Lack of Influence of Fetal Hemoglobin Levels or Erythrocyte Indices on the Severity of Sickle Cell Anemia

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ABSTRACT Persons with sickle cell anemia who have elevated fetal hemoglobin or lowered erythrocyte mean corpuscular volume are reputed to have less severe clinical manifestations and a greater probability of survival. This study examines the relationship between seven clinical indicators of morbidity in sickle cell anemia and seven hematological parameters that were collected from 214 patients. Risks of sickle cell crisis, acute chest syndrome, hospital admissions, cerebrovascular accident, aseptic necrosis, meningitis/septicemia, and death were used as indicators of morbidity. The hematological parameters included percent fetal hemoglobin, absolute fetal hemoglobin, percent hemoglobin A_2, hemoglobin concentration, packed cell volume, mean corpuscular volume, and mean corpuscular hemoglobin concentration. Statistical analyses of the data showed no relationship between the hematological parameters and six of the seven clinical indicators of the severity of sickle cell anemia. The only significant finding was an increased risk of stroke in those patients with lower levels of fetal hemoglobin. Therefore, with this exception, there is no predictable relationship between morbidity and mortality in sickle cell anemia and levels of fetal hemoglobin or erythrocyte indices. Thus, the general belief that there is an association between severity of sickle cell anemia and the levels of fetal hemoglobin has not been established.

INTRODUCTION

The variable clinical expression of sickle cell anemia has initiated a world-wide search for biochemical, hematological, or environmental factors that may influence the severity of the illness. There is a widely held opinion that elevation of fetal hemoglobin (Hb-F)[1] or lowered erythrocyte mean corpuscular volume (MCV) is associated with a decreased severity of clinical manifestations and an increased survival. This paper reports the results of a statistical assessment of the relationship between the hematological factors and the mortality and morbidity of 214 patients with sickle cell anemia.

Background of study. The concept that elevation of Hb-F favorably influences morbidity has support from both biochemical and clinical studies (1). Thus, increased proportions of Hb-F increase the minimum gelling concentration of mixtures of hemoglobins S and F (2, 3), modify the polymerization kinetics (4, 5), and increase the delay time to polymerization in S-F mixtures (3, 6). Hb-F is decreased in irreversibly sickled SS cells (7) and greater numbers of irreversibly sickled SS cells produce increased viscosity of oxygenated whole SS blood (8). However, the kinetics of Hb-S polymerization suggest that the variable clinical expression may result from several kinetic paths to polymerization and to marginal changes in free energy (9). Miale et al. (10) have shown irregular shifts of Hb-F content among the individual erythrocytes and have attempted to demonstrate a correlation of this shift with growth and development of the SS patient. Moreover, according to Dover et al. (11), wide variation exists in the production and survival of F cells in persons with sickle cell anemia.

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[1] *Abbreviations used in this paper*: Hb, hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; SS, sickle cell anemia.

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Those persons with the determinant for the hereditary persistence of Hb-F in the *trans* position to S have essentially an asymptomatic condition (12, 13). Further support for the concept that Hb-F is protective has come from observations of sickle cell anemia in several populations (14–20). In these populations the mean Hb-F was high (10–25%), although within each population the range was wide (a few to 40%). The universal suggestion has been that the relatively mild expression of sickle cell anemia in these populations is caused by the high Hb-F.

Following Lehmann's observations (21) that sickle cell anemia appeared to be a less severe disease in western Africa where α-thalassemia is frequent, it has been postulated that α-thalassemia and sickle cell anemia in the same individual will protect the SS patient because of lowered MCV or mean corpuscular hemoglobin concentration (MCHC) (22, 23). Lowering of the Hb-S concentration does lengthen the time for Hb-S polymerization and decreases the numbers of irreversibly sickled SS cells (24, 25), and Clark et al. (26) report that MCHC is a major determinant of the membrane changes that are associated with sickling.

METHODS

Study population. The study population consists of 214 "S-only" patients whose hematological and clinical data were available for analysis. An S-only patient is defined as one whose hemoglobin, by electrophoresis and column chromatography, has only Hb-S as the major hemoglobin (absence of Hb-A) and whose cells have a positive sickling test. Consequently, not only may this category include patients with homozygous sickle cell anemia, but also those patients with S- β^0-thalassemia, S-$\delta\beta$-thalassemia, S-hereditary persistence of fetal hemoglobin, and any variety of α-thalassemia. Excluded from the study were infants who had not yet attained a reasonably stable level of Hb-F, regularly transfused patients, and SS patients with concomitant α-chain substitutions. Of the 214 patients, 206 have homozygous sickle cell anemia, 6 have S-β^0-thalassemia, and 2 have S-$\delta\beta$-thalassemia.

Clinic and medical care for patients with sickle cell anemia has been provided as part of a continuing program since 1964. Although many SS patients have been known from birth or shortly thereafter, others have been diagnosed at a later age. Consequently, an extensive medical history is available for some hundreds of SS patients. Since 1972, extensive biochemical and genetic characterizations of many of the patients have been made.

Laboratory parameters. The hematological parameters used in the statistical analysis were percent Hb-F, absolute Hb-F, Hb-A_2, hemoglobin concentration, packed cell volume (PCV), MCV, and MCHC. All blood samples were tested with sodium metabisulfite for morphologic evidence of sickling. Erythrocyte indices and hemoglobin levels were determined by standard methodology. Microhematocrit determinations were obtained on all samples.

