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Stability Over Time of Hematological Variables in 197 Children With Sickle Cell Anemia

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One hundred ninety-seven children with sickle cell anemia were followed for 4 years at the Wayne State Comprehensive Sickle Cell Center to evaluate the stability of the hematological variables (Hb, Hct, RBC count, MCV, %HbF and $\%HBA_2$) over time. The mean values of the hematological measurements taken during three separate 16-month intervals were used to represent an individual's values. The correlations of the hematological variables between intervals ranged from a low of 0.46 for $\%HBA_2$ to a high of 0.91 for %HbF. Correlations that spanned two intervals (an average of 32 months) were of the same magnitude as those that spanned only one interval (an average of 16 months), suggesting that there was no decrease in the degree of stability of these variables as the time between measurements increased. The stability of the correlations between variables within intervals, and the stability of the coefficients of the first two principal components of the six hematological variables over time suggested that the relationships among variables were also stable.

In a recent report [Odenheimer et al, 1983], we used the values of the six hematological variables collected at an individual's first visit to the sickle cell center to identify four hematologically distinct subgroups of children. In the current report, we found that as many as 83% of the individuals remained in the same subgroup in at least two of the three follow-up intervals, suggesting that the factors that contributed to this classification were the result of stable, rather than transient phenomena.

Key words: sickle cell anemia, hematology, fetal hemoglobin

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INTRODUCTION

The clinical variability of patients with sickle cell anemia is well known; likewise it is well known that the severity of a patient's condition may flucuate with time. Recently, Odenheimer et al [1983] reported the use of a profile of hematological data (Hb, Hct, RBC count, MCV, %HbF and $\%HBA_2$) to identify heterogeneity in children with sickle cell anemia. Subgroups differed significantly for a number of measures of disease severity, including the proportion that ever had pneumonia, dactylitis, a transfusion, and a hospitalization before enrolling in the Wayne State University Comprehensive Center for Sickle Cell Anemia. The hematological data used in identifying these subgroups were collected at a patient's initial visit to the center. The objective of the current study was to determine whether the age-adjusted hematological variables and the linear relationships among these variables were stable over time. Specifically, our analyses were designed to answer the following: 1) Do individuals maintain their relative rank in the sample over time for each of the hematological variables considered? 2) Are the relationships among variables stable over time? and 3) On follow-up, do individuals tend to remain in the same subgroup to which they were assigned at initial assessment.

If the hematological variables are stable over time, cross-sectional data may be useful for the prediction of subsequent hematological status and the associated clinical symptoms in children with sickle cell anemia. In addition, the stability of the subgroups identified at initial assessment establishes these subgroups as candidates for analyses to identify the genetic and environmental factors that explain the heterogeneity of sickle cell anemia.

MATERIALS AND METHODS

Sample

A description of the large sample of patients seen at initial assessment at the Wayne State Comprehensive Sickle Cell Center has been presented elsewhere [Odenheimer et al, 1983]. The sample consists of 360 children (age 1–18 years) with sickle cell anemia who were first seen at the center between October 1973 and October 1980. Of the 360 patients seen at initial assessment, 225 were included in the cluster analysis [Odenheimer et al, 1983]; the remaining 135 patients were excluded because of incomplete data. The sample studied here consists of 197 patients (136 involved in the cluster analysis sample and 61 others) who were followed for at least 4 years. These 197 individuals were not significantly different from the 163 patients who were not followed with respect to the characteristics: proportion of males or females, average age, or the mean values of Hb, Hct, RBC count, %HbF, $\%HBA_2$, and MCV measured at their initial assessment visit. The main reasons for not being followed include 1) the patient did not enter the study early enough to have been followed for 4 years, 2) the patient reached his or her 18th birthday before being followed for 4 years and was transferred to the adult clinic, 3) a patient's family moved out of the Detroit area, 4) a patient began seeing a private physician, 5) a patient died before 4 years of follow-up were completed, and 6) a patient's hematological profile was measured only once in the 4-year follow-up period. In addition, we excluded 35 patients who were on a special transfusion program for individuals who had experienced a previous stroke.

