Published in final edited form as:
Cancer. 2011 August 1; 117(15): 3485–3492. doi:10.1002/cncr.25904.

Dapsone-Induced Methemoglobinemia: A Dose Related Occurrence?

Adam J Esbenshade, MD, MSCI,
Department of Pediatrics, Vanderbilt University School of Medicine and the Monroe Carell Jr. Children's Hospital at Vanderbilt, Nashville TN, USA

Richard H. Ho, MD, MSCI,
Department of Pediatrics, Vanderbilt University School of Medicine and the Monroe Carell Jr. Children's Hospital at Vanderbilt, Nashville TN, USA

Ayumi Shintani, PhD, MPH,
Department of Biostatistics at Vanderbilt University, Nashville TN, USA

Zhiguo Zhao, MS,
Department of Biostatistics at Vanderbilt University, Nashville TN, USA

Lesley-Ann Smith, BS, and
Department of Pediatrics, Vanderbilt University School of Medicine, the Monroe Carell Jr. Children's Hospital at Vanderbilt

Debra L. Friedman, MD, MS
Department of Pediatrics, Vanderbilt University School of Medicine, the Monroe Carell Jr. Children's Hospital at Vanderbilt and Vanderbilt-Ingram Cancer Center, Nashville TN, USA

Abstract

Objectives—Dapsone, used for Pneumocystis jiroveci (PCP) prophylaxis, is associated with increased risk of methemoglobinemia. Absence of cytochrome b5 reductase enzyme activity (CYB5RA) causes congenital methemoglobinemia, but its role in dapsone-associated methemoglobinemia is unknown. We sought to elucidate drug-related risk factors for dapsone-associated methemoglobinemia in pediatric oncology patients, including contribution of CYB5RA.

Patients and Methods—Among 167 pediatric patients treated for hematologic malignancies or aplastic anemia who received dapsone for PCP prophylaxis, demographic and dapsone treatment data were retrospectively collected. Drug-related risk factors were evaluated by Cox proportional hazards, and in a cross-sectional subgroup of 40 patients, CYB5RA was assessed.

Results—Methemoglobinemia (median methemoglobin level = 9.0% [3.5–22.4]) was documented in 32 patients (19.8%). There was a 73% risk reduction in methemoglobinemia with dosing ≥20% below the target dose 2mg/kg/day (HR = 0.27; 95% confidence interval (CI) 0.09, 0.78; p=0.016), while methemoglobinemia risk was increased with dosing ≥20% above the target dose (HR = 6.25; 95% CI 2.45, 15.93; p<0.001). Sex, body mass index, and age were not associated with increased risk. CYB5RA did not differ by methemoglobinemia status (median 8.6 IU/g Hb; [5.5 – 12.1] vs. 9.1 IU/g Hb; [6.7 – 12.7]). No patient developed PCP on dapsone.

Corresponding Author: Adam Esbenshade, MD, MSCI, 2200 Pierce Avenue, 397 PRB, Vanderbilt University Medical Center, Nashville, TN 37232, Phone: 615-936-1762, Fax: 615-936-1767, adam.esbenshade@vanderbilt.edu.

Financial disclosures/conflicts of interest: None.

Conclusions—Methemoglobinemia occurred in almost 20% of pediatric oncology patients receiving dapsone for PCP prophylaxis. Higher dapsone dosing is associated with increased risk. A cross-sectionally acquired CYB5RA level was not associated with methemoglobinemia risk. Studies are needed to define biologic correlates of methemoglobinemia and evaluate lower dapsone doses for PCP prophylaxis.

Keywords

pediatric oncology; HIV; dapsone; methemoglobinemia; Drug-related complications; cytochrome b5 reductase

Introduction

Dapsone is a synthetic sulfone antimicrobial used as prophylaxis for *Pneumocystsis jiroveci* (PCP) in both cancer and Human Immunodeficiency Virus (HIV) patients 1. It is considered the best alternative treatment for PCP prophylaxis in those who cannot tolerate trimethoprim-sulfamethoxazole (TMP-SMX) 2. However, its use is limited by adverse effects, such as methemoglobinemia, which occurs when hemoglobin iron becomes trapped in the ferric (Fe^{3+}) state. Decreased oxygen carrying capacity leads to severe hypoxia and cyanosis if not treated 3. The prevalence and associated risk factors for dapsone-associated methemoglobinemia in pediatric patients has not been well studied 1, 4–7. Cytochrome b5 reductase is an enzyme that reduces the toxic metabolite of dapsone (dapsone hydroxylamine) in the liver 8. Complete absence of cytochrome b5 reductase enzyme activity (CYB5RA) is associated with congenital methemoglobinemia, and its role in dapsone-associated methemoglobinemia has been postulated in a small case series 4. Using a large retrospective cohort, our study was designed to assess the prevalence of dapsone-assocatied methemoglobinemia as well as drug and host-related risk factors associated with increased risk of methemoglobinemia such as sex, age, body mass index (BMI), and dapsone dosing. In a cross-sectional subset, we sought to evaluate if a random cytochrome b5 reductase level is associated with the development of methemoglobinemia. A secondary aim of the study was to evaluate the efficacy of dapsone in PCP prophylaxis in the cohort.

