Effects of Thalassemia and Microcytosis on the Hematologic and Vasoocclusive Severity of Sickle Cell Anemia

By Martin H. Steinberg, Wendy Rosenstock, Mary B. Coleman, Junius G. Adams, Ovidiu Platica, Marisol Cedeno, Ronald F. Rieder, John T. Wilson, Paul Milner, Stewart West, and the Cooperative Study of Sickle Cell Disease

The characteristic clinical heterogeneity of sickle cell anemia (HbSS) may be, in part, a result of its interactions with α-thalassemia. Although α-thalassemia clearly affects some hematologic features of HbSS, its role in modulating the vasoocclusive severity of disease is not clear. To further explore this relationship, we examined the microcytosis (HbSS) may be, in part, a result of its interactions with α-thalassemia. The effects of a reduced MCV and mean corpuscular hemoglobin concentration (MCHC), of possible benefit by themselves, when accompanied by a reduction in hemolysis and rise in hemoglobin concentration, as in HbSS-α-thalassemia, may cause sufficient rise in blood viscosity in critical vascular beds to impair blood flow and negate any amelioration of vasoocclusive complications in HbSS.

A characteristic of sickle cell anemia (HbSS) is its remarkable clinical heterogeneity. Some patients are constantly plagued by repeated vasoocclusive episodes that cumulatively lead to organ damage and failure and premature death. Others have relatively few of these problems and may have a nearly trouble-free and productive long life. The causes of this clinical diversity are not entirely clear, but candidate modulators of the course of HbSS have been proposed.

Among the factors that have been proposed to influence the severity of HbSS, on both theoretical and experimental grounds, are mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). A reduction in cell size and hemoglobin concentration may alter the dynamics of the sickling process by allowing deformed, but smaller, cells to better traverse the microcirculation. In addition, the quantity of intracellular sickle hemoglobin (Hbs) polymer is reduced as the mean corpuscular HbS concentration falls. The interactions of α-thalassemia and HbSS have been recently studied by Embury et al., Mears et al., and Higgs and coworkers. The association of these two disorders appears to produce microcytic erythrocytes, a low MCHC, fewer dense red cells with greater degrees of cellular deformability, a possible survival advantage for the patient, and fewer episodes of acute chest syndrome and leg ulceration. Powars and associates have reported that MCV and MCHC bear no relationship to a variety of vasoocclusive events in patients with HbSS.

In an effort to gain further insights into the effects of MCV and thalassemia on the vasoocclusive complications of sickle cell disease, we stratified our patients who presented with only HbS, F, and A₂ present upon hemoglobin electrophoresis (“S only”) on the basis of MCV and ascertained the incidence of painful episodes, acute chest syndrome, aseptic necrosis of bone, and leg ulcers in each group. In addition, subsets of patients were examined for the presence of HbSS-α-thalassemia or HbS-β-thalassemia by measuring the relative rates of α- and non-α-globin chain synthesis in reticulocytes and by restriction endonuclease analysis of the α-globin genes.

We found little evidence of a beneficial effect of microcytosis or α-thalassemia on the vasoocclusive complications of HbSS.
MATERIALS AND METHODS

Patients

Patients were recruited from 13 clinical centers representing most geographic areas of the country with a high prevalence of sickle cell disease. Patients attending these clinics prior to the inception of the Cooperative Study of Sickle Cell Disease (CSSCD), as well as new patients presenting at the clinic during the initial 3 yr of the CSSCD, were enrolled after informed consent was obtained. This analysis includes only patients aged 2 yr or more. Patients had over 2 yr of follow-up at the time of data analysis.

Laboratory Studies

The complete blood count (CBC) and erythrocyte indices were determined using Coulter electronic cell counters. Hemoglobin electrophoresis was done on cellulose acetate membranes and citrate agar gels.\(^6\) HbA\(_2\) was measured by DEAE cellulose column chromatography\(^7\) and HbF by alkali denaturation.\(^8\) All hemoglobin studies were done at the Center for Disease Control (CDC), Atlanta, GA.

