Pitfalls of Clinical Trials and Their Interpretation

Session Chair: Mark A. Crowther, MD, MSc, FRCPC
Speakers: Michael R. DeBaun, MD, MPH; Marcie R. Tomblyn, MD, MS; and Deborah J. Cook, MSc, MD FRCPC

Limitations of Clinical Trials in Sickle Cell Disease: A Case Study of the Multi-center Study of Hydroxyurea (MSH) Trial and the Stroke Prevention (STOP) Trial

Michael R. DeBaun[1] and Joshua J. Field[2]

[1] Department of Pediatrics, Division of Genetics, Washington University School of Medicine, St. Louis, MO.
[2] Department of Medicine, Division of Hematology, Washington University School of Medicine, St. Louis, MO.

In the past two decades, two landmark randomized controlled trials (RCT) have been completed among individuals with sickle cell disease (SCD), the Multi-center Study of Hydroxyurea (MSH) trial and the Stroke Prevention (STOP) trial. The MSH trial tested the hypothesis that hydroxyurea will reduce the frequency of painful episodes for adults with hemoglobin SS who had a history of 3 or more painful episodes per year. The STOP trial tested the hypothesis that among children with hemoglobin SS and an elevated transcranial Doppler (TCD) velocity measurement, blood transfusion therapy would decrease the risk of an initial stroke. After completion, both trials have defined standard care for individuals with hemoglobin SS. The purpose of this review is to examine the limitations of the MSH and STOP trials. In the context of these trials, we will examine the effects of narrow inclusion criteria that primarily include participants with hemoglobin SS and secondary analyses that are prone to false-positive results. In addition, we describe how after publication of these two trials use of hydroxyurea and TCD assessment has drifted towards a standard practice without evidence of therapeutic efficacy among groups that were excluded from the trials. Finally, we suggest that rigorously conducted RCTs or at the minimum multicenter observation studies with strong methodology should be performed in these excluded subgroups to confirm a benefit of hydroxyurea or TCD measurement.

Clinical Trials

In the hierarchy of study design, randomized controlled trials (RCTs) are considered the best method to rigorously test a hypothesis and subsequently change clinical practice.[1] As opposed to observational studies, the experimental design of RCTs most effectively minimizes the influence of confounding variables to ascertain a reliable treatment effect. However, even well-designed and executed RCTs have limitations. For this review, we will discuss three limitations of clinical trials (narrow spectrum of inclusion criteria, over-reliance on secondary analyses, and extrapolating trial results to other clinical settings) in the context of the Multi-center Study of Hydroxyurea (MSH) trial and the Stroke Prevention (STOP) trial[2,3] (**Table 1**). Our objectives are to provide insight into the limitations of RCTs so that sickle cell disease (SCD) practitioners may better apply the findings of RCTs to their patient population and to identify areas of investigation requiring additional research.

The first limitation of RCTs pertinent to practitioners caring for individuals with SCD is narrow inclusion criteria. Limiting a clinical trial to a homogeneous group of participants lessens potential confounders, but the end result may be a study group that is representative of only a minority of the targeted patient population.

Second, subgroup analyses or secondary analyses of an RCT are often considered to have the same weight and

Table 1. Multi-center Study of Hydroxyurea (MSH) trial and the Stroke Prevention Trial (STOP) trial.

Study design	Randomized, controlled trial	Randomized, controlled trial
No. patients	299	130
Inclusion criteria	HbSS Age ≥18 y ≥3 painful episodes in the year prior For transfused patients, HbA ≤15%	HbSS or HbS β^0 thalassemia Age 2-16 y TCD velocity ≥200 cm/second
Exclusion criteria	Pregnancy HIV Chronic transfusion therapy Stroke within last 6 y Bone marrow suppression Narcotic abuse or overuse Prior hydroxyurea therapy Use of another antisickling agent	Pregnancy HIV Chronic transfusion therapy History of stroke History of seizures Serum ferritin >500 ng/mL
Primary analysis	Reduction in painful episodes Intention to treat analysis	Redcution in stroke incidence Intention to treat analysis
Level of significance	.05	.05
Secondary outcome measures	Reduction in acute chest syndrome episodes, death, stroke, hepatic sequestration, blood transfusion requirements, and time to first and second painful episode	None
Level of significance	.01	None