Patients and Methods

Study Design and Participant Accrual

Following Human Subjects committee approval from the Vanderbilt University Institutional Review Board, we assembled a retrospective cohort from all pediatric patients 22 years or younger at the time of diagnosis with a hematologic malignancy or aplastic anemia between 1994–2009 and treated by the Division of Pediatric Hematology/Oncology at Vanderbilt University who received dapsone for PCP prophylaxis.

Eligible participants for the retrospective component were identified using a search of the electronic medical record, resulting in a cohort of 167 patients. In addition, we included a cross-sectional subset in which we measured CYB5RA in a group of 20 patients with confirmed methemoglobinemia and 20 patients who never developed methemoglobinemia in order examine the association of a random CYB5RA level with methemoglobinemia development. After IRB approval, 40 patients were consecutively identified patients meeting inclusion criteria that had upcoming clinic visits, who had not received dapsone for a minimum of one month prior to assessment of CYB5RA. Prospective patients eligible for the cross-sectional sub-cohort evaluating CYB5RA were sent a letter and then approached in clinic. Of the 43 patients who were approached, 41 consented. One patient was initially enrolled as a control but was then found to have had recent suspected methemoglobinemia,

and was excluded. G6PD status was also obtained in all patients in the cross-sectional component by qualitative fluorescent spot test.

Definitions and Measurements

Confirmed symptomatic methemoglobinemia was defined as otherwise unexplained cyanosis or hypoxia (02 saturation under ≤95%) together with an elevated methemoglobin level ≥ 3% based on co-oximetry measurement on venous blood. Suspected symptomatic methemoglobinemia included the above symptoms without laboratory-confirmed methemoglobin level. The target dose for dapsone followed the standard pediatric recommendation of 2mg/kg/day with a maximum of 100mg a day 1. Cytochrome B5 reductase levels were measured at the Mayo Medical Laboratories, Rochester, MN with a commercially available assay that uses a kinetic spectrometric method, measuring the oxidation of NADH at 340 nm at 30°C as cytochrome b5 reductase catalyzes the NADH-linked reduction of ferricyanide as previous used by Williams et al 4.The reference range for cytochrome b5 reductase is 8.2–19.2 IU/g Hb. Adult values of this enzyme are obtained by 2–3 months of age (www.mayomedicallaboratories.com).

Data collection

From the medical record, demographics were collected for each participant, as well as baseline height and weight. In addition, cancer diagnosis, dapsone dose, duration of treatment, adverse side effects, including methemoglobinemia occurrence and presentation, measured methemoglobin level and treatment were collected. BMI values were converted to age and sex adjusted z-scores using the Centers for Disease Control and Prevention Year 2000 growth charts for patients 2 – 20 years (Epi Info 3.5). Length for weight z-scores were substituted for patients who had measured values prior to turning 24 months old. In patients over 20 years old, z-score data for age 20 was used. A patient's dapsone dose was considered to be in control at a target range if it fell within 20% of that recommended (2mg/kg/day maximum of 100mg a day).

Statistical considerations

For the retrospective portion of the study, the primary endpoint was time to the development of symptomatic methemoglobinemia. Baseline characteristics were summarized by median with 25^{th} and 75^{th} percentiles for continuous variables. For categorical variables, frequencies and percentages were shown. Mann-Whitney U test was used for continuous variables and Chi-square or Fisher's exact test were used to compare categorical variables between patients with or without methemoglobinemia. Effects of potential risk factors included age, sex, BMI z-score, and dapsone dose were assessed using a Cox proportional hazards regression model. To assess effects of covariates that changed status over the course of follow-up, change in age, BMI z-score and dapsone dosage variables were treated as time-varying covariates. The dapsone dose was adjusted any time a patient changed category (e.g., dosed 20% below control versus dosed at target versus 20% above control). Age and BMI z-score were updated for every 180-day period during follow-up. Only laboratory-confirmed methemoglobinemia cases were included in the final analysis. The reliability of the final regression model was internally validated via bootstrap method $9^{-}10$ by measuring degree of over-fitting quantified by optimism parameter in a calibration plots. One hundred fifty resamples were performed with bootstrap with replacement.