Estimation of the relative rates of \(\alpha\)- to non-\(\alpha\)-globin synthesis was done by incubation of 2 ml of packed erythrocytes in 2 ml of plasma with 100 \(\mu\)Ci of \(^{3}H\)-l-leucine. Following incubation, the cells were washed free of the label, frozen, and sent to a central laboratory, where the globin chains were separated and radioactivity ratios were calculated as previously described.\(^9\)

Restriction enzyme analysis of the \(\alpha\)-globin genes was done using the \(\alpha\)-globin cDNA plasmid, JW101,\(^9\) as the probe of cellular DNA digested with the enzymes EcoRI, EcoR I + BamH I, or Bgl II. The conditions of hybridization and autoradiography have been described previously.\(^9\,10\)

Definition of Clinical Events

The following definitions were used to categorize the vasoocclusive events of interest. At the time clinical events were recorded, the results of globin biosynthesis studies or gene mapping were not known to the involved investigators.

1. Painful episode. Pain in the extremities, back, abdomen, chest or head for which no other explanation could be found, with the following restrictions: (A) the pain should have lasted for at least 2 hr, and (B) if the patient was old enough, he/she should state that the pain is of the type usually associated with crisis.

2. Acute chest syndrome. A new pulmonary infiltrate that is demonstrable on a chest x-ray or by an isotope scan of the lung. An additional criterion was pleuritic chest pain, with or without dyspnea, in the absence of a pulmonary infiltrate on chest x-ray. When pleuritic pain was limited to the chest and the chest film was negative, a perfusion lung scan was done.

3. Chronic leg ulcer. Ulceration of the skin of the lower legs, especially on the medial and/or lateral surfaces with or without trauma, which fails to heal in a period of 2 wk.

4. Aseptic necrosis. Early radiographic findings of subepiphyseal lucency and widening of the joint space or late radiographic changes of flattening of the epiphysis and sclerosis with fragmentation.

Statistical Methods

Three different analyses involving three patient populations were performed here.

Analysis one. Analysis one utilized a group of patients, aged 2 yr and over, who had Hb "S only" upon electrophoresis. The objective of this analysis was to determine whether clinical severity, as measured by frequency of acute and chronic complications, was related to MCV levels. The specific complications considered were:

1. presence of painful crises;
2. presence of aseptic necrosis;
3. presence of leg ulcers;
4. presence of acute chest syndrome;
5. number of painful crises per person; and
6. number of acute chest syndromes per person.

For the analysis on presence of these complications, logistic regression was used, with presence or absence of the complication as the dependent variable; MCV (greater than or equal to 80 \(fL\) versus below 80 \(fL\)) was used as a dichotomous independent variable and age and follow-up time as covariates. For the analysis on mean number of these complications, the number of events was used as a dependent variable in a multiple linear regression model, with dichotomous MCV again used as independent variable and age and follow-up time again as covariates. The analysis was actually performed with and without the square root transform of number of events. This transform is often used to better approximate the assumptions of the statistical tests used.\(^9\) The results were always the same with and without the transform, and the findings presented are those based on the transform. Although MCV was used in the analysis as a dichotomous variable (above or below 80 \(fL\)) (see Table 6), these data are also presented for purposes of completeness, with MCV categorized into more detailed groupings (see Tables 1 and 5).

Analysis two. Analysis two was performed on a group of 183 patients who had globin biosynthesis studies. These patients were classified into one of three genotypes (SS, S-/beta-thalassemia, and SS-alpha-thalassemia) using HbA\(_2\), MCV, and alpha/nonalpha ratio and, where necessary, hemoglobin and hemoglobin F. This classification was originally done at the laboratory where the globin synthesis studies were performed. Table 2 shows the criteria for classification into these groups. The analyses performed on this group had two aims: (1) to find those hematologic variables that differentiate among the three genotypes, and (2) to determine differences in severity among these groups as well as between each of these groups and an age-sex-matched random sample of SS patients who had not had globin synthesis performed. The first of these analyses was performed using a one-way multivariate analysis of variance (MANOVA).\(^22,24\) A model was fit to the data using HbA\(_2\), MCV, HbF, and hemoglobin concentration as dependent variables and genotype (defined as above) as an independent class variable.