influence as the primary analysis.[4] Clinical trials are designed to address a primary hypothesis with a primary analysis. Thereafter, a therapeutic benefit can be sought in any number of secondary analyses. Results from these analyses are inherently less reliable due to a risk of type I (false-positive) errors.[5] By convention, a significance level set at 5% indicates the chance of falsely concluding a statistically significant relationship exists. If multiple comparisons are made, the risk of incorrectly rejecting the null hypothesis increases precipitously. The relationship between the probability of a type I error and the number of comparisons is as follows—probability of type I error = $1 - (1-\alpha)^n$ (α = significance level, most commonly .05, n = number of comparisons).[6] Thus, if 3 separate secondary analyses are performed with an α of .05, the type I error rate increases to 14%. Given the complex nature of clinical trials designed with the intent of addressing a single primary hypothesis but collecting vast amounts of data, ample opportunities exist for secondary analyses and corresponding type I errors.

One strategy to lessen the probability of type I errors in secondary analyses is to use a more stringent alpha level (e.g., <.01). While a reduction in the alpha level may not be necessary for *a priori* secondary hypotheses, adjustments should strongly be considered for post-hoc analyses that involve multiple comparisons. However, these strategies are rarely used. Most commonly, secondary analyses are reported as a primary hypothesis in the same or separate manuscript without adjustment of the level of significance to decrease the probability of a type I error. Without adjusting the level of significance from .05 to a lower threshold, secondary analyses lead to false-positive results. At the very least, secondary analyses should be identified as such and a rationale discussion made as to the pros and cons for why no adjustment of the level of significance was done.

A third limitation of RCTs is not the results of the trial *per se*, but new practice patterns emerging after the completion of the trial. Often, clinicians extrapolate the data from an RCT to other clinical situations that were not originally included in the trial. For example, despite the potential differences in dosing, safety, and efficacy between drug trials in adults when compared with children,[7,8] pediatricians are often forced to rely on data from studies performed in adults to make treatment decisions in children. Based on these known limitations, the US Food and Drug Administration (FDA) developed a program that offered incentives to pharmaceutical companies to perform studies in children. From 1998 to 2004, 253 of these studies were conducted, yet only 50% of the pediatric trials resulted in a new indication for the pediatric setting,[9] highlighting the limitation of therapeutic trials in adults being the basis for treatment in children. Rather than accepting adult SCD trials as sufficient evidence for pediatric practice or relying on pediatric trials for management of adults with SCD, pediatric- and adult-specific trials in SCD should dictate practice patterns of treating hematologists.

Limiting Inclusion to Individuals with Sickle Cell Anemia or S β^0 Thalassemia in Clinical Trials

Most RCTs in SCD have limited the inclusion criteria by hemoglobin phenotype. Both the MSH and STOP trials limited the participants to individuals with hemoglobin SS (and S β^0-thalassemia in the STOP trial) and excluded individuals with hemoglobin SC. Several single-institution, single-arm studies have suggested a therapeutic efficacy of treatment with hydroxyurea among individuals with hemoglobin SC[10,11]; however, no RCT has been completed to assess the safety and efficacy of hydroxyurea among

individuals with this SCD phenotype. Given the marked difference in incidence of pain for persons with SC disease when compared with SS disease—0.4 episodes per year and 0.8 episodes per year, respectively—the potential efficacy and risk benefit ratio of hydroxyurea requires a formal evaluation among individuals with SCD.[12] Even with limited evidence, treatment with hydroxyurea for individuals with hemoglobin SC has received tacit support within the sickle cell community and is slowly emerging as standard clinical practice for individuals with hemoglobin SC and multiple painful episodes. Currently, a National Institute of Health (NIH)–National Heart, Lung and Blood Institute (NHLBI) phase 2 clinical trial is being conducted among individuals with hemoglobin SC disease that includes hydroxyurea and magnesium pidolate (W. Wang, personal communication).