For the cross-sectional component of the study, the primary outcome was CYB5RA level for patients with and without methemoglobinemia. Sample size was determined based on a conservative estimate of difference in mean CYB5RA of 3.0 with a pooled standard deviation of 2.82. Twenty patients were required in each group to achieve 90% power. However, since the data by Williams demonstrated that mean difference in CYB5RA was

3.9 based on a small sample size (10 patients), we estimated the difference conservatively 4. Sample size computation was performed using PS software 11.

For patients in the cross-sectional component, a linear regression was conducted to assess whether age and BMI z-score, at the time the CYB5RA levels were obtained, were associated with measured CYB5RA. Residuals were evaluated for normality. In patients with methemoglobinemia, Spearman correlations were performed to assess correlation of CYB5RA with measured methemoglobin level or the duration of dapsone therapy prior to developing methemoglobinemia. All statistical inferences were assessed at a two-sided 5% significant level.

Results

Methemoglobinemia and Dapsone Administration

All of the patients in our cohort were initially placed on dapsone prophylaxis due to adverse effects from TMP-SMX, of which prolonged neutropenia and rash/allergy were most common. Once treated with dapsone, concern for methemoglobinemia was triggered by cyanosis or hypoxia.

Confirmed methemoglobinemia was present in 32 of 167 individuals (19.8%), and an additional 4 patients had suspected disease. The median measured methemoglobin level was 8.95% [25th, 75th percentiles 5.48%, 13.08%; range 3.5% to 22.4%]. None of these patients had previously developed methemoglobinemia while on TMP-SMX. Of these 32 patients, 34% of the patients had presented with cyanosis and 78% had documented hypoxia with O2 saturation levels under 95%. Of those with hypoxia, 44% had O2 saturation levels < 90%. Presentation with suspected methemoglobinemia most commonly occurred in the outpatient clinic (59.4%) but was also detected in the inpatient patient care unit (34.4%) and far less commonly in the emergency department (6.2%).

Table I describes characteristics of patients treated with dapsone for PCP prophylaxis. Patients with and without methemoglobinemia were similar with regard to age, sex, cancer diagnosis, treatment with hematopoietic cell transplant (HCT), or having a diagnosis of Down syndrome. There was a slight, but not statistically significant, increase in the number of Hispanic patients in the methemoglobinemia group 4/32 (12.5%) vs. 7/131 (5.3%) of controls. Patients without a history of methemoglobinemia had slightly higher median baseline BMI z-scores (0.79 vs 0.58), although this difference was not statistically significant, and were on dapsone for longer periods of time than those who developed methemoglobinemia (median 427 days vs 67 days) ($p<0.001$).

Dosing of dapsone differed by methemoglobinemia status. A greater proportion of patients initially treated with dapsone ≥20% below the target dose of 2mg/kg/day (46.6% vs 12.5%; $p = 0.001$) did not develop methemoglobinemia. Similarly, a higher proportion of patients initially treated with dapsone ≥20% in excess of the target dose 2 mg/kg/day (12.5% vs. 3.1%; $p = 0.043$) developed methemoglobinemia. This relationship between dose and methemoglobinemia occurrence remained constant over time.

Management of Methemoglobinemia

In all cases of methemoglobinemia, dapsone was discontinued when methemoglobinemia was recognized, and only one patient with confirmed methemoglobinemia required treatment with methylene blue. Chest radiographs were performed in 11 patients and no significant abnormalities were noted. Six of the patient were put on supplemental oxygen until the etiology for hypoxia was confirmed. No patients had prolonged hypoxia or cyanosis following discontinuation of the dapsone. In addition to methemoglobinemia, a

number of patients in the cohort had discontinuation of dapsone for other reasons, including rash (1.8%), neutropenia (4.8%), hepatic toxicity (3.0%), oral intolerance (1.2%), and one person each who had nausea (0.6%), hemolytic anemia (0.6%), thrombocytopenia (0.6%), and unsubstantiated concern for methemoglobinemia (0.6%).

Among those who required dapsone discontinuation for methemoglobinemia, they were treated with other alternative therapies for PCP prophylaxis, including TMP-SMX (12.5%), inhaled pentamidine (46.9%), atovaquone (37.5%), and intravenous pentamidine (0.6%) for one patient.

Occurrence of PCP

No patients in our cohort developed breakthrough PCP while on dapsone prophylaxis with 213.4 patient years of follow-up. Two patients had previously developed PCP pneumonia while on TMP-SMX. Among patients removed from dapsone prophylaxis for methemoglobinemia, two patients subsequently developed PCP pneumonia while on treatment with inhaled pentamidine.

