Safety and efficacy of rapidly administered (one hour) one gram of low molecular weight iron dextran (INFeD) for the treatment of iron deficient anemia

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Oral iron is a standard treatment of iron deficient anemia despite high rates of intolerance and nonadherence and may not replenish iron stores rapidly enough to meet ongoing losses [1]. Intravenous (IV) iron has advantages but remains underutilized. Whereas most formulations of IV iron require multiple doses for replacement, low molecular weight iron dextran (LMW ID) may be administered as a total dose infusion, typically over a 4- to 6-hr period [2,3]. A 4-hr infusion for doses up to 4 g was standard in our practice until 2 years ago. However, clinical studies suggest that 1 g of IV iron is an adequate dose for many patients [3–5], and it became apparent that we were frequently infusing doses of at least 1 g in 1 hr without evidence of significant adverse events [3]. Now, our clinical practice routinely infuses 1 g of LMW ID in 250 mL normal saline over 1 hr without premedication as our standard practice. We summarize our experience with the safety and efficacy of this method of administering IV iron in unselected patients with iron deficiency.

From July 11, 2008 to February 25, 2010, a total of 396 consecutive iron deficient patients received 1g LMW ID infusions. The mean age was 50.7 years (range 14 to 90), 84.1% were women, 75.1% were white, and 14.4% had multiple documented drug allergies at baseline. The most common diagnoses were heavy uterine bleeding among women (43.5%) and gastrointestinal bleeding among men (33.3%). The majority (78.9%) included in this study had baseline transferrin saturation (TSAT) $\leq$ 20% (mean: 11.5 ± 8.7%) and serum ferritin $\leq$ 100 ng/mL (median: 11.0 ng/mL, range: <1–1,164 ng/mL). (Demographic characteristics and baseline laboratory parameters are further described in Supplemental Table I, available online.)

A total of 570 infusions were administered over a median time of 63 min [interquartile range: 60–66 min (Fig. 1)].

A total of 41 adverse events (AEs) were reported in 22 patients (5.6%), with six patients requiring a decreased rate or temporary interruption of the infusion. One refused further treatment due to an AE (hives). There were no anaphylactoid reactions and no serious AEs (SAEs).

All AEs were considered mild to moderate in severity, the most common being back pain, headache, myalgia, and nausea (Table I), all of which were self-limiting and completely resolved with no intervention in the majority of patients. A total of seven patients received treatment with methylprednisolone injection ($n = 5$) or acetaminophen ($n = 2$).

Although AEs were relatively more common among female and black patients, these differences were not significant. An independent association was observed between multiple (>2) drug allergies and an increased likelihood of an AE [odds ratio (OR) = 3.40; 95% CI: 1.09–10.63; $P = 0.036$] when controlling for race, gender, and relative dose [estimated body surface area (BSA)] (Figure 2; Supplemental Table II, available online). However, this finding should be interpreted with caution due to the very low absolute incidence rate. Other variables, including baseline iron status and age, were not associated with the incidence of observed AEs.

Premedication with 125 mg of IV methylprednisolone was administered to only 10 patients with a history of multiple drug allergies (4), asthma, active inflammatory bowel disease, and/or a previous reaction to IV iron. Three received granisetron premedication due to anticipatory nausea ($n = 2$) or nausea with prior IV iron therapy ($n = 1$). All premedicated patients subsequently received the infusion without AEs. No patient received premedication with antihistamines.

Clinically significant hypophosphatemia, defined as a serum phosphate level < 2 mg/dL, was not observed. The mean change in serum phosphate level was monitored in a subset of patients (88 infusions in 87 patients). At preinfusion baseline, mean phosphate level was 3.6 ± 0.54 mg/dL (range: 2.0–5.1 mg/dL). After a median follow-up period of 2 weeks postinfusion, mean phosphate level was 3.6 ± 0.52 mg/dL (range: 2.4–5.3 mg/dL), with a mean change from baseline of 0.0 mg/dL (95% CI: −0.15 to 0.08; $P = 0.58$).

Paired preinfusion and postinfusion hemoglobin (Hb) data were available for 434 infusions in 319 patients (Supplemental Table III, available online). Mean preinfusion Hb was 10.8 ± 1.6 g/dL. Treatment resulted in a significant increase in Hb (mean change from preinfusion baseline: +1.1 g/dL; $P <$ 0.0001; 95% CI: 1.0–1.2 g/dL) over a median follow-up time of 4 weeks. An increase in Hb of $\geq$1 g/dL occurred following 222 infusions (51.2%) in 187 patients, and an increase in Hb of $\geq$2 g/dL occurred following 114 infusions (26.3%) in 108 patients. One patient received a blood transfusion following a severe gastrointestinal bleed secondary to angiodysplasia. Four patients received a dose of darbepoetin alfa within a week of the iron infusion, and the follow-up Hb data for these patients were excluded from the efficacy analyses.

Although the majority of patients had preinfusion TSAT $\leq$20% and serum ferritin $\leq$100 ng/mL, 21.1% had TSAT >20% and/or serum ferritin >100 ng/mL. There was no significant difference in magnitude of Hb response between these two subsets (mean difference in Hb increase between patients with TSAT $\leq$ 20% and serum ferritin $\leq$ 100 ng/mL and the others was 0.3 g/dL; 95% CI: −0.0 to 0.5 g/dL; $P = 0.08$). When baseline TSAT $\leq$ 20% and serum ferritin $\leq$ 100 ng/mL, 54% of infusions led to an increase in Hb of at least 1 g/dL, while 37% of infusions when TSAT >20% or serum ferritin >100 ng/mL achieved an increase in Hb of at least 1 g/dL (Supplemental Table IV, available online).

A total of 31 infusions in 43 women with pregnancy-related anemia (second and third trimester, or postpartum), for whom follow-up data were available, were included in the efficacy analysis. In this subgroup, the mean change in Hb from baseline was 1.2 g/dL (95% CI: 0.79–1.65 g/dL; $P <$ 0.0001), and 25.8% resulted in an increase in Hb of >2 g/dL. Four of the patients with pregnancy-related anemia reported AEs. One resolved with a decreased rate and temporary interruption of the infusion and three received treatment with IV methylprednisolone.

Although oral iron is a convenient and inexpensive therapy for iron deficient anemia, it has several important limitations. Even in patients who are not inflamed, and therefore have no problems with absorption, effective treatment requires a long course to correct anemia and completely replenish stores. Significant nonadherence and intolerance abound.

While any of the available IV irons can infrequently cause acute reactions, the incidence and severity of these reactions are far less than perceived [6]. Whereas LMW ID, iron sucrose, ferric gluconate, and ferumoxytol can be administered safely and effectively, only LMW ID can be used to provide total dose repletion in a single setting (a method of administration approved in Europe but not the United States). This method of administration has typically been 2–6 hr in published reports. In some studies, doses of $\geq$3 g (and up to 4.5 g) were infused. Numerous clinical studies suggest that 1 g is an adequate dose for the majority of patients, and several support the use of 1 g/hr, providing a rationale for administering 1 g over 1 hr in this study [3,7–11].

No prospective study has shown benefit of premedication with antihistamines, yet they are often administered empirically. A study of 135 iron deficient patients who received antihistamines before the administration of IV iron reported that the most frequent AE observed was sedation due to the antihistamine [1]. Antihistamines have been associated with flushing, hypotension, supraventricular tachycardia and somnolence, all of which may be misinterpreted as iron-related reactions. In contradistinction to the AEs just described, there is a syndrome occurring in ~1:200 patients described by Fishbane, consisting of arthralgia and myalgia of the chest or flank, usually

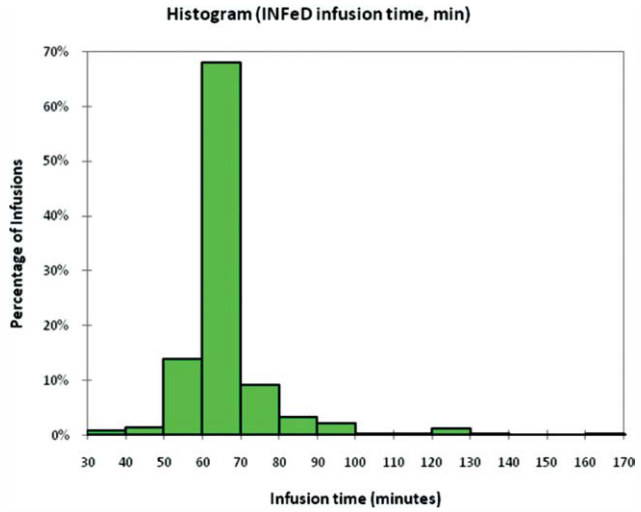

Figure 1. INFeD administration–distribution of infusion time. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

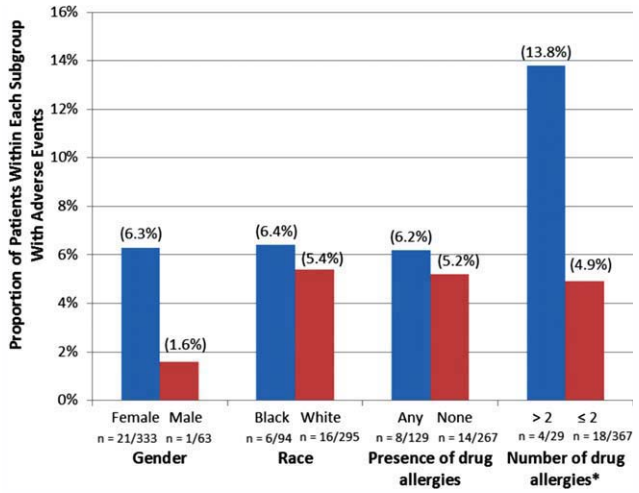

Figure 2. Adverse events by subgroup. When controlling for race, gender, and relative LMW ID dose, there was an independent association between number of drug allergies and the occurrence of adverse events ($P = 0.036$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE I. Adverse Events

	Patients, n (%) (n = 396)
Any AE	22 (5.6)
Any serious AE	0 (0.0)
AEs resulting in discontinuation	1 (0.3)
AEs requiring intervention	9 (2.3)
Decreased infusion rate or temporary interruption alone	2 (0.5)
IV methylprednisolone alone	1 (0.3)
Acetaminophen	2 (0.5)
Temporary infusion interruption plus IV methylprednisolone	4 (1.0)
AEs occurring in > 1 patient[a]	7 (1.8)
Back pain	
Headache	4 (1.0)
Myalgia	3 (0.8)
Nausea	3 (0.8)
Chest discomfort	2 (0.5)
Flushing	2 (0.5)
Nasal congestion	2 (0.5)
Pruritus	2 (0.5)

The following adverse events were reported in 1 patient each: Arthralgia, constipation, cough, decreased appetite, diarrhea, dizziness, dry throat, increased lacrimation, injection site extravasation, neck pain, pain in extremity, pharyngolaryngeal pain, rhinorrhea, sneezing, throat irritation, urticaria.
Abbreviation: AE, adverse event.

occurring with or after the test dose, without associated hypotension, tachypnea, tachycardia, wheezing, stridor, or periorbital edema. This reaction routinely abates without treatment and rarely recurs with rechallenge [6]. Inappropriate intervention with antihistamines or pressors can convert this minor reaction to one which is hemodynamically significant. On the basis of these observations, we avoid the use of antihistamine premedication.

A history of multiple drug allergies has been associated with an increase in AEs to IV iron [12]. Our results were consistent with these observations in that AEs were relatively more common among those with more than two drug allergies. Four patients with multiple drug allergies received premedication with methylprednisolone, none of whom subsequently experienced an AE. Nonetheless, minor AEs were observed in 22 (5.6%) patients, and no SAEs were observed throughout this study. The reported AEs usually consisted of mild to moderate back pain, headache, and nausea. All AEs occurring during the infusion resolved within minutes, and all received the total planned dose, with the one exception who discontinued due to hives.

Over the limited range of values observed in this study, we observed that Hb response, among these iron-deficient patients, was not associated with baseline iron status (TSAT $\leq$ 20% and serum ferritin $\leq$ 100 ng/mL vs. TSAT $>$ 20% and/or serum ferritin $>$ 100 ng/mL). This finding is consistent with that observed in anemia of chronic disease, where total iron binding capacity (TIBC, a negative acute phase reactant) is often depressed and serum ferritin (a positive acute phase reactant) can be increased. In fact, in hemodialysis- and chemotherapy-related anemias, serum ferritin has been shown to be a poor predictor of Hb responsivesness to IV iron [13,14], particularly in settings of inflammation, infection, and chronic diseases.

This study included 43 women who had pregnancy-related anemia. Anemia in pregnancy, a known risk factor for maternal and fetal morbidity and mortality, continues to be managed primarily with oral iron. However, gastrointestinal adverse effects and intolerance significantly limit the use of oral iron in pregnancy, as the effect of pregnancy on the gastrointestinal tract is often aggravated by the adverse effects of oral iron [15,16]. These results are consistent with the study published by Ayub in 2008, where infusion of LMW ID was a safe and effective method of iron supplementation in pregnancy-related iron deficient anemia [17].

Ferric carboxymaltose has been given as a 1g infusion over 15 min to patients with chronic kidney disease, inflammatory bowel disease, and iron deficient anemia due to menorrhagia or pregnancy [18,19]. However, in February 2008, the FDA delayed approval of ferric carboxymaltose for distribution in the United States, due to an increased number of adverse cardiac events, an imbalance in death rates in the treatment arm compared to the control arm, and hypophosphatemia, a marker of renal tubular injury, 2 weeks following administration [20]. As a result of these findings, we monitored serum phosphate levels prior to and 2 weeks following the infusion of 1 g LMW ID and found no decrease.

Subsequent to this analysis, we have administered another 696 1-hr infusions of 1 g LMW ID in 492 consecutive, nonselected patients. The safety and efficacy data from these additional patients are consistent with the results reported in this study. Only one patient did not receive the planned dose due to refusal to be rechallenged after a transient myalgia of the chest. In the integrated safety analysis of 888 patients, no SAEs have been observed in a total of 1,266 infusions. However, this retrospective analysis is limited in its ability to detect serious events as the reported rate of SAEs is so rare that even if the underlying rate were as high as 1 in 1,000, we would need to study 3,000 infusions to have a 95% chance of detecting one such event.

The data in this large population of consecutive, unselected patients with iron deficient anemia provide support for the safety and effectiveness of replacement doses of 1 g LMW ID given IV over 1 hr, without premedication. This method of administration has the advantages of a shorter treatment period and assured compliance compared to oral iron. While future prospective, randomized studies are needed to confirm these findings, the results of this study question the paradigm that oral iron should be standard first line therapy for all conditions associated with iron deficiency.

Methods

Data were collected from consecutive, unselected patients with iron deficient anemia not actively receiving erythropoiesis-stimulating agents (ESAs) or chemotherapy, who were treated with 1 g LMW ID. All patients included were referred from internal medicine, family practice, obstetrics and gynecology, and gastroenterology practices. With the exception of patients with inflammatory bowel disease, where oral iron has been shown to exacerbate the inflammation of the intestinal epithelium [21], all had either previously failed or were intolerant of oral iron.

After obtaining informed consent for data collection and treatment, patients underwent a baseline evaluation including medical history, physical examination, and laboratory testing (hematology and biochemistry profiles). Following initial consultation and confirmation of iron deficiency (TSAT $\leq$ 20% and/or serum ferritin $\leq$ 100 ng/mL), all patients received an infusion of 1 g LMW ID (INFeD, Watson Pharma, Morristown, NJ) diluted in 250 mL of normal saline. A test dose was administered to all IV iron-naïve patients by withdrawing 10 mL from the bag and administering it as a 5-min IV push. After a 15-min observation period, the remainder of the dose was administered at 300 mL/hr. If an adverse reaction occurred, the infusion was stopped, and the patient was observed for up to 1 hr. In these instances, the infusion was restarted at 100 mL/hr and adjusted as needed. Subsequent infusions, and those administered to non-IV iron-naïve patients, were administered at a rate to infuse the IV iron over a total of 1 hr.

The primary outcome of this study was defined as the safety of administering 1 g LMW ID infusions over 1 hr. Safety was assessed by recording all signs or reports of AEs temporally associated with an infusion, and details of their treatment and resolution. AEs were categorized by Medical Dictionary for Regulatory Activities (MedDRA) preferred term. Additionally, due to concerns about hypophosphatemia observed with other IV iron formulations, mean change in phosphate level from baseline was evaluated in a subset of patients.

The secondary efficacy variables were the mean change in Hb from preinfusion baseline, the proportion of patients achieving an increase in Hb $\geq$ 2g/dL, and number of transfusions administered. The following additional data were extracted from patients' charts: age, gender, height, weight, diagnosis, tests of iron status (serum ferritin, TIBC, serum iron, and TSAT), history of drug allergies and/or iron allergies, dose of iron dextran, and infusion rate.

Statistical analysis. Demographic data, baseline laboratory values, and infusion data were summarized using descriptive statistics. Association between patient characteristics and the likelihood of experiencing an AE were explored using multiple logistic regression. Significance of change from baseline in Hb and serum phosphorus levels was analyzed using the paired student's *t*-test. The relationship between baseline iron status (preinfusion TSAT $\leq$ 20% and serum ferritin $\leq$ 100 ng/mL) and change in Hb was explored using analysis of covariance model including baseline value and length of follow-up period ($<$4 weeks or $\geq$4 weeks) as covariates.

Acknowledgments

The authors thank Kristin Vaneekhoven for excellent editorial assistance. *Financial Disclosures*: Dr. Auerbach has not served as a paid consultant for any company manufacturing intravenous iron, been a member of any speaker bureau, or accepted honorarium since September 2007. Dr. Dahl and Dr. Pappadakis are salaried employees of Watson Pharmaceuticals. Drs. Bahrain, Ballard, and S. Auerbach have no financial disclosures to report.

Additional Supporting Information may be found in the online version of this article.

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Contract grant sponsor: Watson Pharmaceuticals
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Conflict of interest: Nothing to report.
Published online 27 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22153

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When children with sickle-cell disease become adults: lack of outpatient care leads to increased use of the emergency department

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Young adults with sickle-cell disease have increased emergency department (ED) utilization and increased risk of mortality for unclear reasons. Emergency Department Reliance (EDR) differentiates excessive ED use due to increased need in chronic illness from increased use due to limited access to outpatient care. A higher EDR has been used to define excessive reliance on the ED and thus access to care issues leading to increased ED utilization. We conducted a retrospective cohort study of sickle-cell disease patients within the Wisconsin Medicaid database over a 5-year period to examine EDR during the transition period from childhood to adulthood. The study population included four distinct groups: (1) children, (2) patients transitioning from pediatric to adult providers, (3) young adults, and (4) adults age 31–45. Rates of visits per year were calculated for ED visits and outpatient visits for all diagnoses and sickle-cell disease-related diagnoses. Overall, we found increased EDR among the transition group and young adults compared to children and adults for sickle-cell disease-related diagnoses. These findings suggest access to care issues play a significant role in the increased ED utilization seen during the transition period from pediatric to adult providers in sickle-cell disease.