The hemoglobin type of all patients has been examined by one or more of three electrophoretic procedures (cellulose acetate or starch gel at alkaline pH or agar at acid pH) and by chromatography. Hb-F, Hb-A_2, and other components were determined quantitatively by microchromatographic procedures (27–29). These procedures for Hb-F have the advantage of accuracy at all Hb-F levels and are applicable to large numbers of determinations with small samples of blood. The quantity of Hb-F has been calculated both as the percentage of total hemoglobin and as the absolute value in milligrams per decaliter of whole blood. Staining for Hb-F by the Kleihauer-Betke method (30) has been applied to the cells of 144 patients.

In no instance has a hemoglobin of the study group been examined by amino acid sequencing.

Clinical parameters. Collection of morbidity data accompanies an ongoing study of the demographic characteristics of the population. Data from medical history were transferred to computer cards and current data are collected regularly from each patient. Regardless of the reason for which the patient may be seen, blood is collected so that appropriate hematological and biochemical data for the visit may be incorporated into the data base. At the same time, the diagnosis of any clinical complications and an assessment of the state of well-being are recorded.

The clinical indicators of morbidity used in this study were total hospital admissions for any cause, total hospitalized vaso-occlusive sickle cell crisis, acute chest syndrome (which includes proven bacterial pneumonia, strongly suspect but nonbacteriologically proven pneumonia, viral pneumonia, proven pulmonary infarcts, and multiple microthrombotic chest syndrome), bacterial meningitis and septicemia, aseptic necrosis in any major bony site, stroke (cerebral infarct and subarachnoid hemorrhage), and death.

Statistical methods. The seven clinical parameters were studied in two categories. The first category provided measures of morbidity through multiple occurrences of sickle cell crisis, acute chest syndrome, and hospital admission. Each parameter in this group was analyzed using both multiple regression and discriminant analysis techniques (31). The second category of clinical parameters included cerebrovascular accident, aseptic necrosis, meningitis/septicemia, and death. The first occurrence of these clinical parameters, which often significantly changes the subsequent course of the patient, was analyzed by survival analysis techniques (32, 33). In the multiple regression analysis, the dependent variable is the risk of the recurrent clinical event, defined as the number of episodes per person-year of observation or rate of occurrence. The independent variables are age at entry and the laboratory parameter under study.

In the discriminant analysis, strata of each laboratory parameter were used. Cut points of the strata were chosen either to separate normal from abnormal levels, or to divide the parameter into equal groups with high, medium, and low values. The dependent variable in the analysis was the laboratory parameter and the independent variables were age at entry and the risk of the clinical parameter as defined above. In view of the marked effect of age on clinical manifestation (34), age at entry was "forced" into the discriminant equation to adjust for differences in average age at entry among the strata defined by each laboratory parameter.

The survival analysis also used strata of the laboratory parameters as in the discriminant analysis. The analysis compared the risk of the clinical parameter, defined as the probability of "termination" by the first occurrence of the clinical event, among patients in different strata. Here, "termination" refers to the end point of the patient's observation for that particular event under analysis. The log rank test was used in the survival analysis to determine statistical significance among the strata (35).

TABLE I
Summary of the Hematologic Laboratory Parameters

Laboratory parameters	Number of persons	Mean±SD	Range
% Hb-F	214	12±7	3–36
Absolute Hb-F, *gm/dl*	214	1,080±717	240–3,888
MCV, *fl**	179	88±10	55–117
Hb-A_2, %*	212	3.2±0.6	1.5–5.9
Hb, *gm/dl*	214	8.5±1.4	5.4–13.7
PCV, %	214	25.4±4.3	16–41
MCHC, *gm/dl*	203	33.9±1.5	28–38

* These data include results from individuals with S-β^0- and S-$\delta\beta$-thalassemia.

RESULTS

Characteristics of the study population. The study population of 214 persons had an average age of 19.2 yr. The sex distribution was 114 males (53%) and 100 females (47%). 19% of the patients are currently <10 yr of age, 37% are between 10 and 19, 31% are in the third decade, 9% are in the fourth decade, and 4% are >40 yr of age. 94% (202) are currently alive. The cumulative number of years of observation in the 214 patients is 2,126 person yr. A total of 34% (72 patients) entered the study before age 3 and have an average (±SD) of 12.6±7.7 yr of observation; 32% (70 patients) entered between ages 3 and 10 and have an average of 10.2±6.6 yr of observation; 16% (34 patients) entered between ages 11 and 20 and have an average of 7.3±4.6 yr of observation and 18% (38 patients) entered between ages 21 and 56 and have an average of 6.7±4.5 yr of observation.

Description of the laboratory parameters. The cells of all patients in this study had the expected morphologic changes with sodium metabisulfite, and their hemoglobins showed the anticipated electrophoretic and chromatographic behaviors. A representative laboratory value was chosen for each patient in an attempt to select values that reflect his "steady-state" level. Thus, some patients have had as many as 10 determinations of Hb-F over a period of several years. In the 144 patients to whose cells the test was applied, the Kleihauer-Betke method (30) detected a heterogeneous distribution of Hb-F with markedly variable staining in individual erythrocytes.

Of the various parameters, hemoglobin concentration perhaps tends to vary the most with time in the individual patient. The most recent steady-state hemoglobin level and hematocrit have been used for this analysis. Table I summarizes various aspects of the laboratory parameters that were used in the study. Figs. 1 and 2 depict the frequency distributions of the percentage of Hb-F and of the MCV of the cases.