Similar to the MSH trial, participants with hemoglobin SC were excluded from the STOP trial. The prevalence of overt strokes among children with hemoglobin SC is substantially lower than in children with SS, 0.01 versus approximately 0.04,[11,13] but still higher when compared with children without SCD.[14] No RCT has been performed, and only scant data exist describing the relationship between transcranial Doppler (TCD) measurement and the risk of strokes among children with hemoglobin SC. Despite this limitation, at least one report has used TCD measurement to assess velocity in the middle cerebral artery in this population.[15] Although this report does not advocate screening individuals with hemoglobin SC disease, no explicit guidelines state that TCD measurement should be limited to patients with hemoglobin SS or S β^0-thalassemia. Recommendations from the American Academy of Neurology advocate TCD screening in all children with SCD regardless of phenotype.[16] However, in the event that the TCD measurement is greater than 200 cm/second in a child with SC disease, no data exist to estimate the incidence of an initial stroke without blood transfusion or the benefit of blood transfusion therapy in preventing primary strokes. The relative merits of screening for elevated TCD measurements among children with hemoglobin SC is worthy of formal evaluations, else this practice will continue without evidence.

Over-Reliance on Secondary Analyses

Both the MSH and the STOP trials collected significant data that allowed for secondary analyses, analyses that were not based on the primary hypothesis. Due to the strength of the original trials, these secondary studies are often given the same weight of credibility as the primary analysis. However, when closely evaluated, these secondary analyses are rarely performed with the same rigor as the primary analysis. Specifically, intention-to-treat analyses are often not included, and the adjudication process for the secondary outcomes are commonly not as well defined. Further, these secondary analyses are often unplanned and adjustment in the threshold for significance, from .05 to a lower level, is commonly not done (**Table 2**).

Following completion of the MSH trial, participants were given the option to continue, discontinue, or initiate hydroxyurea therapy if they were in the observation arm. Observational data was then prospectively collected for up to 9 years. A total of 299 participants elected to receive hydroxyurea, and 233 individuals were able to be evaluated. Using an intention-to-treat analysis, the original placebo group was compared to the hydroxyurea group, and no difference in survival (P = .35) was noted between the two assigned groups. However, a 40% reduction in mortality was found when individuals were compared based on total duration of hydroxyurea therapy without attention to the original study grouping (P = .04).

Several limitations weaken the evidence that hydroxyurea increases overall survival in adults. First, the follow-up was an observational study, not a randomized trial. Study participants self-selected whether they wanted to continue, discontinue, or start hydroxyurea treatment. The process of self-selection may have resulted in nonsystematic bias. For example, individuals who elected to continue receiving hydroxyurea or start therapy could be less likely to have a comorbid condition such as renal failure, a risk factor for premature death[17] and a relative contra-indication for the use of hydroxyurea; or they may be more likely to have private insurance or come from a higher social economic group. Any one or all of these factors may contribute to an improvement in overall survival in those who elected to take hydroxyurea when compared with those who did not. Second, no direct evidence was available that individuals actually took the medication. Specifically, the measures that were used in the MSH study to assess adherence to hydroxyurea were not used in the observational study, and no other evidence was offered to support that participants were compliant with therapy throughout the observational study period.

Perhaps the strongest limitation of the post-hoc analysis of the MSH survival data was the exploratory nature of the analysis coupled with the marginal level of significance for the survival analysis (P = .04). The authors of the primary analysis were aware of the problem associated with multiple comparisons and planned for an adjustment of the level of significance for the secondary analysis. In the primary manuscript the following statement was included in the Statistical Analysis section, "To adjust for multiple tests of the data in secondary analyses, two-sided tests with P values between .01 and .001 were considered to provide some evidence of significance differences between the groups, and the tests with P values below .001 were considered to provide strong evidence of such differences."[2] Despite recognition of the risk of a type I error, the post-hoc analysis did not adhere to previously stated level of significance. If a significance level of .001 or even .01 was adhered to as designated in the primary manuscript, the results of the trial would have received far less attention. Further, in recognition of the marginal statistical signifi-

Table 2. Secondary analyses of the Multi-center Study of Hydroxyurea (MSH) trial and the Stroke Prevention (STOP) trial.