Multivariable analysis

In the multivariable Cox model, dapsone dose was associated with risk of methemoglobinemia (Table II). Compared to the target dose of 2mg/kg/day, doses ≥20% lower than target dose resulted in a protective effect, with 73% reduction in the hazard of methemoglobinemia (Hazard Ratio (HR) = 0.27; 95% confidence interval (CI) 0.09, 0.78; p=0.016). Conversely, doses ≥20% over the target dose significantly increased risk for methemoglobinemia (HR = 6.25; 95% CI 2.45, 15.93; p<0.001). Age, sex, and BMI z-score were not significant risk factors for methemoglobinemia. The optimism for the final model was estimated as 0.146, meaning the degree of over-fitting was 14.6%, providing no evidence of over-fitting.

Cross sectional component

The potential association of CYB5RA and dapsone-associated methemoglobinemia was assessed in a sub-cohort of 20 patients with confirmed methemoglobinemia and 20 patients without methemoglobinemia. These patient groups did not differ with regard to age, sex, diagnosis, treatment with HCT, and initial BMI z-score (Table III). As was true for the entire study cohort described above, patients who did not develop methemoglobinemia received dapsone for longer periods of time than did those with methemoglobinemia (median 798 versus 70 days). The association between dapsone dose and prevalence of methemoglobinemia that was observed in the entire cohort was also evident in the sub-cohort.

The 20 patients in the methemoglobinemia group had a median measured methemoglobin level of 9.85% 25th, 75th quartile (5.13, 11.68). The G6PD screen revealed one patient in the methemoglobinemia group and none in the control group to be G6PD deficient. The patient with G6PD deficiency had the highest measured methemoglobin level in the cohort at 22.4. Cytochrome b5 reductase levels were similar between groups with the median level of 8.6 IU/g Hb (range 5.5 – 12.1) in patients with methemoglobinemia vs 9.1 IU/g Hb (range 6.7 – 12.7) in patients without methemoglobinemia. Six patients in the methemoglobinemia group (5.5;6.1;6.1;7.1;7.2;8.1) and 5 patients in the control group (6.7; 7.1; 7.5;7.6; 8.1) had CYB5RA levels below the reference range. No patients were receiving dapsone at the time the levels were drawn. Cytochrome b5 reductase levels did not correlate with measured methemoglobin levels (Spearman $\rho = -0.51$, p=0.83) or time treated with dapsone prior to methemoglobinemia (Spearman $\rho = 0.191$, p=0.23). When included in the Cox proportional hazards model, CYB5RA was not found to be a risk factor for methemoglobinemia and a

linear regression model confirmed that age and BMI z-score were not associated with the CYB5RA level.

Discussion

Dapsone is an effective second line agent for PCP prophylaxis for those who cannot tolerate TMP-SMX 1. This study is confirmatory of this efficacy as there was no PCP breakthrough in over 234 patient-years of follow-up. However, despite widespread use, few studies have examined the prevalence of and risk factors for dapsone-associated methemoglobinemia, and, to our knowledge, this represents the largest cohort of pediatric oncology patients in which this has been examined. Most other reports of dapsone-associated methemoglobinemia have not analyzed potential risk factors 5, 12–15

Our prevalence of confirmed methemoglobinemia cases approaches 20%, which is higher than what has been reported in the adult literature 3, but consistent with a rate of 20% previously observed in a much smaller cohort (N = 15) of pediatric oncology patients 4.

Age, sex, and BMI z-score were not risk factors for dapsone-induced methemoglobinemia in our cohort, consistent with that of Ash-Bernal et al. 3.

In our cohort, we identified dapsone dosing to be a strong risk factor for methemoglobinemia, with a dose-response evident, a finding that, to our knowledge, has not been reported previously in children, although it was suggested in some early adult pharmacokinetic studies 16. In addition, there were no cases of PCP in our cohort who received dapsone, even amongst those who received lower doses. These data raise the question of whether dapsone could be effectively used in PCP prophylaxis in pediatrics at a lower dose, where methemoglobinemia risk was much lower. Pharmacokinetic data from HIV patients suggest that this is indeed possible, with efficacy and adequate lung penetration evident when given once per week17–18. Several subsequent studies in pediatric HIV have suggested that 2–3 times weekly dosing of 2mg/kg results in acceptable drug levels and lung penetration19–21. One study comparing dapsone dosing (1mg/kg/day vs. 4 mg/kg/week vs. 2mg/kg/day) in 94 pediatric HIV positive children demonstrated no statistically significant difference in PCP breakthrough 22. There have been no studies evaluating alternative schedules of dapsone dosing in pediatric oncology patients, where PCP occurs less commonly than in HIV patients.