Sickle-cell disease is the most common inherited blood disorder in the United States, affecting ~90,000 Americans [1,2]. Complications of sickle-cell disease begin in early childhood and historically have contributed to a significantly shortened life expectancy [3–5]. With advances in clinical care, however, the life expectancy of children with sickle-cell disease has increased significantly, and, now, the majority of individuals with sickle-cell disease live well into adulthood [6]. With improved survival, the need for transition from pediatric centered care to adult-centered care for patients with sickle cell disease has garnered increased attention in the recent years. Much of the literature has focused on patient and provider perceptions of this transition, determination of patient readiness, or components of a good transition program [7–11]. Recent studies have demonstrated that young adults 18–30 years have increased emergency department (ED) utilization [12]. In addition, young adults have a higher risk of mortality during this transition period [6,13]. The reasons for increased utilization and mortality are not entirely clear. The transition of adolescents with chronic illnesses to adult providers is associated with many challenges, including loss of a medical home, decreased access to ambulatory care providers, and loss of insurance coverage [14]. Adolescents with sickle-cell disease face similar challenges as they transition from pediatric care, and such issues may impact their clinical course. Alternatively, a worsening of disease pathology may itself lead to more severe complications in this age group. The relative role of these factors in relation to utilization patterns in patients with sickle-cell disease is not known.

Emergency Department Reliance (EDR) has been used to differentiate increased ED use due to need for care from increased ED use secondary to access issues [15]. EDR is defined as the number of ED visits divided by the number of ED and outpatient visits and therefore views ED visits in relation to all ambulatory visits. Patients with more severe disease, who utilize the ED more, should also have more outpatient visits, while those without adequate outpatient clinic access simply use the ED more without a rise in outpatient clinic visits, thus increasing EDR. The objectives of our study were to examine health care utilization patterns and reliance on ED care among patients with sickle-cell disease transitioning from pediatric to adult care. We hypothesized that patients transitioning from pediatric to adult centered care would have increased reliance on the ED for acute care as reflected by an increased EDR during this time period.

Our final study population included 687 patients: 345 children, 65 patients in the transition group, 139 young adults, and 138 adults age 31–45. The study population had a mean of 47 months of continuous Medicare enrollment. Ambulatory visits (32,258) have occurred over the 5-year study period including 20,418 outpatient visits and 11,840 ED visits. The most frequent diagnosis for all groups was sickle-cell disease unspecified (for outpatient visits) and sickle-cell disease with crisis (for ED visits) in both the sickle-cell-related and all diagnoses categories. Significant differences were found among the study groups for outpatient and emergency department visit rates for all diagnostic categories (Table I).

EDR was highest in the transition and young adult groups across all diagnoses categories. Differences in EDR were significant between the four age groups for any diagnoses ($P < 0.001$) and for SCD-related diagnoses ($P < 0.001$) (Fig. 1). Upon pairwise comparisons, for sickle-cell disease-related diagnoses the transition group had a significantly higher EDR compared to children [mean (SE) of 0.46 (0.03) vs. 0.38 (0.01), $P = 0.013$] as well as older adults [mean (SE) of 0.46 (0.03) vs. 0.33 (0.02), $P < 0.001$]. Similarly, for sickle-cell-related diagnoses, the young adult group had a significantly higher EDR than both children [mean (SE) of 0.47 (0.03) vs. 0.38 (0.01), $P = 0.002$] and adults [mean (SE) of 0.47 (0.03) vs. 0.33 (0.02), $P < 0.001$]. A similar pattern emerged when computing EDR based on all diagnoses, with the EDR for the transition group being significantly higher than for children [mean (SE) of 0.39 (0.03) vs. 0.29 (0.01), $P < 0.001$] as well as older adults [mean (SE) of 0.39 (0.03) vs. 0.30 (0.02), $P = 0.003$]. Again, the young adult group had similar results for all diagnoses with a significantly higher EDR than both children [mean (SE) of 0.41 (0.02) vs. 0.29 (0.01), $P < 0.001$] and adults [mean (SE) of 0.41 (0.02) vs. 0.30 (0.02), $P < 0.001$]. There were no significant differences in EDR between the transition group and young adults for either diagnostic category.

Our data show a higher utilization of the ED in patients with sickle-cell disease who are transitioning from pediatric to adult care when compared with children. The EDR was highest for the transition group and young adults across all diagnoses categories, suggesting these patients depend on the ED for care. The high-ED utilization rates are similar to findings of others [12,13]. However, our study uniquely includes a transition group of patients who made the transition from pediatric to adult providers during the study period. Inclusion of this group allows a direct comparison of the transition group of patients to their older and younger counterparts.

Use of the EDR allows rates of ED utilization to be viewed in relation to all ambulatory care encounters. By doing so, the EDR provides additional information on the underlying reasons behind these patterns. The increased EDR for the transition group across all diagnoses categories suggests that the increase in ED utilization is at least in part due to limitations in access to primary care providers. The return of the EDR for sickle-cell disease-related diagnoses in the older adult group to those levels seen in childhood suggests a stabilization of a medical home following the transition period. The increased ED utilization might also be explained by a worsening of disease. However, as disease worsens and the need for more frequent ED visits develops, one would also expect these patients to be followed in clinic on a more regular basis to optimize their medical care through interventions such as hydroxyurea. Increased ambulatory visits would then serve to maintain a lower EDR for this group of patients. The finding of an elevated EDR in the transition and young adult groups for all diagnostic categories therefore suggests that even if their underlying sickle-cell disease is indeed worsening, patients transitioning to adult providers are unable to access adequate ambulatory care outside of the emergency room setting. The return of the EDR in the adult group to those levels seen in childhood suggests a stabilization of a medical home as patients leave the transition period.

Although our data suggest access to care or changes in the medical home contribute to increased utilization for patients with sickle-cell disease transitioning to adult care, the mere presence of a comprehensive clinic or other form of medical home for patients during the transition period may itself not be the only answer to these issues. Even if such resources exist, additional challenges such as school, work, and family obligations may make access to care

TABLE I. Emergency Department and Outpatient Visits Per Year for Children, the Transition Group, Young Adults, and Adults

			Emergency Department		Outpatient	
		N	Mean (standard error)	*P*-value	Mean (standard error)	*P*-value
Rate of visits with any diagnoses	Children	345	2.66 (0.13)	<0.001	6.97 (0.31)	<0.001
	Transition group	65	4.10 (0.71)		5.51 (0.50)	
	Young adults	139	7.81 (1.18)		8.33 (0.87)	
	Adults	138	5.68 (0.93)		10.33 (0.63)	
Rate of visits with SCD or pain diagnoses	Children	345	2.10 (0.12)	<0.001	3.59 (0.23)	<0.001
	Transition group	65	3.55 (0.69)		3.27 (0.37)	
	Young adults	139	6.48 (1.15)		4.88 (0.80)	
	Adults	138	4.57 (0.92)		5.84 (0.49)	

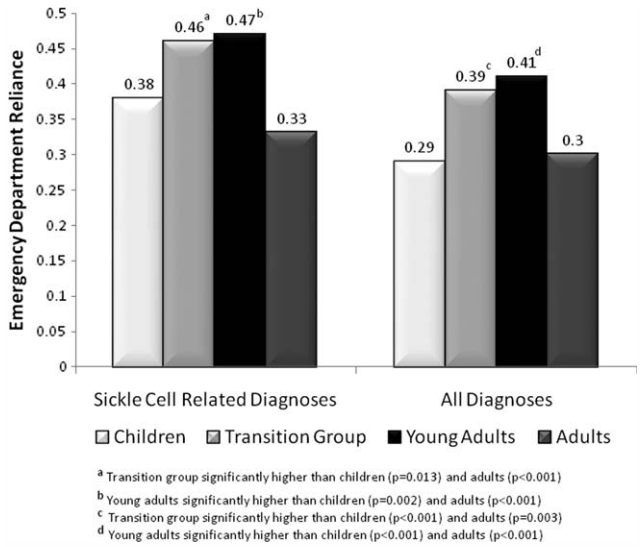

Figure 1. Mean Emergency Department Reliance for children, the transition group, young adults, and adults for sickle-cell disease related and all diagnoses.

issues not simply about whether a clinic or provider is available. One study examining the correlation between the proximity of a comprehensive sickle-cell disease center to patient's homes found no improvement in access to care based on location alone [16], highlighting the importance of additional barriers to access to care. Further research is needed to identify such barriers and appropriate interventions to improve access to care.

Access to care issues and reliance on the ED shown in our study is yet another challenge facing adolescents with sickle-cell disease. This challenge adds to the documented increased risk of mortality during this time period described by others [4]. Whether improving the transition of patients with sickle-cell disease from pediatric to adult providers will decrease ED utilization or lower the risk of mortality in this vulnerable period is an unanswered but critical question in ensuring the best ongoing care for these patients.

This study has additional limitations that must be addressed. First, the database is limited to a single state and availability of expert sickle-cell-related care will vary by state and institution. Second, the database includes only individuals cared for within the Wisconsin State Medicaid system, excluding those with private insurance. However, nationally, 67% of patients with sickle-cell disease have public insurance meaning that our database includes the majority of sickle cell disease patients in Wisconsin [17]. Additionally, our analysis relied on ICD-9-CM coding and any coding errors would affect our analysis. However, the method of selecting sickle-cell patients using previously established criteria for large database studies limits the inclusion of many such errors. Last, numerous other factors associated with the transition period such as decreased parental involvement, insurance coverage changes, and evolving work, social, and family commitments may contribute to the decreased use of outpatient resources and therefore affect our analysis.

In conclusion, these findings support our hypothesis that for sickle-cell disease patients, the transition of care from pediatric to adult providers is associated with increased reliance on the ED for acute care as reflected by an increased EDR during this time period. These findings that suggest access to care issues play a significant role in the increased ED utilization seen during the transition period from pediatric to adult providers. Further research should focus on the access to care issues of these patients as well as how such access issues affect quality of care and outcomes in sickle-cell disease for patients transitioning to adult providers.

Methods

Study design. We conducted a retrospective cohort study utilizing Wisconsin State Medicaid data from January, 2003 to December, 2007. This database includes 825 patients of any age with sickle-cell disease who are part of the Wisconsin Medicaid system. Consistent with previous literature, having sickle-cell disease was defined by having at least one inpatient hospitalization, or two outpatient visits at least one month apart, with a diagnosis of sickle-cell disease [13,18]. Although this definition may exclude some patients with sickle-cell disease who are infrequent users of medical care, it also protects against including misdiagnosed or miscoded patients (such as patients with sickle cell trait diagnosis) who do not have sickle-cell disease. All encounters during the study period were extracted from the database and linked by unique anonymous identifiers. Information extracted included age, all diagnosis codes, and classification as ED visit, outpatient visit, or inpatient hospitalization. Provider types that were not representative of acute medical care, such as dentists, physical therapists, and home health agencies, were excluded (see Supporting Information Appendix Table AI for complete details on encounter and provider types). To protect against multiple charges originating from a single patient encounter, only a single visit type (ED, inpatient, or outpatient) on any given day was included in the final analysis. Time of enrollment was defined as the number of months between the first and last encounters in the database. Individuals with a gap in enrollment of 3 months or greater were excluded from the analysis as these individuals likely received significant amounts of care outside the Medicaid system. Similarly, individuals with only a single encounter over the 5-year period were also excluded as their length of enrollment in the database could not be calculated.

Study population. The study population was divided into three mutually exclusive age groups: (1) children: age $\leq$ 18 years old for the entire study period, (2) transition group: individuals who turned 19 years old during the 5-year study period; and (3) young adults: age 19–45 years at first encounter. The age of 19 was selected for the transition period as our institutional practice involves transitioning patients to adult providers when they turn 19 years old. Adults age $\geq$ 46 at first encounter were eliminated from the analysis as the goal was to compare the transition group to the age groups immediately above and below. Patients with only a single encounter in the database, those with a gap of 3 months or greater in enrollment, and those patients over the age of 45 were excluded from the analyses. Encounter data were examined for all diagnoses and for all sickle-cell disease-related diagnoses. The sickle-cell disease-related diagnoses category included all visits with an ICD-9-CM code for sickle-cell disease (28,241; 28,260; 28,261; 28,263; 28,268) or sickle cell with crisis (28,242; 28,262; 28,264; 28,269), as well as ICD-9-CM codes for pain likely related to sickle-cell disease regardless of additional coding (see Supporting Information Appendix Table AII for complete list of ICD-9CM codes included in the analysis).

Measures. The primary outcomes measured were rates of visits per year and EDR. Rates were calculated for each age group for ED visits and outpatient encounters. Two diagnostic categories based on ICD-9-CM coding were examined for these visits: sickle-cell disease-related diagnoses and all diagnoses. The mean EDR (ED visits/[ED + outpatient visits]) was computed for each age group for both diagnostic categories. A higher EDR value

is indicative of higher reliance on the ED for acute care issues, with no increased proportional rise in utilization of a primary care provider in an outpatient setting. Prior studies of ED utilization in infant and general pediatric populations have defined an excessive EDR as >0.33 [15,19,20].

Statistical analysis. For each patient in the database, the rate of visits per year was calculated using the number of visits divided by the number of enrollment years. EDR was calculated for each patient as described earlier. Mean visit rates and mean EDR were compared after log transformation among the four age groups using the analysis of variance and pairwise comparisons were performed between each group using the *t*-test. The study and a waiver of patient consent were approved by the Medical College of Wisconsin institutional review board.

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Additional Supporting Information may be found in the online version of this article.
Conflict of interest: Nothing to report.
Published online 14 June 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22106

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TABLE AI. Provider Types Included/Excluded from Analyses

Provider types included	Provider types excluded
Day treatment	Ambulance
Federally Qualified Health Clinic	Anesthetist
Health Check	Audiologist
Hospital	Case Management
Nurse Practitioner	Chiropractor
Physician	Dentist
Physician Assistant	Facility for developmentally disabled
Physician Group	Family planning clinic
	Home health/personal care agency
	Independent lab
	Medical equipment vendor
	Mental health and substance abuse services
	Nurse service
	Optician
	Optometrist
	Pharmacy
	Physical therapy
	Podiatrist

TABLE AII. ICD-9-M Codes Included in Sickle-Cell-Related Diagnostic Category

ICD-9CM code	Diagnosis
282.41	Sickle cell thalassemia without crisis
282.42	Sickle cell thalassemia with crisis
282.60	Sickle cell disease unspecified
282.61	Hb SS disease without crisis
282.62	Hb SS disease with crisis
282.63	Sickle cell/Hb C disease without crisis
282.64	Sickle cell/Hb C disease with crisis
282.68	Other sickle cell disease without crisis
282.69	Other sickle cell disease with crisis
102	Nonspecific chest pain
251	Abdominal pain
607.3	Priapism
719.40–719.49	Pain in joint
723.1	Cervicalgia
724.1	Pain in thoracic spine
724.2	Lumbago
724.5	Backache unspecified
729.5	Pain in limb

Possible new *LNK* mutations in myeloproliferative neoplasms

Jung-Sook Ha and Dong-Seok Jeon

Recently, lymphocyte adaptor protein (*LNK* or *SH2B3*) has been reported as a new genetic abnormality in *BCR-ABL* negative myeloproliferative neoplasms (MPN). We performed *LNK* sequence analysis in 42 chronic phase MPN patients (14 polycythemia vera, 15 essential thrombocythemia, 9 primary myelofibrosis, and 4 myeloproliferative neoplasm, unclassified) and detected total three types of genetic mutations (7.1%) including p.Q423X, p.R551W and p.I568T. These mutations were accompanied by a *JAK2* V617F mutation. The p.Q423X is a nonsence mutation located on exon 7 encoding the Src homology 2 domain. The p.R551W and p.I568T are missense mutations located on exon 8 encoding the C-terminal region. Our study suggests that *LNK* mutations occur in low frequency in human MPN, and can occur in several regions of the *LNK* gene not only on a pleckstrin homology domain which have been regarded as a 'hot spot'.

Recently, the mutation of lymphocyte adaptor protein (*LNK* or *SH2B3*) was detected in MPN. LNK is a negative regulator of thrombopoietin (TPO)-MPL and erythropoietin (EPO)-EPO receptor-mediated JAK2 activation [10,11]. LNK belongs to a family of adaptor proteins possessing several domains: a proline-rich N-terminal dimerization domain, a pleckstrin homology (PH) domain, a Src homology 2 (SH2) domain, and a conserved C-terminal tyrosine residue [12]. Via its SH2 domain, LNK binds to JAK2 and inhibits its downstream signal transduction. LNK is also capable of regulating the aberrant signaling of *MPL*-W515L and *JAK2* V617F *in vitro* [13,14]. *LNK*-deficient mice show a phenotype consistent with MPN, involving an expanded hematopoietic stem cell compartment, megakaryocytic hyperplasia, splenomegaly, leukocytosis, and thrombocytosis [15]. In human MPN, three studies about *LNK* mutations have been reported, in which 12 of 102 patients were shown to harbor 14 different *LNK* mutations [16–18].

In this study, we performed *LNK* mutation analysis in 42 chronic-phase MPN patients to study additional mutations in human MPN. We also performed *JAK2* and *MPL* W515L/K mutation analyses to identify the relationship with the *LNK* mutation.

The patients comprised 27 males and 15 females with average ages of 63 and 64 years, respectively. All patients were on initial diagnosis with chronic phase, and the subtypes were 14 PV, 15 ET, 9 PMF, and 4 MPN, unclassified (MPN-U) (See Supporting Information I). Six genetic variations were detected including p.P242S, p.W262R, p.Q423X, p.A536T, p.R551W, and p.I568T (Fig. 1). The p.P242S (c.724C>T) were detected in six patients and was an already known polymorphism (rs78894077) listed in the National Center for Biotechnology Information Single Nucleotide Polymorphism (SNP) Database (dbSNP; http://ncbi.nlm.nih.gov/projects/SNP/). The p.W262R (c.784T>C) was detected in all 42 patients as a homozygote, was also known polymorphism (rs3184504). The p.A536T (c.1606G>A) variation was detected in a PV patient. Although it was not listed in the dbSNP, we regarded it as one of polymorphisms, because it was detected in one of 50 normal controls. In contrast, the other three variations, p.Q423X (c.1267C>T), p.R551W (c.1651C>T), and p.I568T (c.1703T>C), which were detected in each ET, PMF, and PV patient, were not found in 50 control samples. Moreover, those had not been reported in previous literatures or listed in the dbSNP, either. The p.Q423X is located on exon 7 of *LNK* gene and results in a premature stop codon in the SH2 domain. The p.R551W and p.I568T are missense variations located on exon 8, which encodes the C-terminal region of the LNK protein. Because we could not perform germline study with the paired normal cell in each patient, we could not definitely rule out the possibility that these variations were not true somatic mutations, especially for p.R551W and p.I568T. However, when we regarded these three variations as new mutations based on the study using 50 normal controls, the prevalence of *LNK* mutation in our study could be estimated as 7.1% in chronic-phase MPN. The evaluation of the subtype-specific prevalence of *LNK* mutation was not performed due to small numbers of cases harboring the mutations. Previous estimates of *LNK* mutation prevalence have ranged from 6.1 to 25% [16–18]. Oh et al. reported two types of *LNK* mutations in 33 (6.1%) *JAK2 V617F*-negative MPN patients [17], and Lasho et al. identified two mutations (25.0%) in eight *JAK2 V617F*-negative patients with unexplained erythrocytosis [19]. Pardanani et al. studied a relatively large cohort of 61 patients with blast-phase MPN, and postulated that *LNK* mutations are more prevalent in the blast-phase (13.1%) than in the chronic phase (4.9%).