Secondary analysis	Title	Level of significance
MSH		
Ballas et al[36] (2006)	Hydroxyurea and sickle cell anemia: effect on quality of life	.01
Steinberg et al[37] (2003)	Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment	.05
Moore et al[38] (2000)	Cost-effectiveness of hydroxyurea in sickle cell anemia	.05
Steinberg et al[39] (1997)	Fetal hemoglobin in sickle cell anemia: determinants of response to hydroxyurea	.01
STOP		
Kwiatkowski et al[40] (2006)	Elevated blood flow velocity in the anterior cerebral artery and stroke risk in sickle cell disease: extended analysis from the STOP trial	.05
Lee et al[41] (2006)	STOP: extended follow-up and final results	Not stated
Lezcano et al[42] (2006)	Regular transfusion lowers plasma free hemoglobin in children with sickle cell disease at risk for stroke	.05
Wang et al[43] (2005)	Effect of long-term transfusion on growth in children with sickle cell anemia: results of the STOP trial	.05
Abboud et al[44] (2004)	Magnetic resonance angiography in children with sickle cell disease and abnormal transcranial doppler ultrasonography findings enrolled in the STOP study	.05
Adams et al[45] (2004)	Stroke and conversion to high risk in children screened with transcranial doppler ultrasound during the STOP study	Not stated
Jones et al[46] (2004)	Can peak systolic velocities be used for prediction of stroke in sickle cell anemia?	Not stated
Hsu et al[47] (2003)	Alpha thalassemia is associated with decreased risk of abnormal transcranial doppler ultrasonography in children with sickle cell anemia	.05
Files et al[48](2002)	Longitudinal changes in ferritin during chronic transfusion: a report from the STOP trial	Not stated
Miller et al[49] (2001)	Impact of chronic transfusion on incidence of pain and acute chest syndrome during the STOP trial in sickle cell anemia	.05
Wang et al[50] (2000)	Multicenter comparison of magnetic resonance imaging and transcranial doppler ultrasonography in the evaluation of the central nervous system in children with sickle cell disease	Not stated

cance of the findings, the data may have been used as preliminary evidence for a formal RCT to test the hypothesis that hydroxyurea improves overall survival. However, 4 years after the publication of the post-hoc analysis, consensus now exists that hydroxyurea therapy prolongs survival in adults with hemoglobin SS. A future trial to formally test if hydroxyurea prolongs survival in adults, and if so, whether the risks outweigh the benefits, is unlikely to be initiated due to the lack of equipoise in the sickle cell community. There are published guidelines that can help clinicians determine the reliability of secondary analyses[5] (**Table 3**). In general, secondary analyses are most useful for generating new hypotheses, or replicating results.

Another example of a secondary analysis of a landmark study occurred in the STOP trial, when findings were published demonstrating that children who received transfusion therapy had a significant decrease in the number of pain and acute chest syndrome (ACS) episodes. Miller et al used an intention-to-treat analysis to show a reduction in the frequency of ACS episodes ($P = .0027$); however, this analysis did not demonstrate significant reductions for pain ($P = .13$). Thereafter, the authors limited the analysis to

Table 3. An example of guidelines for interpreting secondary or subgroup analyses using the mortality analysis from the Multi-Center Study of Hydroxyurea (MSH) trial (adapted from Oxman, et al).[5]

1. Is the magnitude of the difference clinically important?	Yes, 40% reduction in mortality.
2. Was the difference statistically significant?	No, *P* values were not less than .01.
3. Did the hypothesis precede rather than follow the analysis?	No, mortality was not mentioned in the primary manuscript.
4. Was the subgroup analysis one of a small number of hypotheses tested?	No, several were tested (for example, episodes of acute chest syndrome, time to pain episodes, transfusion requirements).
5. Was the difference suggested by comparisons within rather than between studies?	Yes, comparisons were within the study.
6. Was the difference consistent across studies?	There are no other studies to compare.
7. Is there indirect evidence that supports the hypothesized difference?	Yes, it makes biologic sense that hydroxyurea could improve survival.

children in the treatment arm who had been adherent to transfusion therapy. When the compliant group was analyzed, the authors were able to demonstrate a decrease in the number of pain episodes versus the nontransfused group (P = .014). Despite the limitations of the secondary analysis, the results are consistent with a previous study[18] and confirm the prevailing belief that blood transfusion prevents subsequent pain and ACS episodes among individuals with hemoglobin SS disease. Still, the benefit of blood transfusion therapy to prevent pain and ACS episodes when measured against the risks has not been formally defined.

