up was 11 yr for a total of 3,011 patient-years of clinic observation. HbF was determined quantitatively by microchromatographic procedures: the mean for HbF was 10% ± 6% with a range from 2% to 32%. For major organ failure, analyzed as termination events of morbidity, such as stroke or aseptic necrosis, the threshold appears to be 10%, whereas for recurrent clinical events, such as crisis or pulmonary disorders, it is 20%. No linear trend was found between HbF levels and morbidity. If a threshold exists, it is important to recognize this fact when attempts are made to raise the level of HbF in patients with sickle cell disease.

The assumption that the elevation of fetal hemoglobin (HbF) can favorably influence the pattern of morbidity for patients with sickle cell anemia (SS) has been supported from observations of “mild” sickle cell anemia in several populations and is suggested by polymerization kinetic studies of hemoglobin mixtures. However, our statistical analysis of 7 clinical indicators of morbidity in 214 SS patients showed no relationship between fetal hemoglobin and these indicators, with the single exception of stroke, where a positive association was seen. The lack of association with clinical severity in our population might be due to a lower mean HbF (12% ± 7%) when compared to a mean of 25% (range 5%–45%) for the Arabian population. Is there a “threshold” value of HbF above which a patient would be protected from morbidity of the disease? In order to investigate this possibility, data on levels of HbF and the clinical indicators of severity were examined to determine if there was a level of HbF at which a change in the risk of clinical complications occurred.

In view of the reports of increased HbF in baboons and in patients with sickle cell anemia or thalassemia after injections of 5-azacytidine, the concept of a threshold level may have clinical relevance. If the treatment does not raise the HbF above the threshold, it may be ineffective.

MATERIALS AND METHODS

Study Population

The study population consisted of 272 patients with sickle cell anemia (3,011 patient-years of observation) who have had repeated HbF determinations and about whom large numbers of hematologic indices and clinical data are available. There were 148 males and 124 females. The ages at entry ranged from birth to 56 yr, and the average length of follow-up was 11 yr. The collection of data on HbF began in 1974. At present, 216 patients are still in active follow-up, 37 have been lost to follow-up, and 19 patients have died. The patients are those in whose blood hemoglobin A (HbA) was absent and whose cells showed morphological evidence of sickling. Therefore, 7 Sβ-thalassemia patients are included. Patients were excluded from the study if they had been followed for less than 6 mo or had not yet attained a stable level of HbF, that is, anyone currently less than 5 yr of age.

Laboratory Data

The levels of HbF were determined quantitatively by microchromatographic procedures, which have the advantage of equal accuracy at all levels of HbF and are applicable to large numbers of determinations with small samples of blood. A HbF value for each patient was chosen to represent the patient’s “steady state” level of HbF. The mean and standard deviation for HbF were 10% ± 6% and the range was 2%–32%.

Clinical Parameters

For purposes of analysis, six commonly occurring events that were used as measures of severity were divided into two categories on the basis of the nature of the episode. The first category, called "recurrent events," provided measures of morbidity through multiple occurrences of sickle cell crisis, acute chest syndrome, meningitis/sepsis, and hospital admissions. In this article, the second category, called "termination events," included cerebrovascular accident (CVA) and aseptic necrosis. For the latter type of episodes, the measure of severity was based on the presence or absence of the disorder, because the first occurrence of these clinical events inevitably changes the subsequent course of the patient. Examples of such conditions are strokes with neurologic deficit, subsequent transfusion problems, seizures and restrose potential; aseptic necrosis of one bone with progression to include many bony sites; leg ulcers that never really heal; and renal failure with the innumerable subsequent complications of dialysis or kidney transplantation. Death is not reported here, because it was not considered a reliable measure in this study due to: (1) the rapidly shifting pattern of age of death during the last 8 yr and (2) an increasing fraction (nearly one-

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quarter) of deaths that are unrelated to sickle cell disease (trauma, medical mishaps, SLE, malignant lymphoma, murder, etc.).