MANOVA was used in order to utilize the underlying correlation structure inherent in measuring several variables on the same individual. The standard assumptions of multivariate normality and equal variance–covariance matrices were made. In order to identify the specific variables that are different for each comparison, the multivariate analogue of Scheffe confidence intervals was calculated for each variable within each comparison. Age was considered as a possible confounding factor; therefore, the analysis was repeated using ages as a covariate. Table 3 presents the data used for these analyses.

The second of these analyses used ANOVA and chi-squared tests to determine severity differences among the three genotypes. A square root transformation was performed on the count variables for the acute events. Results of these analyses are presented in Table 7. Each of the three groups was then compared with a matched sample of SS patients with MCV >80 \(fL\) and HbA\(_2\) <3.5% for presence or absence of painful crises, acute chest syndrome, leg ulcers, and aseptic necrosis. These comparisons were done using the Mantel-Haenszel test statistic.\(^25\)

Analysis three. This was performed on a patient population consisting of 125 patients on whom \(\alpha\)-globin gene mapping was done. The gene mapping identified groups of patients with 2, 3, or 4 alpha genes. The hematologic data associated with these groups (HbA\(_2\), MCV, HbF, and hemoglobin level) were analyzed using MANOVA, as was done with the globin synthesis data. Severity was also analyzed with ANOVA (again using the square-root transformation on counts for acute events) and chi-squared tests for chronic events.
Table 7. Results of the analysis of severity are shown in Table 4. Patients under age 10 and age 10 and greater were also analyzed separately. Baseline hematologic data for these patients is shown in Table 1. Number of α-genes present was used as the group variable for the analysis. Patients under age 10 and age 10 and greater were also analyzed separately. Baseline hematologic data for these patients is shown in Table 7.

RESULTS

Hematologic and Electrophoretic Data

These data are shown in Table 1 and Tables 3 and 4. Two thousand, one hundred and forty-one patients, over age 2, with HbSFA2 upon electrophoresis, were stratified according to MCV, and the data collected are shown in Table 1. Table 1A presents data from the thalassemia groups. These data are shown in Table 1 and Tables 3 and 4. There is a significant relationship between MCV and HbS-α-thalassemia and HbSS-α-thalassemia in these groups. There is a significant relationship between HbA2 and MCV (r = 0.43; p < 0.0001), again reflecting the influence of β and α-thalassemia on both of these measurements. This observation was previously noted by Higgs et al. Globin biosynthesis studies were done on 183 patients who had either an MCV <80 fl or a HbA2 of >3.5%. Hematologic and electrophoretic measurements, grouped according to these presumptive genotypes, are shown in Table 3. As a reduced MCV was one of the selection criteria for globin biosynthesis study, the HbSS group in Table 3 has an MCV that is lower than expected. Patients with HbS-β-thalassemia have higher HbA2 levels and lower MCVs than the HbSS and HbSS-α-thalassemia patients (p < 0.01).

A comparison of data from the thalassemia groups of Table 3 with the patients having an MCV of <80 fl (Table 1) suggests that α-thalassemia patients reside mainly in the 79–70 fl MCV group, whereas HbS-β-thalassemia patients are in the 69–60 fl MCV group.