Extrapolating the Results of the Trial to Other Clinical Settings Resulting in a Clinical Drift to Change Practice Without Direct Evidence

In the MSH trial, there was significant evidence for a reduced rate of pain and ACS among adults. Subsequently, these results have been extrapolated as evidence to treat children with multiple painful episodes or repetitive ACS episodes. The most rigorous hydroxyurea study performed in pediatrics was a phase 2 study funded by NHLBI that enrolled 84 children between the ages of 5 and 15 years.[19] This study and others[20,21] have demonstrated that when hydroxyurea was administered to children, similar hematologic responses to those seen in adults occur, such as a rise in fetal hemoglobin and mean cell volume, along with a reduction in WBC and reticulocyte count. Further, no significant short-term toxicity was demonstrated. In a prospective infant cohort study (n = 21) with a mean follow-up of 4.9 years, Hankins et al reported a reduction in morbidity for children treated with hydroxyurea.[20] Episodes of ACS occurred 7.5 times per 100 patient-years in the group treated with hydroxyurea compared with 24.5 episodes per 100 patient-years observed in historic controls. Ferster et al conducted a randomized trial (n = 25) also evaluating the clinical benefit of hydroxyurea in children.[21] A reduction in the number of hospitalizations for painful episodes (P = .0016) and number of days in the hospital (P = .0027) was observed. No benefit was determined for decreasing the rate of ACS; however, given the small sample size and the limited follow-up period, the lack of demonstrated efficacy was expected. No other RCTs have evaluated the efficacy of hydroxyurea in children with SCD.

Among pediatric hematologists, the greatest clinical drift for establishing a new clinical practice without substantial evidence is the common use of hydroxyurea to prevent ACS episodes among children with multiple ACS episodes. Limited data are available to suggest that the same therapeutic benefit in reducing the incidence of ACS in adults participating in the MSH trial will be observed in children. On the contrary, strong evidence is available to question the potential efficacy of hydroxyurea to treat children with multiple ACS episodes. In children, the peak age for ACS is between 2 and 4 years.[22] This age group is marked by a high incidence of respiratory viruses with bronchiolitis.[23] Given the nonspecific definition of ACS (increased respiratory symptoms, new radiodensity on chest radiograph, and fever) there is significant overlap between bronchiolitis and ACS. Further, asthma occurs in approximately 17% of children with SCD. Asthma exacerbation also has significant overlap with ACS (low-grade fever, increased respiratory effort, wheezing and new radiodensity, most often atelectasis) and children with asthma have at least a two times greater rate of ACS when compared with children without asthma,[24] highlighting the importance of identifying asthma among children with SCD.

Based on the significant clinical overlap between viral-associated bronchiolitis or asthma exacerbation and ACS coupled with the higher rate of ACS among children when compared to adults, significant pause should be given before starting hydroxyurea for repetitive ACS episodes in children. At the very least, children with asthma and multiple ACS episodes should be evaluated by a pediatric asthma specialist for appropriate treatment of asthma according to the NHLBI guidelines[25] prior to initiating hydroxyurea.

Another example of clinical practice that has drifted from the original trial is the timing of TCD assessment. The STOP trial had well-defined criteria as to the clinical setting in which measurements could be obtained. These settings included, but were not limited to, children without evidence of an acute illness.[26] Despite the established criteria, clinicians have decided to perform TCD measurements when patients are hospitalized and acutely ill.[27] The relationship between pain or ACS episodes and TCD velocity has not been studied in a clinical trial setting. However, the increase in TCD velocity associated with a lower hemoglobin has been well documented.[28,29] The expected drop in hemoglobin among children with pain[30-32] or ACS episodes[33] would likely result in a higher-than-expected baseline measurement. Thus, the practice of obtaining TCD measurements while the patient is acutely ill may lead to a subgroup with falsely elevated velocities.