The previously reported mutations were variable and differed across patients, except for one mutation (c.644C>T) that was detected in three cases (Table I). Interestingly, most of the mutations were located on the PH domain, which have led investigators to regard the PH domain as a mutational "hot spot" [18]. The PH domain of LNK is involved in the colocalization of LNK to the plasma membrane. These mutations are expected to disrupt the PH domain and consequently mislocalize LNK in the cytoplasm abrogating its function to JAK2. However, the mutations we detected were on the SH2 domain or C-terminus. The SH2 domain of LNK is known to bind JAK2 and do a critical role in negative regulation of the downstream signal transduction. So, we expected the p.Q423X mutation might result in the disruption of LNK function and have a role in MPN pathogenesis. If we perform the expression study for the production of truncated protein or no protein synthesis by this mutation, it would be more helpful to understand its effect to LNK function. Regarding the p.R551W and p.I568T in C-terminal region, actually we cannot expect the functional consequences because those are not located in the main domain. When we performed the conservation study, we could find these location were well-conserved interspecies, so we just guessed these amino acid changes might have effect on the LNK structure or its function (See Supporting Information II). According to previous investigations using transgenic mice, the mutational effect of *LNK* could be different according to the mutation type. The SH2-mutated type showed most severe disruption of LNK function compared to the mutated form in PH domain or C-terminal tyrosine residue [10,19]. If large number of cases with *LNK* mutations would be collected in the future, the phenotypic difference according to mutation type could be evaluated.

The *JAK2* V617F (exon 14) was detected in 26 of 42 patients (61.9%), but the exon 12 mutation was not detected in this study. All of three patients with *LNK* mutation also harbored *JAK2* V617F mutation. The *MPL* W515L was identified in two ET patients, and neither of them harbored *LNK* or *JAK2* V617F mutations. The frequent cooccurrence of *LNK* mutation and *JAK2* V617F was also observed in previous study, which reported *LNK* mutations were found at similar frequencies between *JAK2* V617F-negative and positive patients [17]. Now we do not know whether the *LNK* mutation and *JAK2* V617F occur in the same clone or not, and the effect of those

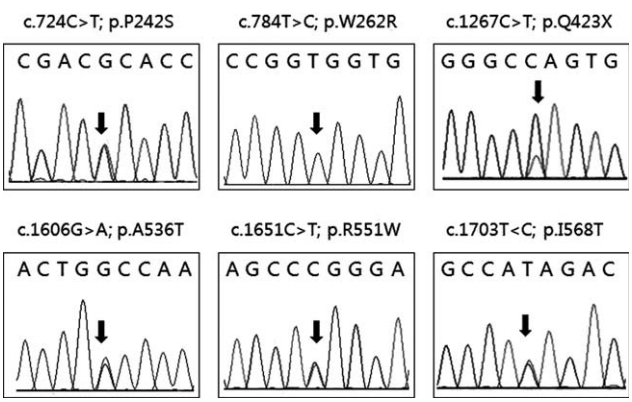

Figure 1. Nucleotide variations found in our study. Arrows in each DNA sequence trace are indicating the substituted position. Three variations are new mutations composed of two missense mutations (p.R551W and p.I568T) and one nonsense mutation (p.Q423X). The others (p.P242S, p.W262R and p.A536T) are known SNPs.

TABLE I. *LNK* mutations reported in previous literatures including our cases

No.	Diagnosis	Exon	Nucleotide change	Mutation location	Mutation Effect/AA change	*JAK2* V617F	*MPL* W515L/K	Other Gene mutation	Reference
1	PMF	2	c.[603_607delGCGCT; 613C>G]	PH domain	Frameshift leading premature stop codon	Negative	Negative		Oh et al.[16]
2	ET	2	c.622G>C	PH damain	Misssense/E208Q	Negative	Negative		Oh et al.[16]
3	PMF→AML	2	c.658G>A	PH damain	Misssense/G220R	Positive	Negative		Pardanani et al.[17]
4	PMF→AML	2	c.644C>T	PH damain	Misssense/A215V	Negative	Negative		Pardanani et al.[17]
5	PMF→AML	2	c.644C>T	PH damain	Misssense/A215V	NA	NA		Pardanani et al.[17]
6	PMF→AML	2, 5	c.[685_691delGGCCCCG]+ [955delA]	PH damain	Frameshift leading premature stop codon	Positive	Negative	IDH2 R140Q	Pardanani et al.[17]
7	PMF→AML	2	*c.[668C>T(+)700G>A]*	PH damain	Misssense/A223V, D234N	Negative	Negative		Pardanani et al.[17]
8	PMF→AML	2	c.659G>T	PH damain	Misssense/G220V	Negative	Negative		Pardanani et al.[17]
9	PMF→AML	2	c.685G>A	PH damain	Misssense/G229S	Positive	Negative		Pardanani et al.[17]
10	PMF→AML	2	c.624G>A	PH damain	Synonymous/E208E	Positive	NA		Pardanani et al.[17]
11	Unexplained erythrocytosis	2	c.622G>T	PH damain	Nonsense/E208X	Negative	Negative		Lasho et al.[18]
12	Unexplained erythrocytosis	2	c.644C>T	PH damain	Misssense/A215V	Negative	Negative		Lasho et al.[18]
13	PV	8	c.1703T<C	C-terminal region	Misssense/I568T	Positive	Negative		This study
14	PMF	8	c.1651C>T	C-terminal region	Misssense/R551W	Positive	Negative		This study
15	ET	7	c.1267C>T	SH2 domain	Nonsense/Q423X	Positive	Negative		This study

Abbreviations: AA, amino acid; AML, acute myeloblastic leukemia; ET, essential thrombocythemia; MPN, myeloproliferative neoplasm; NA, not available; PH domain, pleckstrin homology domain; SH2 domain, Src homology 2 domain; PMF, primary myelofibrosis; PV, polycythemia vera.

cooccurrence on MPN pathogenesis or phenotype. We expected more severe phenotype will result in cases with two mutations than those with only one mutation, based on a previous study that reported the augmented ability of oncogenic JAK2 to expand myeloid progenitors in *LNK*-deficient mice [20].

Although only a few studies, including ours, have been published regarding *LNK* mutations in human MPN, the collective data does favor some suggestions. The prevalence of *LNK* mutations is not high in human MPN, especially among patients with chronic-phase MPN (<10% mutated). Second, *LNK* mutations can target several regions of the *LNK* gene, not only in the PH domain. Whether mutations in different domains contribute differently to phenotypic expression needs to be addressed in larger studies. Finally, the *LNK* mutation could be accompanied by *JAK2* V617F. We cannot be sure if *LNK* mutations and consequent functional disruption are solely responsible or only provide a supportive role for MPN pathogenesis by other genetic changes like *JAK2* V617F. Larger-scale studies of *LNK* mutation in human MPN are warranted.

Methods

Sample collection. This study was approved by the Dongsan Medical Center's Institutional Review Board. Total 42 DNA samples that had been extracted and stored from peripheral blood or bone marrow of MPN patients between 2007 and 2010 were used. All samples were obtained on initial diagnosis and chronic phase of MPN. For control study, total 50 DNA samples from healthy controls were acquired.

Mutation analysis. The primers for *LNK* (exon 2-8) and *JAK2* (exon 12 and 14) were designed using Primer3 (http://frodo.wi.mit.edu/primer3/), and the sequencing was conducted using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) on an ABI 3730XL DNA analyzer (Applied Biosystems) (See Supporting Information III). The detection of *MPL* W515L or W515K mutation was performed using a Real-QTM MPL W515L/K screening kit (BioSewoom, Seoul, Korea) according to the manufacturer's instructions.

Acknowledgments

The authors thank KS Park, Ph.D. and WA Bae, Ph.D. for good assistance with sample collection, DNA extraction, and sequencing analysis.

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Additional Supporting Information may be found in the online version of this article.
Conflict of interest: Nothing to report.
Published online 14 June 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22107

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Concomitant lupus anticoagulant and monoclonal IgMκ antibody in a patient with bleeding tendency: a case report and literature review

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Antiphospholipid antibodies (aPL) are a heterogeneous population of autoantibodies including lupus anticoagulant (LAC), anticardiolipin antibodies and antiβ_2-glycoprotein I antibodies. aPL may be associated with systemic lupus erythematosus, other autoimmune disorders, connective tissue disorders, malignancies, drug use and/or infections. They are usually associated with thrombotic complications (thromboembolism, spontaneous abortion, livedo reticularis). Bleeding is generally not a feature of aPL, but uncommonly, bleeding can occur if significant thrombocytopenia or prothrombin deficiency develop. An aPL-monoclonal gammopathy association has been described, particularly in patients suffering from lymphoid proliferations, including Waldenström's macroglobulinemia and lymphoma. Here we report a strong association between LAC and hemostasis abnormalities with monoclonal immunoglobulin Mκ in a 71-year-old woman who experienced excessive bleeding after dental extraction. Our findings suggest that the monoclonal component induced spontaneous platelet aggregation. Unlike previous reports, Waldenström's macroglobulinemia or lymphoma was not found in this patient.

The patient was referred by her general practitioner to our Hematology Department because of abnormal bleeding after dental extraction, not requiring transfusion. Several years ago, the patient had undergone surgery, e.g. complete hysterectomy, cholecystectomy, and sclerotherapy for varicose veins, without complications. She had no personal history of abnormal bleeding or family history of coagulation disorders. No clinical abnormality was found. She was not taking any drug known to increase the hemorrhage risk.

Complete blood count was normal (erythrocytes 4.25 $\times$ 10^{12}/L, leukocytes: 3.9 $\times$ 10^9/L, platelets: 156 $\times$ 10^9/L). Her bleeding time was prolonged (11.5 min, normal range $<$8 min), as was the closure time measured with the Platelet Function Analyzer (PFA100) with the cartridge containing epinephrine (170 sec, normal range $<$160 sec), but normal with the ADP cartridge. Repeated coagulation tests showed normal prothrombin time, thrombin time, and fibrinogen level but a markedly prolonged activated partial prothrombin time (aPTT ratio 2.4, normal range $<$1.2). Mixing aPTT test of the patient's plasma with normal plasma did not correct the abnormal aPTT and the Rosner index was positive (50, normal range $<$15). The presence of a lupus anticoagulant (LAC), according to International Society on Thrombosis

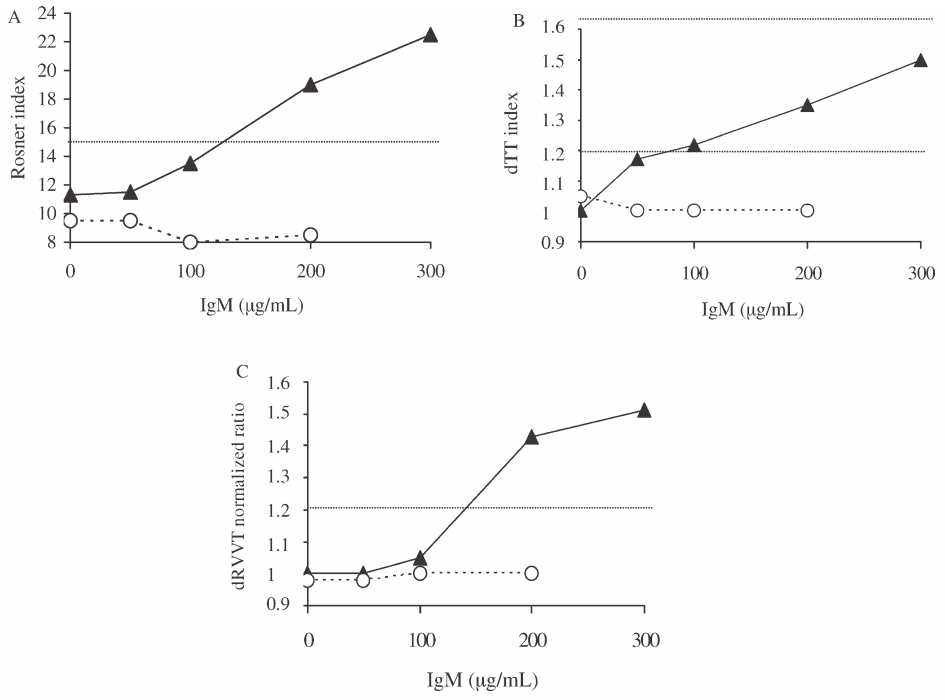

Figure 1. The lupus anticoagulant activity of the patient's IgMκ. Effect of increasing concentrations of the patient's (▲) or control (○) monoclonal IgMκ in normal plasma on the aPTT (A), dTT (B) and dRVVT (C). Index were calculated as follow: Rosner: (mixing aPTT test - normal plasma aPTT)/patient aPTT, dTT: mixing dTT test/normal plasma dTT, dRVVT normalized ratio: patient plasma LA1 Screening Reagent/mean normal LA1 Screening reagent) x (mean normal LA2 Confirmation Reagent/patient plasma LA2 Confirmation reagent). LAC is positive when its value is above the horizontal dotted line.

TABLE I. Monoclonal Immunoglobulins With Antiphospholipid Specificity Previously Described

References	Patient	Isotype	Clinical context	Bleeding or thrombosis	Specificity
Thiagarajan, 1980 [1]	1	IgMλ	Waldenström's macroglobulinemia	No	LAC, PS, PI, PA
Andes, 1982 [8]	1	IgMκ	Lymphocytic lymphoma	No	LAC
Wisloff, 1987 [2]	1	IgMλ	Waldenström's macroglobulinemia	No	LAC, PS, PE
Bellotti, 1989 [4]	1st	IgG1κ	MGUS	No	LAC
	2nd	IgG3κ	Myeloma	Bleeding	LAC, PI
	3rd	IgMλ	Lymphocytic lymphoma	No	LAC, PA
Skjonsberg, 1990 [3]	1	IgMλ	Waldenström's macroglobulinemia	No	LAC
Yasin, 1999 [9]	1	κ light-chain	Multiple myeloma	DVT	LAC
Gallart, 2002 [5]	1	IgMλ	B-cell lymphocytic lymphoma	No	LAC, aCL, PS
von Landenberg 2002 [7]	1	IgMκ	Non-Hogkin lymphoma	No	LAC
Taher, 2003 [10]	1	IgGκ	MGUS Acquired hemophilia	Bleeding	LAC FVIII inhibitor
Colovic, 2006 [6]	2	IgMλ IgGλ	Splenic lymphoma with villous lymphocytes	DVT and pulmonary embolism	LAC, RF

LAC, lupus anticoagulant; aCL, anticardiolipin antibody; PS, phosphatidylserine; PE, phosphatidylethanolamine; PI, phosphatidicinositol; PA, phosphatidic acid. RF, rheumatoid factor; DVT, deep venous thrombosis; MGUS, monoclonal gammopathy unknown significance.

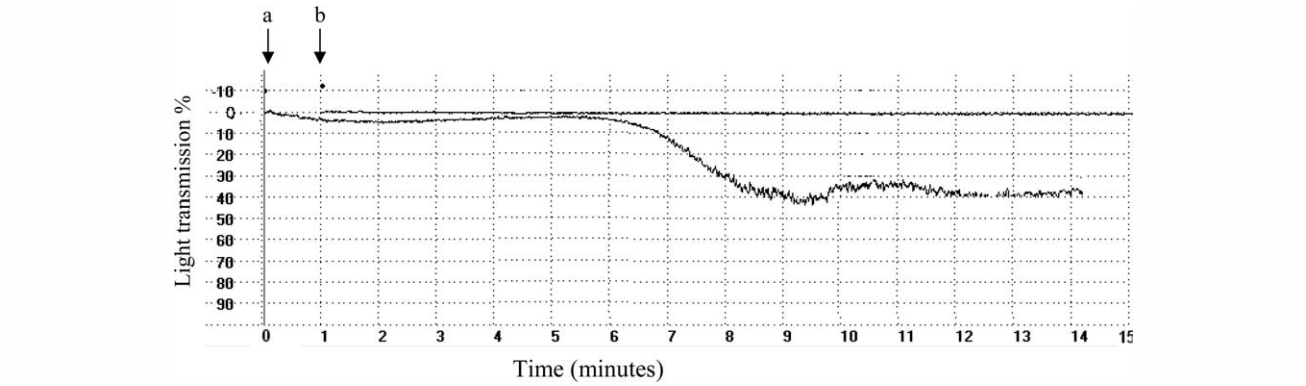

Figure 2. Platelet aggregation in the presence of IgMκ. Optical transmission changes were recorded for 20 min after adding 100 μg/mL of IgMκ from our patient (a) or a control patient (b) to a washed control platelet suspension under stirring at 37°C. Representative of three experiments.

and Hemostasis criteria was screened by the dilute thromboplastin time index (dTT index: 2.70, normal range <1.2) and the dilute Russel viper venom time (dRVVT) (patient LA1 Screening Reagent/mean normal LA1 Screening reagent ratio: 2.17, normal range <1.2). The use of the LA2 Confirmation Reagent, which is similar to LA1 Screening Reagent but contains a high phospholipid concentration, largely corrects the clotting time, thus confirming the presence of the LAC (normalized ratio 2.2, normal range < 1.2). Factors II, V, VII and X were normal. Despite high dilutions (up to 1:640), factors VIII, IX, XI and XII levels could not be determined with a one-stage modified aPTT assay, because there was strong LAC interference in these assays. Factor IX tested with antigen assay was normal. Chromogenic or antigen assay was not available for other factors. A Bethesda assay showed no evidence for a factor VIII inhibitor. Von Willebrand factor (VWF) antigen (221%) and ristocetin-cofactor activity (207%) were elevated, consistent with an acute phase reaction and not suggestive of von Willebrand disease. Previous surgeries without any complications were reported in the patient's medical file. Thus, a congenital factor deficiency is unlikely. Her serum was positive for various antiphospholipid specificities with a low titer in enzyme-linked immunosorbent assay (ELISA anticardiolipin, antiprothrombin, phosphatidylinositol, and phosphatidylserine). The antinuclear antibodies, anti double stranded DNA, and autoantibodies to extractable nuclear antigens were negative.

Owing to an accelerated erythrocyte sedimentation rate (ESR) without inflammatory syndrome, serum protein electrophoresis and immunofixation were performed and revealed the presence of a monoclonal IgMκ (9.5 g/L). The serum viscosity was normal (1.014 g/mL). There was a weak monoclonal kappa light chain in urine. Results of serum κ and λ free light chains were normal (16.1 and 14.1 mg/L respectively with a κ/λ ratio: 1.14, normal range 0.26–1.65). Total serum value of IgM was increased (8.2 g/L, normal range [0.22–2.93]); serum values of IgG and IgA were normal (10.1 and 0.80 g/L, respectively). Bone-marrow examination was normal, with no lymphoplasmacytoid infiltration.