The pathogenesis of dapsone-associated methemoglobinemia is not well understood. Cytochrome b5 reductase is an enzyme that is important in restoring methemoglobin back to its ferrous state 23.When the enzyme is congenitally absent, it causes cyanosis from birth. There is a Type-1 variant of this disorder in which the cytochrome b5 reductase is only decreased in the red blood cells and leads to occasional cyanosis23. It has therefore been hypothesized that low levels of this enzyme may predispose individuals to dapsone-associated methemoglobinemia. Williams and colleagues found a non-statistically significant difference in cytochrome b5 reductase levels between 3 patients with methemoglobinemia (CYB5RA = 8.6 IU/g Hb) compared with 7 patients without methemoglobinemia (CYB5RA = 12.5 IU/g Hb) 4. Our study, which was designed with appropriate statistical power to detect such a difference, failed to confirm this finding. Our study demonstrated slightly decreased cytochrome b5 reductase levels in patients with and without methemoglobinemia and only one patient had a level that was 40% below the reference range indicating a true heterozygous state. Thus we cannot conclusively state if a more robust deficiency in CYB5RA is a risk factor for methemoglobinemia. However, as only one patient out of 20 with methemoglobinemia is our cohort had a 40% reduction in CYB5RA, and mildly decreased levels were seen amongst patients without

methemoglobinemia, CYB5RA level is unlikely to be a useful screening test to predict most patients at risk for dapsone-induced methemoglobinemia. Due to the known association of methemoglobinemia resulting from hemolysis in individuals with G6PD deficiency 3, 24, patients should be screened for this prior to the onset of dapsone therapy, as is evidenced by our patient with G6PD deficiency who had the highest methemoglobin level in the cohort.

There are multiple medications that are associated with methemoglobinemia, mostly notably benzocaine, chloroquine, eutectic mixture of local anesthetics (EMLA) cream, lidocaine, metoclopromide, nitrates, and phenazopyridine3. In our cohort, 38 patients received metoclopromide; of these, one patient had confirmed methemolglobinemia, and another patient had suspected methemoglobinemia. An additional patient with methemoglobinemia received both dapsone and metoclopromide concurrently, but not at the time of methemoglobinemia diagnosis. Notably no patient with methemoglobinemia was on drugs that conferred increased risk when methemoglobinemia was diagnosed and there were no differences in the exposure to methemoglobin-inducing medications in those with and without methemoglobinemia. Sepsis and gastrointestinal infection have also been associated with methemoglobinemia3, however these conditions were not present in any of our cohort at the time of the methemoglobinemia event.

Another risk factor for methemoglobinemia is G6PD deficiency 3. Patients with G6PD deficiency are at increased risk of oxidative stress due to depletion of reduced glutathione. Exogenous oxidizing agents can then overwhelm the cytochrome b5 reductase system, leading to increased production of methemoglobin 24. No patient in our cohort had a known family history of G6PD and only one patient in the cross-sectional component of the study was found to be deficient.

Better defining those at risk for methemoglobinemia has clear implications for the choice of second-line alternatives to TMP-SMX for PCP prophylaxis. If a dose could be identified that is associated with a much lower risk of methemoglobinemia, dapsone could have more widespread use, and it is more cost-effective than other alternatives, which include aerosolized or intravenous pentamidine and atovaquone. Monthly aerosolized pentamidine has been shown to be effective in preventing PCP with minimal side effects 1, but PCP breakthrough is higher than with dapsone or TMP-SMX25. In our cohort, two patients developed PCP after being switched to aerosolized pentamidine because of inability to be treated with TMP-SMX or dapsone. In addition to the concern for efficacy, aerosolized pentamidine is an expensive agent (approximate one year therapy = $1200 with additional financial, technical and personnel resources required. Younger pediatric patients also may not be able to adequately inhale an effective dose1, 26. Intravenous pentamidine is also used, but efficacy remains a concern, as radioisotope-imaging data has shown intravenous pentamidine has poor lung penetration vs. the inhaled formulation 27–29. A previous smaller study from our institution showed PCP breakthrough in two of 12 (16.7%) pediatric oncology patients 30 and Kim et al recently demonstrated 1.3% PCP breakthrough rate in a study of 232 pediatric oncology patients 26. A 5.5% breakthrough rate was identified in a study of 30 pediatric HIV patients27.

Atovaquone is another alternative to dapsone and has been shown to be effective in pediatric cancer patients with an acceptable toxicity profile31. However, it is much more expensive than dapsone with an atovaquone having a US retail daily cost of $52 a day for an adolescent or adult ($18,980 dollars a year) and $26 dollars a day for 25kg child ($9,490 dollars a year) 1, 31. This is in contrast to a daily cost of $1.30 to an adult taking daily dapsone ($474.50 dollars a year) and $0.65 a day for a 25kg child ($237.25 dollars a year). TMP-SMX still remains the most cost effective at a cost of only $0.29 per double strength

tablet ($60.32 dollars a year if dosed BID twice a week) and thus remains first-line treatment.