Statistical Analyses

Initially, plots showed that the relationship between the level of HbF and the clinical indicators were examined in an effort to determine if there existed a level of HbF above which the risk of the episode appeared to decline. A lessening of the risk was noted only at higher HbF levels. For further investigation, the data were divided into 5 groups with 0%-4%, 5%-9%, 10%-14%, 15%-19%, and ≥20% HbF, and the age-adjusted incidence rates per 100 person-years of observation were derived for each type of complication within each HbF group.18

To derive the age-adjusted rate, each age-specific rate for every complication was multiplied by the total patient population, which served as the standard in that age interval. The resulting numbers represent the expected numbers of complications for the given age distribution of the total patient population. The expected numbers of each complication were then summed over all age intervals. The age-adjusted rate was calculated as the ratio of this sum to the total number of person-years, multiplied by 100. The use of age-adjusted rates allows for comparison of the rates across groups, removing the effect of any differences in age composition. The actual magnitude of the age-adjusted rates are not necessarily the same as the original overall crude rates, however.

For each complication, a regression analysis was performed on the age-adjusted incidence rate to determine whether any linear association between the risk of the complication and the HbF groups existed. If a significant linear association were to be found, this would negate the possibility of a threshold level of HbF. Once the theory of a threshold level was substantiated by the regression analysis, the ratios of the age-adjusted incidence rates (risk ratios) were calculated to measure the relative risk of the group above the threshold to the group below the threshold. Although the primary intention of this study was not hypothesis testing, we calculated approximate 95% confidence intervals for the risk ratios for descriptive purposes,30 assuming that the events were independent and followed a Poisson distribution.

As a final step, the effect of alteration of the threshold level on the risk ratios was examined. Risk ratios were calculated for thresholds of 5%, 10%, 15%, 20%, and 25% HbF. In addition, histograms of the age-adjusted incidence rate above and below the threshold were plotted.

RESULTS

Determination of Threshold Level

The chosen HbF groups resulted in the following distribution of sample sizes: 48 patients (565 patient-years) with 0%-4% HbF; 105 patients (1,292 patient-years) with 5%-9% HbF; 61 patients (653 patient-years) with 10%-14% HbF; 31 patients (244 patient-years) with 15%-19% HbF; and 27 patients (257 patient-years) with ≥20% HbF. Regression analyses of the age-adjusted incidence rates with the median HbF values for each group (Table 1) demonstrated no significant linear trends. Shown in the tables are the estimates of the regression coefficients or slopes ($\beta$), their standard errors, confidence intervals for the slope, and the levels of significance. Note that each confidence interval includes the value “0,” indicating no linear trend exists.

The histograms in Fig. 1 demonstrated no consistent downward trend of incidence rates with increasing HbF levels for recurrent events. Below 15% HbF, no linear pattern in the age-adjusted incidence rates could be discerned. However, a consistent pattern did emerge above 15% for sickle cell crisis, chest syndrome, and hospitalizations. For these episodes, it was noted that patients with HbF values ≥20% consistently had lower incidence rates than patients in the next lower HbF group, between 15% and 19%. Therefore, it was decided that 20% was the potential threshold value for recurrent events.

The only recurrent event that did not fall into this pattern completely was meningitis/sepsicemia. For this episode, the patients with HbF ≥20% had the second lowest incidence rate (the group with 0%-4% was slightly lower). This irregular pattern is related to the exclusion of children less than 5 yr of age because of their failure to attain a steady-state HbF, which in turn resulted in a significant reduction in the number of analyzable cases with meningitis/sepsicemia. The risk of this clinical complication is manyfold greater among younger children, and the fewer cases of adult type infections might have caused the variability of the pattern of incidence rates. It was therefore concluded that this slight deviation from the general pattern did not constitute a reliable counter example of the threshold effect.