The monoclonal component was isolated and its potential activity on coagulation tests was assessed. When added to normal plasma, the purified monoclonal IgMκ caused a dose-dependent prolongation of LAC screening test (aPTT, dTT, dRVVT LA1 Screening test) and generated positive Rosner and dTT indexes, and dRVVT screening ratio. In the dRVVT confirmatory steps, we observed a shortening of the clotting time when excess phospholipids were added (positive dRVVT normalized ratio when 100 μg/mL of patient IgMκ was added to normal plasma) (see Fig. 1). The monoclonal IgMκ of 3 control patients with Waldenström's macroglobulinemia were studied, and no LAC activity was observed when they were tested under the same conditions. Such an association between monoclonal Ig and LAC activity has mainly been reported in patients with Waldenström's macroglobulinemia [1–3] or lymphoma [4–7] (Table I) without any clinical complications. Intriguingly, two myeloma patients were symptomatic, one with bleeding events [4] and the other with deep venous thrombosis [9]. But in comparison with those cases, no myeloma or lymphoma was found in our patient. Coexistence of LAC and acquired hemophilia was described in a monoclonal gammopathy of unknown significance (MGUS) patient [10]. Our patient had no detected specific factor VIII inhibitor in a Bethesda assay. Hemorrhagic symptoms associated with the presence of antiphospholipid antibodies have been mainly reported in the presence of decreased prothrombin levels [11,12]. Our patient's factor II activity was normal but the purified monoclonal IgMκ had weak antiprothrombin specificity in ELISA. The other phospholipid-antigen specificities tested, i.e., β_2-glycoprotein I, cardiolipin, cardiolipin + β_2-glycoprotein I, phosphatidylcholine, phosphatidylinositol, phosphatidylserine, and sphingomyelin, were negative (when tested using the purified monoclonal IgMκ protein).

Monoclonal immunoglobulins, high amounts of immunoglobulins or high viscosity can cause nonspecific interference with coagulation, causing PT and/or aPTT prolongations, prolongations in LAC screening assays (dilute phospholipid assays), noncorrection in mixing studies, difficulty in determining factor level, and if viscosity is increased, bleeding. However, viscosity is normal in this case, and the positive dRVVT confirmatory assay is consistent with LAC rather than nonspecific interference.

Platelet dysfunctions were described in plasma-cell dyscrasia, particularly when associated with a monoclonal IgM [13]. The abnormal bleeding after dental extraction in the setting of normal VWF activity and prolonged bleeding time led us to study patient's platelet function by light-transmission aggregometry (LTA) in platelet-rich plasma (PRP), as recommended for the diagnosis of bleeding disorders [14]. Spontaneous platelet aggregation (not

shown) prevented any further evaluation of platelet sensitivity to various agonists. Therefore, we assessed the effect of adding our patient's IgMκ (100 μg/mL) to washed platelets from normal subjects. It induced delayed aggregation (see Fig. 2) whereas the IgMκ from the three controls tested under the same conditions had no impact on platelet aggregation. Surprisingly, the monoclonal component seems to have induced platelet-activation rather than inhibition, as reported previously [13,15]. This effect of the monoclonal component on platelet could modify platelet sensitivity to physiological agonist. Thus, a link between patient's transient bleeding disorder and impaired platelet function induced by the monoclonal IgMκ cannot be excluded.

Discovery of a LAC activity concomitant with the presence a monoclonal gammopathy, although usually asymptomatic or associated with thrombotic complications, does not exclude a bleeding risk. Careful clinical and laboratory evaluation is mandatory to evaluate that risk, especially when the patient is exposed to any kind of surgery.

Methods

Patient and controls. Our 71-year-old female patient provided informed consent for this study. Four control subjects (blood donors from the Etablissement Français du Sang), who had not taken any medication during the 10 days preceding blood sampling, were enrolled with their written informed consent.

Sample collection and biological assays. Blood was collected from the antecubital vein on EDTA with Vacutainer® system (Becton Dickinson, Franklin Lakes, NJ) for hematocrit and blood-cell counts determined on an XE2100 automatic cell counter (Sysmex corporation, Kobe, Japan), and on 0.129 M trisodium citrate (9:1) for PFA100 and clotting tests. Blood specimens were analyzed within 1 h after collection.

The PFA100 system is a high-shear system that simulates primary hemostasis after injury to a small vessel [16]. Whole citrated blood samples were analyzed with both PFA100 cartridges containing a nitrocellulose membrane with a central aperture (Siemens, Marburg, Germany). One cartridge is coated with collagen and 10 μg of epinephrine, the other is coated with collagen and 50 μg of adenosine 5-diphosphate. The system measures closure time, i.e., the time required to obtain full occlusion of the membrane aperture. This time is dependent on platelet activation and aggregation.

Bleeding time was determined with the Surgicutt device (International Technidyne Edison, NJ).

For clotting tests, blood samples were centrifuged at 2,500 g for 20 min at 12°C to obtain platelet-poor plasma (PPP). Coagulation tests (prothrombin time, activated thromboplastin time and fibrinogen, and measurement of coagulation factor II, V, VII, and X levels) were performed on an STA analyzer using standard techniques. Factors VIII, IX, XI, and FXII were determined on KC10μ (Amelung, North Rhine-Westphalia, Germany) using a one-stage modified aPTT assay with CK-Prest cephalin (dilution 1:8) and factor-depleted plasmas (Diagnostica Stago, Asnières, France).

Factor IX antigen was determined using Asserachrom IX: AG (Diagnostica Stago).

After a second centrifugation, PPP was aliquoted and stored at −80°C; it was used later to perform LAC assays, VWF antigen (STA-liatest VWF; Diagnostica Stago) and VWF ristocetin-cofactor activity (VWF: RCo) on an optical aggregometer (Regulest Aggregometer, AFFI-Bio, Nancy, France). For LAC testing, mixing aPTT test with 1:1 dilution of the patient's plasma was performed with normal lyophilized plasma (CoagNorm, Diagnostica Stago). The Rosner index was calculated as followed: (mixing aPTT test—normal plasma aPTT)/patient aPTT. The dTT index was determined with 1:500 diluted Neoplastin CI (Diagnostica Stago) and calculated as followed: mixing dTT test/normal plasma dTT. The dRVVT (LA1/LA2, Siemens, Marburg, Germany) was carried out according to the manufacturer's recommendations. Briefly, the LA1 Screening Reagent clotting time being more than 2 SD longer than the mean of normal plasma, the LA2 Confirmation Reagent time was measured. LA2 Confirmation Reagent was similar to LA1 Screening Reagent but contains a high phospholipid concentration, which counteracts the LAC antibody and largely corrected the clot time. Results were expressed in normalized ratio [(patient LA1 Screening Reagent/mean normal LA1 Screening reagent) × (mean normal LA2 Confirmation Reagent/patient LA2 Confirmation reagent)]. If ratio was greater than 1.2, LAC was present.

For platelet function, PRP was obtained after a 10-min centrifugation of whole blood at 100 g at 20°C. The platelet count was adjusted to 2.5×10^8/mL with autologous PPP and then studied by LTA. Platelet aggregation was quantified as the percentage of maximal optical transmission change. Spontaneous platelet aggregation in PRP under stirring (1,100 rpm) at 37°C prevented any further evaluation of platelet sensitivity to the different agonists usually tested [14]. Control subjects' platelets were isolated from acid citrate dextrose (BD Vacutainer®) anticoagulated whole blood. Briefly, PRP were obtained as described above and then consecutively subjected to differential centrifugations in presence of 0.2 μM prostaglandin E1 and 0.06 IU/ml of apyrase (Sigma Aldrich, Saint Louis, MO). The platelet pellets were resuspended at 2.5×10^8 platelets/mL in 10 mM Hepes pH 7.35, 140 mM NaCl, 3 mM KCl, 5 mM $NaHCO_3$, 0.5 mM $NaHCO_3$, and 10 mM glucose. Platelet stability was controlled under stirring at 37°C in the absence of any inducer at the beginning and the end of each experiment. IgMκ 100 μg/mL from our patient or a control were added to 0.250 mL of platelet suspension in the aggregometer under stirring (1,100 rpm) at 37°C. Platelet aggregation was quantified as the percentage of maximal optical transmission change recorded 20 min after addition of IgMκ. Primary platelet-aggregation slopes and maximal aggregation were analyzed.

The monoclonal component was isolated using the euglobulins property to precipitate in low ionic force medium [17]. Purity of isolated paraprotein was assessed by electrophoresis and by immunofixation, which demonstrated that the purified protein had the same migratory properties as the original.

The antiprothrombin activity of the purified monoclonal component was detected with Asserachrom anti-prothrombin, (Diagnostica Stago). The other phospholipid antigen specificities, i.e. β_2-glycoprotein I, cardiolipin + β_2-glycoprotein I, phosphatidylcholine, phosphatidyl inositol, phosphatidylserine and sphingomyelin were determined with AESKULISA Phospholipid-8PRO-GM (Aesku Diagnostics, Wendelsheim, Germany).

Acknowledgments

The authors are grateful to Dr A. Appert-Flory for patients' platelet-function studies by light-transmission aggregometry in platelet-rich plasma.

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Conflict of interest: Nothing to report.
Published online 21 June 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22113

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Treatment of vitamin D deficiency in transfusion-dependent thalassemia

Ellen B. Fung,* Catherine Aguilar, Ida Micaily, Drucilla Haines, and Ashutosh Lal

The survival of patients with thalassemia major has progressively improved with advances in therapy; however, osteoporosis remains a frequent, unresolved issue [1]. Adequate circulating levels of vitamin D are essential for optimal skeletal health and reducing fracture risk [2]. Vitamin D insufficiency is reported in the majority of patients with thalassemia in the USA [3] and elsewhere [4–10], despite routine prescription of 400–1,000 IU vitamin D per day. In this study, assessment of serum 25-hydroxy vitamin D (25-OH D) levels in 96 patients with thalassemia revealed that 70 (73%) were either deficient (<20 ng/ml, 43%) or insufficient (20–29 ng/ml, 30%). Significantly more transfusion-independent patients were deficient compared with the transfusion-dependent group (60% versus 33%, $P = 0.014$). Supervised administration of high-dose (50,000 IU) oral vitamin D_2 every 3 weeks during transfusion visits in 32 transfusion-dependent patients increased the 25-OH D level from 18.4 to 24.2 ng/ml ($P < 0.001$) over a 4-month period. Each dose of vitamin D_2, given at 3-week intervals, increased 25-OH D levels by 1.4 ± 2.0 ng/ml. These results show that vitamin D deficiency remains widespread despite daily low-dose supplementation. Supervised high-dose oral vitamin D supplementation is a safe and noninvasive method for predictable improvement of vitamin D status in thalassemia.

The risk of vitamin D deficiency in thalassemia increases with age [9,10], and older patients with thalassemia have significantly worse vitamin D status compared with age-matched healthy controls [10]. One-third of healthy adults consuming vitamin D-fortified milk and multivitamin supplementation remain vitamin D-deficient [11]. Similarly, despite greater awareness and routine prescription of daily vitamin D, the problem of vitamin D deficiency in thalassemia remains intractable. The alternative to daily supplementation is intermittent supervised therapy with high-dose vitamin D [12]. Oral therapy is desirable to maintain long term acceptance of the therapy. The objective of this study was to evaluate the effect of high-dose (50,000 IU) oral vitamin D_2 administered at the time of transfusion on serum levels of 25-OH D.

We screened 96 patients between 3.6 and 57.5 years of age (mean ± SD: 25.2 ± 12.9 years; Table I-online material) with various types of thalassemia for vitamin D status. Exactly half of this sample was male and 61 (64%) of the patients were transfusion-dependent. There were significantly more patients with Asian ethnic background in the transfusion-independent than in the transfusion-dependent category (80% vs. 62.3%, $P = 0.002$). Serum 25-OH D levels were sufficient in only 26 (27%) patients, whereas 41 (43%) were deficient and 29 (30%) were insufficient. There were no significant differences in age, gender, or season of sample collection between those with deficient and sufficient levels of 25-OH D. The mean parathyroid hormone level in patients with 25-OH D<20 ng/ml was 38.6 ± 21.4 pg/ml compared with 27.3 ± 12.6 pg/ml in those with 25-OH D ≥ 20 ng/ml ($P < 0.001$).

TABLE I. Baseline Characteristics of Subjects with Thalassemia ($n = 96$)

Mean age, years	25.2 (range: 3.6–57.5)
Gender	
Female	48
Male	48
Ethnic group, Asian	66 (69%)
Caucasian	23 (24%)
Other/mixed	7 (7%)
Type of thalassemia	
β or E,B thalassemia transfused	61 (63.5%)
β or E,B thalassemia non-transfused	13 (13.5%)
Hb H or H Constant Spring	22 (22.9%)
Mean 25-OH vitamin D (ng/ml)	23.9 (range: 5–68)
Mean Parathyroid hormone (pg/ml)	31.8 (range: 6–115)

Mean ± SD. Frequency (% of total or range provided) is given either in range or percentage.

There was a trend toward a lower mean 25-OH D level among patients with Asian ethnic background (mean 22.4 ± 12.3 ng/ml) compared with Caucasian ethnic background (26.8 ± 9.7 ng/ml, $P = 0.12$). Additionally, deficient vitamin D status was significantly more prevalent in patients with Asian ethnic background than the Caucasian ethnic group (56% vs. 9.7%, $P = 0.002$). There was no difference in the mean 25-OH D level among patients with Hemoglobin H or Hemoglobin H Constant Spring disease (mean 22.2 ± 12.9 ng/ml), and those with other types of nontransfusion-dependent thalassemia (23.2 ± 12.9 ng/ml, $P = 0.82$), or transfusion-dependent thalassemia (24.6 ± 10.8 ng/ml, $P = 0.39$). However, a majority (60%) of the nontransfused group had deficient levels of 25-OH D compared with the transfusion-dependent group (32.8%, $P = 0.014$, Fig. 1).

Thirty-two transfusion-dependent patients with 25-OH D <30 ng/ml were placed on intermittent high-dose oral vitamin D_2 supplementation for a total of 66 unique supplementation periods. These patients received a mean of 5 (1–15) doses of 50,000 IU vitamin D_2 over 129 (14–521) days. The mean daily dose of vitamin D_2 delivered according to this protocol was 2,118 IU/day. The baseline 25-OH D level increased from 18.4 ± 5.9 ng/ml to 24.3 ± 8.8 ng/ml following supplementation ($P < 0.001$, Fig. 2). Administration of

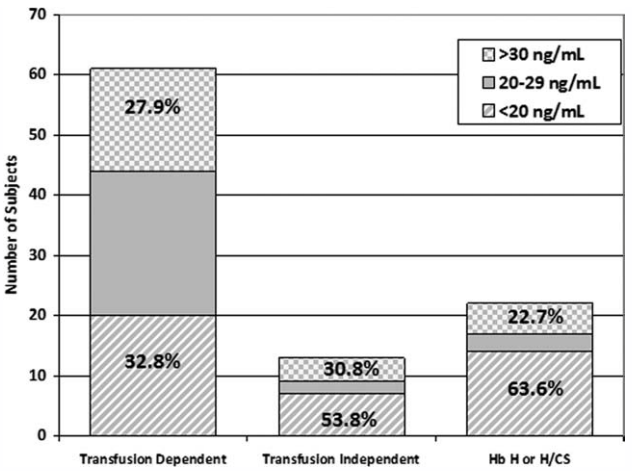

Figure 1. Categorization of patients with transfusion-dependent thalassemia ($n = 61$: β-thalassemia 43, E-β thalassemia 18), transfusion-independent ($n = 35$: β-thalassemia 8, E-β thalassemia 5, hemoglobin H or hemoglobin H Constant Spring disease 22) by baseline vitamin D level. Vitamin D sufficiency (serum 25-OH $D \geq 30$ ng/ml) is noted by hatched bars, insufficiency (20–29 ng/ml) by gray bars, and deficiency (<20 ng/ml) by striped bars. There was a significant difference in the prevalence of vitamin D deficiency by transfusion status ($P < 0.014$).

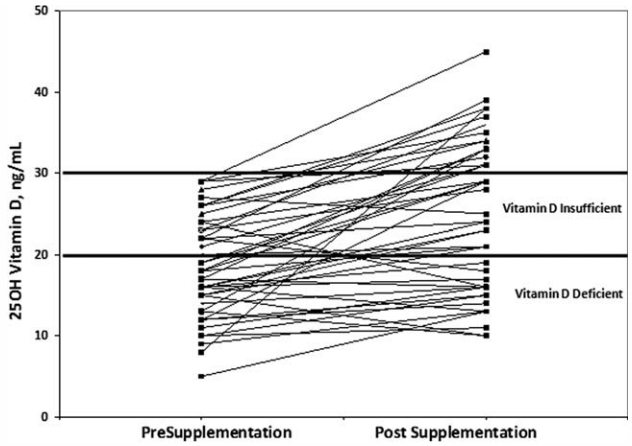

Figure 2. Serum 25-hydroxy vitamin D levels before and after supplementation with intermittent high-dose oral vitamin D_2 (50,000 IU given every 3 weeks) in transfusion-dependent thalassemia (32 patients, 66 unique observations). The average length of supplementation was 130 ± 93 days. The two horizontal lines represent vitamin D deficiency (<20 ng/ml) and insufficiency (<30 ng/ml).

each dose of 50,000 IU vitamin D_2 increased serum 25-OH vitamin D by 1.4 ± 2.0 ng/ml. Regardless of the baseline vitamin D level or the duration of the supplement regimen, no 25-OH D level >80 ng/ml was observed over the course of the observation period.

There were 18 individuals who attained a 25-OH D level >30 ng/ml at the end of their supplementation period and continued daily supplementation of 400–1,000 IU vitamin D thereafter. When retested at an average of 8 months (1–21 months) later, the serum 25-OH D had dropped significantly and collectively for all but two of them, from a mean of 34.4 ± 3.7 to 26.9 ± 6.8 ng/ml ($P < 0.001$). The rate of decline was on an average 1.5 ng/ml per month. Hence, patients who had inadequate vitamin D status on screening were likely to require ongoing high-dose supplementation. In contrast, the 35 patients who had normal vitamin D status on screening did not show a similar decline in 25-OH D when maintained on daily oral low-dose supplementation. Twenty (57%) had a decrease in their 25-OH D level by ≥5 ng/ml, whereas 15 (43%) had either stable or higher level during follow up.

Our data demonstrate that the problem of inadequate vitamin D status in thalassemia has persisted despite routine daily supplementation of 400–1,000 IU vitamin D. Though hyperparathyroidism in thalassemia is rare, vitamin D deficiency was correlated with increased parathyroid hormone level in this and a previous study [12]. Identifying the optimal strategy for replacing vitamin D in patients with thalassemia is critical because adequate circulating levels of vitamin D are essential for optimal skeletal health and reducing fracture risk [13]. A significant negative correlation exists between 25-OH D levels and spine bone mineral density in thalassemia [1]. Moreover, the association between vitamin D deficiency and myocardial iron deposition and cardiac ejection fraction in thalassemia is of increasing interest [4,14]. Patients with elevated myocardial iron were noticed to have a 40% higher level of serum parathyroid hormone [4]. Removing this modifiable risk factor may improve long-term bone health and quality of life in aging patients with thalassemia.

In our experience, the intermittent high-dose supplementation with oral 50,000 IU vitamin D_2 every 3 weeks proved to be an effective and safe strategy for increasing 25-OH D levels. One other group has studied the efficacy of high-dose vitamin D_3 (10,000 IU/kg) in thalassemia given as a single intramuscular injection. Although a majority of the patients showed an improvement in 25-OH D level to >20 ng/ml, the effect did not persist at 6 months [12,15].

This observation is similar to our own data which suggest that repeated courses of high-dose vitamin D will be necessary for most individuals with thalassemia who are diagnosed with vitamin D deficiency. Even patients with normal vitamin D status on screening were at risk of developing insufficiency during followup visits during our study. Though seasonality and assay variability can affect measurements, we suggest that 25-OH D levels should be monitored annually in all patients with thalassemia.