Description of the clinical parameters. The percentage of the 214 patients with at least one episode of each of the seven selected clinical indicators of severity of illness is depicted in Fig. 3. Table II summarizes the risk of sickle cell crisis, chest syndrome, and hospital admission in terms of frequency of episodes per person-year observed, and the risk of stroke, aseptic necrosis, meningitis/septicemia, and death in terms of the cumulative probability of termination by the first occurrence of these episodes at various ages.

Statistical analysis. No significant differences were observed in the multiple correlations of the risk of the clinical parameters with age at entry and the laboratory parameters. Neither the partial correlation of the laboratory parameter with risk, given age at entry, nor of age at entry with risk, given the laboratory parameter, was significant. The maximum value of the square of the multiple correlation coefficient, which measures the goodness-of-fit of the analysis, was found to be <3%. Thus, no laboratory parameter was found

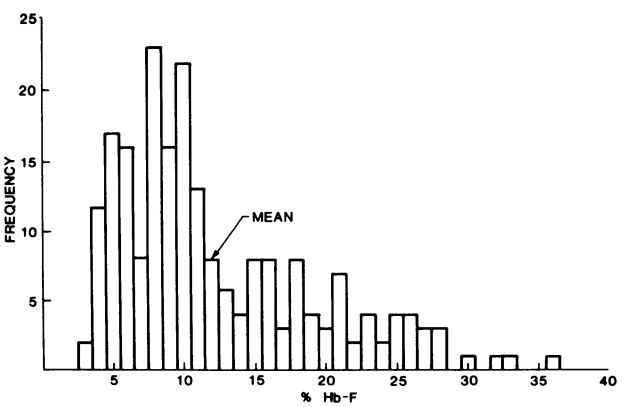

FIGURE 1 The frequency distribution of the percentage of Hb-F in 214 persons with sickle cell disease.

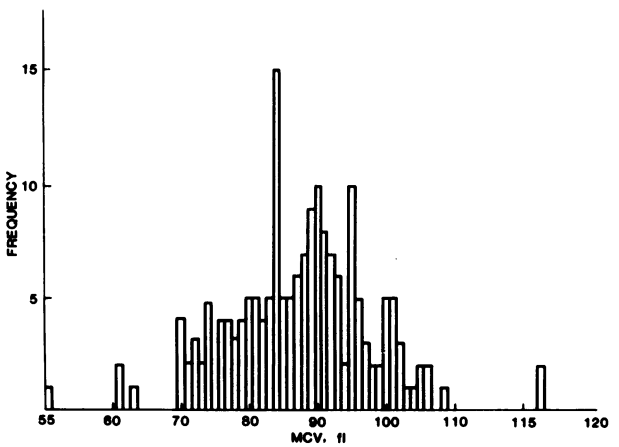

FIGURE 2 The frequency distribution of MCV values in 179 persons with sickle cell disease.

to predict the risk of sickle cell crisis, chest syndrome, or hospitalization.

In the discriminant analyses, no significant differences were detected in the average age at entry or in the risk of the clinical parameters across the groups defined by the laboratory parameters. Table III presents the average age at entry and the average age of occurrence of sickle cell crisis, chest syndrome, and hospitalization for the selected groupings of each laboratory parameter.

Table IV summarizes the results of the survival analysis of stroke, aseptic necrosis, meningitis/septicemia, and death for the same groupings of each laboratory parameter. No significant differences were evident for the risk of aseptic necrosis, meningitis/septicemia, or death among groups of patients defined by any one of the laboratory parameters. No significant differences were found for the risk of stroke among groups of patients defined by levels of MCV, Hb-A_2, Hb, PCV, and MCHC. Fig. 4 presents the survival curves of aseptic necrosis for the three groups of patients with different levels of percent Hb-F. Fig. 5 presents the survival curves of stroke for the same groups. The probability of stroke was found to be significantly different among groups of patients defined by percent Hb-F and absolute Hb-F; a decreased probability of stroke was associated with increased levels of Hb-F.

DISCUSSION

There are major difficulties in developing an index of clinical severity for sickle cell anemia (35) although various indices of severity have been used (19, 36–38). These indices, in our opinion, have usually not been applicable. Age separates the population of sickle cell anemia patients by the nature of the associated conditions and the outcome (39). In general, children

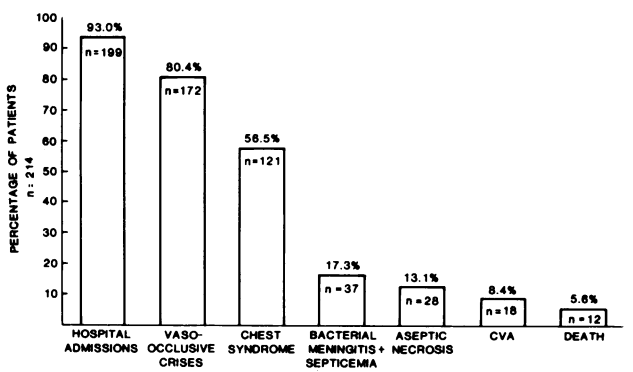

FIGURE 3 Percentage of patients with one or more episodes of the seven clinical indicators of morbidity. Sickle cell crisis, chest syndrome, and hospital admission are frequently recurrent clinical events, whereas death, aseptic necrosis, stroke, and septicemia are analyzed as one-time (termination) events.