The termination events in Fig. 1 also demonstrated no consistent trend of the incidence rate with the lower HbF levels. However, the threshold levels appeared to

Table 1. Regression Analyses of the Linear Relationship of Age-Adjusted Incidence Rate on HbF Levels

<table>
<thead>
<tr>
<th>Episode</th>
<th>Regression Coefficient ($\hat{\beta}$)</th>
<th>SE ($\hat{\beta}$)</th>
<th>95% CI for $\hat{\beta}$</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningitis/sepsicemia</td>
<td>0.027</td>
<td>0.091</td>
<td>(-0.264, 0.317)</td>
<td>0.79</td>
</tr>
<tr>
<td>Crisis</td>
<td>-0.379</td>
<td>0.787</td>
<td>(-2.883, 2.125)</td>
<td>0.66</td>
</tr>
<tr>
<td>Chest syndrome</td>
<td>-0.093</td>
<td>0.120</td>
<td>(-0.474, 0.288)</td>
<td>0.49</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>-0.395</td>
<td>1.147</td>
<td>(-4.044, 3.254)</td>
<td>0.75</td>
</tr>
<tr>
<td>CVA</td>
<td>-0.051</td>
<td>0.038</td>
<td>(-0.171, 0.069)</td>
<td>0.27</td>
</tr>
<tr>
<td>Aseptic Necrosis</td>
<td>-0.035</td>
<td>0.025</td>
<td>(-0.114, 0.044)</td>
<td>0.25</td>
</tr>
</tbody>
</table>

* $\hat{\beta}$ is the estimate of the slope of the line.

† CI, confidence interval.


be somewhat lower, with the incidence beginning to decrease at HbF levels between 10% and 14%. Aseptic necrosis followed a pattern similar to the recurrent events, with the highest rate occurring just below 10%, and the lowest risk occurring at 10%–14%. The pattern for CVA suffered from small sample sizes; only 3 CVAs occurred in patients with HbF over 10% (compared to 20 CVAs in those with HbF below 10%), and all 3 occurred between 15% and 19% HbF. This caused the relatively high incidence rate at this level. However, in view of the small number of events above 10%, this was chosen to be the potential threshold value for these termination events.

**Impact of Threshold Levels on Risk Ratios**

The number of episodes and the age-adjusted incidence rates for recurrent events for the groups above and below the threshold HbF value of 20% are presented in Table 2. The risk ratios of the age-adjusted incidence rates (≥20% HbF/<20% HbF) are also given, along with their approximate 95% confidence intervals, as a descriptive measure of the range of their relative risk. If the risk ratio were 1.00, incidence rates for low and high HbF would be equal, whereas if the risk ratio were under 1.00, risk for the low HbF group would be higher. The relative risks from 0.59 to 0.81 (Table 2) are indicative of greater risk in those with HbF under 20. For crises and hospitalizations, the confidence intervals do not include 1.00, indicating that the risk might be higher in patients below the threshold than those above the threshold.

When data for the termination events in Table 2 were examined using 10% HbF as the threshold value, the relative risks were below 1.00, 0.20 for CVA, and 0.45 for aseptic necrosis. The 95% confidence intervals for CVA remained below 1.00, and the upper limit of the interval for aseptic necrosis was slightly above 1.00.

**Effect of Varying the Threshold Level**

In order to compare the results from the chosen threshold values to those that would be obtained from other choices, the risk ratios for five HbF cutpoints were calculated. These risk ratios (high HbF/low

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**Table 2. Age-Adjusted Incidence Rates and Risk Ratios Above and Below Threshold HbF Levels**

<table>
<thead>
<tr>
<th>Recurrent Events</th>
<th>HbF &lt;20% (n = 245, Patient-Years = 2,754)</th>
<th>HbF ≥20% (n = 27, Patient-Years = 257)</th>
<th>Approximate 95% CI for Risk Ratio†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Episodes</td>
<td>Age-adjusted Incidence Rate</td>
<td>No. of Episodes</td>
</tr>
<tr>
<td>Meningitis/septicemia</td>
<td>70</td>
<td>2.58</td>
<td>5</td>
</tr>
<tr>
<td>Crisis</td>
<td>1,736</td>
<td>62.91</td>
<td>104</td>
</tr>
<tr>
<td>Chest syndrome</td>
<td>416</td>
<td>15.16</td>
<td>31</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>2,839</td>
<td>102.97</td>
<td>188</td>
</tr>
</tbody>
</table>

| Termination Events | HbF <10% (n = 153, Patient-Years = 1,857) | HbF ≥10% (n = 119, Patient-Years = 1,154) |                  |
|                   | Cases | Age-adjusted Incidence Rate | Cases | Age-adjusted Incidence Rate | Risk Ratio* | 95% CI for Risk Ratio       |
| CVA               | 20    | 1.24                       | 3     | 0.24                        | 0.20        | (0.06, 0.67)               |
| Aseptic necrosis  | 24    | 1.45                       | 7     | 0.66                        | 0.45        | (0.19, 1.06)               |