A 4-month-period of supplementation proved inadequate to bring the 25-OH D to a level >30 ng/ml for many patients in our study. Since the average increment in serum level of 25 OH-D with each 50,000 IU dose of vitamin D_2 administered at 3-week-intervals was 1.4 ng/ml, a patient with a starting 25-OH D level of 10 ng/ml would require 14 or more doses to achieve sufficiency. Patients with severe deficiency may benefit from larger doses of vitamin D than used in this report. While both vitamin D_2 and vitamin D_3 are efficacious, the latter possesses three times more biological activity in maintaining serum 25-OH D levels [16]. Thus, the dose-effect of 50,000 IU vitamin D_2 observed by us may be replicated by a lower dose of vitamin D_3. The difference in potency is likely to be clinically unimportant since the supplement is being provided as repeated intermittent doses, instead of a single large dose.

Transfusion-independent patients, especially those with Hemoglobin H and Hemoglobin H Constant Spring disease, were at particularly high risk for vitamin D deficiency. These patients have fewer clinic visits and receive less nutritional counseling compared with transfusion-dependent patients. A combination of poor dietary and supplemental intake, darker skin, or less sun exposure could place them at increased risk for deficiency. Optimal strategies for vitamin D supplementation in nontransfused patients should be addressed in future studies.

The present study shows for the first time the efficacy and tolerability of intermittent high-dose oral vitamin D supplementation in thalassemia given in a simple, noninvasive regimen that is convenient and acceptable to patients. This regimen, provided at the time of transfusion, alleviates burden to the patient (daily dosing) and assures adherence for the clinician (supervised therapy). The safety of this regimen is demonstrated by the nontoxic levels of 25-OH D at the end of the period of supplementation. The average daily dose of vitamin D_2 used in this study (2,100 IU/day) is lower than the upper limit for children and adults, which was recently revised by the U.S. Institute of Medicine to 4,000 IU/day for all forms of vitamin D [13]. We recommend that 25-OH D levels should be monitored every 6 months in patients on high-dose supplementation to ensure adequacy of therapy and to monitor for toxicity.

Methods

Patients with thalassemia attending Children's Hospital & Research Center, Oakland (CHRCO) are routinely prescribed daily vitamin D 400–1,000 IU and checked for adequacy of vitamin D status every year. Beginning in January 2007, new treatment guidelines recommended supervised therapy with 50,000 IU of ergocalciferol (vitamin D_2) in the form of a gel capsule on the day of the transfusion (every 3 weeks) for patients with low vitamin D status. Blood samples were obtained at baseline and after 4–6 months of supplementation to calculate the rate of correction of serum 25-OH D level. If the 25-OH D level remained <30 ng/ml at the end of this period, an additional cycle of supplementation was begun. The post-supplementation 25-OH D level was obtained within 3 months of the last dose of vitamin D_2.

Data collected from chart review included age, gender, type of thalassemia, and number of doses of vitamin D supplement. The 25-OH D level was measured using a chemiluminescent immunoassay (ARUP, Salt Lake City, Utah), which quantifies the sum of 25-OH D_2 and 25-OH D_3. The serum 25-OH D level was used to define abnormal vitamin D status as deficiency (<20 ng/ml), insufficiency (20–29 ng/ml), sufficiency (30–80 ng/ml), and toxicity (>80 ng/ml). This study was considered exempt by the Institutional Review Board as all information was de-identified prior to statistical review.

Data were analyzed using STATA 9.2 (College Station, TX), and the *P*-value <0.05 was considered statistically significant. Paired *t*-test was performed to evaluate the efficacy of high-dose intermittent vitamin D supplementation, and other differences by diagnostic category in continuous variables. Chi-squared test was used to describe differences in the prevalence of vitamin D deficiency by gender, race, diagnostic category, and transfusion status.

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Grant sponsor: Maternal and Child Health Bureau, Health Resources and Services Administration, Department of Health and Human Services; Grant number: 57480
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Conflict of interest: Nothing to report.
Published online 27 June 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22117

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Cyclophosphamide and prednisone induction followed by cyclophosphamide mobilization effectively decreases the incidence of engraftment syndrome in patients with POEMS syndrome who undergo stem cell transplantation

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High-dose chemotherapy with autologous stem cell transplantation (ASCT) can achieve excellent clinical responses in patients with POEMS syndrome (Jimenez Zepeda et al., Blood 2010;116:2403; Gertz et al., Am J Hematol 2005;79:319–328; Gherardi et al., Ann Neurol 1994;35:501–505; Gattinoni et al., Nat Rev Immunol 2006;6:383–393; Salem et al., J Immunol 2009;182:2030–2040; Salem et al., Cancer Immunol Immunother 2010;59:341–353; Salem et al., Cell Immunol 2010;261:134–143). However, High-dose melphalan with ASCT should be considered carefully due to its treatment-related morbidity (Vuckovic et al., Blood 2003;101:2314–2317), especially in patients with poor performance status owing to polyneuropathy and multiorgan involvement, such as cardiac, respiratory, and renal failure. Significant increases in the concentration of circulating macrophage colony-stimulating factor, erythropoietin, IL-6, and TNF-α, reach near maximal values at approximately day +12, predating neutrophil engraftment, and clinically manifest with fever, rash and edema (Dispenzieri et al., Eur J Haematol 2008;80:397–406). Depending on the definition used, ∼50% of patients satisfied criteria for engraftment syndrome (ES) (Vuckovic et al., Blood 2003;101:2314–2317). ES occurs in 27–47% of patients who undergo ASCT; mortality rate is reported from 8% to 18% (Gattinoni et al., Nat Rev Immunol 2006;6:383–393; Vuckovic et al., Blood 2003;101:2314–2317). We have therefore reviewed our experience with ASCT in patients with POEMS syndrome who were treated with cyclophosphamide and prednisone as induction therapy followed by cyclophosphamide mobilization with an emphasis on treatment-related morbidity and frequency of ES. Our study confirms that ASCT is a feasible and efficacious treatment for patients with POEMS syndrome. In addition, the use of CP followed by cyclophosphamide mobilization decreases the incidence of PES leading to less morbidity and mortality rates.

Between March 2000 and February 2011, Eight patients with POEMS syndrome underwent autologous stem cell transplantation (ASCT) at our Institution. Of the eight patients transplanted, 75% were female. Patient characteristics are shown in Table I. Polyneuropathy, edema, organomegaly, endocrine changes, and sclerotic lesions were present in all patients. Splenomegaly was seen in three cases. (37.5%) Median time to transplant from onset of symptoms was 31 months. Eastern cooperative group performance score was 3 in one patient and 1 or 2 in the remainders. Pulmonary function showed decreased diffusion capacity for carbon monoxide (DLCO $<$ 70%) in one patient (12.5%). All patients received oral cyclophosphamide and prednisone (CP) before undergoing ASCT; a median of three cycles was given (ranging from 3 to 7). Peripheral blood stem cells were collected using intravenous cyclophosphamide (2.5 g/m^2) and granulocyte colony-stimulating factor (G-CSF). The median number of CD34 cells collected was 4.99 $\times$ 10^6/kg (range 2.73–9.95) and the median number of stem cell apheresis required was 2 (1–4). Six patients were conditioned using Melphalan 200 mg/m^2, and two received melphalan 140 mg/m^2. Standard supportive care with prophylactic antibiotics was provided to all patients. Transplanted patients had a median time to higher or equal to ANC 0.5 $\times$ 10^9/L of 12 days (range 11–14). The time to platelets higher or equal to 20 $\times$ 10^9/L was

TABLE I. Clinical Characteristics of Patients With POEMS Syndrome who Undergo ASCT

Clinical characteristic (N = 8)	Median	Range	%
Age (years)	57	42–69	
Hemoglobin (g/L)	127	97–162	
Creatinine (μmol/L)	83	53–103	
Male			25
Female			75
IgG			12.5
IgA			87.5
Kappa			0
Lambda			100
Polyneuropathy			100
Sclerotic lesion			100
Organomegaly			100
Edema			100
Endocrine disease			100
Castleman's			25
Thrombocytosis			37.5
Splenomegaly			37.5
Abnormal PFT (DLCO $<$ 70%)			12.5

PFT, pulmonary function test.

TABLE II. Induction Therapy and Transplant Related Outcomes for Patients With POEMS Syndrome who Undergo ASCT

Characteristic ($N = 8$)	Median	Range	%
Cyclophosphamide and Prednisone (cycles)	3	3–7	100%
Time to ANC $\geq 0.5 \times 10^9$/L (days)	12	11–14	
Time to platelets $\geq 20 \times 10^9$/L (days)	14	14–22	
Time to discharge (days)	19	16–24	
Engraftment syndrome			0%
Fever			37.5%
Diarrhea			12.5%
Weight gain $\geq$3% of baseline			25%
Rash			50%
Transplant-related mortality			0%

ANC, absolute neutrophil count.

TABLE III. Response Assessment and Outcomes for Patients With POEMS Syndrome Who Undergo ASCT

Characteristic ($N = 8$)	%
Peripheral neuropathy improvement	100
Performance status improvement	100
*Overall response rate ($\geq$PR)	87.5%
Complete hematological response	62.5%
Partial hematological response	25%
Disease-free survival at 3 years	100%
Overall survival at 3 years	100%

*Response assessment at 3 months post ASCT.

14 days (range 14–22). Median time to discharge was 19 days (range 16–24). Most importantly, no patients exhibited engraftment syndrome (ES). At a median follow-up of 54 months (4–166), all patients are alive and progression-free with the exception of one case exhibiting progression at 100 months from transplant. Treatment-related mortality with this approach was 0%. (Table II) The median number of platelet and erythrocyte transfusions was three apheresis units (1–5) and four units (0–8), respectively [similar to that seen in multiple myeloma (MM) and AL transplants at our center] [1]. All patients were admitted to the hospital for ASCT. Rates of fever, diarrhea, weight gain $\geq$3% of baseline and rash were occurring in 37.5%, 12.5%, 25%, and 50% of patients, respectively. Grade 2 oral mucositis was reported in two patients (25%). No patients required temporary or permanent dialysis. Only one patient required supplemental oxygen due to increase in preexisting pleural effusion. Three patients developed fever and blood cultures were persistently reported as negative. According to response criteria cited by light chain amyloidosis, [2] 62.5% have achieved a complete HR, and 25% attained PR. Free light-chain assays were abnormal in six patients and only two patients exhibited a higher than 10 g/L of monoclonal protein in serum. All evaluable patients have derived clinical benefit, including those who achieved less than PR (Table III). Only one patient received bolus corticosteroids as prophylaxis for ES due to high-risk features including abnormal pulmonary function test and splenomegaly. At a median follow-up of 54 months (4–166), all patients are alive. One patient progressed at 100 months and received oral CP re-treatment exhibiting complete response after 16 months and remains on observation only. Treatment-related mortality with this approach was 0%. Peripheral neuropathy improved in all of the cases and in some, it dramatically leaded to a change on the performance status (PS). Before treatment with CP was given, three patients were not considered eligible for transplant but after a median of three cycles of treatment their PS dramatically improved and became transplant-eligible. No patients have developed long-term complications related to ASCT. It has been suggested that pleiotropic cytokines, which act in synergy on the immune, nervous, and endocrine systems, could play a pathogenic role in POEMS syndrome [3]. Increased serum levels of interleukin-6 (IL-6) have been occasionally reported in patients with POEMS syndrome, but whether the finding was relevant to POEMS syndrome itself or to an associated Castleman's disease was debated. Gherardi et al. reported that serum concentrations of IL-1B, TNF-α, and IL-6, but not IL-2 and IFNγ, were higher in POEMS syndrome than in multiple myeloma (MM) without neuropathy [3]. These results confirmed an increased release of pro-inflammatory cytokines in patients with POEMS syndrome [3]. The strong activation of the pro-inflammatory cytokine network in POEMS syndrome and the imbalance between productions of cytokines and their antagonists support the view that cytokines may be implicated in the expression of the disease. Given the significance of cytokines in the pathogenesis of POEMS, new therapeutic approaches are targeted to these cytokines. Cyclophosphamide is one of the drugs that has been shown to be effective to decrease the pro-inflammatory cytokine network and also to effectively augment the anti-tumor efficacy of adoptively transferred T cells [4]. Lymphodepletion induced by cyclophosphamide increases the relative and absolute numbers of dendritic cells with an immature phenotype during the restoration phase after cyclophosphamide-induced lymphodepletion [5–7]. This observation is consistent with recent studies that reported increases in number of dendritic cells during the restoration phase in the peripheral blood of cancer patients receiving combinatorial treatment with cyclophosphamide and the growth factors [8]. The potential clinical significance of this observation is evidenced by the substantial increases in the tumor efficacy of adoptively transferred CD8+ T cells. In this study we aimed to evaluate the role of CP as induction therapy in decreasing the incidence of ES after ASCT. Interestingly, we were able to decrease the rate of fever, diarrhea, weight gain $\geq$3% of baseline and rash from 93%, 77%, 53%, and 43% reported by Dispenzieri et al. [9] to 37.5%, 12.5%, 25%, and 50% of patients, respectively, by using CP induction with a median of three cycles followed by cyclophosphamide mobilization. ES prophylaxis was given in only one patient considered to be high risk due to the presence of splenomegaly and pulmonary function test abnormalities. Treatment-related mortality with this approach was 0% and at a median follow-up of 74 months, all patients are alive and progression-free with the exception of one patient who progressed at 100 months after ASCT. Our study confirms efficacy and feasibility of ASCT in the treatment of patients with POEMS syndrome. The use of cyclophosphamide either as induction or mobilization is responsible for a decrease in the incidence of ES. Whether CP or cyclophosphamide mobilization is directly responsible for this benefit remains to be elucidated. Dispenzieri et al. showed a significant decrease of ES in patients receiving cyclophosphamide either as part of previous regimens or mobilization (0/3 and 0/5, respectively) [9] which encourage the use of cylophosphamide before ASCT. In addition, oral CP is a well-tolerated regimen with minimum toxicity which was able to give a great clinical benefit in all the patients treated and transformed the transplant candidacy in three cases after a median of three cycles. Our findings raise many questions as answers, but provide insights about the use of novel strategies aiming to decrease morbidity associated to ASCT. We speculate that the use of cyclophosphamide before ASCT may modulate marrow reconstitution, decreasing the incidence of ES, and subsequently lead to a reduction in peri-transplant morbidity and mortality. Based on this report, we may consider using immunomodulatory drugs or proteasome inhibitors as induction therapy known to have anti-proliferative, anti-cytokine, and anti-angiogenic properties. Larger studies with correlative biology are necessary.

Patients and Methods

Between March 2000 and February 2011, 16 pts with POEMS syndrome were seen at Princess Margaret Hospital (PMH). Eight patients underwent ASCT, and we conducted a retrospective chart review approved by the Institutional Review Board at Princess Margaret Hospital. Peripheral blood stem cells were collected using cyclophosphamide and G-CSF.

POEMS syndrome definition. A diagnosis of POEMS was given according to the Mayo Clinic Criteria [10]. POEMS syndrome was defined by the presence of a peripheral neuropathy (P), a monoclonal plasma cell disorder (M), and other paraneoplastic features, the most common of which include organomegaly (O), endocrinopathy (E), skin changes (S), papilledema, edema, effusions, ascites, and thrombocytosis. Virtually all patients will have either sclerotic bone lesions (s) or co-existent Castleman's disease. Not all features of the disease are required to make the diagnosis.

Treatment schedule and mobilization. All patients received oral cyclophosphamide (300 mg/m^2) and prednisone (100 mg every other day) (CP) before undergoing ASCT. Standard supportive care with prophylactic antibiotics was provided to all patients. As part of the ASCT the stem cells were obtained from the peripheral blood with G-CSF (5 μg per kilogram of body weight subcutaneously, twice daily) and intravenous cyclophosphamide (2.5 g/m^2). Patients were conditioned, infused, and monitored on an inpatient basis and were hospitalized for these purposes. Patients received HDM, given intravenously on

day −1, and stem cells were infused on day 0. Pre-transplant assessments were performed according to our center guidelines and data was extracted from the medical charts.

ES criteria. Spitzer and Maiolino (Bone Marrow Transplant 2001 and 2003) criteria were used for defining ES [11,12]. Spitzer criteria included major and minor features. The major criteria are temperature >38.3C with no identifiable infectious etiology; erythroderma involving >25% of body surface area and not attributable to a medication and non-cardiogenic pulmonary edema and hypoxia. The minor criteria are: hepatic dysfunction as characterized by a bilirubin >2 mg/dL or a doubling of the serum creatinine; weight gain ≥2.5% over baseline body weight or transient encephalopathy. To be classified as ES, all three major criteria or two major and one minor criteria are required within 96 hr of neutrophil engraftment [absolute neutrophil count (ANC) 0.5×10^9/L]. The second published definition for ES used was that by Maiolino et al., which requires fever within 24 hr of first appearance of neutrophil along with any of the following: cutaneous rash, pulmonary infiltrates, or diarrhea. A third engraftment entity examined was the periengraftment respiratory distress syndrome. This is defined as fever >38.3°C and evidence of pulmonary injury in the form of hypoxia and/or pulmonary infiltrates on chest radiographs in the absence of clinical cardiac dysfunction that has to occur within 5 days of neutrophil engraftment [13].

Response assessment. The assessments of M protein by serum and urine protein electrophoresis, serum, and urine immunofixation electrophoresis, serum-free light chain assay, and quantitation of light chain in 24-hr urine samples were performed every 3 months. A hematologic response was defined according to the response criteria cited by light-chain amyloidosis [2]. Briefly, a complete hematologic response (CR) was defined as the complete disappearance of the monoclonal immunoglobulin or light chain in a serum or urine specimen; a partial hematologic response (PR) was defined as >50% reduction in these proteins; a progressive disease (PD) was defined as >50% increase in these proteins or the reappearance of the proteins after CR; and a stable disease (SD) included all the status other than CR, PR, and PD.

Statistical analysis. Endpoints for the analyses included: ES, need for corticosteroid bolus during transplant course and time from ASCT to hospital discharge. Demographic and baseline clinical and laboratory data were recorded. Fisher's exact test was used to test differences in categorical variables. For the purpose of outcomes analyses, transplant related events included radiographic changes, fever, weight gain, diarrhea, positive blood cultures, admission to the intensive care unit and administration of bolus corticosteroids. Survival was calculated from the time of transplant and survival curves were constructed according to the Kaplan-Meier method. A value of $P < 0.05$ was considered to be statistically significant.

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Conflict of interest: Nothing to report.
Published online 27 June 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22115

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Circulating cytokine levels, Epstein-Barr viremia, and risk of acquired immunodeficiency syndrome-related non-Hodgkin lymphoma

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Cytokine dysregulation and decontrol of Epstein-Barr virus (EBV) latency by human immunodeficiency virus (HIV) infection are potential mechanisms for acquired immunodeficiency syndrome (AIDS)-related non-Hodgkin lymphoma (NHL). We therefore assessed circulating blood levels in pre-diagnosis plasma or serum from 63 AIDS-related NHL cases 0.1–2.0 (median 1.0) years pre-NHL and 181 controls matched for CD4+ T-cell count. Cytokines were measured by Millipore 30-plex Luminex assays and cell-free EBV DNA detected by polymerase chain reaction (PCR). Correlations in multiplex cytokine levels were summarized by factor analysis. Individual cytokines and their principal factors were analyzed for associations with NHL by conditional logistic regression. Cases had higher levels for 25 of the 30 cytokines. In analyses of cytokine profiles, cases had

significantly higher scores for a principal factor primarily reflecting levels of interleukin (IL)-4, IL-5, IL-13, and granulocyte-macrophage colony stimulating factor (four gene products with coordinated transcription in vitro), as well as IL-1alpha. Epstein-Barr viremia was not significantly associated based on 113 evaluable samples without PCR inhibition. We found increases of T-helper Type 2 interleukins and generalized elevations of other inflammatory cytokines and growth factors up to 2 years before AIDS-NHL. Cytokine-mediated hyperstimulation of B-cell proliferation may play a role in AIDS-related lymphomagenesis.