TABLE II
Summary of the Clinical Parameters

Multiple episode clinical parameters	Average rate of occurrence per person-year	SD
Sickle cell crisis	0.71	1.23
Chest syndrome	0.17	0.30
Hospitalization	1.17	1.68

Single episode clinical parameters	Cumulative probability of termination at Age 5	Age 10	Age 15	Age 20	Age 30	Age 40
Stroke	0.06	0.11	0.16	0.17	0.19	0.32
Aseptic necrosis	0.06	0.10	0.15	0.19	0.27	0.57
Meningitis/ septicemia	0.43	0.46	0.49	0.51	0.55	0.63
Death	0.01	0.01	0.02	0.04	0.12	0.37

<7 yr of age often have acute infectious illnesses, whereas, in adults, overwhelming problems of specific organ damage become the predominant feature of the manifestations of sickle cell anemia. Once permanent damage to a vital organ occurs, the problem of the malfunctioning organ overshadows those that can be related simply to sickle cell anemia itself. Consequently, we have chosen the seven major clinical complications already listed as measures of clinical severity.

Fetal hemoglobin and morbidity. The results of the present study do not agree with the generally held opinion that high percentages of Hb-F and low values of MCV have an ameliorating effect upon the severity of sickle cell anemia. Thus, the statistical analyses have failed, with one exception, to uncover any relationship between the severity of sickle cell anemia (as measured by the stated indicators) and the levels of Hb-F, Hb-A_2, or other hematological parameters. The single exception, to be discussed below, is the association between Hb-F and stroke. Contrary to the general views expressed in the literature, conclusions similar to ours were drawn by Ozsoylu and Altinoz (40) from a study of 95 Turkish cases. They felt that these cases, with less than one crisis per year, were clinically milder than "African cases." However, in our group of Black Americans, the risk of crisis was 0.71±1.23/yr of observation. Turkish and American SS patients seem to be similar clinically, hematologically, and in range of Hb-F. Serjeant (41) reported on 60 SS patients over 30 yr of age who were alive, reasonably well, and did not have elevated Hb-F levels (41). Recently he and his collaborators (42) report mean percent Hb-F of 5.18±3.59 among 123 SS cases and 6.50±3.73 among 41 probable S-β^0-thalassemia cases. Although these values are some-

TABLE III

Discriminant Analysis of the Risk of Sickle Cell Crisis, Chest Syndrome, and Hospitalization

Laboratory parameters	Number of patients	Mean age at entry	Sickle cell crisis		Chest syndrome		Hospitalization	
			Mean rate per person-year	*P* value	Mean rate per person-year	*P* value	Mean rate per person-year	*P* value
%Hb-F ≤ 8	78	11.4	0.73		0.20		1.13	
8 < %Hb-F ≤ 13	65	9.0	0.74	0.97	0.16	0.55	1.13	0.85
%Hb-F > 13	71	8.8	0.68		0.16		1.24	
Total	214	9.8	0.71		0.17		1.17	
Abs Hb-F ≤ 650	70	10.8	0.73		0.19		1.15	
650 < Abs Hb-F ≤ 1150	73	8.9	0.79	0.64	0.19	0.44	1.25	0.82
Abs Hb-F > 1150	71	9.8	0.61		0.14		1.10	
Total	214	9.8	0.71		0.17		1.17	
MCV ≤ 75	20	9.2	1.25		0.11		1.65	
75 < MCV ≤ 95	125	9.1	0.62	0.11	0.20	0.48	1.07	0.28
MCV > 95	34	14.0	0.87		0.18		1.50	
Total	179	10.0	0.74		0.18		1.22	
HbA_2 < 2.5	25	8.8	0.78		0.22		1.60	
2.5 ≤ HbA_2 ≤ 4.0	171	9.8	0.68	0.70	0.17	0.64	1.08	0.28
HbA_2 > 4.0	16	12.0	0.95		0.13		1.43	
Total	212	9.9	0.71		0.17		1.17	
Hb < 8	71	8.3	0.73		0.19		1.13	
8 ≤ Hb < 9	73	10.1	0.73	0.91	0.18	0.85	1.30	0.67
Hb ≥ 9	70	11.1	0.68		0.16		1.07	
Total	214	9.9	0.71		0.17		1.17	
PCV ≤ 23	71	8.8	0.71		0.19		1.20	
23 < PCV ≤ 26	68	8.4	0.73	0.91	0.17	0.74	1.24	0.66
PCV > 26	75	12.1	0.69		0.16		1.07	
Total	214	9.8	0.71		0.17		1.16	
MCHC ≤ 32	29	9.4	0.53		0.09		0.90	
MCHC > 32	174	9.3	0.69	0.52	0.18	0.09	1.15	0.46
Total	203	9.3	0.66		0.16		1.11	

what lower than our mean percent Hb-F of 12.3±7.0, the methodologies for Hb-F determination were different. In general, alkaline denaturation methods as used in Jamaica and Turkey give lower readings particularly at higher levels (above 20%) of Hb-F so that these apparent differences may not be significant. Wrightstone and Huisman (43) reported a mean Hb-F (alkaline denaturation) of 6.6% in 117 Black SS individuals.

The strongest support for the concept that a high level of Hb-F ameliorates the severity of sickle cell disease comes from the ongoing reports of Arabian sickle cell anemia patients (14–19). Their mean Hb-F among 183 children was 25% and among 54 adults was 24%. Nevertheless, 95% of their SS cases covered a range of 12 to 40% Hb-F whereas in our group 40% of the cases fall within this range. As reported by Perrine et al. (18), the Arabian SS cases show considerable variability of clinical expression. Information is not currently available regarding the correlation of risk of sickle crisis, stroke, chest syndrome, etc., and Hb-F levels in the Arabian SS population. Note that 75% of the Arabian SS patients were less than 20 yr of age when reported (18), including many adolescent patients, an age range in which sickle cell anemia tends to be most quiescent in its manifestations. The 15 mildly affected Indian SS patients had characteristics much like those of the Saudi Arabians; the age range was from 8 to 26 yr and only two were >20 (20).