Study Design

The follow-up period was divided into three 16-month intervals that began at a patient's initial assessment visit. A 16-month interval was chosen because it maximized the number of patients whose hematological status was measured at least once per interval. The number of times a blood sample was assayed during the follow-up varied markedly among individuals. For example, the number of blood samples taken during the first interval ranged from one to 13 (mean 4.9). However, in no case was an individual's mean value for a variable within an interval significantly correlated with the number of measurements taken. Furthermore, there was no significant difference in the mean number of follow-up measurements when comparing those with and without pain crises, hospitalizations, pneumonia, or dactylitis before enrolling at the center. However, there was a significant increase ($P < 0.05$) in the number of measurements taken on individuals who had had at least one transfusion (mean 5.2) as compared to those who had never been transfused (mean 4.5). For all subsequent analyses, we used the means of the hematological measurements taken during an interval to represent an individual's values.

The hematologic values were obtained using the Coulter Counter, model S. HBA_2 was measured either by elution from cellulose acetate electrophoresis strips [Schneider, 1974] or radial immunodiffusion assay [Chudwin and Rucknagel, 1974]. Initially HbF was measured by alkali denaturation [Singer et al, 1951], but subsequently by radial immunodiffusion assay of whole blood [Chudwin and Rucknagel, 1974]. The two sets of values were merged separately for each sex by adding the overall HbF mean to the residuals from the linear regression of each method on age [Neter and Wasserman, 1974].

Statistical Analysis

We used the linear regression of an individual's mean value on his average age for the interval to remove the age variation in these data. The bivariate relationships between the mean values of the age-adjusted hematological variables within a follow-up interval were evaluated using the Pearson product-moment correlation. The relationship between an individual's mean values for a variable at two different intervals was evaluated using the Pearson product-moment correlation and Spearman's rank correlation. The results using these two methods were very similar; thus, results of only the Pearson product-moment correlations will be reported here.

The primary linear relationships among the six hematological variables were evaluated in each follow-up period using the first two principal components of the correlation matrix [Morrison, 1976]. The consistency of the coefficients of the principal components are a measure of the stability of the multivariate relationships among the hematological variables. The first principal component accounts for the greatest percentage of variability represented by this set of variables, and the other five principal components each explain a progressively smaller percentage of the variability. Each principal component is uncorrelated with all other principal components.

Linear discriminant analysis was used to determine whether the 136 individuals included in both the cluster analysis at initial assessment and the sample studied here tended to remain in the same subgroup on follow-up as they were assigned to at initial assessment. Discriminant scores were calculated for each individual at the three

TABLE I. Descriptive Statistics: Age-Adjusted Hematological Variables

		Interval 1	Interval 2	Interval 3	Significance[b] level	N
Males						
HB	1, 2, 3	8.4 ± 0.9[c]	8.4 ± 0.9	8.3 ± 0.9	0.50	95
	1, 2[a]	7.5 ± 0.8	8.1 ± 0.8			2
	1, 3	8.6		9.7		1
	2, 3		7.6 ± 0.9	7.5 ± 0.5		3
HCT	1, 2, 3	24.6 ± 2.8	24.7 ± 2.8	24.3 ± 3.1	0.66	95
	1, 2	21.4 ± 1.7	22.8 ± 0.9			2
	1, 3	25.5		28.7		1
	2, 3		21.7 ± 2.3	22.1 ± 1.1		3
RBC	1, 2, 3	2.8 ± 0.5	2.8 ± 0.4	2.8 ± 0.4	0.46	94
	1, 2	3.0 ± 0.8	2.9 ± 0.5			3
	1, 3	2.9		3.2		1
	2, 3		2.5 ± 0.5	2.6 ± 0.6		3
MCV	1, 2, 3	88.0 ± 6.7	89.4 ± 7.0	89.2 ± 5.8	0.28	94
	1, 2	78.2 ± 9.9	86.5 ± 6.7			3
	1, 3	86.8		91.5		1
	2, 3		87.2 ± 7.8	87.8 ± 4.5		3
%HbF	1, 2, 3	10.0 ± 5.2	9.4 ± 4.7	10.0 ± 5.3	0.74	65
	1, 2	8.5 ± 3.0	8.5 ± 3.8			15
	1, 3	13.6 ± 4.9		12.4 ± 3.9		18
	2, 3		0.7	2.4		1
%HBA_2	1, 2, 3	2.6 ± 0.4	3.0 ± 0.4	2.7 ± 0.5	0.0003	53
	1, 2	2.8 ± 0.4	2.9 ± 0.5			26
	1, 3	2.6 ± 0.3		2.6 ± 0.5		18
	2, 3		3.5	2.8		1
Females						
HB	1, 2, 3	8.4 ± 0.9	8.4 ± 0.9	8.4 ± 0.9	0.93	92
	1, 2					0
	1, 3	9.0 ± 0.8		8.8 ± 0.5		4
	2, 3					0
HCT	1, 2, 3	25.0 ± 2.9	24.8 ± 2.8	25.0 ± 2.8	0.88	91
	1, 2					0
	1, 3	27.8 ± 0.9		26.4 ± 1.2		4
	2, 3	21.3		23.3		1
RBC	1, 2, 3	2.9 ± 0.4	2.8 ± 0.5	2.8 ± 0.5	0.95	91
	1, 2					0
	1, 3	3.0 ± 0.0		2.9 ± 0.2		3
	2, 3	2.9		2.6		1
MCV	1, 2, 3	88.5 ± 5.6	88.6 ± 7.1	89.5 ± 7.4	0.53	91
	1, 2					0
	1, 3	92.0 ± 2.2		91.0 ± 8.1		3
	2, 3		74.0	89.1		1
%HBF	1, 2, 3	10.0 ± 4.2	9.1 ± 4.1	9.4 ± 4.6	0.53	62
	1, 2	9.5 ± 4.4	9.0 ± 4.6			16
	1, 3	10.6 ± 4.2		10.1 ± 5.0		12
	2, 3	9.2 ± 4.1		8.9 ± 5.1		5
%HBA_2	1, 2, 3	2.5 ± 6.3	2.9 ± 0.4	2.8 ± 0.5	0.0001	56
	1, 2	2.7 ± 0.5	2.7 ± 0.4			20
	1, 3	2.7 ± 0.4		2.9 ± 0.4		13
	2, 3		3.7	3.5		1

