Functional Asplenia in Hemoglobin SC Disease

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The incidence of functional asplenia in sickle-hemoglobin C (SC) disease has not been defined, and the use of prophylactic penicillin to prevent life-threatening septicemia in this disorder is controversial. The percentage of red blood cells with pits (pit count) is a reliable assay of splenic function in other disorders but has not been validated in hemoglobin SC disease. To address these issues, we conducted a prospective, multicenter study of splenic function in persons with hemoglobin SC disease. Baseline clinical data were recorded, and red blood cell pit counts were performed on 201 subjects, aged 6 months to 90 years, with hemoglobin SC; 43 subjects underwent radionuclide liver-spleen scanning. Pit counts greater than 20% were associated with functional asplenia as assessed by liver-spleen scan, whereas pit counts less than 20% were found in subjects with preserved splenic function. Pit counts greater than 20% were present in 0 of 59 subjects (0%) less than 4 years of age, in 19 of 86 subjects (22%) 4 to 12 years of age, and in 25 of 56 subjects (45%) greater than 12 years of age. Other subjects with hemoglobin SC, who had previously undergone surgical splenectomy, had higher pit counts (59.7% ± 9.5%) than splenectomized subjects without hemoglobinopathy (38.5% ± 8.8%) or with sickle cell anemia (20.5% ± 1.5%; P < .001). Two subjects with hemoglobin SC disease (not splenectomized), ages 14 and 15 years, with pit counts of 40.3% and 41.7% died from pneumococcal septicemia. These data indicate that functional asplenia occurs in many patients with hemoglobin SC disease, but its development is usually delayed until after 4 years of age. The pit count is a reliable measure of splenic function in hemoglobin SC disease, but values indicative of functional asplenia (>20% in our laboratory) are higher than in other disorders. The routine administration of prophylactic penicillin to infants and young children with hemoglobin SC disease may not be necessary.

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ity of this conclusion in hemoglobin SC disease has not been established because pit count results in patients with hemoglobin SC disease have never been correlated with any other measure of splenic function.

The overall objective of the present study was to determine the prevalence of functional asplenia in hemoglobin SC disease. Specifically, we sought to determine whether the RBC pit count can be used to accurately assess splenic function in hemoglobin SC disease and whether the development of functional asplenia correlates with other clinical features of the disease. Finally, because exposure to high altitude sometimes causes other splenic complications in sickle disorders, we sought to determine whether residence at a moderately high altitude adversely affects splenic function in hemoglobin SC disease.

MATERIALS AND METHODS

Patient population. All patients with hemoglobin SC disease who were older than 6 months of age and were seen at one of the four participating centers in Denver, CO; Dallas, TX; Kansas City, MO; and New York City, NY were eligible for inclusion in the study. Subjects who had received blood transfusions within the previous 4 months were excluded. Hemoglobin phenotype had been previously documented by hemoglobin electrophoresis on cellulose acetate and citrate agar or by isoelectric focusing. Baseline clinical data that were recorded included age, residence at altitude ≥1,600 m above sea level, (ie, Colorado subjects), past history of splenic sequestration, presence or absence of a palpable spleen, hemoglobin level, and platelet count.

RBC pit counts. Pit counts were performed as previously described. 29 One drop of fresh capillary or venous blood, anticoagulated in EDTA, was mixed by gentle inversion and fixed in 0.5 mL of isotonic 1% buffered glutaraldehyde. Samples were stored at 4°C and shipped in batches to Denver, where all pit counts were determined by one of us (J.L.O.), who was blinded to liver-spleen scan results and had little or no knowledge of clinical data. Nomarski differential interference contrast microscopy was used to count 1,000 cells per sample. The same individual had previously performed pit counts on 88 normal individuals and determined an upper limit of normal (mean ± 2 SD) of 3.6%. To establish the reliability of the pit count assay, pit counts were repeated 1 to 6 months later on the original samples by the same individual, blinded to the original results. Linear regression analysis for these repeated determinations on 20 subjects with pit count values of 0.3% to 47.0% yielded a slope of 1.03 ± 0.04 (r = 0.987). Pit counts also were found to be highly reproducible when repeated in Dallas on 13 samples originally counted in Denver (pit count values, 3.5% to 37.4%) by a second individual, who was blinded to liver-spleen scan results and had little or no knowledge of clinical data. Nomarski differential interference contrast microscopy was used to count 1,000 cells per sample. The same individual had previously performed pit counts on 88 normal individuals and determined an upper limit of normal (mean ± 2 SD) of 3.6%. To establish the reliability of the pit count assay, pit counts were repeated 1 to 6 months later on the original samples by the same individual, blinded to the original results. Linear regression analysis for these repeated determinations on 20 subjects with pit count values of 0.3% to 47.0% yielded a slope of 1.03 ± 0.04 (r = 0.987). Pit counts also were found to be highly reproducible when repeated in Dallas on 13 samples originally counted in Denver (pit count values, 3.5% to 37.4%) by a second individual, who was also blinded to the original results (linear regression: slope, 1.00 ± 0.04; r = .992).