Some of our S-only cases as well as those observed by others (18, 19) have remarkably high Hb-F levels (above 30%). Nevertheless, such individuals demonstrate the hematologic values and other characteristics of classic sickle cell anemia. Several such patients in the present study have had serious illness. Contrariwise, some cases with Hb-F of the order of 5% have had minimal clinical problems. Some questions are unanswered: Are the Saudi Arabian or

TABLE IV

Analysis of Termination Probabilities of Stroke, Aseptic Necrosis, Meningitis/Septicemia, and Death

Laboratory parameters	Number of patients	Stroke: Number with 1+ eipsodes*	Stroke: P value‡	Aseptic necrosis: Number with 1+ episodes*	Aseptic necrosis: P value‡	Meningitis/septicemia: Number with 1+ episodes*	Meningitis/septicemia: P value‡	Death	Death: P value‡
%Hb-F ≤ 8	78	14 (788)		15 (788)		15 (744)		7 (893)	
8 < %Hb-F ≤ 13	65	3 (670)	0.003	9 (651)	0.59	13 (603)	0.68	1 (709)	0.21
%Hb-F > 13	71	1 (518)		4 (485)		9 (502)		4 (524)	
Total	214	18 (1,976)		28 (1,924)		37 (1,849)		12 (2,126)	
Abs Hb-F ≤ 650	70	15 (698)		13 (712)		13 (688)		6 (813)	
650 < Abs Hb-F ≤ 1150	73	3 (739)	0.0001	11 (713)	0.46	15 (644)	0.85	3 (773)	0.85
Abs Hb-F > 1150	71	0 (540)		4 (500)		9 (518)		3 (540)	
Total	214	18 (1,977)		28 (1,925)		37 (1,850)		12 (2,126)	
MCV ≤ 75	20	1 (203)		2 (191)		5 (177)		2 (205)	
75 < MCV ≤ 95	125	11 (1,065)	0.47	17 (1,068)	0.80	24 (998)	0.52	7 (1,173)	0.80
MCV > 95	34	2 (360)		5 (327)		3 (332)		2 (376)	
Total	179	14 (1,628)		24 (1,586)		32 (1,507)		11 (1,754)	
HbA_2 < 2.5	25	1 (179)		2 (162)		4 (182)		0 (185)	
2.5 ≤ HbA_2 ≤ 4.0	171	16 (1,576)	0.83	21 (1,579)	0.98	29 (1,484)	0.91	10 (1,718)	0.71
HbA_2 > 4.0	16	1 (201)		5 (163)		3 (173)		2 (203)	
Total	212	18 (1,956)		28 (1,904)		36 (1,839)		12 (2,106)	
Hb < 8	71	9 (598)		7 (633)		10 (599)		5 (676)	
8 ≤ Hb < 9	73	7 (706)	0.11	11 (653)	0.64	13 (683)	0.29	3 (764)	0.50
Hb ≥ 9	70	2 (672)		10 (639)		14 (568)		4 (686)	
Total	214	18 (1,976)		28 (1,925)		37 (1,850)		12 (2,126)	
PCV ≤ 23	71	8 (592)		7 (610)		11 (570)		3 (656)	
23 < PCV ≤ 26	68	7 (651)	0.77	11 (613)	0.55	11 (645)	0.51	4 (720)	0.98
PCV > 26	75	3 (734)		10 (702)		15 (633)		5 (750)	
Total	214	18 (1,977)		28 (1,925)		37 (1,848)		12 (2,126)	
MCHC ≤ 32	29	1 (303)		5 (279)		6 (265)		2 (305)	
MCHC > 32	174	17 (1,576)	0.22	21 (1,559)	0.61	30 (1,493)	0.86	10 (1,724)	0.98
Total	203	18 (1,879)		26 (1,838)		36 (1,758)		12 (2,029)	

* Entry in parentheses is the total number of patient-years of observation.

‡ The log rank test was applied to the survival analysis using life table technique.

Indian SS patients different from the American, Jamaican, or Turkish cases? If so, is the Hb-F responsible? What is the genetic explanation?

Erythrocyte indices and morbidity. Our data have been unable to show any association of Hb-A_2, MCV, or MCHC values with the expression of clinical disease. Although the number of patients with S-β^0-thalassemia is small, the severity of expression in them seems equal to that in sickle cell anemia. Although Hb-A_2 influences the polymerization kinetics of Hb-S in the same way as Hb-F (6), the concentration required is much above the 5–6% in the patient with S-β^0-thalassemia (42). Consequently, as do other investigators, we conclude that interacting α- or β-thalassemia in an S-only patient does not sufficiently change the rheology of the erythrocyte to affect the clinical course in any demonstrable way (42, 44–48). We would not be inclined to support the concept that lowered MCV, which is induced by iron deficiency, changes the course of the illness (49).

Fetal hemoglobin and stroke. From the statistical analysis of 18 stroke patients (Table IV), it follows that the risk of stroke increases in those patients with lowered Hb-F (*P* 0.003 and 0.0001). A previous study of 12 stroke patients, some of whom are included in this study, failed to show a significant difference in Hb-F between the group of stroke patients and the group of nonstroke patients when age-matched cohort analysis was used (50). It should be emphasized that this opposite conclusion was derived from a smaller sample. In contrast, the other indicators of morbidity, which are based on large numbers of data, show no relationship between Hb-F or the other laboratory parameters.