[a]Individuals in the (1, 2), (1, 3), and (2, 3) categories had blood assayed in only two of three intervals.
[b]Test of mean difference between intervals.
[c]Mean ± SD.

follow-up periods using the initial assessment classification as the stratum for the analysis and the six hematological variables measured on follow-up as the independent variables to estimate the coefficients of the discriminant functions. Using these functions, and a priori probabilities that were equal for each stratum, we calculated the probability that an individual was classified into groups 1, 2, 3, and 4 on follow-up and assigned an individual to the group for which this probability was the greatest. An individual's classification on follow-up was then compared with that established by the cluster analysis at initial assessment.

RESULTS

The hematological data were age-adjusted separately by sex. The percent variability explained by age was approximately 5% for Hb, Hct, RBC count, and MCV; 7.5% for $\%HBA_2$; and 23% for %HbF. The age-adjusted hematological data at each interval are presented in Table I. With the exception of $\%HBA_2$, the means do not change significantly over time. Although mean $\%HBA_2$ values are significantly higher at intervals 2 and 3 than at interval 1, the size of the difference is small. For all subsequent analyses, the data on the few individuals whose hematological status was evaluated in only two of three intervals were pooled with the data on the individuals evaluated in all three intervals.

The correlations of the hematological variables between intervals are presented in Table II. They range from a low of 0.46 for $\%HBA_2$ in males (interval 2 and

TABLE II. Correlations of the Hematological Variables Between Intervals

		Females					Females		
	HB-1[a]		0.80	0.79		HCT-1		0.83	0.77
			(92)	(96)				(91)	(95)
Males	HB-2	0.70		0.82	Male	HCT-2	0.73		0.80
		(97)[b]		(92)			(97)		(92)
	HB-3	0.64	0.72			HCT-3	0.73	0.77	
		(96)	(96)				(96)	(98)	
		HB-1	HB-2	HB-3			HCT-1	HCT-2	HCT-3
		Female					Female		
	RBC-1		0.87	0.88		MCV-1		0.61	0.62
			(91)	(94)				(91)	(94)
Males	RBC-2	0.74		0.87	Male	MCV-2	0.55		0.76
		(97)		(92)			(97)		(92)
	RBC-3	0.71	0.76			MCV-3	0.56	0.64	
		(95)	(97)				(95)	(97)	
		RBC-1	RBC-2	RBC-3			MCV-1	MCV-2	MCV-3
		Female					Female		
	%HBF-1		0.91	0.86		$\%HBA_2$-1		0.55	0.53
			(78)	(74)				(76)	(69)
Males	%HBF-2	0.88		0.90	Male	$\%HBA_2$-2	0.50		0.55
		(80)		(67)			(79)		(57)
	%HBF-3	0.82	0.90			$\%HBA_2$-3	0.48	0.46	
		(83)	(66)				(71)	(54)	
		%HBF-1	%HBF-2	%HBF-3			$\%HBA_2$-1	$\%HBA_2$-2	$\%HBA_2$-3

[a]Interval.
[b]Number of individuals.

interval 3), to a high of 0.91 for %HbF in females (interval 1 and interval 2). All correlations are significantly different from zero at the 0.0001 level of probability. In almost all instances, the correlations are stronger in females than in males. For Hb, Hct, and RBC count, these differences between sexes are statistically significant ($P < 0.05$). The correlations that span two intervals (ie, interval 1 with interval 3) are of the same magnitude as those that span only one interval (ie, interval 1 with interval 2, and interval 2 with interval 3).