Some subjects with hemoglobin SC disease had a pit count measured on more than one occasion. To determine the relationship of pit count to age and to other clinical features of the disease, the last pit count obtained on each of these subjects was used along with the baseline clinical data from that clinic visit. For comparison, RBC pit counts were also performed in patients with homozygous hemoglobin C disease and in subjects who had previously undergone surgical splenectomy for a variety of indications.

Radionuclide scans. Radionuclide liver-spleen scans were performed according to protocols approved by the institutional review boards at each institution. Informed consent was obtained from the subjects and/or the parents or guardians. Efforts were made to obtain the scans on as many subjects with hemoglobin SC as possible. Subjects who received scans were not selected by any clinical criteria other than the presence of hemoglobin SC disease. The scans were performed in a standard manner by the nuclear medicine departments at each institution after intravenous infusion of 5 μCi/kg technetium-99 metastable sulphur colloid. Scans were then forwarded to one of us (J.L.L.) who interpreted all of them in one sitting, blinded to clinical data and to pit count results. Splenic uptake was compared with hepatic uptake and classified as normal, slightly decreased, markedly decreased, or absent (Fig 1). Absent uptake of sulphur colloid by the spleen was taken to indicate functional asplenia.

Statistical analysis. The relationship between pit count and spleen scan results as well as the effect of hemoglobin phenotype on the pit count were analyzed using one-way analysis of variance. Two-sample t test was used to compare pit counts from subjects with and without palpable spleens and with and without a history of previous splenic sequestration. Linear regression analysis was used to examine the relationship between pit count results and baseline hemoglobin levels and platelet counts. We used analysis of covariance to determine whether residence at altitude ≥1,600 m above sea level influenced the relationship between age and pit counts.

RESULTS

Pit counts were performed in 201 subjects with hemoglobin SC disease who ranged in age from 6 months to 90 years (median age, 7.3 years). Of the 201 subjects with hemoglobin SC, 43 also had liver-spleen scans performed. Spleen scans were performed within 1 month of obtaining the pit count sample in 38 subjects (in 34, the scans and pit count samples were obtained on the same day). In 5 cases, the spleen scans were obtained 1 to 4 months after the pit count; a subsequent pit count was obtained more than 4 months after the spleen scan in 3 of these 5 and was not substantially changed from the first value. Figure 2 shows the relationship between pit count results and splenic reticuloendothelial function as judged by uptake of sulfur colloid. All 28 individuals with normal or slightly decreased splenic uptake had pit counts less than 20%, whereas all 11 subjects with absent splenic uptake (ie, functional asplenia) had pit counts greater than 20%. Markedly decreased splenic uptake was noted in 4 subjects with pit counts from 11.6% to 27.1%.

Pit counts were determined on more than one occasion in 103 subjects with hemoglobin SC disease, aged 6 months to 35 years. The results generally varied little over a 1- to 2-year period. The median rate of change in the pit count for subjects with multiple values was an increase of 0.9% per year. Seven subjects, aged 5 to 15 years, with pit counts less than 20% initially, showed pit counts greater than 20% 6 to 26 months later. In most cases, this progression to functional asplenia, as indicated by the pit count, was not associated with splenic symptoms or with a change in the physical examination of the spleen.

To explore the possibility that hemoglobin phenotype affects the pit count by mechanisms unrelated to splenic reticuloendothelial function, we also performed the test on subjects who had previously undergone surgical splenectomy. After splenectomy, subjects with hemoglobin SC disease had higher pit counts (59.7% ± 9.5%, n = 6) than spleenectomized subjects without hemoglobinopathy (38.5% ± 8.8%, n = 9) or with sickle cell anemia (20.5% ± 1.9%, n = 5; see Fig 3). Subjects with homozygous hemoglobin C disease
(not splenectomized) also had markedly elevated pit counts (mean, 41.8% ± 20.3%, n = 8).

Figure 4 shows the relationship of the RBC pit count to age for all 201 subjects (not splenectomized) with hemoglobin SC disease. Most subjects (79%) had pit counts greater than 3.6%, the upper limit for normal individuals. Functional asplenia, defined by a pit count greater than 20%, occurred in 0 of 59 subjects (0%) less than 4 years of age, in 19 of 86 subjects (22%) 4 to 12 years of age, and in 25 of 56 subjects (45%) greater than 12 years of age.