Cytokines are a diverse group of secreted proteins that are produced and regulated by cells of the immune system [1]. Their biological activities are both pleiotropic and redundant (i.e., each cytokine may act upon multiple molecular targets and multiple cytokines may elicit the same cellular response), in part due to overlapping features of their receptors and signal-transduction pathways. Moreover, expression and effects vary depending upon such factors as cellular characteristics, surrounding microenvironment, and concentrations of other cytokines.

Disruption of the cytokine signaling network is a key pathophysiologic feature of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS) [2]. Concomitant with the declining function of CD4 T helper cells, increasing levels of various cytokines stimulate B cell activation and proliferation. Replication of HIV and opportunistic infections, particularly the Epstein-Barr virus (EBV), provides additional B cell stimulation, both by generating cytokine response as well as by direct actions of microbial antigens. Hence, blood levels of cytokines and EBV are markers of HIV disease progression as well as mediators of AIDS pathogenesis.

Apart from interfering with immune function, B cell hyperstimulation also has tropic consequences [3]. Generalized lymphadenopathy is an early sign of HIV infection. Increased DNA replication also generates opportunities for genetic errors, in part due to the lymphocyte-specific mechanisms for chromosomal rearrangement and somatic hypermutation that generate antibody diversity and specificity. Accumulation of errors that provide growth advantage and abrogation of apoptosis can lead to malignant transformation and clonal outgrowth, which manifests as non-Hodgkin lymphoma (NHL). As the initiating factors in this process, alterations of cytokine signaling and EBV control may indicate severity of B cell hyperstimulation and risk of subsequent lymphoma.

Accordingly, we assessed these markers in blood samples obtained prior to diagnosis of AIDS-related NHL. Fifty-six cases had at least one matched control, with mean (median) CD4/mm3 of 176 (85) for cases and 166 (91) for controls (Table I). All but three cytokines (G-CSF, MIP-1beta and GM-CSF) were detectable in at least 50% percent of samples, taking cases and controls together. Geometric means for detectable samples ranged from 1 pg/ml for IL-5 to over 24,000 pg/ml for RANTES (Table II). EBV was detectable in 21 (19%) of 113 sera and acid-citrate-dextrose plasma samples, but the remaining plasma samples with heparin or other anticoagulants could not be assayed due to PCR inhibition.

For 25 of the 30 cytokines, NHL cases had higher levels than their matched controls. The mORs significantly exceeded 1.0 for greater than median IL-6, IL-15, and GM-CSF ($P < 0.01$), as well as IL-1alpha, IL-12p70, IL-13, IP-10, and VEGF ($P < 0.05$) (Table II). EBV PCR-positivity had a mOR of 1.6 (95% CI: 0.5–5.0), based on serum and evaluable plasma samples only.

TABLE I. Selected Characteristics of AIDS-Related Non-Hodgkin Lymphoma Cases and Controls

Characteristic	Cases ($n = 63$)	Controls ($n = 181$)
Male sex, *n* (%)	59 (94)	169 (93)
Age in years, median (IQR[a])	38 (29–44)	38 (32–44)
White race, *n* (%)	51 (81)	151 (83)
CD4 /mm^3, median (IQR)	85 (31–230)	91 (16–252)
Year of NHL, median (IQR)	1994 (1991–1998)	
NHL subtype, *n* (%)		
Diffuse large B-cell	27 (43)	–
Primary brain	11 (17)	–
Burkitt	4 (6)	–
Other/unknown	21 (33)	–

[a]Interquartile range.

Factor analysis identified four principal cytokine factors in controls that had little overlap of salient loadings after rotation. Thirteen cytokines had salient loadings on Factor 1, nine on Factor 2 (including two from Factor 1), seven on Factor 3 (including one from Factor 1 and two from Factor 2), and five on Factor 4 (including one from Factor 1); two cytokines did not have salient loadings on the rotated factors (Fig. 1).

In analyses of the cytokine principal factors, only Factor 4 was significantly associated with risk of subsequent NHL, with mOR 1.5 (95% CI: 1.1–2.2) per unit change. Associations with Factor 4 were similar for cases with blood collected less than ($n = 31$) vs. greater than ($n = 32$) 1.0 year pre-NHL, with mOR 1.5 (95% CI: 0.92–2.5) and 1.6 (95% CI: 0.96–2.6) per unit change, respectively. Associations of the other three principal factors were not statistically significant, with mORs 1.5 ($P = 0.1$) for Factor 1, 1.3 ($P = 0.2$) for Factor 2, and 1.3 ($P = 0.4$) for Factor 3. Analyzing the factors as quartiles, there was a significant linear trend only for Factor 4 with mOR vs. quartile 1 of 2.4 (95% CI: 0.7–8.0) for quartile 2, 2.9 (95% CI: 0.9–8.8) for quartile 3 and 3.3 (95% CI: 1.0–10.2) for quartile 4, adjusted for the other three factors (mOR 1.4 per quartile; 95% CI: 1.0–1.9). The cytokines with salient factor loadings for Factor 4 were IL-1alpha, IL-4, IL-5, IL-13, and GM-CSF (Fig. 1).

Factor 4, which was significantly associated with risk for AIDS-NHL, includes three classic T-helper type 2 (Th2) cytokines (IL-4, IL-5, and IL-13), pro-inflammatory IL-1alpha, and hematopoeitic growth factor GM-CSF. With the exception of the gene for IL-1alpha, the genes for the other four are all clustered in tandem orientation in a 650 kilobase DNA segment at chromosome 5q31, clonal deletion of which may occur in myelodysplastic syndrome and acute myelogenous leukemia associated with alkylator chemotherapy [4]. A conserved non-coding sequence called *CNS1* is a coordinate regulator of transcription at this locus [5]. Thus, the common regulation of circulating levels of their four gene products, as identified by our factor analysis, may have a chromosomal basis.

IL-4, also known as B-cell stimulatory factor 1 (BSF1), promotes proliferation and differentiation of activated B-cells and is a prime driver of Th2 polarization of CD4-positive T cells [6]. IL-5, also known as eosinophil differentiation factor (EDF), stimulates production and differentiation of eosinophils [7].

TABLE II. Prediagnosis Cytokine Levels and Matched Odds Ratios (mOR) for AIDS-Related Non-Hodgkin Lymphoma Cases and Matched Controls ($N = 244$)

		Geometric mean (S.D.) pg/ml[a]		
	Percent detectable	Cases	Controls	
Cytokine	N = 244	n = 63	n = 181	mOR (95% CI)
IL-1alpha	71%	131 (*x*/4.6)	66 (*x*/4.5)	1.6 (1.0–2.5)
IL-1beta	70%	2 (*x*/3.7)	2 (*x*/3.1)	1.2 (0.8–1.8)
IL-1ra	87%	334 (*x*/3.5)	272 (*x*/3.4)	1.4 (0.8–2.4)
IL-2	76%	17 (*x*/4.1)	13 (*x*/3.8)	1.4 (0.8–2.4)
IL-4	52%	102 (*x*/4.8)	54 (*x*/4.9)	1.4 (1.0–2.1)
IL-5	64%	1 (*x*/4.6)	1 (*x*/2.3)	1.1 (0.7–1.8)
IL-6	96%	14 (*x*/5.2)	10 (*x*/5.3)	2.5 (1.3–4.8)
IL-7	90%	6 (*x*/2.7)	5 (*x*/2.6)	1.1 (0.6–2.1)
IL-8	100%	20 (*x*/6.6)	15 (*x*/5.3)	1.8 (0.8–3.8)
IL-10	98%	26 (*x*/2.8)	20 (*x*/2.6)	1.2 (0.6–2.1)
IL-12p40	75%	218 (*x*/2.5)	218 (*x*/2.6)	1.2 (0.8–1.8)
IL-12p70	51%	5 (*x*/3.3)	3 (*x*/2.8)	1.7 (1.1–2.7)
IL-13	63%	48 (*x*/3.5)	27 (*x*/3.6)	1.5 (1.0–2.3)
IL-15	61%	14 (*x*/2.5)	12 (*x*/2.6)	2.0 (1.3–3.1)
IL-17	56%	28 (*x*/2.5)	23 (*x*/3.2)	1.4 (0.9–2.1)
EGF	78%	82 (*x*/2.7)	78 (*x*/2.6)	1.2 (0.7–2.0)
Eotaxin	96%	148 (*x*/2.4)	154 (*x*/2.3)	1.4 (0.7–2.9)
Fractalkine	57%	243 (*x*/3.1)	191 (*x*/2.7)	1.4 (0.8–2.3)
G-CSF	35%	136 (*x*/2.7)	187 (*x*/3.9)	1.2 (0.8–1.8)
GM-CSF	48%	5 (*x*/3.3)	3 (*x*/2.7)	1.8 (1.2–2.9)
IFN-g	64%	11 (*x*/4.1)	8 (*x*/3.3)	1.0 (0.6–1.5)
IP-10	100%	1,650 (*x*/2.8)	1,270 (*x*/2.6)	2.3 (1.1–4.6)
MCP-1	100%	341 (*x*/3.0)	305 (*x*/2.8)	1.3 (0.6–2.5)
MIP-1a	62%	29 (*x*/4.8)	38 (*x*/5.5)	1.0 (0.6–1.5)
MIP-1b	42%	174 (*x*/4.5)	164 (*x*/5.3)	1.0 (0.6–1.5)
RANTES	100%	24,600 (*x*/3.1)	24,500 (*x*/3.1)	0.9 (0.4–1.8)
sCD40L	99%	7,100 (*x*/3.2)	7,600 (*x*/3.1)	0.9 (0.4–2.0)
TGFa	76%	22 (*x*/4.0)	14 (*x*/2.5)	1.5 (1.0–2.3)
TNFa	100%	13 (*x*/2.5)	10 (*x*/2.3)	1.4 (0.7–2.7)
VEGF	88%	105 (*x*/2.3)	98 (*x*/2.4)	1.7 (1.0–2.9)

[a]Excluding non-detectables.

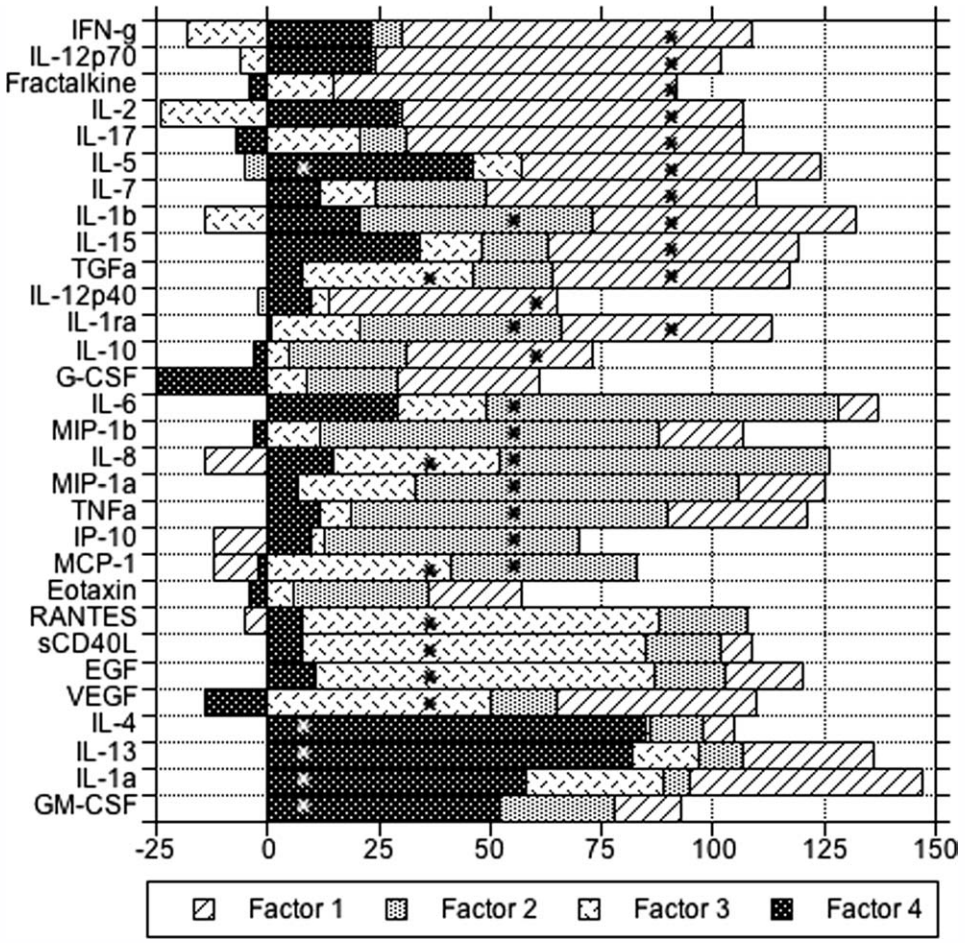

Figure 1. Rotated loadings (×100) on four principal factors for log-transformed cytokine levels among controls in descending order of salient loadings within factors. Asterisks indicate loadings greater than 0.36 for a given factor (i.e., salient).

IL-13 is an important down-regulator of pro-inflammatory cytokines and induces immunoglobulin synthesis by B cells [8]. GM-CSF is an essential regulator of neutrophils and macrophages [9]. IL-1alpha is a potent pro-inflammatory mediator that is also produced by stimulated B cells [10]. While our analysis associated lymphoma risk with this group of markers collectively, it may be the case that only some have causal effects related to B-cell hyperproliferation and malignant transformation.

Several cytokines have been previously found to be increased prior to AIDS-lymphoma diagnosis in the Multicenter AIDS Cohort Study (MACS). The B-cell growth factor sCD23, the anti-inflammatory IL-10 and the pleiotropic IL-6 have each been associated with NHL risk in various analyses [11–13]. While we did not measure sCD23, our non-significantly positive association with IL-10 and strongly significant association with IL-6 are consistent with these reports. Increases of circulating sCD27 and sCD30, two cytokine receptor molecules, have also been associated with NHL risk in the MACS [14,15].

HIV infection has profound effects on the immune system and AIDS-NHL is relatively homogeneous as compared to NHL in the general population. Nevertheless, blood levels of cytokines and other immune stimulatory molecules have also been investigated as predictors of NHL risk in the absence of HIV, with varying results. In the EPIC Italy cohort, there was a significant association with levels of CD54 and an inverse association with IL2 and gamma interferon among 68 NHL cases with plasma drawn 2.1–10.4 years prior to diagnosis [16]. In the New York University Women's Health Study, there was a significant association with sIL2r and an inverse association with IL13 among 92 NHL cases with serum drawn a median of 8.2 years prior to diagnosis [17]. In the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, there was a significant association with sCD30 among 234 NHL cases with serum drawn 1–10 years prior to diagnosis [18]. The discrepancies among these studies may reflect the heterogeneity of NHL, highlighting both the utility and the limitations of AIDS-NHL as a model system for lymphoma etiology.

In summary, we found generalized elevations of Th1, Th2, and pro-inflammatory cytokines and growth factors up to 2 years before AIDS-NHL, although only one of four principal factors was statistically significant. That the Factor 4 association was somewhat stronger (although not statistically significant) in the earlier samples drawn more than 1 year prior to NHL argues against disease bias as an explanation. While EBV viremia was not significantly predictive, our study had little statistical power in part due to PCR inhibition in heparin plasma samples. Cytokine hyperstimulation of B-cell proliferation may be an important etiologic pathway to AIDS-related lymphomagenesis.

Methods

Patients. We identified AIDS-NHL cases ($n = 63$) from three HIV-infected cohorts followed at the U.S. National Cancer Institute (NCI). Eight occurred in a cohort of 133 HIV-1-infected homosexual men [19], 39 were among 2126 HIV-1-infected hemophilia patients [20], and 16 were in a study of 2,803 patients at AIDS treatment and clinical trial sites [21]. Institutional review boards at the NCI and collaborating institutions approved each cohort study, and all participants gave written informed consent.

The NHLs had been diagnosed between 1985 and 2004 (median, 1994; Table I). We identified archived plasma ($n = 53$ cases) or serum ($n = 10$ cases) samples collected roughly 1 year before NHL diagnosis (range 0.1–2.0 years). Up to three lymphoma-free controls per case ($n = 181$ samples) were matched for cohort and sample type (i.e., plasma or serum) within the same time period, as well as age at diagnosis (±5 years), race, sex, and CD4+ cells/mm^3 at diagnosis (categorized as 0-, 100-, 200-, and 500-).

Cytokine and EBV assays. Cytokine levels were determined by Luminex fluorescent bead human cytokine immunoassays (MILLIPLEX™ MAP, Millipore, Billerica, MA). Thirteen cytokines were part of a high-sensitivity multiplex panel (GMCSF, IFNg, interleukin (IL)-1beta, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, and TNFalpha), 16 were in a standard-sensitivity multiplex panel (EGF, eotaxin, fractalkine, G-CSF, IL-1alpha, IL-1ra, IL-12 (p40, free form), IL-15, IL-17, IP-10, MCP-1, MIP-1alpha, MIP-1beta, soluble CD40L, TGFalpha, and VEGF), and one was measured as a single analyte (RANTES). Values below the limit of detection were assumed to be half of the minimum detectable level. Measurements on blinded replicate samples included with test samples had coefficients of variation between 10 and 46%.

Cell-free EBV DNA was detected by extracting 200 μl of sample using the total nucleic acid isolation protocol on the MagnaPure LC instrument (Roche Diagnostics, Penzberg, Germany) and performing quantitative real-time PCR for the p143 gene *BNRF* [22]. Amplification was considered to be inhibited when the average cycle threshold (Ct) for a spiked internal control sequence was more than two standard deviations from the longstanding average.

Statistical analysis. Geometric means and standard deviations of cytokine measurements greater than the limit of detection were calculated with Stata/SE 10.1 for Macintosh software (Stata, College Station, TX). To account for correlations among cytokines, we performed factor analysis to extract independent factors related to levels among controls, using SAS v9.2 software (SAS Institute, Cary, NC). The number of principal factors was based on examination of scree plots and Kaiser's rule [23]. Correlations of cytokine levels with particular factors were determined as factor loadings, with a higher value indicating greater correlation and a negative loading meaning that the cytokine was inversely related to that factor. Factors were rotated by orthogonal Varimax transformation to obtain a simpler structure for interpretation; rotated values exceeding the root mean square factor loading (0.36) were considered to be salient.

Factor scores for principal factors were calculated for each subject as the sum of their standardized (mean 0, variance 1) cytokine levels times the respective standardized scoring coefficients from factor analysis. Since factor scores were themselves standardized, the parameter estimates are interpretable as multiples of their standard deviations [24].