If it is correct that a decreased level of Hb-F increases the risk of stroke, then this briefly described hypothesis may explain why Hb-F levels, but none of the other indicators of morbidity, do influence the risk of stroke. In sickle cell anemia the distorted damaged erythrocyte must be a factor in stroke. As the cell becomes less deformable, increased hemolytic breakdown occurs, particularly in the small arterial capillary bed, with the resultant local increase in ionized and nonionized calcium concentration. Those patient's with less Hb-F would have a greater population of erythrocytes with reduced flexibility. Oxygenated sickle cells preferentially agglutinate to capillary endothelial cells in tissue cultures in a similar manner to barnacles on a ship hull (51). Consequently, one could envision a specific phenomenon only in the brain that would be related to the combined effects of less flexible, but still oxygenated erythrocytes, and increased localized calcium ionization with resultant neurovascular spasm and increased platelet function. The brain capillary system, which is exquisitely sensitive to calcium ion, would interact with the calcium overloaded erythrocytes, and barnacle like plaques of erythrocytes would accumulate and produce an obstructed circulation. Thus, it may be suggested that there is sufficient variability of erythrocyte vascular interrelationships in different organs to account for a variability of predictive factors. Therefore, a given factor might be influential in producing damage to one organ but not to another. The statistical analyses implicate Hb-F concentration in producing the risk of stroke but not of aseptic necrosis. The anatomy and pathophysiology of brain and bone are sufficiently different that different predictive factors are not surprising. In sickle cell anemia, where the pathophysiology is related to many interdependent physicochemical and functional factors, it may be simplistic to expect to find a single factor that would reproducibly produce the same risk of damage in all situations.

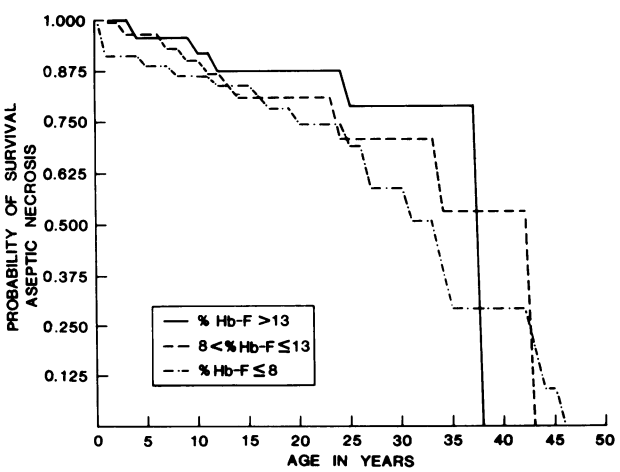

FIGURE 4 Survival curves for aseptic necrosis in three groups of patients as defined by percent Hb-F levels demonstrating no significant association.

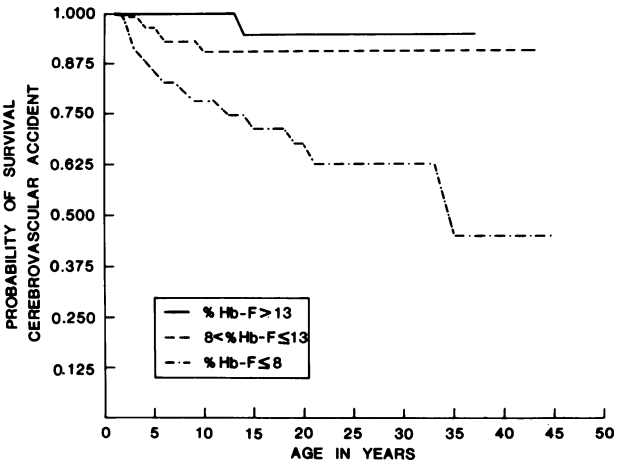

FIGURE 5 Survival curves for cerebral vascular accident in three groups of patients as defined by percentage of Hb-F levels demonstrating a significant association between the risk of cerebral vascular accident and levels of fetal hemoglobin below 13%.

The extraordinarily small values of the square of multiple correlations of the risk of the clinical variables with age at entry and the laboratory variable indicate that other factors not considered in this study may have an influence on the risk. The inability of the analyses to reveal significant relationships may be due to small sample sizes, especially when subgroups of patients were studied. Large collaborative studies are called for. The cumulative effects of environmental, sociological, physiological, and physicochemical factors, coupled with the remarkable age-related clinical variability that is observed in sickle cell anemia, overshadow the influence of identifiable hematological or biochemical parameters on the overall disease process and the clinical severity.