The correlations between variables within each interval are presented in Table III. In general, the correlations are similar in the three intervals. For example, the correlation between Hb and %HbF in males is 0.40 in interval 1, 0.48 in interval 2, and 0.49 in interval 3. In the sample of males, the correlation matrices of the six hematological variables in the three intervals are not significantly different from one another ($P > 0.40$). In the female sample, the correlations are also generally stable. However, in a few instances there are differences in the magnitude of the correlations. For example, the correlations between Hb and %HbF are similar at interval 1 ($r = 0.47$) and interval 2 ($r = 0.51$), but lower at interval 3 ($r = 0.25$). Although these correlations between Hb and %HbF are not significantly different from one another ($P > 0.10$), there is evidence that the correlations matrices in the female sample are not homogenous ($P = 0.06$). This difference is primarily attributable to the different correlation structure at interval 3 from that at intervals 1 and 2.

The primary linear relationships among the hematological variables are identified by the first two principal components presented in Table IV. These principal components account for approximately 50% and 30% respectively, of the total variability represented by all six hematological variables at each interval considered. In general, the coefficients of the principal components are stable across intervals, suggesting that the multivariate relationships among variables are approximately the same in each of the three follow-up intervals.

TABLE III. Correlations Between Variables Within Each Interval

		Interval	Males			Females		
	HB	1		0.94 (94)[a]	0.79 (94)	0.01 (94)	0.47 (89)	−0.23 (88)
		2		0.94 (92)	0.81 (92)	−0.26 (92)	0.51 (83)	−0.22 (77)
		3		0.96 (95)	0.78 (95)	−0.22 (95)	0.25 (78)	−0.06 (69)
	HCT	1	0.91 (98)		0.86 (94)	−0.04 (94)	0.48 (89)	−0.20 (87)
		2	0.94 (100)		0.85 (92)	−0.24 (92)	0.53 (83)	−0.19 (77)
		3	0.96 (99)		0.84 (95)	−0.27 (95)	0.30 (78)	−0.03 (69)
	RBC	1	0.80 (97)	0.84 (97)		−0.52 (94)	0.30 (88)	0.01 (86)
		2	0.77 (100)	0.80 (100)		−0.70 (92)	0.28 (83)	0.11 (77)
		3	0.85 (98)	0.89 (98)		−0.73 (95)	0.12 (78)	0.20 (69)
Males	MCV	1	−0.25 (97)	−0.21 (97)	−0.66 (98)		0.19 (88)	−0.36 (86)
		2	−0.03 (100)	0.01 (100)	−0.54 (100)		0.18 (83)	−0.46 (77)
		3	−0.08 (98)	−0.10 (98)	−0.51 (98)		0.21 (78)	−0.42 (69)
	%HBF	1	0.40 (95)	0.43 (95)	0.33 (95)	0.04 (95)		−0.56 (87)
		2	0.48 (79)	0.50 (79)	0.26 (79)	0.24 (79)		−0.60 (77)
		3	0.49 (84)	0.49 (84)	0.32 (83)	0.19 (83)		−0.55 (70)
	$\%HBA_2$	1	−0.5 (94)	−0.07 (94)	0.04 (94)	−0.23 (94)	−0.51 (96)	
		2	−0.10 (78)	−0.09 (78)	0.10 (78)	−0.29 (78)	−0.45 (78)	
		3	−0.20 (72)	−0.19 (72)	0.03 (71)	−0.41 (71)	−0.41 (71)	
			HB	HCT	RBC	MCV	%HBF	$\%HBA_2$

[a]Number of individuals.