Rationale for Additional and Specific Clinical Trials for Hemoglobin SC Disease and Children with SCD

This review highlights the need for RCTs as a cornerstone to improve the care of individuals with SCD. The findings from RCTs are the highest form of evidence for a treatment intervention, and these findings rightfully direct clinical practice. Limited resources do not permit RCTs to address every major clinical problem in SCD. In the absence of a RCT, rigorously designed observational studies (cohort or case-control design) can provide reasonable evidence to guide clinical practice.[1] Nevertheless, additional RCTs are needed for more precise recommendations for all individuals with SCD regardless of phenotype or age group.

A major, but appropriate, limitation in the two landmark SCD clinical trials is the exclusion of individuals with hemoglobin SC. Consequently, results from these trials do not provide evidence that individuals with hemoglobin SC disease should be treated in the same manner as

their hemoglobin SS counterparts. Based on the estimate that 70,000 Americans have SCD,[34] at least 20,000 have hemoglobin SC disease, approximately 10 times more individuals than with β-thalassemia major,[35] yet no formal phase 3 trial has been completed among individuals with hemoglobin SC disease. Rather than making inferences from trials that are designed for participants with hemoglobin SS, resources should be allocated to answer major clinical questions for both individuals with hemoglobin SS and those with hemoglobin SC.

As well, drugs such as hydroxyurea with proven efficacy in adults with SCD should be investigated thoroughly in children. In the general population, the vast majority of drugs for children lack appropriate dosing, safety, and efficacy data. Given strong evidence indicating different toxicities and benefit for drugs in children compared with adults,[8,9] pediatric therapeutic trials in SCD are necessary after completion of adult studies to appropriately assess investigational agents. When there is limited market value in conducting such trials, the NIH becomes one of the few alternatives available to sponsor such RCTs. The lack of sufficient efficacy data for the use of hydroxyurea in children, coupled with the limited marketing value of hydroxyurea, underscores the importance of conducting trials that ultimately must be sponsored by the NIH, or else they are unlikely to ever be completed.

For the current and future generation of individuals with SCD, RCTs that build on existing knowledge and expand to more diverse subgroups of individuals with SCD are required to enhance our knowledge about the best strategies to provide care for this vulnerable population.

Acknowledgments

Supported in part by the National Institutes of Health, National Heart, Lung and Blood Institute, RO1 HL079937 (MRD), K12HL08710 (JJF).

Correspondence

Michael R. DeBaun, MD MPH, Washington University School of Medicine in St Louis, CB 8519, St Louis, MO 63108; phone (314) 286-1174; fax (314) 286-2609; debaun_m@kids.wustl.edu