*Risk ratio = Age-adjusted incidence rate for high HbF group divided by the age-adjusted incidence rate for low HbF group.
†Based on assumptions that events are independent and follow a Poisson distribution.
HbF) for the cutpoints 5%, 10%, 15%, 20%, and 25% HbF are shown in Table 3. For the 4 recurrent events, the risk ratios do indeed decrease at a cutpoint of 20%, and they decrease even further when 25% is used. However, due to the small number of cases with HbF > 25% (n = 11), it was decided that the findings based on 20% have a higher level of confidence. The termination events show a decrease in the risk ratios at a cutpoint of 10%. The apparent rise in the ratio for CVA at a cutpoint of 15% is due to 3 cases. Aseptic necrosis is the only episode where the ratio is approximately the same at a cutpoint of 5% as it is at 25%, dropping only at intermediate levels.

Figure 2 depicts the relative age-adjusted incidence rates above and below the threshold levels, at 20% for recurrent events, 10% for termination events, and, for comparative purposes, at 25% for all episodes. Note that those patients above the threshold consistently have a lower risk than those below the threshold.

Hematologic Correlates

The mean corpuscular volume (MCV) of the low HbF group (<20%) ranged from 61 to 117 fl, with a mean of 88 fl. The MCV of the high HbF group ranged from 61 to 117 fl, with a mean of 90 fl. The mean steady-state Hb concentration was 8.4 g/dl for the low HbF group, and was 9.4 g/dl for the high HbF group. This difference of 1.0 g/dl is significant (p < 0.001). Among our high HbF cases, most of the red cells (80%) contained stainable fetal hemoglobin (Kleihauer-Betke method), but the F distribution was uneven with marked heterogeneity. No correlative analyses of F cell populations were performed.

DISCUSSION

On the basis of many studies, it is believed that the alteration of certain hematologic or biochemical characteristics of the red cell would improve the clinical course and general well being of patients with sickle cell anemia. These parameters include a reduction of MCV below 72 fl, a reduction of MCHC below 32 g/dl, an increase in intracellular water, and an elevation of HbF and HbA2. Support for such a belief stems from observations in certain populations with a reportedly more benign clinical course. Thus, Arabian and Indian SS populations,1-3 who are said to have a relatively benign course despite all the manifestations of the illness, have HbF levels on the order of 25%, and 40% of patients demonstrate alpha thalassemia. No studies describe clinical associations of these interacting factors operative in an additive manner. The overall clinical severity of the illness tends to overwhelm the power of any single given biochemical or hematologic factor on the clinical course. Of course, the manifestation of vasoocclusive crises in sickle cell anemia becomes secondary after the occurrence of such catastrophic complications as cerebral vascular

Table 3. The Influence of Alteration of Threshold HbF Levels on the Risk Ratio

<table>
<thead>
<tr>
<th>Episode</th>
<th>Risk Ratios*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HbF &gt; 5%</td>
</tr>
<tr>
<td>Meningitis/septicemia</td>
<td>2.56</td>
</tr>
<tr>
<td>Crisis</td>
<td>1.30</td>
</tr>
<tr>
<td>Chest syndrome</td>
<td>0.94</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>1.27</td>
</tr>
<tr>
<td>CVA</td>
<td>0.38</td>
</tr>
<tr>
<td>Aseptic necrosis</td>
<td>0.79</td>
</tr>
</tbody>
</table>

*Risk ratio = Age-adjusted incidence rate for high HbF group divided by the age-adjusted incidence rate for low HbF group.
accidents or damaging infarctive episodes of any vital organ, such as kidney or lung.