TABLE IV. The First Two Principal Components for Each of the Three Follow-up Intervals

	N	% Variance explained					
Principal component 1							
Males							
Interval 1	100	52.02	0.52 (HB)	+0.53 (HCT)	+0.53(RBC)	−0.25(MCV)	+0.30(HBF)	−0.09(HBA_2)
Interval 2	77	49.79	0.55 (HB)	+0.55 (HCT)	+0.51(RBC)	−0.14(MCV)	+0.33(HBF)	−0.09(HBA_2)
Interval 3	70	52.03	0.54 (HB)	+0.55 (HCT)	+0.51(RBC)	−0.09(MCV)	+0.36(HBF)	−0.13(HBA_2)
Females								
Interval 1	91	51.75	0.53 (HB)	+0.54 (HCT)	+0.51(RBC)	−0.12(MCV)	+0.34(HBF)	−0.17(HBA_2)
Interval 2	80	53.47	0.53 (HB)	+0.54 (HCT)	+0.52(RBC)	−0.25(MCV)	+0.31(HBF)	−0.08(HBA_2)
Interval 3	69	50.80	0.52 (HB)	+0.54 (HCT)	+0.55(RBC)	−0.34(MCV)	+0.13(HBF)	−0.07(HBA_2)
Principal component 2								
Males								
Interval 1	100	26.25	0.2 (HB)	+0.04 (HCT)	−0.22(RBC)	+0.51(MCV)	+0.52(HBF)	−0.65(HBA_2)
Interval 2	77	29.20	0.03 (HB)	+0.03 (HCT)	−0.31(RBC)	+0.59(MCV)	+0.48(HBF)	−0.57(HBA_2)
Interval 3	70	28.40	0.01 (HB)	−0.001(HCT)	−0.31(RBC)	+0.64(MCV)	+0.36(HBF)	−0.61(HBA_2)
Females								
Interval 1	91	28.64	−0.005(HB)	−0.02 (HCT)	−0.31(RBC)	+0.61(MCV)	+0.43(HBF)	−0.59(HBA_2)
Interval 2	80	32.68	0.07 (HB)	+0.06 (HCT)	−0.25(RBC)	+0.55(MCV)	+0.48(HBF)	−0.63(HBA_2)
Interval 3	69	31.75	0.17 (HB)	+0.17 (HCT)	−0.12(RBC)	+0.45(MCV)	+0.59(HBF)	−0.61(HBA_2)

In a previous report [Odenheimer et al, 1983], we used cluster analysis techniques and the hematological data collected at initial assessment to identify four hematologically distinct subgroups of patients. The mean values of the hematological data within these subgroups are presented in Table V. In all instances, the differences in mean values among groups are highly significant ($P < 0.0001$).

To assess whether individuals tend to maintain their relative rank in the sample with respect to these four subgroups, we calculated discriminant scores for each individual at each of the three follow-up intervals using the cluster assignment at initial assessment as the stratum for the analysis. In each follow-up interval, an individual was assigned to the group for which the probability of assignment was the greatest. The comparison of the classification at follow-up with the classification established at initial assessment is given in Table VI. Of the individuals with complete data at all three intervals, 46% of the males and 49% of the females were assigned to the same group at each of the follow-up periods as they had been assigned to at initial assessment, while 81% of the males and 83% of the females were assigned to the same group in at least two of the three intervals. Previously [Odenheimer et al, 1983] we showed that individuals in subgroups 1 and 2 had relatively mild disease severity, whereas individuals in subgroups 3 and 4 had relatively severe manifestations of the disease. When hypothesizing that subgroups 1 and 2 represent one severity class and that subgroups 3 and 4 represent a second severity class, 86% of the males and 76%

TABLE V. Distribution of Hematological Variables in the Four Subgroups Identified at Initial Assessment

Subgroup	N		HB	HCT	RBC	%HBF	MCV	%HBA_2
Males								
1	16	Mean 1	7.32	21.33	2.30	9.84	92.54	2.46
2	36	Mean 2	8.46	25.03	3.01	9.18	83.26	2.77
3	20	Mean 3	9.43	27.47	3.09	16.53	88.86	2.30
4	7	Mean 4	9.70	29.01	4.13	14.91	71.21	2.42
Overall mean			8.59	24.25	2.99	11.68	85.49	2.56
Significance level			0.0000	0.0000	0.0000	0.0000	0.0000	0.0008
Intraclass correlation			0.60	0.56	0.80	0.52	0.57	0.23
Females								
1	21	Mean 1	7.73	22.65	2.58	7.86	88.00	2.82
2	15	Mean 2	8.48	24.97	3.29	10.24	76.25	2.84
3	31	Mean 3	8.76	25.88	2.89	14.17	90.13	2.33
4	7	Mean 4	10.02	30.40	3.62	15.75	84.55	2.33
Overall mean			8.53	25.20	2.95	11.73	86.19	2.57
Significance level			0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Intraclass correlation			0.61	0.63	0.67	0.53	0.58	0.42