We used conditional logistic regression analyses on matched sets to estimate odds ratios (mORs) and 95% confidence intervals for association of case status with individual cytokines (categorized as undetectable, low and high based on median values for controls), and with the rotated principal factors as continuous measures. To account for possible non-linear associations, we also analyzed the factors in quartiles based on their values among controls, both as categorical variables and in tests for trend. Unconditional analyses adjusted for the matching variables yielded generally similar estimates of associations, so only results for the conditional models are presented. Regression analyses were performed using Stata and SAS software. All significance tests were two-sided and *P*-values less than 0.05 were considered significant.

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Contract grant sponsor: U.S. National Cancer Institute (Intramural Research Program)
Conflict of interest: Nothing to report.
Published online 28 June 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22119

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Peritransplant palifermin use and lymphocyte recovery after T-cell replete, matched related allogeneic hematopoietic cell transplantation

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Allogeneic hematopoietic cell transplantation (allo-HCT) is often the only curative option for people with otherwise fatal hematologic malignancies. As the number of allo-HCT procedures continues to increase [1], it is increasingly clear that a major obstacle to success is delayed immune recovery, which puts patients at risk for a wide variety of opportunistic infections [2–8]. Additionally, rapid early lymphocyte recovery may serve as a surrogate predictor of better transplant outcomes. Robust recovery of absolute lymphocyte counts (ALC) early after transplantation is associated with improved survival following autologous, sibling, unrelated bone marrow, peripheral blood, and umbilical cord blood transplantation [9–15]. There is a clear need to develop strategies to accelerate and improve immune reconstitution (IR). Several novel approaches have been successfully tested in preclinical animal models and early human clinical trials. These include pretransplant androgen ablation, keratinocyte growth factor (KGF), and a p53 inhibitor or post-transplant administration of interleukin (IL)-7, IL-15, growth hormone, or insulin-like growth factor-1 [16–20].

KGF is a potent growth factor belonging to the fibroblast growth factor family that stimulates epithelial growth and differentiation, without affecting the nonepithelial cells that lack the fibroblast growth factor receptor 2-IIIb receptor. In the thymus, KGF is produced locally by mesenchymal cells and acts on thymic epithelial cells (TECs) [18,21]. Through its trophic effects on TECs, KGF facilitates normal thymopoiesis, which is required for naïve T cell generation [22]. In murine allo-HCT models, KGF treatment improved thymopoiesis and enhanced T cell IR [18,23]. In a non-human primate model of autologous hematopoietic cell rescue following myeloablation, peritransplant KGF administration led to the preservation of thymic architecture for up to 12 months after HCT [24]. In the same study, KGF-treated animals had higher frequencies of naïve T cells compared to the control group. Another preclinical study that combined KGF with androgen depletion led to enhanced thymopoiesis characterized by a broad V-beta T cell repertoire [25].

To assess the GVHD protective potential of KGF, a phase I/II randomized, placebo-controlled trial of recombinant human KGF (palifermin) was performed from 2000 to 2003 in 100 patients undergoing T-cell replete, matched related donor (MRD) allo-HCT following myeloablative conditioning [26,27]. The focus of this study was to determine the impact of KGF on GVHD. Detailed IR was not studied within the trial. Because ALC is associated with transplant outcomes [9–15], we questioned whether there might be differential effects of KGF on early ALC recovery. Here, we report our retrospective analysis using data from our earlier trial to assess the effects of peritransplant use of palifermin on ALC recovery at following HCT. This is the first report analyzing the results of palifermin and lymphocyte recovery in humans.

We tested ALCs after transplant in the palifermin and placebo treatment groups. As shown in Fig. 1a, the median ALC at days 30, 60, and 100 after transplant did not significantly differ in the palifermin versus placebo arms (600×10^6/L vs. 600×10^6/L; $P = 0.26$, 500×10^6/L vs. 600×10^6/L; $P = 0.86$, 600×10^6/L vs. 700×10^6/L, $P = 0.19$). Likewise, there was no influence of the palifermin dose and subsequent ALC recovery post-transplant ALC (Fig. 1b). As well, palifermin did not impact ALC based on the conditioning regimen (Bu/Cy vs. Cy/total body irradiation [TBI]), recipient age, and the presence of acute GVHD (aGVHD; data not shown). As previously reported, in this trial, palifermin use was not associated with shorter time to engraftment, aGVHD, survival, or infectious complications after transplant. However, palifermin modestly decreased mucositis [26,27].

Based on prior studies [9–15], we also examined whether differential lymphocyte counts were associated with transplant outcomes. Patients with an ALC above the median at D+30 ($>600 \times 10^6$/L) showed a trend for improved progression-free survival (PFS) (49% [95% confidence interval (CI), 32–65%] vs. 29% [95% CI 16–43%], $P = 0.07$; Fig. 2a). There was no association between D+30 ALC and disease recurrence (28% [95% CI 14–42%] vs. 23% [95% CI 10–36%], $P = 0.6$; Fig. 2b). There were trends toward less 2-year treatment related mortality (TRM) (18% 95% CI [6–30%] vs. 35% [95% CI 20–50%], $P = 0.1$) and grade II–IV aGVHD (31% [95% CI 17–45%] vs. 47% [95% CI 32–62%], $P = 0.14$) in patients with an ALC > 600 at D+30 after transplant (Fig. 2c,d). These rates were unaffected by KGF therapy.

In this first allotransplant palifermin dose escalation trial, using ALC as a marker for IR, we found that peri-transplant palifermin had no impact on ALC at days 30, 60, or 100 after allo-HCT. These results differ from rodent studies [22,25] and a non-human primate study [24] where KGF was associated with protection of TECs from radiation-induced damage, resulting in improved thymopoiesis and peripheral IR after transplant. Numerous explanations may account for our findings including suboptimal palifermin dose or dosing schedule, existing pretransplant chemotherapy-induced TEC damage or a limited potency of this agent on human TECs. ALC is a relatively crude marker for IR and the lack of information on various T cell subsets, T cell receptor Vβ repertoire, and newly formed T cells (i.e., T Cell Receptor Excision Circles) limits our ability to detect any effects of palifermin on the naïve versus homeostatically expanded T cells. It is possible that the early lymphocyte recovery after T-cell replete HCT is not reflective of thymic output and may be more related to homeostatic proliferation of existing T cells. In this scenario, ALC may be unaffected by palifermin. In addition, significant aGVHD occurred in nearly half the patients in this study. This complication is associated with thymic injury and requires extended immunosuppressive therapy. Thus aGVHD (or its treatment) might have confounded any potential of palifermin to augment IR. However, there was no significant difference in ALCs between the palifermin- and placebo-treated groups, even after excluding patients with aGVHD (data not shown). However, it is still possible that the GVHD prophylaxis itself (which were not used in the preclinical models) may have blunted any potential benefit of palifermin on lymphocyte recovery, considering that most GVHD prophylactic agents inhibit T cell production and expansion.

Previous studies show a positive correlation between early ALC and transplant with outcome measures [9,14,28]. Although the clinical impact of ALC recovery was only a secondary aim of this study, we observed a trend toward improved PFS and lower TRM in patients above the median ALC at day 30, consistent with other reports [13–15,28]. Interestingly, a lower day 30 ALC was associated with a trend toward more frequent grade II–IV aGVHD. Thus, it is possible that before becoming clinically evident, aGVHD is heralded by delayed recovery of ALC.

Delayed IR continues to be a major problem after allo-HCT. Based on this analysis, palifermin alone is unlikely to significantly improve post-allo-HCT immune recovery, at least following T-cell replete allo-HCT. In recent years, there have been significant advances in understanding the mechanisms behind delayed IR, and some novel interventions have been successful in preclinical models (reviewed in Ref. 29). Perhaps, strategies combining two or more agents, shown to be promising in preclinical models, may be needed to overcome the profound immune deficiency which follows allo-HCT.

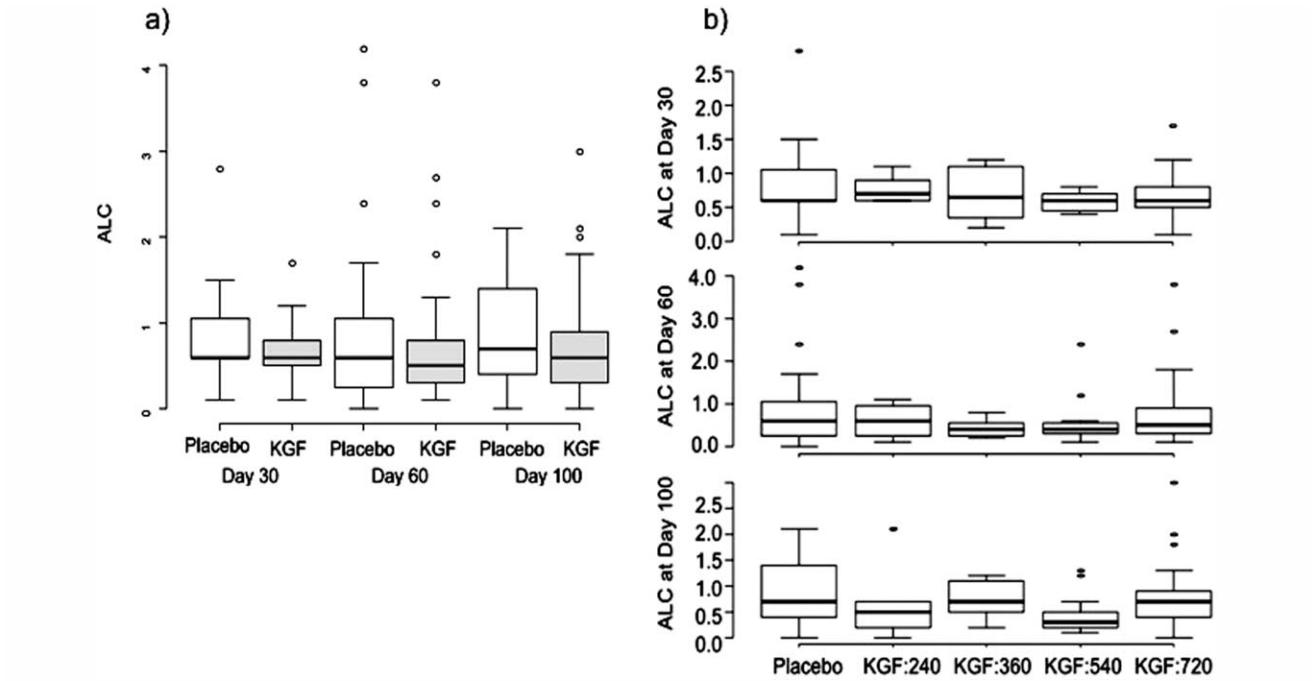

Figure 1. Post-transplant ALC in palifermin and placebo groups. (a) Median and interquartile range of ALC for the placebo (open bars) and palifermin (shaded bars) at days 30, 60, and 100 after transplantation. The median ALC at the three time points in the two groups were 600×10^6/L versus 600×10^6/L ($P = 0.26$), 600×10^6/L versus 500×10^6/L ($P = 0.86$), and 700×10^6/L versus 600×10^6/L ($P = 0.19$), respectively. (b) Comparison of median and interquartile ranges of days 30, 60, and 90 ALCs at various doses of palifermin with placebo.

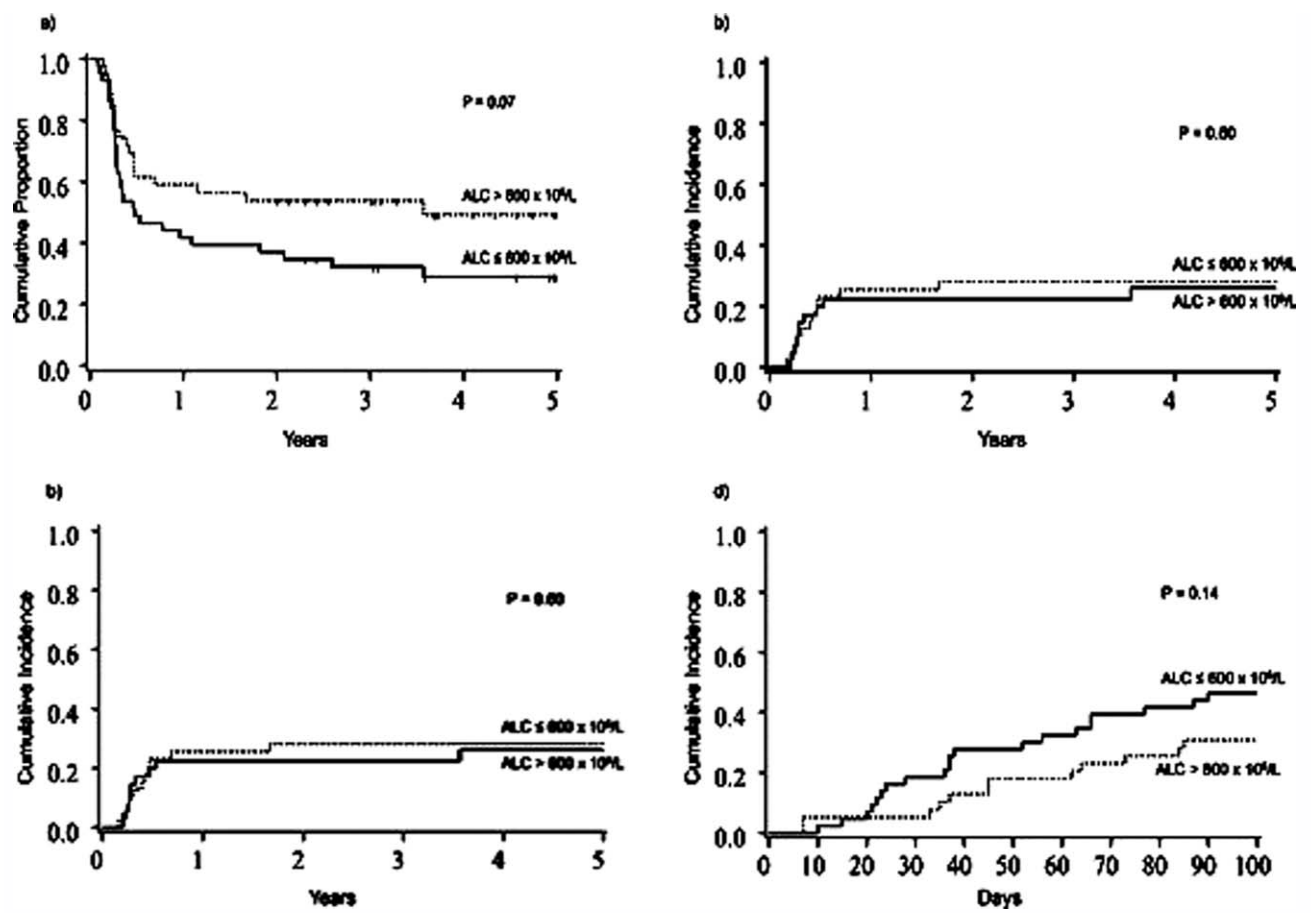

Figure 2. Transplant outcomes based on ALC at day 30.Outcomes were measured for patients above or below the median ALC (i.e., $>600 \times 10^6$/L or ≤ 600) at day 30. (a) PFS at day 30 was 49% versus 29%, respectively ($P = 0.07$). (b) The cumulative incidence of relapse was 28% versus 23%, respectively ($P = 0.6$). (c) Two-year TRM was 18% versus 35%, respectively ($P = 0.1$). (d) Grade II-IV aGVHD was 31% versus 47% ($P = 0.14$).

TABLE I. Patient Baseline Demographics and Disease Characteristics

	N	Palifermin	Placebo	*P*
Center				0.91
Michigan	46	32	14	
Minnesota	54	37	17	
Gender				0.99
Male	58	40	18	
Female	42	29	13	
Median age, years (range)		46 (7–65)	46 (7–63)	0.58
Diagnosis				0.08
AML	36	24	12	
CML	15	7	8	
MDS	12	6	6	
NHL	14	13	1	
ALL	9	8	1	
Hodgkin's disease	1	1	0	
Other malignancies	13	10	3	

AML: acute myelogenous leukemia; CML: chronic myelogenous leukemia; MDS: myelodysplastic syndrome; NHL: non-Hodgkin lymphoma.

Patients and Methods

Patient and transplant characteristics have been reported previously [26,27]. A total of 100 patients were enrolled in this study and randomized to receive placebo ($n = 31$) or palifermin ($n = 69$). Two patients in the palifermin group did not undergo transplantation and were excluded. As shown in Table I, patient demographics and underlying diagnosis were well matched between the two groups. Median follow-up among survivors is 4.1 years (range: 2–6.1 years) for both groups.

All patients received allo-HCT from HLA-identical sibling donors after myeloablative conditioning, which included 120 mg of cyclophosphamide and 1,320 cGy of fractionated TBI at University of Minnesota or 16 mg/kg of oral busulfan and 60 mg/kg of cyclophosphamide (Bu/Cy) at the University of Michigan. A calcineurin inhibitor (cyclosporine or tacrolimus) and methotrexate (15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11) were used for GVHD prophylaxis.

This was a randomized, double-blind, placebo-controlled, dose-escalation study. Three cohorts receiving increasing palifermin doses were sequentially enrolled, with each cohort randomized to achieve balance within each study site and in each cohort. Stratification was based on conditioning regimen and patient age. A total of 98 patients completed this study, with a 1:2 randomization between placebo ($n = 31$) and study drug ($n = 67$). All treatment cohorts received 3 days of palifermin at 40 mg/kg ($n = 8$) or 60 mg/kg ($n = 61$) prior to the start of conditioning (days -11 to -9) and then received the same palifermin dose on 3 consecutive days weekly (first 3 days of a week) staring on day 0 and extending for 1 ($n = 18$), 2 ($n = 14$), or 3 ($n = 37$) weeks after transplant. Thus, the total palifermin dose was 240 mg/kg in the lowest dose cohort and 720 mg/kg for the highest dose cohort. For the current analysis, ALCs were extracted from the patient medical records on days 30, 60, and 100 (±7 days) after obtaining institutional review board approvals at both study sites.

Statistical comparisons of the distribution of ALC over time were performed by examining the median, interquartile ranges, and ranges of ALC at days 30, 60, and 100 post-transplant and performing either a general Wilcoxon or Kruskal-Wallis test by a number of factors: KGF group (placebo vs. KGF), placebo dose, presence of grade II-IV aGVHD, conditioning and age.

Outcomes by ALC were analyzed by simply dividing cohorts by the median ALC and estimating the outcomes of the two groups using Kaplan-Meier estimates for overall survival and PFS (34) and the cumulative incidence (CI) for relapse, nonrelapse mortality, and GVHD (35). Analyses were performed using SAS 9.2 (SAS Institute) and R 2.4 statistical software.

Author Contributions

Romee Rizwan and Michael R. Verneris planned data analysis, reviewed and complied data, and wrote paper; Todd DeFor performed statistical analysis; John Levine planned the original trial, provided data, and edited the manuscript; and James Ferarra, Daniel Weisdorf, and Bruce R. Blazar planned the original clinical trial and edited the manuscript.