Our study has some limitations. It is a retrospective study and thus adherence with prescribed dosing could not be assessed nor could the full clinical presentation of methemoglobinemia be verified in all cases. We were also unable to obtain CYB5RA serum levels at the time of the methemoglobinemia event and thus cannot fully disregard its role in the pathogenesis of methemoglobinemia. As there is no reason to believe that the enzyme levels we obtained were associated with age or BMI, it still appears that a random one-time level will not be an adequate screening test. Patients enrolled in the cross-sectional component of the study were consecutively as opposed to randomly selected, but these patients did not differ in any discernable factors from those not enrolled, with the exception that those who were lost to follow-up or deceased could not be included in the subcohort.

Conclusion

In conclusion, dapsone is an effective drug for PCP prophylaxis that remains the most inexpensive of second line alternatives to TMP-SMX and is well tolerated by the majority of patients. However its use is limited by the common occurrence of methemoglobinemia as an adverse event, which can be life threatening and results in increased medical costs and distress to families. Given the dose related association and lack of PCP breakthrough at the lower doses, a prospective trial of lower doses or dosing at less frequent intervals is warranted to improve the utility of dapsone, better define the dose-related occurrence of methemoglobinemia, and identify biologic correlates which may help predict those at highest risk for methemoglobinemia.

Acknowledgments

Supported in part by Vanderbilt CTSA grant 1 UL1RR024975 from NCRR/NIH

References

1. Hughes WT. Use of dapsone in the prevention and treatment of Pneumocystis carinii pneumonia: a review. Clin Infect Dis. 1998; 27:191–204. [PubMed: 9675476]
2. Hughes, WalterÂ T.; Armstrong, D.; Bodey, GeraldÂ P., et al. 2002 Guidelines for the Use of Antimicrobial Agents in Neutropenic Patients with Cancer. 2002:730–751.
3. Ash-Bernal R, Wise R, Wright SM. Acquired methemoglobinemia: a retrospective series of 138 cases at 2 teaching hospitals. Medicine (Baltimore). 2004; 83:265–273. [PubMed: 15342970]
4. Williams S, MacDonald P, Hoyer JD, Barr RD, Athale UH. Methemoglobinemia in children with acute lymphoblastic leukemia (ALL) receiving dapsone for pneumocystis carinii pneumonia (PCP) prophylaxis: a correlation with cytochrome b5 reductase (Cb5R) enzyme levels. Pediatr Blood Cancer. 2005; 44:55–62. [PubMed: 15390276]
5. Zosel A, Rychter K, Leikin JB. Dapsone-induced methemoglobinemia: case report and literature review. Am J Ther. 2007; 14:585–587. [PubMed: 18090884]
6. Blum RN, Miller LA, Gaggini LC, Cohn DL. Comparative trial of dapsone versus trimethoprim/sulfamethoxazole for primary prophylaxis of Pneumocystis carinii pneumonia. J Acquir Immune Defic Syndr. 1992; 5:341–347. [PubMed: 1548570]
7. Kemper CA, Tucker RM, Lang OS, et al. Low-dose dapsone prophylaxis of Pneumocystis carinii pneumonia in AIDS and AIDS-related complex. Aids. 1990; 4:1145–1148. [PubMed: 2282188]
8. Coleman MD, Jacobus DP. Reduction of dapsone hydroxylamine to dapsone during methaemoglobin formation in human erythrocytes in vitro. Biochem Pharmacol. 1993; 45:1027–1033. [PubMed: 8461032]
9. Efron, B.; Tibshirani, R. An introduction to the bootstrap. New York: Chapman & Hall; 1993.