Because only subjects greater than 4 years of age had pit counts indicative of functional asplenia (>20%), we explored the relationship of the pit count to other clinical features of the disease in this older age group. Subjects greater than 4 years of age with palpable splenomegaly had lower pit counts than those without splenomegaly (9.9% ± 9.5%,
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Fig 4. The relationship of the RBC pit count to age for subjects with hemoglobin SC disease (n = 201).

n = 52, v 20.3% ± 17.1%, n = 86; P < .001). However, there was much overlap between the two groups, and splenomegaly was recorded in some subjects with pit counts greater than 20%. Pit count results from subjects greater than 4 years of age with previously documented episodes of splenic sequestration were not different from those without history of splenic sequestration (17.5% ± 14.3%, n = 9, v 16.8% ± 15.7%, n = 112; 95% confidence interval [CI] for difference in means, −10.0% to 11.4%). The RBC pit count in subjects greater than 4 years of age was directly related to the baseline platelet count (r = .33; P < .001; see Fig 5), but there was no relationship between the pit count results and baseline hemoglobin levels (r = .032; 95% CI, −0.14 to 0.20; data not shown).

The possible effect on splenic function of residence at ≥1,600 m above sea level was examined by comparing pit counts from subjects greater than 4 years of age (Colorado subjects, n = 24, versus subjects near sea level, n = 118). After adjusting for age, subjects from Colorado had pit counts 5.5% higher than subjects near sea level, but the difference was not significant (P = 0.16; 95% CI, −2.2% to 13.2%).

Two of the subjects, aged 14 and 15 years, with pit counts of 40.3% and 41.7% died from pneumococcal septicemia. Details of their fatal illnesses are reported elsewhere.†

DISCUSSION

Uptake of technetium-labeled sulphur colloid by the spleen has been widely used to assess splenic reticuloendothelial function and is considered the "gold standard" for documentation of functional asplenia in sickle cell disease.25 The data reported here showed a clear association between the liver-spleen scan and RBC pit count results in subjects with hemoglobin SC disease. Pit counts greater than 20% were indicative of functional asplenia, whereas pit counts less than 20% were associated with normal or near normal splenic function, as assessed by radionuclide scan. These results differed strikingly from an earlier report by Pearson et al of infants and children with sickle cell anemia which showed that a pit count ≥3.5% correlated with functional asplenia.3 We believe the different results are due, in large part, to inherent differences between SS and SC erythrocytes.

RBC membranes become pitted where large endocytic vacuoles, generated during the process of erythrocyte aging, are tethered to the RBC membrane.23 RBCs "groomed" in the spleen by macrophages that remove these endocytic vacuoles. Thus, the percentage of RBCs containing pits is increased by splenic dysfunction or asplenia. The pit count would also be affected by any factors intrinsic to RBCs that altered the rate of vacuole formation, the erythrocyte life span, or the RBC's susceptibility to grooming by the spleen. The possibility (which is confirmed by this report) that hemoglobin phenotype might affect the pit count was suggested by previous studies that found markedly elevated pit counts in patients with homozygous hemoglobin C, a disorder associated with normal splenic function.22,25 To further assess the influence of hemoglobin phenotype on the RBC pit count, we studied patients who had previously undergone surgical splenectomy. Splenectomized patients with hemoglobin SC disease had significantly higher pit counts than those without hemoglobinopathy, and splenectomized patients with sickle cell anemia had even lower values. The strikingly elevated results in persons with hemoglobin SC after splenectomy, as well as the increased pit counts in homozygous hemoglobin C disease, suggest that hemoglobin C containing RBCs may form large endocytic vacuoles more readily than normal RBCs. The relatively low pit counts in splenectomized subjects with sickle cell anemia probably result from the mark-

Fig 5. The relationship of the RBC pit count to platelet count in subjects with hemoglobin SC disease who are greater than 4 years of age (r = .33; P < .001).
edly shortened survival of their erythrocytes. The results clearly show that erythrocyte abnormalities, as well as altered splenic reticuloendothelial function, affect pit count results. Thus, values shown to indicate functional asplenia in one disorder (sickle cell anemia) cannot be extrapolated to indicate functional asplenia in another (hemoglobin SC disease).

We also considered the possibility that variations in pit count methodology might account, in part, for the marked difference between our findings in hemoglobin SC disease and those reported by Pearson et al in sickle cell anemia.3 Results of the pit count vary somewhat among laboratories because of differences in the glutaraldehyde buffer or in the optical systems used to perform the assay.2 Our pit count results for subjects without hemoglobinopathy who had previously undergone surgical splenectomy were the same or slightly lower than those reported by others5,28,29 but were higher than those reported by Pearson et al.2 Our results for children with hemoglobin SC were also higher than previously reported by Pearson’s laboratory31 but were similar to28 or only slightly higher than19 the results of others. In this study, when samples from subjects with hemoglobin SC were recounted in a second laboratory in Dallas,32 the results were unchanged. Thus, whereas our pit counts were reproducible in one other laboratory, we recognize that methodological differences between laboratories may alter clinically significant cutoff values. This is important because the use of pit count values greater than 20% to indicate functional asplenia in hemoglobin SC disease, validated for our laboratory by comparison with liver-spleen scans, might not be valid for pit counts performed in some other laboratories.