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REFERENCES

1. Bertles, J. F. 1974. Human fetal hemoglobin: significance in disease. *Ann. N.Y. Acad. Sci.* **241:** 638.
2. Bertles, J. F., R. Rabinowitz, and J. Dobler. 1970. Hemoglobin interaction: modification of solid phase composition in the sickling phenomenon. *Science (Wash. D. C.).* **169:** 375.
3. Bookchin, R. M., and R. L. Nagel. 1973. Conformational requirements for the polymerization of hemoglobin S: studies of mixed liganded hybrids. *J. Mol. Biol.* **76:** 233.
4. Hofrichter, J., P. D. Ross, and W. A. Eaton. 1976. A physical description of hemoglobin S gelation. *In* Proceedings of the Symposium on Molecular and Cellular Aspects of Sickle Cell Disease. J. I. Hercules, G. L. Cottam, M. R. Waterman, and A. N. Schechter, editors. Department of Health, Education, and Welfare publication number 76-1007, Bethesda, Md. 185.
5. Cottam, L., K. Shibata, and M. R. Waterman. 1978. Effectors of the rate of deoxyhemoglobin S polymerization. *In* Biochemical and Clinical Aspects of Hemoglobin Abnormalities. W. S. Caughey, editor. Academic Press, Inc., New York. 695.
6. Nagel, R. L., R. M. Bookchin, J. Johnson, D. Labie, H. Wajcman, W. A. Isaac-Sodeye, G. R. Honig, G. Schiliro, J. H. Crookston, and K. Matsutomo. 1979. Structural bases of the inhibitory effects of hemoglobin F and hemoglobin A_2 on the polymerization of hemoglobin S. *Proc. Natl. Acad. Sci. U. S. A.* **76:** 670–672.
7. Bertles, J. F., and P. F. A. Milner. 1968. Irreversibly sickled erythrocytes: a consequence of the heterogeneous distribution of hemoglobin types in sickle cell anemia. *J. Clin. Invest.* **47:** 1731.
8. Chien, S., S. Usami, and J. F. Bertles. 1970. Abnormal rheology of oxygenated blood in sickle cell anemia. *J. Clin. Invest.* **49:** 623.
9. Briehl, R. 1978. Sickle cell: a metastable disease. *Nature (London)* **276:** 666.
10. Miale, T. D., D. L. Lawson, L. S. Rotolo, and F. S. Rotolo. 1978. Semiquantitative computerized image analysis of fetal hemoglobin distribution patterns in sickle cell anemia and its variants. *Hemoglobin.* **2:** 291.
11. Dover, G. J., S. H. Boyer, S. Charache, and K. Heintzelman. 1978. Individual variation in the production and survival of F cells in sickle cell disease. *N. Engl. J. Med.* **299:** 1428.
12. Conley, C. L., D. J. Weatherall, S. N. Richardson, M. K. Shepard, and S. Charache. 1963. Hereditary persistence of fetal hemoglobin: a study of 79 affected persons in 15 Negro families in Baltimore. *Blood* **21:** 261.
13. Fessas, P., and G. Stamotoyannopoulos. 1964. Hereditary persistence of fetal hemoglobin in Greece. A study and a comparison. *Blood* **24:** 223.
14. Ali, S. A. 1970. Milder variant of sickle-cell disease in Arabs in Kuwait associated with unusually high level of foetal haemoglobin. *Br. J. Haematol.* **19:** 613.
15. Haghshenass, M., F. Ismail-Beigi, J. B. Clegg, and D. J. Weatherall. 1977. Mild sickle-cell anaemia in Iran associated with high levels of fetal haemoglobin. *J. Med. Genet.* **14:** 168.
16. Gelpi, A. P. 1970. Sickle cell disease in Saudi Arabs. *Acta Haematol. (Basel).* **43:** 89.
17. Perrine, R. P., M. J. Brown, D. J. Weatherall, J. B. Clegg, and A. May. 1972. Benign sickle cell anaemia. Lancet. **II:** 1,163.
18. Perrine, R. P., M. E. Pembrey, P. John, S. Perrine, and F. Shoup. 1978. Natural history of sickle cell anemia in Saudi Arabs: a study of 270 subjects. *Ann. Intern. Med.* **88:** 1.
19. Pembrey, M. E., W. G. Wood, D. J. Weatherall, and R. P. Perrine. 1978. Fetal haemoglobin production and the sickle gene in the oases of eastern Saudi Arabia. *Br. J. Haematol.* **40:** 415.
20. Brittenham, G., B. Lozoff, J. W. Harris, S. M. Mayson, A. Miller, and T. H. J. Huisman. 1979. Sickle cell anemia and trait in Southern India: further studies. *Am. J. Hematol.* **6:** 107–123.
21. Lehmann, H., and R. G. Huntsman. 1974. Man's Haemoglobins. J. B. Lippincott Co., Philadelphia, Pa. 2nd edition. 268.
22. Weatherall, D. J., J. B. Clegg, J. Blankson, and J. R. McNeil. 1969. A new sickling disorder resulting from interaction of the genes for haemoglobin S and α-thalassemia. *Br. J. Haematol.* **17:** 517.
23. Van Enk, A., A. Lang, J. M. White, and H. Lehmann. 1972. Benign obstetric history in women with sickle-cell anaemia associated with α-thalassemia. *Br. Med. J.* **4:** 524.
24. Seakins, M., W. N. Gibbs, P. F. Milner, and J. F. Bertles. 1973. Erythrocyte Hb-S concentration. An important factor in the low oxygen affinity of blood in sickle cell anemia. *J. Clin. Invest.* **52:** 422.
25. Serjeant, G. R., B. E. Serjeant, P. Desai, K. P. Mason, A. Sewill, and J. M. England. 1978. The determinants of irreversibly sickled cells in homozygous sickle cell disease. *Br. J. Haematol.* **40:** 431.
26. Clark, M. R., N. Mohandas, and S. B. Shohet. 1978. Deformability of irreversibly sickled cells, influence of MCHC. *Blood.* **52**(Suppl. 1.): 96. (Abstr.)
27. Schroeder, W. A., L. Evans, L. Grussing, E. C. Abraham, T. H. J. Huisman, H. Lam, and J. B. Shelton. 1976. Quantitative microchromatographic determination of hemoglobin F in patients with hemoglobins S and/or C. *Am. J. Hematol.* **1:** 331–342.
28. Schroeder, W. A., L. A. Pace, and T. H. J. Huisman. 1978. Microchromatography of hemoglobins. VIII. A general qualitative and quantitative method in plastic drinking straws and the quantitative analysis of Hb-F. *J. Chromatogr.* **145:** 203.
29. Huisman, T. H. J., W. A. Schroeder, A. N. Brodie, S. M. Mayson, and J. Jakway. 1975. Microchromatography of hemoglobins. III. A simplified procedure for the determination of hemoglobin A_2. *J. Lab. Clin. Med.* **86:** 700.
30. Kleihauer, E., H. Braun, and K. Betke. 1957. Demonstration von fetalem hämoglobin in den erythrocyten eines blut ausstriches. *Klin. Wochenschr.* **35:** 637.
31. Afifi, A. A., and S. P. Azen. 1979. Statistical Analysis: A Computer Oriented Approach. Academic Press, Inc., New York. 2nd edition.
32. Peto, R., M. C. Pike, P. Armitage, N. E. Breslow, D. R. Cox, S. V. Howard, N. Mantel, K. McPherson, J. Peto, and P. G. Smith. 1976. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. I. Introduction and design. *Br. J. Cancer.* **34:** 585.
33. Peto, R., M. C. Pike, P. Armitage, N. E. Breslow, D. R. Cox, S. V. Howard, N. Mantel, K. McPherson, J. Peto, and P. G. Smith. 1977. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. *Br. J. Cancer.* **35:** 1.
34. Powars, D. R., and W. A. Schroeder. 1978. Progress in the natural history studies of the clinical severity of sickle cell disease: epidemiologic aspects. *In* Bio-