The present exploratory analysis suggests that there may indeed exist a threshold level of HbF above which the severity of sickle cell anemia decreases. A lower risk above the threshold level was observed for each type of illness. The dissimilarities in anatomic vascularity, rate of blood flow, blood viscosity, tissue pH, and the propensity of damaged blood vessels to develop intimal hyperplasia in concert with variability of red cell vascular interrelationship account for the mutability of predictive factors. Therefore, a given factor has greater influence in producing damage to one organ but not to another.

Our study was aimed at constructing the hypothesis of a threshold level of HbF above which patients are likely to be protected from complications of sickle cell anemia. Due to the nature of this study, and the derivation of threshold levels from this data set, the hypothesis should be tested for confirmation in another patient population; the Saudi Arabian population or the present cooperative study of sickle cell disease in the United States could provide this opportunity.

Stevens et al.‘s observation of an increased risk of dactylitis, hypersplenism, and death in SS children at 6 mo of age if HbF levels fell below 21% supports the concept of a meaningful threshold of HbF at 20%.21 In a discriminant analysis that related splenic function, as measured by red cell vesicle (pock) formation, to HbF levels, a positive association between retained splenic function and elevated HbF levels greater than 18% has been reported.22 In effect, these observations equate clinically to the mild syndrome in patients with S-β′-thalassemia, who usually have 25% non-S hemoglobin (combination of A, A2, and F) and are recognized to have considerably less morbidity and rare organ failure.

Because 245 of our patient population had HbF less than 20%, and 27 had HbF 20% or greater, 90% of our patients have HbF levels that are below the apparent protective range. The S-β-thalassemia patients segregated proportionately between the two groups; one of the S-β-thalassemia patients had HbF greater than 20%. The addition of more patients to a prior study11 did not recruit a higher proportion of patients with HbF above 20%; apparently, the population in Los Angeles is stable at about 10% of the patients with more than 20% HbF. The genetic determinants that result in elevated fetal hemoglobin levels are not resolved, although there is evidence that an as yet unidentified hereditary factor(s) may be present.23,24

Noguchi and Schecter have developed a biophysical model system according to which an increase in the hemoglobin concentration in the individual red cell would not be expected to increase the time of polymerization (delay time) of HbS and HbF mixtures.25 Our data provide support for their model. The relationship between the HbF value and the recurrent clinical events, as depicted in Fig. 1 and Table 3, demonstrates an erratic pattern of correlative risks for those with HbF values under 20%. Clearly, there is no linear continuous downward trend of risk with a rising HbF. Rather, these data support the concept of a threshold level and deny the concept of a linear change, that is, that 12% is somewhat better than 8%. As we sought the threshold level for each specific clinical complication, a distinct threshold level was found for each given clinical problem. With Noguchi and Schecter, one may conclude that HbF must replace the HbS in the individual red cell in order to provide amelioration of clinical symptomatology. It has been assumed that certain normoblastic red cells continue to synthesize γ-chains; the high HbF red cells thus released into the circulation preferentially survive. In addition, data suggest that the preferential survival of high HbF cells is not uniform, i.e., cells with identical biochemical constituency of HbF do not survive in the circulation equally.23

Therapeutic means to reverse the normal postnatal switch from HbF to an adult hemoglobin have been much sought after as a method to ameliorate the problems of sickle cell anemia. In studies with baboons, 5-azacytidine has produced such a temporary reversal.13 When 5-azacytidine was used in a few SS patients,14,15 it induced decreased production of β5-chains and replaced them with γ-chains.15 One patient attained an HbF level of 8.9%, and F-containing cells rose to 52%, along with a slight rise in MCV.14 In a long trial with 5-azacytidine (more than 260 days) in a patient with sickle cell anemia, Dover et al.16 report no dramatic rise in HbF (8.9%), but hemoglobin concentration ranged between 10 and 13.5 g/dl after 200 days of treatment. Although the patient had vasoocclusive crises during 70 of 166 days prior to treatment and had little improvement early in treatment, he had only two episodic crises between days 130 and 260. Our data suggest that the attainment of 20% HbF would be necessary to effect an improvement in clinical status; other undetermined factors, as well as the small increase in HbF, may have produced the apparent improvement.

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REFERENCES


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