Restriction endonuclease analysis of the α-globin genes done to detect deletion α-thalassemia was done in 125 patients from 3 clinical centers. The data are

Table 2. Criteria for Genotype Assignment Using Globin Biosynthesis Ratios, MCV, and HbA2 Values

<table>
<thead>
<tr>
<th>MCV</th>
<th>HbSS</th>
<th>HbS-β-thalassemia</th>
<th>HbSS-α-thalassemia</th>
<th>A2</th>
<th>α/Non-α Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;80</td>
<td>&lt;80</td>
<td>&lt;80</td>
<td>&lt;80</td>
<td>&lt;3.5</td>
<td>&gt;3.5</td>
</tr>
<tr>
<td>&lt;80</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>&gt;3.5</td>
<td>&lt;3.5</td>
</tr>
</tbody>
</table>

Table 3. Hematologic Data of Patients Classified by Globin Biosynthesis Ratios*

<table>
<thead>
<tr>
<th>Number</th>
<th>Age</th>
<th>Sex</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Hb (g/dl)</th>
<th>PCV</th>
<th>HbA2 (%)</th>
<th>HbF (%)</th>
<th>Reticulocytes (%)</th>
<th>A2/Non-α Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>74</td>
<td>14.3 ± 10.7</td>
<td>38 (51.4)</td>
<td>36 (48.6)</td>
<td>8.4 ± 1.4</td>
<td>25.1 ± 4.3</td>
<td>82.0 ± 8.4</td>
<td>3.3 ± 0.6</td>
<td>11.8 ± 7.5</td>
<td>0.95 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>15.6 ± 10.5</td>
<td>22 (53.7)</td>
<td>19 (46.3)</td>
<td>9.3 ± 1.5</td>
<td>28.4 ± 4.7</td>
<td>69.4 ± 9.1</td>
<td>4.7 ± 0.9</td>
<td>9.5 ± 5.5</td>
<td>1.50 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>19.7 ± 12.4</td>
<td>41 (60.3)</td>
<td>27 (39.7)</td>
<td>8.7 ± 1.3</td>
<td>25.9 ± 4.1</td>
<td>79.3 ± 9.1</td>
<td>4.6 ± 0.7</td>
<td>9.7 ± 5.5</td>
<td>0.75 ± 0.09</td>
</tr>
</tbody>
</table>

*Globin biosynthesis was done only on patients with HbSFA2 who had either an MCV of <80 fl or a HbA2 of >3.5%.
summarized in Table 4. These results show graded increments in hemoglobin levels and HbA2 values and decrements in MCV with the presence of 4, 3, or 2 \(a\)-globin genes, but this is not statistically significant.

**Incidence of Vasoocclusive Episodes**

Analyses of the frequency of painful episodes, aseptic necrosis of bone, acute chest syndrome, and leg ulcers in patients stratified by MCV, globin synthesis studies, and \(a\)-globin gene mapping are shown in Tables 5, 6, 7, and 8.

When patients are stratified by MCV (Table 6), patients with MCV below 80 fl had a lower incidence of leg ulcers \((p = 0.02)\) but a higher incidence of aseptic necrosis \((p = 0.007)\). No significant differences were observed with respect either to incidence of acute chest syndrome or incidence of painful episodes. Likewise, there were no differences between patients with MCV <80 and those with MCV \(\geq 80\) fl with respect to mean number of painful episodes or mean number of acute chest syndromes.

When hemoglobin genotype was classified by the results of globin biosynthesis study, comparisons among the 3 groups showed no significant differences. Separate analysis of patients less than 10 yr of age and age 10 or over did not show differences among the groups. However, when each patient category was compared to the age- and sex-matched HbSS controls without microcytosis or elevated HbA2, controlling for length of follow-up, there was a higher incidence of acute chest syndrome \((p < 0.05)\) and prevalence of aseptic necrosis \((p < 0.025)\) in HbSS-\(\alpha\)-thalassemia than expected. In addition, patients with HbS-\(\beta\)thalassemia had more painful episodes than did controls \((p < 0.05)\). Separate analysis of patients less than 10 yr of age and age 10 or more did not show differences among the groups.