The most important finding of the present study was that the development of functional asplenia in hemoglobin SC disease is greatly delayed compared with that for sickle cell anemia.2 No child with hemoglobin SC under 4 years of age had functional asplenia, as judged by the pit count or by liver-spleen scan. However, functional asplenia did occur in many older subjects and was present in 45% of those greater than 12 years of age. These results were similar to a previous study that found 9 of 25 adults with hemoglobin SC (36%) had functional asplenia by liver-spleen scan.30 The potential clinical significance of functional asplenia in older children and adults was dramatically illustrated by the deaths of 2 adolescent patients from pneumococcal sepsis.

Our study also sought to identify clinical features of hemoglobin SC disease that might correlate with the development of functional asplenia. After the age of 4 years, patients with palpable spleens had lower pit counts than those without palpable spleens, an observation previously reported by others.31 However, the presence of splenomegaly did not reliably exclude the possibility of functional asplenia, probably because congestion of the red pulp of the spleen with sickled erythrocytes can result simultaneously in both splenomegaly and functional asplenia.2,10,32 The RBC pit count was directly related to the platelet count in subjects with hemoglobin SC disease. This finding is consistent with the concept that thrombocytosis in sickle cell disease is caused, in part, by splenic dysfunction.33

Exposure to mild environmental hypoxia has been associated with splenic complications in sickle disorders. Travel from sea level to altitudes ≥1,600 m may precipitate splenic sequestration in persons with hemoglobin SC disease.34 Rarely, even individuals with the sickle cell trait may experience splenic infarction at mountain altitudes.35 We found no significant differences in pit count results between Colorado subjects with hemoglobin SC and subjects who resided at or near sea level. These data suggest that residence at a moderately high altitude does not adversely affect splenic function in hemoglobin SC disease, an impression underscored by the 90-year-old subject, a Denver resident, whose pit count was 4.2% and whose spleen scan was normal.

We believe that the results of this study have important implications for the clinical management of patients with hemoglobin SC disease. Our data indicate that the vast majority of children with hemoglobin SC disease do not develop functional asplenia during the first 4 years of life, the period when pneumococcal bacteremia occurs most frequently, even in normal children.35,36 Thus, despite reports of bacteremia in young children with hemoglobin SC,5,16 the risk of life-threatening sepsis may be minimal. This impression is supported by the medical literature, which contains only three cases of fatal bacterial infection in children with hemoglobin SC less than 4 years of age, an 8-month-old, a 1-year-old who also had cyanotic congenital heart disease, and a 3½-year-old.18,19

Some concern about infection in young children with hemoglobin SC disease is still warranted. Partial impairment of splenic function was seen by liver-spleen scan in some of our subjects less than 4 years of age, and this might increase somewhat the risk of infection. Transient functional asplenia also may occur in hemoglobin SC disease,36,37 and we recognize that this phenomenon may not exist long enough to cause a significant elevation in the pit count.36 Thus, febrile illness in young children with hemoglobin SC disease should still raise suspicion of serious bacterial infection. However, the balance of available data suggest that the risk for life-threatening sepsis is extremely low; therefore, the routine use of prophylactic penicillin in this age group may not be necessary. Furthermore, we are concerned that prophylactic penicillin could be harmful if prolonged antibiotic administration during infancy and early childhood impaired the development of naturally acquired antibodies against Streptococcus pneumoniae.38-40 Such immunity would provide important protection later in life, especially if functional asplenia subsequently develops. Needless administration of prophylactic penicillin might also lead to the emergence of penicillin-resistant organisms.41

Our data, as well as published case reports of fatal sepsis,16-19 show that some older children and adults with hemoglobin SC disease are at increased risk of life-threatening infection. The current study does not permit estimation of the risk of fatal sepsis in older patients, but certainly all patients with hemoglobin SC disease should be immunized with pneumococcal and Hemophilus influenzae vaccines2,24 and possibly with meningococcal vaccine as well.42 In addition, we believe that such patients should be advised to seek prompt medical attention at the onset of all febrile illnesses. Health care providers confronted with such patients...
should suspect sepsis and should strongly consider the prompt administration of a parenteral antibiotic, such as ceftriaxone, that is effective against \textit{S pneumoniae} and other encapsulated organisms.\textsuperscript{1,2,3} The use of penicillin prophylaxis in older children and adults with functional asplenia, whether caused by hemoglobin SC disease or sickle cell anemia, remains controversial.

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