10. Harrell, FE. Regression modeling strategies : with applications to linear models, logistic regression, and survival analysis. New York: Springer; 2001.
11. Dupont WD, Plummer WD Jr. Power and sample size calculations. A review and computer program. Control Clin Trials. 1990; 11:116–128. [PubMed: 2161310]
12. Dunford LM, Roy DM, Hahn TE, et al. Dapsone-induced methemoglobinemia after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2006; 12:241–242. [PubMed: 16443522]
13. Hamirani YS, Franklin W, Grifka RG, Stainback RF. Methemoglobinemia in a young man. Tex Heart Inst J. 2008; 35:76–77. [PubMed: 18427660]
14. Ward KE, McCarthy MW. Dapsone-induced methemoglobinemia. Ann Pharmacother. 1998; 32:549–553. [PubMed: 9606476]
15. Mandrell BN, McCormick JN. Dapsone-induced methemoglobinemia in pediatric oncology patients: case examples. J Pediatr Oncol Nurs. 2001; 18:224–228. [PubMed: 11588763]
16. Manfredi G, De Panfilis G, Zampetti M, Allegra F. Studies on dapsone induced haemolytic anaemia. I. Methaemoglobin production and G-6-PD activity in correlation with dapsone dosage. Br J Dermatol. 1979; 100:427–432. [PubMed: 454569]
17. Cruciani M, Gatti G, Mengoli C, et al. Penetration of dapsone into pulmonary lining fluid of human immunodeficiency virus type 1-infected patients. Antimicrob Agents Chemother. 1997; 41:1077–1081. [PubMed: 9145873]
18. Gatti G, Merighi M, Hossein J, et al. Population pharmacokinetics of dapsone administered biweekly to human immunodeficiency virus-infected patients. Antimicrob Agents Chemother. 1996; 40:2743–2748. [PubMed: 9124833]
19. Gatti G, Fioredda F, Lorusso C, Cruciani M, Bassetti D. Alternative dapsone dosage regimen for prophylaxis of Pneumocystis carinii pneumonia. Pediatr Infect Dis J. 1996; 15:183–184. [PubMed: 8822303]
20. Gatti G, Loy A, Casazza R, Miletich F, Cruciani M, Bassetti D. Pharmacokinetics of dapsone in human immunodeficiency virus-infected children. Antimicrob Agents Chemother. 1995; 39:1101–1106. [PubMed: 7625796]
21. Gatti G, Loy A, Lorusso C, Rossi G, Bassetti D. Penetration of dapsone into lung of human immunodeficiency virus-infected children. Pediatr Infect Dis J. 1997; 16:523–524. [PubMed: 9154550]
22. McIntosh K, Cooper E, Xu J, et al. Toxicity and efficacy of daily vs. weekly dapsone for prevention of Pneumocystis carinii pneumonia in children infected with human immunodeficiency virus. ACTG 179 Study Team. AIDS Clinical Trials Group. Pediatr Infect Dis J. 1999; 18:432–439. [PubMed: 10353516]
23. Fermo E, Bianchi P, Vercellati C, et al. Recessive hereditary methemoglobinemia: two novel mutations in the NADH-cytochrome b5 reductase gene. Blood Cells Mol Dis. 2008; 41:50–55. [PubMed: 18343696]
24. Schuurman M, van Waardenburg D, Da Costa J, Niemarkt H, Leroy P. Severe hemolysis and methemoglobinemia following fava beans ingestion in glucose-6-phosphatase dehydrogenase deficiency: case report and literature review. Eur J Pediatr. 2009; 168:779–782. [PubMed: 19263080]
25. Vasconcelles MJ, Bernardo MV, King C, Weller EA, Antin JH. Aerosolized pentamidine as pneumocystis prophylaxis after bone marrow transplantation is inferior to other regimens and is associated with decreased survival and an increased risk of other infections. Biol Blood Marrow Transplant. 2000; 6:35–43. [PubMed: 10707997]
26. Kim SY, Dabb AA, Glenn DJ, Snyder KM, Chuk MK, Loeb DM. Intravenous pentamidine is effective as second line Pneumocystis pneumonia prophylaxis in pediatric oncology patients. Pediatr Blood Cancer. 2008; 50:779–783. [PubMed: 17635000]
27. Gupta M, Stephenson K, Gauar S, Frenkel L. Intravenous pentamidine as an alternate for Pneumocystis carinii pneumonia prophylaxis in children with HIV infection. Pediatr Pulmonol Suppl. 1997; 16:199–200. [PubMed: 9443273]
28. Milstone AM, Balakrishnan SL, Foster CB, Chen A. Failure of intravenous pentamidine prophylaxis to prevent pneumocystis pneumonia in a pediatric hematopoietic stem cell transplant (HSCT) patient. Pediatr Blood Cancer. 2006; 47:859–860. [PubMed: 16568443]

29. Thomas SH, Page CJ, Blower PJ, et al. Disposition of intravenous 123iodopentamidine in man. Nucl Med Biol. 1997; 24:327–332. [PubMed: 9257331]

30. Prasad P, Nania JJ, Shankar SM. Pneumocystis pneumonia in children receiving chemotherapy. Pediatr Blood Cancer. 2008; 50:896–898. [PubMed: 17458875]

31. Madden RM, Pui CH, Hughes WT, Flynn PM, Leung W. Prophylaxis of Pneumocystis carinii pneumonia with atovaquone in children with leukemia. Cancer. 2007; 109:1654–1658. [PubMed: 17345613]