The prevalence and incidence of selected vasoocclusive complications in patients who had \(\alpha\)-globin gene mapping completed are shown in Table 8. Neither

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### Table 4. Hematologic Data of Patients Classified by \(\alpha\)-Globin Gene Mapping

<table>
<thead>
<tr>
<th>(\alpha)-Globin Genotype</th>
<th>(aa/aa) (Normal)</th>
<th>(-a/-a) (Heterozygous)</th>
<th>(-a/-\alpha) (Homozygous)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>73</td>
<td>39</td>
<td>13</td>
</tr>
<tr>
<td>Age</td>
<td>26.3 ± 8.8</td>
<td>22.9 ± 12.6</td>
<td>18.7 ± 10.2</td>
</tr>
<tr>
<td>Sex</td>
<td>Male (%)</td>
<td>31 (42.5)</td>
<td>19 (48.7)</td>
</tr>
<tr>
<td></td>
<td>Female (%)</td>
<td>42 (57.5)</td>
<td>20 (51.3)</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>8.4 ± 1.1</td>
<td>9.0 ± 1.2</td>
<td>9.5 ± 1.5</td>
</tr>
<tr>
<td>PCV (g/dl)</td>
<td>25.1 ± 3.9</td>
<td>27.0 ± 4.0</td>
<td>28.5 ± 4.1</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>93.9 ± 7.9</td>
<td>81.5 ± 9.3</td>
<td>72.3 ± 4.2</td>
</tr>
<tr>
<td>HbA2 (%)</td>
<td>2.8 ± 0.5</td>
<td>3.5 ± 0.9</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>HbF (%)</td>
<td>4.9 ± 3.5</td>
<td>4.4 ± 2.8</td>
<td>6.4 ± 5.3</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>10.8 ± 5.9</td>
<td>8.8 ± 5.8</td>
<td>6.9 ± 5.4</td>
</tr>
</tbody>
</table>

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### Table 5. Number of Patients With Selected Events Stratified by Mean Corpuscular Volume

<table>
<thead>
<tr>
<th>MCV (fl)</th>
<th>110–100</th>
<th>99–90</th>
<th>89–80</th>
<th>79–70</th>
<th>69–60</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Total patients</td>
<td>242</td>
<td>701</td>
<td>827</td>
<td>283</td>
<td>88</td>
</tr>
<tr>
<td>Patients with Painful episode</td>
<td>90 (37)</td>
<td>280 (40)</td>
<td>332 (40)</td>
<td>109 (39)</td>
<td>42 (48)</td>
</tr>
<tr>
<td>Mean number events ± 1 SD</td>
<td>1.8 ± 4.6</td>
<td>1.6 ± 4.2</td>
<td>1.5 ± 3.5</td>
<td>1.5 ± 4.4</td>
<td>2.1 ± 3.8</td>
</tr>
<tr>
<td>Aseptic necrosis</td>
<td>19 (8)</td>
<td>57 (8)</td>
<td>64 (8)</td>
<td>26 (9)</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Acute chest syndrome</td>
<td>22 (9)</td>
<td>105 (15)</td>
<td>145 (18)</td>
<td>51 (18)</td>
<td>15 (17)</td>
</tr>
<tr>
<td>Mean number events ± 1 SD</td>
<td>0.1 ± 0.4</td>
<td>0.2 ± 0.7</td>
<td>0.3 ± 0.6</td>
<td>0.3 ± 0.5</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>Leg ulcer</td>
<td>12 (5)</td>
<td>61 (9)</td>
<td>38 (5)</td>
<td>7 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>(B) Total patients (&gt;20 yr)</td>
<td>65</td>
<td>110</td>
<td>84</td>
<td>23</td>
<td>8</td>
</tr>
<tr>
<td>Patients with Painful episode</td>
<td>29 (45)</td>
<td>63 (57)</td>
<td>47 (56)</td>
<td>15 (65)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Mean number events ± 1 SD</td>
<td>3.0 ± 7.0</td>
<td>3.1 ± 8.0</td>
<td>2.7 ± 5.0</td>
<td>5.4 ± 12.7</td>
<td>1.1 ± 1.4</td>
</tr>
<tr>
<td>Aseptic necrosis</td>
<td>11 (7)</td>
<td>20 (22)</td>
<td>21 (25)</td>
<td>10 (43)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Acute chest syndrome</td>
<td>7 (11)</td>
<td>12 (11)</td>
<td>22 (26)</td>
<td>1 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mean number events ± 1 SD</td>
<td>0.2 ± 0.5</td>
<td>0.2 ± 0.7</td>
<td>0.4 ± 0.8</td>
<td>0.04 ± 0.2</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Leg ulcer</td>
<td>4 (6)</td>
<td>26 (24)</td>
<td>17 (20)</td>
<td>3 (13)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*Number (percent).*
Table 7. Number of Patients With Selected Events Stratified by Globin Biosynthesis Ratios