References

1. Concato J, Shah N, Horwitz RI. Randomized, controlled trials, observational studies, and the hierarchy of research designs. N Engl J Med. 2000;342:1887-1892.
2. Charache S, Terrin ML, Moore RD, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. N Engl J Med. 1995;332:1317-1322.
3. Adams RJ, McKie VC, Hsu L, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. N Engl J Med. 1998;339:5-11.
4. Brookes ST, Whitley E, Peters TJ, Mulheran PA, Egger M, Davey Smith G. Subgroup analyses in randomised controlled trials: quantifying the risks of false-positives and false-negatives. Health Technol Assess. 2001;5:1-56.
5. Oxman AD, Guyatt GH. A consumer's guide to subgroup analyses. Ann Intern Med. 1992;116:78-84.
6. Curran-Everett D. Multiple comparisons: philosophies and illustrations. Am J Physiol Regul Integr Comp Physiol. 2000;279:R1-R8.
7. Berlin CM Jr. Advances in pediatric pharmacology, therapeutics, and toxicology. Adv Pediatr. 2001;48:439-464.
8. Roberts R, Rodriguez W, Murphy D, Crescenzi T. Pediatric drug labeling: improving the safety and efficacy of pediatric therapies. JAMA. 2003;290:905-911.
9. Benjamin DK Jr, Smith PB, Murphy MD, et al. Peer-reviewed publication of clinical trials completed for pediatric exclusivity. JAMA. 2006;296:1266-1273.
10. Steinberg MH, Nagel RL, Brugnara C. Cellular effects of hydroxyurea in Hb SC disease. Br J Haematol. 1997;98:838-844.
11. Miller MK, Zimmerman SA, Schultz WH, Ware RE. Hydroxyurea therapy for pediatric patients with hemoglobin SC disease. J Pediatr Hematol Oncol. 2001;23:306-308.
12. Platt OS, Thorington BD, Brambilla DJ, et al. Pain in sickle cell disease. Rates and risk factors. N Engl J Med. 1991;325:11-16.
13. Ohene-Frempong K, Weiner SJ, Sleeper LA, et al. Cerebrovascular accidents in sickle cell disease: rates and risk factors. Blood. 1998;91:288-294.
14. Earley CJ, Kittner SJ, Feeser BR, et al. Stroke in children and sickle-cell disease: Baltimore-Washington Cooperative Young Stroke Study. Neurology. 1998;51:169-176.
15. Verlhac S, Bernaudin F, Tortrat D, et al. Detection of cerebrovascular disease in patients with sickle cell disease using transcranial Doppler sonography: correlation with MRI, MRA and conventional angiography. Pediatr Radiol. 1995;25:S14-19.
16. Sloan MA, Alexandrov AV, Tegeler CH, et al. Assessment: transcranial Doppler ultrasonography: report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology. 2004;62:1468-1481.
17. Platt OS, Brambilla DJ, Rosse WF, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med. 1994;330:1639-1644.
18. Styles LA, Vichinsky E. Effects of a long-term transfusion regimen on sickle cell–related illnesses. J Pediatr. 1994;125:909-911.
19. Kinney TR, Helms RW, O'Branski EE, et al. Safety of hydroxyurea in children with sickle cell anemia: results of the HUG-KIDS study, a phase I/II trial. Pediatric Hydroxyurea Group. Blood. 1999;94:1550-1554.
20. Hankins JS, Ware RE, Rogers ZR, et al. Long-term hydroxyurea therapy for infants with sickle cell anemia: the HUSOFT extension study. Blood. 2005;106:2269-2275.
21. Ferster A, Vermylen C, Cornu G, et al. Hydroxyurea for treatment of severe sickle cell anemia: a pediatric clinical trial. Blood. 1996;88:1960-1964.
22. Vichinsky EP, Styles LA, Colangelo LH, et al. Acute chest syndrome in sickle cell disease: clinical presentation and course. Cooperative Study of Sickle Cell Disease. Blood. 1997;89:1787-1792.
23. Jhawar S. Severe bronchiolitis in children. Clin Rev Allergy Immunol. 2003;25:249-257.
24. Boyd JH, Macklin EA, Strunk RC, DeBaun MR. Asthma is associated with acute chest syndrome and pain in children with sickle cell anemia. Blood. 2006;108:2923-2927.
25. National Asthma Education and Prevention Program. Expert Panel Report 2: Guidelines for the Diagnosis and Management of Asthma. NIH Publication No. 97-4051. Bethesda, MD: National Heart Lung, and Blood Institute; 1997.
26. Adams RJ, McKie V, Nichols F, et al. The use of transcranial ultrasonography to predict stroke in sickle cell disease. N Engl J Med. 1992;326:605-610.
27. Ross CS, Hennessy JM, Nelson SC. Subarachnoid