chemical and Clinical Aspects of Hemolgobin Abnormalities. W. S. Caughey, editor. Academic Press, Inc., New York. 151.
35. Mantel, N. 1966. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother. Rep.* **50:** 163.
36. Steinberg, M. H., B. J. Drieling, F. S. Morrison, and T. F. Necheles. 1973. Mild sickle cell disease. Clinical and laboratory studies. *JAMA (J. Am. Med. Assoc.).* **224:** 317.
37. Karayalcin, G., F. Rosner, K. Y. Kim, P. Chandra, and A. J. Aballi. 1975. Sickle cell anemia. Clinical manifestations in 100 patients and review of the literature. *Am. J. Med. Sci.* **269:** 51.
38. Konotey-Ahulu, F. I. D. 1971. Computer assisted analysis of data on 1697 patients attending the sickle cell/haemoglobinopathy clinic of Korle Bu Teaching Hospital, Accra, Ghana. Clinical features: I. Sex, genotype, age rheumatism and dactylites frequencies. *Ghana Med. J.* **10:** 241.
39. Powars, D. R. 1975. Natural history of sickle cell disease: the first ten years. *Semin. Hematol.* **12:** 267.
40. Ozsoylu, S., and N. Altinoz. 1977. Sickle-cell anaemia in Turkey. Evaluation of 97 cases (with parents' findings). *Scand. J. Haematol.* **19:** 85.
41. Serjeant, G. R., R. Richards, P. R. H. Barbor, and P. F. Milner. 1968. Relatively benign sickle cell anaemia in 60 patients aged over 30 in the West Indies. *Br. Med. J.* **3:** 86.
42. Serjeant, G. R., A. M. Sommereux, M. Stevenson, K. Mason, and B. E. Serjeant. 1979. Comparison of sickle cell-β^0-thalassemia with homozygous sickle cell disease. *Br. J. Haematol.* **41:** 83.
43. Wrightstone, R. N., and T. H. J. Huisman. 1974. On the levels of hemoglobins F and A_2 in sickle-cell anemia and some related disorders. *Am. J. Clin. Pathol.* **61:** 375.
44. Natta, C. 1978. Failure of the α-thalassemia gene to decrease the severity of sickle cell anemia. *Blood.* **51:** 1163.
45. Joishy, S. K., P. F. Griner, and P. T. Rowley. 1976. Sickle β-thalassemia: identical twins differing in severity implicate nongenetic factors influencing course. *Am. J. Hematol.* **1:** 23.
46. Honig, G. R., M. Koshy, R. G. Mason, and L. N. Vida. 1978. Sickle cell syndromes. II. The sickle cell anemia-α-thalassemia syndrome. *J. Pediatr.* **92:** 556.
47. Steinberg, M. H., and B. J. Dreiling. 1976. Clinical, hematologic and biosynthetic studies in sickle cell-β^0-thalassemia: a comparison with sickle cell anemia. *Am. J. Hematol.* **1:** 35.
48. Felice, A. E., B. Webber, A. Miller, S. M. Mayson, H. F. Harris, J. B. Henson, M. E. Gravely, and T. H. J. Huisman. 1979. The association of sickle cell anemia with heterozygous and homozygous α-thalassemia-2: in vitro HB chain synthesis. *Am. J. Hematol.* **6:** 91–106.
49. Lincoln, T. L., J. Aroesty, and P. Morrison. 1973. Iron-deficiency anemia and sickle cell disease: a hypothesis. *Lancet.* **II:** 260.
50. Powars, D., B. Wilson, C. Imbus, C. Pegelow, and J. Allen. 1978. The natural history of stroke in sickle cell disease. *Am. J. Med.* **65:** 461.
51. Hebbel, R. P., O. Yamada, C. F. Moldow, J. G. White, and J. W. Eaton. 1978. Clinical adherence of sickle erythrocytes to vascular endothelium. *Blood.* **52**(Suppl. 1.): 98. (Abstr.)