<table>
<thead>
<tr>
<th></th>
<th>HbSS</th>
<th>HbS-δ²-Th</th>
<th>HbSS-α-Th</th>
<th>Overall p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>74</td>
<td>41</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Patients with</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Painful episode*</td>
<td>36 (48.6)</td>
<td>24 (58.5)</td>
<td>34 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Aseptic necrosis</td>
<td>6 (8.1)</td>
<td>5 (12.2)</td>
<td>13 (19.1)</td>
<td>0.18</td>
</tr>
<tr>
<td>Acute chest syndrome</td>
<td>13 (17.6)</td>
<td>10 (24.4)</td>
<td>18 (26.5)</td>
<td></td>
</tr>
<tr>
<td>Leg ulcer</td>
<td>4 (5.4)</td>
<td>0 (0)</td>
<td>9 (13.2)</td>
<td>0.07</td>
</tr>
<tr>
<td>Mean number of events ± 1 SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Painful episode</td>
<td>3.6 ± 9.2</td>
<td>3.2 ± 4.1</td>
<td>3.4 ± 5.6</td>
<td>0.81</td>
</tr>
<tr>
<td>Acute chest syndrome</td>
<td>0.3 ± 0.71</td>
<td>0.4 ± 0.62</td>
<td>0.7 ± 1.3</td>
<td>0.15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Control Group 1 (for SS)</th>
<th>Control Group 2 (for S-δ²-Th)</th>
<th>Control Group 3 (for SS-α-Th)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>79</td>
<td>46</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Patients with</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Painful episode*</td>
<td>29 (36.7)</td>
<td>16 (34.8)†</td>
<td>28 (38.9)</td>
<td></td>
</tr>
<tr>
<td>Aseptic necrosis</td>
<td>3 (3.8)</td>
<td>1 (2.2)</td>
<td>5 (6.9)‡</td>
<td></td>
</tr>
<tr>
<td>Acute chest syndrome</td>
<td>15 (19.0)</td>
<td>10 (21.7)</td>
<td>10 (13.9)§</td>
<td></td>
</tr>
<tr>
<td>Leg ulcer</td>
<td>9 (11.4)</td>
<td>1 (2.2)</td>
<td>13 (18.1)</td>
<td></td>
</tr>
</tbody>
</table>

*Number (percent).
†Difference between S-δ²-Th and control significant (p < 0.05).
‡Difference between HbSS-α-Th and control significant (p < 0.025).
§Difference between HbSS-α-Th and control significant (p < 0.05).

heterozygous nor homozygous α-thalassemia-2 appeared to favorably affect the incidence or prevalence of those events examined, and the combined groups of patients with α-thalassemia had a higher prevalence of aseptic necrosis when compared to normal α-globin genotype patients (p < 0.025).

Three individuals were encountered who had five α-globin genes. Hematologic data from these patients are shown in Table 9. During the period of observation, none of these patients had any vasoocclusive events.