hemorrhage in a young child with sickle cell disease: is transcranial Doppler helpful? Pediatrics. 2007;119:1249-1250; author reply 1250-1241.
28. Adams RJ, Nichols FT, Figueroa R, McKie V, Lott T. Transcranial Doppler correlation with cerebral angiography in sickle cell disease. Stroke. 1992;23:1073-1077.
29. Prohovnik I, Pavlakis SG, Piomelli S, et al. Cerebral hyperemia, stroke, and transfusion in sickle cell disease. Neurology. 1989;39:344-348.
30. Ballas SK, Marcolina MJ. Hyperhemolysis during the evolution of uncomplicated acute painful episodes in patients with sickle cell anemia. Transfusion. 2006;46:105-110.
31. Neely CL, Wajima T, Kraus AP, Diggs LW, Barreras L. Lactic acid dehydrogenase activity and plasma hemoglobin elevations in sickle cell disease. Am J Clin Pathol. 1969;52:167-169.
32. Naumann HN, Diggs LW, Barreras L, Williams BJ. Plasma hemoglobin and hemoglobin fractions in sickle cell crisis. Am J Clin Pathol. 1971;56:137-147.
33. Vichinsky EP, Neumayr LD, Earles AN, et al. Causes and outcomes of the acute chest syndrome in sickle cell disease. National Acute Chest Syndrome Study Group. N Engl J Med. 2000;342:1855-1865.
34. The Management of Sickle Cell Disease (4th ed). NIH Publication No. 02-2117. Bethesda, MD: National Heart Lung and BIood, Division of Blood Diseases and Resources; 2002.
35. Vichinsky EP, MacKlin EA, Waye JS, Lorey F, Olivieri NF. Changes in the epidemiology of thalassemia in North America: a new minority disease. Pediatrics. 2005;116:e818-e825.
36. Ballas SK, Barton FB, Waclawiw MA, et al. Hydroxyurea and sickle cell anemia: effect on quality of life. Health Qual Life Outcomes. 2006;4:59.
37. Steinberg MH, Barton F, Castro O, et al. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. JAMA. 2003;289:1645-1651.
38. Moore RD, Charache S, Terrin ML, Barton FB, Ballas SK. Cost-effectiveness of hydroxyurea in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. Am J Hematol. 2000;64:26-31.
39. Steinberg MH, Lu ZH, Barton FB, et al. Fetal hemoglobin in sickle cell anemia: determinants of response to hydroxyurea. Blood. 1997;89:1078-1088.
40. Kwiatkowski JL, Granger S, Brambill DJ, Brown RC, Miller ST, Adams RJ. Elevated blood flow velocity in the anterior cerebral artery and stroke risk in sickle cell disease: extended analysis from the STOP trial. Br J Haematol. 2006;134:333-339.
41. Lee MT, Piomelli S, Granger S, et al. Stroke Prevention Trial in Sickle Cell Anemia (STOP): extended follow-up and final results. Blood. 2006;108:847-852.
42. Lezcano NE, Odo N, Kutlar A, Brambilla D, Adams RJ. Regular transfusion lowers plasma free hemoglobin in children with sickle-cell disease at risk for stroke. Stroke. 2006;37:1424-1426.
43. Wang WC, Morales KH, Scher CD, et al. Effect of long-term transfusion on growth in children with sickle cell anemia: results of the STOP trial. J Pediatr. 2005;147:244-247.
44. Abboud MR, Cure J, Granger S, et al. Magnetic resonance angiography in children with sickle cell disease and abnormal transcranial Doppler ultrasonography findings enrolled in the STOP study. Blood. 2004;103:2822-2826.
45. Adams RJ, Brambilla DJ, Granger S, et al. Stroke and conversion to high risk in children screened with transcranial Doppler ultrasound during the STOP study. Blood. 2004;103:3689-3694.
46. Jones A, Granger S, Brambilla D, et al. Can peak systolic velocities be used for prediction of stroke in sickle cell anemia? Pediatr Radiol. 2005;35:66-72.
47. Hsu LL, Miller ST, Wright E, et al. Alpha thalassemia is associated with decreased risk of abnormal transcranial Doppler ultrasonography in children with sickle cell anemia. J Pediatr Hematol Oncol. 2003;25:622-628.
48. Files B, Brambilla D, Kutlar A, et al. Longitudinal changes in ferritin during chronic transfusion: a report from the Stroke Prevention Trial in Sickle Cell Anemia (STOP). J Pediatr Hematol Oncol. 2002;24:284-290.
49. Miller ST, Wright E, Abboud M, et al. Impact of chronic transfusion on incidence of pain and acute chest syndrome during the Stroke Prevention Trial (STOP) in sickle-cell anemia. J Pediatr. 2001;139:785-789.
50. Wang WC, Gallagher DM, Pegelow CH, et al. Multicenter comparison of magnetic resonance imaging and transcranial Doppler ultrasonography in the evaluation of the central nervous system in children with sickle cell disease. J Pediatr Hematol Oncol. 2000;22:335-339.