Table I

Characteristics of DIMS Cohort by Methemoglobinemia Event Status

Variable	No Event (n=131)	Suspected Event (n=4)	Confirmed Event (n=32)
Age when starting dapsone(years)			
Median (25^{th},75^{th} percentile)	6 (3,11)	4 (3, 6)	6 (3,11)
Sex, n (%)			
Male	78 (60)	2 (50)	21 (66)
Female	53 (41)	2 (50)	11 (34)
Race, n (%)			
Caucasian	113 (86)	3 (75)	25 (78)
African American	11 (8)	1 (25)	2 (6)
Hispanic	7 (5)		4 (13)
Asian			1 (3)
Diagnosis, n (%)			
Pre B ALL	80 (61)	3 (75)	23 (72)
Other ALL	6 (5)		3 (9)
Aplastic Anemia	6 (5)	1 (25)	
Hodgkin Lymphoma	6 (5)		1 (3)
NHL	14 (11)		2 (6)
AML	15 (12)		1 (3)
Other	4 (3)		2 (6)
Down syndrome, n (%)			
Yes	3 (2)	0 (0)	1 (3)
Had Bone Marrow Transplant, n (%)			
Yes	31 (24)	0 (0)	7 (22)
Initial BMI z score			
Median (25^{th},75^{th} percentile)	0.79 (−0.14, 1.7)	0.29 (−0.62, 1.49)	0.58 (−0.40, 1.26)
Total number of days on Dapsone			
Median (25^{th},75^{th} percentile)	427 (197, 820)	120 (27 to 415)	67 (34 to 223)
Total	71,186	749	5,936
Initial dapsone dose, n (%)			
Dosed 20% below target	61 (47)	1 (25)	4 (13)
Dosed at target	63 (48)		22 (69)
Dosed 20% above target	4 (3)	3 (75)	4 (13)
Missing (not recorded)	3 (2)		2 (6)
Dapsone dose at event or censor, n (%)			
Dosed 20% below target	70 (53)		5 (16)
Dosed at target	55 (42)	2 (50)	19 (59)
Dosed 20% above target	3 (2)	2 (50)	6 (19)

Abbreviations: DIMS= Dapsone Induced Methemoglobinemia Study, ALL= acute lymphoblastic leukemia, Other ALL= T cell ALL, very high risk ALL or Infant ALL, NHL= non Hodgkin lymphoma, AML= acute myeloblastic leukemia, Other= chronic myelocytic leukemia, post transplant lymphoproliferative disease (PTLD), or biphenotypic leukemia.

Table II

Cox Regression Model for Confirmed Methemoglobinemia

Variable	Hazard Ratio	95% CI	p-value
Age	1.0	0.99, 1	0.941
Sex (Male)	0.98	0.46, 2.02	0.924
Body Mass Index z-score	0.96	0.74, 1.25	0.783
Dosed 20% below vs. correct dose	0.27	0.09, 0.78	0.016
Dosed 20% above vs. correct dose	6.24	2.45, 15.93	<0.001

Table III

Characteristics of the DIMS Cross-sectional Cohort: (n=20 in each group)

Variable	Methemoglobinemia group	Control group
Age when starting Dapsone(years),		
Median (25^{th},75^{th} percentile)	6 (3,9)	5 (3,9)
Sex, n (%)		
Male	15 (75)	15 (75)
Female	5 (25)	5 (25)
Diagnosis, n (%)		
Pre B ALL	15 (75)	12(60)
Other	5(25)	8(40)
Had Bone Marrow Transplant, n (%)		
Yes	3(15)	5(25)
Initial BMI z score		
Median (25^{th},75^{th} percentile)	0.58 (−0.38, 1.46)	0.55 (−0.26, 1.67)
Total number of days on Dapsone		
Median (25^{th},75^{th} percentile)	70 (35, 215)	798 (324, 1126)
Initial dapsone dose, n (%)		
Dosed 20% below target	3(15)	8 (40)
Dosed at target	13 (65)	12 (60)
Dosed 20% above target	4 (20)	
Dapsone dose at event or censor n (%)		
Dosed 20% below target	3 (15)	13 (65)
Dosed at target	12 (60)	7 (35)
Dosed 20% above target	5 (25)	

Abbreviations: DIMS= Dapsone Induced Methemoglobinemia Study, ALL= acute lymphoblastic leukemia, Other = T cell ALL, very high risk ALL, infant ALL, non Hodgkin lymphoma, acute myeloblastic leukemia, chronic myelogenous leukemia, post transplant lymphoproliferative disease (PTLD), or biphenotypic leukemia. Event= methemoglobinemia