DISCUSSION

The role of microcytosis and α-thalassemia, which is accompanied by microcytosis, in modulating the clinical course of sickle cell disease has been shrouded in a mantle of controversy for many years. The controversy was due, in part, to lack of precision in the ascertainment of α-thalassemia, uncontrolled case reports, small numbers of patients studied, lack of systematic and structured collection of clinical data, and insufficient definition of the patient groups examined. There have been reports that promote14,15,26-28 or refute11,29-31 the contribution of microcytosis and α-thalassemia to the clinical severity of HbSS.

We took advantage of the large numbers of patients being studied by the CSSCD to question (1) whether microcytosis plays a role in the clinical vasoocclusive complications of patients with sickle cell disease who have the "S only" pattern upon hemoglobin electrophoresis, and if these same clinical complications are related to genotypic diagnosis based on (2) hematologic and globin biosynthesis data or (3) restriction endonuclease mapping of the α-globin genes. Because of a large sample size, standardized clinical and laboratory data collection, and three separate approaches to the analysis of the role of microcytosis and thalasse-
severity. The HbA2 level is higher in both a-thalassemia-2 homozygous or homozygous a-thalassemia-2 have higher incidence of leg ulcers and increased incidence of aseptic exclusive events was similar in all groups when patients with HbSS-a-thalassemia, when compared to their controls, had a greater prevalence of aseptic necrosis of bone and incidence of acute chest syndrome. Hawker and coworkers also noted a lower MCV in men with HbSS and aseptic necrosis when compared to patients without bone necrosis.34

Although globin biosynthesis studies may accurately define patients with HbS-β*thalassemia,35 and may have reasonable reliability for detecting HbSS-α-thalassemia-2 homozygotes, there is sufficient overlap between normals and α-thalassemia-2 heterozygotes to make the detection of this latter group unreliable.36 In addition, synthesis studies cannot reliably distinguish between α-thalassemia-2 homozygotes and heterozygotes.37 To obviate these difficulties, we performed restriction endonuclease analysis of α-globin genes in 125 patients. In some clinics, patients were randomly selected for study, and in others, patients were studied because of the suspicion that they had HbSS-α-thalassemia. This method of analysis permits the division of HbSS patients into three categories: (1) four α-globin genes, (2) three α-globin genes, and (3) two α-genes.37 Although gene mapping does not detect nondeletion types of α-thalassemia,39 such lesions appear to be relatively uncommon in blacks. It is likely that patients classified as HbSS-α-thalassemia on the basis of globin biosynthesis studies consist of both heterozygotes and homozygotes for α-thalassemia-2. Consistent with our other two methods of ascertainment, α-globin genotype by restriction enzyme gene mapping does not favorably affect the frequency of most vasoocclusive complications in HbSS and may increase the incidence of aseptic necrosis.

On the basis of our data, it is difficult to link the presence of α-thalassemia (or β*-thalassemia) with any decrease in the vasoocclusive severity of HbSS. In fact, our studies consistently show an increased prevalence of aseptic necrosis in α-thalassemia and patients with microcytosis.

These results differ from those recently reported by Higgs and associates in their examination of the role of α-thalassemia in Jamaicans with HbSS.15 This difference may have a number of explanations that are not mutually exclusive. The incidence of vasoocclusive events in our patients is less than in the Jamaican cohort. This may result from a varying definition of events, although it seems unlikely that it would account for the marked difference in the prevalence of

### Table 9. Hematologic Data in Three Patients With HbSS and Five α-Globin Genes

<table>
<thead>
<tr>
<th>Age</th>
<th>31.0 ± 13.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2</td>
</tr>
<tr>
<td>Female</td>
<td>1</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>9.3 ± 2.0</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>93.3 ± 13.1</td>
</tr>
<tr>
<td>A2 (%)</td>
<td>3.2 ± 0.21</td>
</tr>
<tr>
<td>HbF (%)</td>
<td>6.4 ± 0.7</td>
</tr>
</tbody>
</table>

microcytosis, we hoped to obtain more definitive data on the role of these entities in the determination of clinical severity.