TABLE VI. Cluster Assignments Based on Discriminant Functions on Follow-up

No. intervals where assignment was the same as initial assessment		Four clusters		Two clusters[a]	
		Males	Females	Males	Females
Measurements in all three intervals	3 of 3	17	20	32	31
	2 of 3	13	14	4	4
	1 of 3	5	5	1	5
	0 of 3	2	2	0	1
Measurements in two intervals of three	2 of 2	19	16	27	22
	1 of 2	10	4	4	2
	0 of 2	2	7	0	3

[a]Clusters 1 and 2 combined, and clusters 3 and 4 combined.

of the females measured in all three intervals were classified into the same class at each interval as they had been assigned to at initial assessment, while 97% of the males and 85% of the females were classified into the same class in at least 2 of 3 intervals.

Since the maximum probability of assignment could be rather low (any probability greater than 50% could be the maximum when the strata were defined by the two severity classes), we also evaluated the distribution of these assignment probabilities. Of the individuals assigned to the same severity class on follow-up as initial assessment, the median probability of assignment was 95% in males and 87% in females.

DISCUSSION

A number of investigators have asked whether a patient's hematological status is associated with his or her clinical manifestations of the disease [Odenheimer et al, 1983; Serjeant and Ashcroft, 1972; Serjeant, 1975; Stevens et al, 1981; Steinberg et al, 1973; Powars et al, 1980; Harkness, 1980]. However, there have been no studies of the stability of the hemotological data over time. Longitudinal studies are a prerequisite for gaining a clear understanding of the role of these data in explaining the natural history of the clinical and hematological course of the disease. We have shown that the hematological variables are stable over time. The correlations of the hematological variables between intervals range from a low of 0.46 for $\%HBA_2$ in males to a high of 0.91 for %HbF in females ($P < 0.0001$ for all correlations). The correlations that span two intervals (an average of 32 months) are of the same magnitude as those that span only one interval (an average of 16 months), suggesting that there is no decrease in the degree of stability of these variables as the time between measurements increases. Additionally, the stability of the bivariate correlations and the stability of the coefficients of the principal components over time suggests that the relationships among variables are stable.

Of the six hematological variables considered in this paper, %HbF has been most often hypothesized as being associated with variation in the clinical severity of sickle cell anemia [Serjeant and Ashcroft, 1972; Stevens et al, 1981; Steinberg et al, 1983; Powars et al, 1980; Ali, 1970; Perrine et al, 1972; Haghshenass et al, 1977]. In our previous study examining the role of the hematological data in predicting disease severity, we found that %HbF (and its inverse relationship to $\%HBA_2$) was most highly associated with disease severity in this sample of children.

It is well documented that %HbF levels drop with increasing age, at least up until age 35 [Rucknagel et al, 1979]. In our sample, age accounted for approximately 25% of the variability in %HbF. Age-adjusted %HbF levels were very highly correlated over time, ranging from 0.82 to 0.91. Thus, although the %HbF level of an individual younger than 20 years is likely to drop with time, his or her relative rank in the sample is very stable. The relationship between %HbF and disease severity reported previously, and its high stability reported here, suggests that a single %HbF measurement may be useful in predicting the clinical course of patients with sickle cell anemia. Since most of the hematologic measurements used were gathered in an outpatient setting, this analysis does not preclude short-term changes, such as may occur when patients enter crises.

The relatively low stability of the proportion of HBA_2 is a curious observation, given the inverse correlation between the proportion of HbF and HBA_2. It is difficult to attribute it to technical factors since elution of the hemoglobin components from cellulose acetate strips is regarded as an accurate and reproducible method of analysis.

A more likely explanation is the well-known relationship between serum iron concentration and HBA_2. Iron deficiency, prevalent in growing children, would lower proportion of HBA_2. However, a corresponding positive correlation between MCV and HBA_2 is not present. Therefore other environmental factors, such as folate deficiency, may be involved.

The identification of heterogeneous subgroups of patients is an important first step in identifying groups of individuals that can be compared for genetic and environmental factors that contribute to the clinical heterogeneity of sickle cell anemia. Our finding that the subgroups identified at initial assessment are generally stable over time demonstrates that the factors that contributed to this classification are the result of stable, rather than transient phenomena. Although these factors are still unknown, the identification of stable hematological phenotypes based on a multivariate profile of hematological data provides a powerful framework for conducting the studies that will establish the links between genetic and environmental factors and disease heterogeneity.

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