Our results confirm most of the hematologic data reported by other investigators examining HbSS-α-thalassemia.12,13,32 Patients with HbSS and either heterozygous or homozygous α-thalassemia-2 have higher hemoglobin levels and smaller erythrocytes than HbSS controls. The HbA2 level is higher in both α-thalassemia-2 homozygotes and heterozygotes than in individuals with four α-globin genes, whereas HbF levels appear to be similar in all groups. Patients with HbS-β*-thalassemia have greater degrees of microcytosis and HbA2 elevation than do individuals with HbSS-α-thalassemia; however, there is overlap between these groups.

These hematologic differences have been noted before, and the major focus of this study was to ascertain the effects of thalassemia on the clinical severity of sickle cell disease. The number of vasoocclusive events was similar in all groups when patients were stratified by MCV, other than a reduced incidence of leg ulcers and increased incidence of aseptic necrosis in the microcytosis groups (Table 6). If only those patients over age 20 are examined, those with an MCV of less than 80 fl have a higher prevalence of aseptic necrosis than do patients with MCV of >80 fl. These differences cannot be explained by age. It should be noted that the groups with MCV <80 fl contain patients with HbS-β*-thalassemia and HbSS-α-thalassemia, accounting for their higher hemoglobin levels and HbA2 values. In addition, occasional patients with microcytosis may be iron deficient. It can be argued that combining these three diagnoses into a single category with microcytosis obscures any beneficial effect on clinical severity that might exist in a single diagnostic group. Yet it does indicate that microcytosis, as a sole variable, affords little protection against those vasoocclusive complications we examined.

To obtain a more precise definition in “S only” patients with microcytosis, reticulocyte globin biosynthesis ratios were measured on 183 patients with either an MCV <80 fl or a HbA2 >3.5%. The hematologic characteristics of patients with HbS-β*-thalassemia resembled those reported by Serjeant and coworkers.33 There was no indication that either α- or β-thalassemia ameliorated vasoocclusive complications; in fact, patients with HbSS-α-thalassemia, when compared to their controls, had a greater prevalence of aseptic necrosis of bone and incidence of acute chest syndrome.
leg ulcers, the determination of which does not depend on subjective interpretation. Our population includes patients from most regions of the country where HbSS is common, whereas the study of Higgs examined individuals from a small subtropical island. It is not known whether the age and sex distributions of our respective study groups are comparable. Our findings do confirm in part those of Powars et al., who found no relationship between erythrocyte indices and such vasoocclusive episodes as painful crises, aseptic necrosis, and acute chest syndrome in 214 "S-only" patients with an average age of 19.2 yr. Serjeant has noted that selected clinical events occur with equal frequency in high and low MCV groups of HbSS.

Microcytosis, α-, or β-thalassemia do not beneficially influence the observed vasoocclusive severity of HbSS. We have recently shown that α-thalassemia fails to reduce the prevalence of proliferative retinopathy and aseptic necrosis of the hip in HbSC disease, although it does cause microcytosis. A complicated relationship among MCV, MCHC, hemoglobin concentration, HbF levels, and, perhaps, the interaction of erythrocytes with their environment may determine the severity of HbSS. Changes in any single parameter, without corresponding changes in others, may not be beneficial to the patient. The reduction in MCHC and MCV in HbSS-α-thalassemia or HbS-β-thalassemia, accompanied by less hemolysis and a rise in packed cell volume, may lead to a sufficient increase in blood viscosity to impair flow in critical vascular beds. The crucial determinant of the pathophysiology of HbSS is not the reduction in red cell mass. The vasoocclusive episode, which leads to cumulative organ damage and failure, is the major cause of morbidity and mortality. Therapeutic strategies that act to alter selected erythrocyte characteristics, reduce hemolysis, and raise packed cell volume may not reduce and may even promote vasoocclusive complications unless this occurs in concurrence with a considerable reduction in the intracellular concentration of HbS.

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