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Contract grant sponsor: National Institutes of Health; Grant number: NCI P01-CA65493 (to B.R.B. and M.R.V.). Contract grant sponsor: American Cancer Society; Grant number: RSG-08-181-LIB (to M.R.V.). Contract grant sponsor: Leukemia Research Fund (to M.R.V.). Contract grant sponsor: Children's Cancer Research Fund (to M.R.V. and B.R.B.)
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Received for publication 1 March 2011; Revised 30 June 2011; Accepted 5 July 2011
Conflict of interest: Nothing to report.
Published online 14 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22136

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Lenalidomide-associated pneumonitis in patients with plasma cell dyscrasias

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Lenalidomide is an immunomodulatory drug (IMiD) that has been approved for the treatment of relapsed or refractory multiple myeloma (MM) [1,2]. Common side effects of lenalidomide treatment include myelosupression, diarrhea, fever, muscle cramps, neuropathy, constipation, rash, fatigue, and deep vein thrombosis [1–4]. Pulmonary complications are believed to be uncommon. So far, three cases of lenalidomide-induced pulmonary toxicity have been reported, commonly presented with dyspnea, fever, and hypoxia [5–7]. To assess the incidence, characteristics and outcome of pneumonitis associated with lenalidomide, we assessed the incidence and outcome of lenalidomide-induced pneumonitis in 237 consecutive unselected patients with MM or AL amyloidosis. We identified eight (3.37%) patients with clinical radiographic and laboratory criteria of lenalidomide-induced pneumonitis. In all patients lenalidomide was discontinued and most received systemic corticosteroids. In four of eight patients lenalidomide was reinstituted after symptom resolution. These data suggest that clinicians should be alert for the early recognition and prompt management of this complication.

In an expanded access program of lenalidomide with dexamethasone in 1,438 patients, respiratory symptoms such as dyspnea of unspecified nature, cough or pneumonia were recorded in about 10–15% of patients each [4]. So far, three cases of lenalidomide-induced pulmonary toxicity have been reported [5–7]. In these cases, presentation may have been confused with lower respiratory tract infection, given that these patients presented with dyspnea, fever, and hypoxia [5–7]. Pulmonary toxicity has also been recorded in patients receiving thalidomide [8] or bortezomib [8,9] or pomalidomide [10]. Drug-associated pulmonary diseases vary in their pathophysiology, clinical and radiographic presentation, and prognosis and a high index of suspicion are required for the diagnosis [11,12].

Among 237 consecutive patients, who received lenalidomide, (myeloma: 190 patients, AL amyloidosis: 47 patients) we identified eight patients (3.37%) who fulfilled all the criteria for the diagnosis of lenalidomide-related pneumonitis. The patients' characteristics are summarized in Table I. Five patients received lenalidomide combined with cyclophosphamide and dexamethasone, two patients received lenalidomide with melphalan and prednisone and one lenalidomide with dexamethasone. Only one patient (12.5%) had a history of a prior respiratory disorder (asthma). Symptoms of lenalidomide-related pneumonitis developed within 0.5 to 24 (median 5) months from the initiation of lenalidomide-based treatment and the most common were dyspnea (7/8 patients), dry cough (5/8), and fever (3/8), followed by fatigue (2/8) and skin rash (2/8 patients). Auscultation revealed bilateral lung field fine crackles in all patients. Leukocyte counts varied (mean 8,210/ml, range 1,120–12,400/ml), C-reactive protein levels were elevated (mean 10.1 mg/dl, range 1.4–20.3 mg/dl, ULN 0.5 mg/dl), and were reduced within normal range after the resolution of the symptoms. Peripheral blood eosinophilia was not found in any patient.

Arterial blood gas analysis revealed hypoxemia in all patients with normal/low pCO2. Pulmonary function tests revealed a restrictive pattern with low $T_L CO$ (corrected for the degree of anemia). Chest radiography showed no gross abnormalities. Chest CT high resolution computed tomography (HRCT) scanning displayed symmetrical, bilateral interstitial abnormalities with areas of ground glass opacity, predominantly involving middle lung fields and lung bases compatible with nonspecific interstitial pneumonitis (Figure 1). In all patients cardiac echocardiography was performed to exclude a cardiac cause of interstitial lung disease.

Three out of the eight patients (37.5%) received antibiotics, whereas seven of the eight (87.5%) patients were hospitalized but none required mechanical ventilation. Lenalidomide was held in all patients during the acute event and corticosteroid therapy (prednisolone at a starting dose of 25–75 mg per day) was administered in 7/8 (87.5%) of patients. Standard supportive care (oxygen, bronchodilators, hydration) was given, while in one out of eight patients prolonged (>2 weeks) oxygen therapy was needed. An infectious cause of respiratory dysfunction was excluded in all patients as indicated by negative blood and sputum cultures and negative serology and PCR for certain fungi and viruses. Clinical symptoms of pneumonitis resolved in all patients within 9–50 days after discontinuation of lenalidomide: fever and dyspnea resolved within 2–4 days, whereas dry cough and hypoxia lasted longer. Repeated HRCTs, 2–3 months after the resolution of symptoms, showed significant improvement without residual interstitial fibrosis. Lenalidomide was discontinued permanently in four cases: its dose reduced in two cases, while in two patients lenalidomide was reintroduced without dose modification, but with concomitant low-dose corticosteroids.

Drug-induced pneumonitis is a side effect of several drugs, including cytotoxic, immune-modifying, antiarrhythmic drugs, etc. [12]. Our report is the first systematic effort to record this complication of lenalidomide-based therapy in an unselected population of consecutive patients with plasma cell dyscrasias. In our series of 237 patients, we found that 3.4% of patients developed clinical and radiographic evidence of pneumonitis. However, given the nonspecific presentation of drug-induced pneumonitis, we cannot exclude the possibility that the diagnosis of lenalidomide-induced pneumonitis may have been missed in some patients. The development of pneumonitis in combination with concomitant use of cyclophosphamide needs further evaluation. Cyclophosphamide, in high doses, has been reported to be related with the development of interstitial pneumonitis [13,14]. However, single agent low dose oral cyclophosphamide, only rarely, has been reported to cause interstitial pneumonitis [15]. Whether, its combination with lenalidomide may increase the risk of interstitial pneumonitis requires further evaluation.

The development of lenalidomide-induced pneumonitis is a relatively early complication [5–7], with a median time to the development of symptoms of about 3–5 months in our series, indicating a hypersensitivity-related mechanism, similar to that seen in patients with hypersensitivity pneumonitis. The

TABLE I. Characteristics of Patients Who Were Diagnosed With Lenalidomide-Associated Pneumonitis

Gender	Age (Years)	Disease	Prior therapies	Lenalidomide regimen at onset of pneumonitis	Time to pneumonitis	Number of cycles of lenalidomide before onset of pneumonitis	History of respiratory disorder	Symptoms at presentation	Pulmonary function tests	Treatment	Lenalidomide after event	Hospitalization
1. M	57	AL (IgGλ)	1. Melphalan-\ Dexamethasone 2. CTX- Dexa 3. Bortezomib-Dexamethasone	Lenalidomide-CTX– Dexa	10 months	10	No	Fever Cough Dyspnea	FEV1 = 84.93 FEV1%FVC = 104.76 TLCOc = 63.75	Corticosteroids	D/C	Yes
2. F	61	AL (λ-light chains)		Lenalidomide-CTX– Dexa	0.5 months	1	No	Cough Dyspnea Rash	FEV1 = 81.22 FEV1%FVC = 90.53 TLCOc = 57.67	Corticosteroids	D/C	Yes
3. F	78	MM (IgGl, ISS II)		Melphalan-Prednisone-Lenalidomide	1.5 months	2	No	Cough Dyspnea Fever	FEV1 = 79.85 FEV1%FVC = 97.73 TLCOc = 21.47	Antibiotics Corticosteroids	Dose reduction	Yes
4. M	68	MM (IgGk, ISS III)		Lenalidomide-Dexa	2 months	2	No	Cough Fever Rash	FEV1 = 98 FEV1%FVC = 96 TLCOc = 52	Corticosteroids Antibiotics	D/C	Yes
5. F	69	MM (kLC, ISS III)		Melphalan-Prednisone-Lenalidomide	11 months	10	Allergic asthma	Dyspnea	FEV1 = 20.92 FEV1%FVC = 53.56 TLCOc = 26.48	Antibiotics, Bronchodilators	Dose reduction	No
6. M	50	MM/ AL (IgGk)		Lenalidomide-CTX- Dexa	7 months	8	No	Dyspnea Cough	FEV1 = 99.14 FEV1%FVC = 108.67 TLCOc = 53.17	Bronchodilators, corticosteroids	No Dose reduction	Yes
7. F	69	AL (λLC, IgGλ)		Lenalidomide-CTX-Dexa	3 months	4	No	Dyspnea	FEV1 = 87.29 FEV1%FVC = 91.08 TLCOc = 83.26	Bronchodilators, corticosteroids	No Dose reduction	Yes
8. F	69	MM, (ISSI, kLC)	1. Autologous Transplantation 2. Bortezomib-Dexamethasone-Thalidomide 3. Lenalidomide-Dexamethasone	Lenalidomide-CTX– Dexa	24 months	24 (9 RD followed by 15 RCD)	No	Dyspnea	FEV1 = 52.24 FEV1%FVC = 92.85 TLCOc = 55.37	Bronchodilators, corticosteroids	D/C	Yes

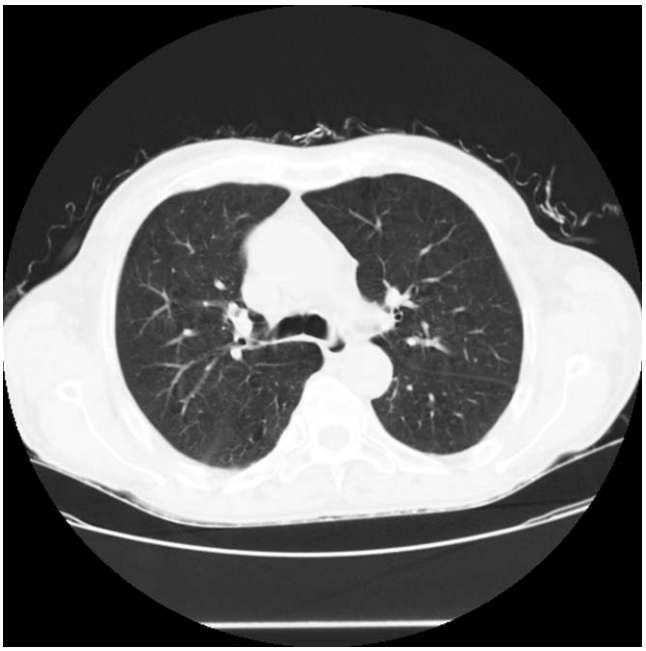

Figure 1. Chest CT scanning showing symmetrical, bilateral interstitial abnormality with areas of ground glass opacity predominantly involving middle lung fields and lung bases.

development of rash in two of our patients supports an underlying hypersensitivity-induced mechanism. However, in three patients symptoms developed after 10–24 months of lenalidomide treatment—thus, a different mechanism perhaps associated with total dose or time of exposure may also coexist.

Management of drug-induced interstitial pneumonitis is based on the discontinuation of the presumed causal agent and in some cases systemic corticosteroids, depending on the severity of hypoxemia and extent of pulmonary infiltrates, after the exclusion of an active infection [12]. This strategy was also followed in our patients. In our experience, therapy with systemic corticosteroids resulted in rapid symptom resolution and improvement of oxygenation. There are no data to support routine use of antibiotics. However, in immunocompromised patients such as those with MM receiving antimyeloma therapy, broad spectrum antibiotics may be used until the diagnosis has been established, since pulmonary infections with several infectious agents may present with a radiographic pattern of interstitial pneumonitis.

The decision to restart therapy with lenalidomide is based on the severity of pneumonitis and the availability of other treatment options. Our data indicate that with appropriate dose reduction and with the concomitant administration of corticosteroids, treatment with lenalidomide may be reinstituted in some patients. It is difficult to make firm recommendations based on the limited available data. However, it would be prudent to stop permanently lenalidomide treatment for patients with severe pneumonitis (such as those with life-threatening respiratory compromise). A strategy involving dose reduction in patients with less severe pneumonitis may be indicated, based on careful assessment of the individual patient. For patients with only mild symptoms initial addition of corticosteroids with close surveillance with or without dose reduction may be considered. However, if symptoms recur then lenalidomide should be held and dose reduced or even discontinued. Data from ongoing clinical studies and the increasing experience with the use of lenalidomide will help to refine these recommendations.

In conclusion, within the limitations of this retrospective study, our data indicate that although an unusual complication, clinicians should be alert for the early recognition of pneumonitis associated with lenalidomide. Moreover, these results suggest a need for the monitoring of pulmonary adverse events and the necessity for guidelines regarding the management of pneumonitis in future studies involving lenalidomide.

Materials and Methods

We reviewed the medical records of 237 patients with MM or AL amyloidosis, who were treated with lenalidomide-based therapy in our centre to identify patients with the diagnosis of lenalidomide-induced interstitial pneumonitis. All patients received lenalidomide in combination with dexamethasone, or with melphalan and prednisone, or with dexamethasone and low dose oral cyclophosphamide.

The causal relationship of pulmonary symptoms to lenalidomide was based on World Health Organization's classification of causal criteria for the adverse effects of medications [16]. The diagnosis of pneumonitis was based on clinical, radiographic, and laboratory criteria that required the presence of all of the following: (1) dyspnea, dry cough, fever, and fatigue; (2) patchy shadows on chest radiograph and ground glass shadows and/or reticular opacities on HRCT; (3) a restrictive pulmonary functional impairment pattern (as assessed by pulmonary function tests) with hypoxemia; (4) absence of pulmonary infection with bacteria, fungi, acid fast bacilli, and viruses and congestive heart failure; (5) symptomatic improvement of pneumonitis after the discontinuation of lenalidomide.

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Conflict of interest: Nothing to report.
Published online 14 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22135

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Managing cardiac amyloidosis: Auto or allotransplant?

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Patients with cardiac amyloidosis are difficult to manage and have high mortality. There is no standard treatment for primary cardiac amyloidosis that is considered curative. We present two cases of cardiac amyloidosis: one treated with reduced intensity allogeneic hematopoietic cell transplant (alloHCT) and the other with autologous (auto)HCT and a summary of alloHCT in the management of primary amyloidosis.

Case 1

A 45-year-old African-American male presented with dyspnea and was found to have congestive heart failure (CHF). A two-dimensional echocardiogram (2D ECHO) demonstrated interventricular septal and left ventricular thickening, and he underwent a cardiac biopsy demonstrating extensive interstial and endocardial kappa light chain amyloid light chain (AL) amyloidosis. Workup demonstrated serum and urine kappa light chains and 10% plasma cells on bone marrow biopsy. Treatment with lenalidomide and dexamethasone was stopped at 3 weeks because of intolerance. At 3 months after presentation, he underwent peripheral blood stem cell (PBSC) collection followed by autoHCT with melphalan at a dose of 120 mg/m^2 and attained a partial response (Table I). He had multiple marrow biopsies, routine labs, and repeat 2D ECHO. His disease progressed at 11 months and became progressively pancytopenic in the postautologous transplant period. A 2D ECHO at 1year postautotransplant showed significant improvement in diastolic dysfunction. At evaluation for a second autoHCT, he was found to have myelodysplastic syndrome (MDS). Multiple marrow biopsies in this period were negative for amyloid.There was minimal dysplasia on his marrow aspirates. There were 1.5% bone marrow blasts and a cellularity of 15%, 11 months postautologous transplant. However, fluorescence in-situ hybridization (FISH) revealed del 12p13 in 51/200 interphase nuclei, consistent with secondary MDS. There was an abnormal chromosome 12 with deletions of portions of both the short and the long arms in 10 of 21 metaphases on conventional cytogenetic analysis with a constitutional pericentric inversion of chromosome 9 in all the cells examined. The peripheral blood counts at the time of diagnosis were: hemoglobin 10.5 g/dL, platelet count 79,000/μ L, and a white blood cell count (WBC) of 4,400/μL with an ANC of 1.8.

His serum kappa:lambda ratio decreased from 16.88 before autotransplant to 3.18 at the time of MDS diagnosis. The duration between his autotransplant and MDS diagnosis was 11 months. He underwent an human leukocyte antigen (HLA)-matched sibling alloHCT with fludarabine 160 mg/m^2, Melphalan 50 mg/m^2, and 400 cGy of total body irradiation for conditioning. He required a cardiac pacemaker because of heart block within the first 100 days post-alloHCT. His CHF symptoms improved, with improvement in diastolic function by 2D ECHO (Table I). Brain natriuretic peptide (BNP) levels were not obtained serially. He developed mild chronic graft-versus-host disease (GvHD) of the skin, treated with topical steroids. A bone marrow biopsy at 1 year post-alloHCT was without plasmacytosis, and he had full lymphoid and myeloid engraftment. He is 21 months post-alloHCT without CHF.

Case 2

A 53-year-old white male with history of diabetes mellitus presented with dyspnea and was found to have CHF and atrial flutter. Workup revealed nephrotic range proteinuria and abdominal fat, renal, and bone marrow biopsies showed lambda amyloidosis, the latter with $<$5% plasma cells. 2D ECHO revealed normal systolic function with enlarged atria and borderline septal thickening (Table II). The patient was started on bortezomib, tolerating only one cycle because of orthostatic hypotension. He underwent PBSC

TABLE I. Patient 1: Laboratory and Echocardiographic Results

Parameter (normal range)	Pre-autoPBSCT	Day + 104 post-autoHCT	Pre-alloPBSCT	Day + 100 post-alloHCT	Day + 180	Day + 360
Serum kappa/lambda ratio (0.26–1.65)	13	4.4	4	10	1	0.86
LV hypertrophy	Concentric	Concentric	Concentric, severe	Concentric, severe	Concentric, severe	Concentric, moderate
Diastolic dysfunction	Stg 2, no restriction	Stg 2, no restriction	Stg 2, no restriction	Stg 3 restriction+	Stg1	
Left atrium (1.9–4.0 cm)	5.8	5.3	5.2 (increased pressure)	5.2	5.0	4.9
Right atrium (3.0–4.6 cm)	Enlarged	Enlarged	Normal	Normal	Normal	Normal
LV internal dimension diastolic (3.5–5.7 cm)	3	3.6	3.8	3.9	4.1	5.4
IV septum, diastolic (0.6–1.1 cm)	2.6	2.5	1.8	2	1.8	1.4
LV posterior wall, diastolic (0.6–1.1)	2.8	2.5	1.9	2.2	1.8	1.4

TABLE II. Patient 2: Laboratory and Echocardiographic Results

Parameter (normal)	Baseline	Postbortezomib	Pre-autoHCT	Day + 100 post-autoHCT	Day + 180	Day + 220	Day + 265
BNP (0–100 pg/mL)	2,179		1,056	1,807	2,865		3,170
Kappa/lambda ratio (0.26–1.65)	0.03	0.39	0.38	0.69			
24 hr urinary protein (41–225 mg)	17,136	12,028	13,364	9,416	7,018	7,742	
LV hypertrophy/EF	Absent/50%	Mild, inc. echogenicity/45%	Mild/45%	Mild/60%		Mild/55%	Mild/40%
Diastolic dysfunction	Unable to be assessed	Grade 2	Grade 3	Grade 3		Grade 3 with restriction	Grade 3
Left atrium (1.9–4.0 cm)	5.5	5.6	5.0	6.0		5.8	5.2
Right atrium	Enlarged	Enlarged	Enlarged	Enlarged		Enlarged	Enlarged
LV internal dimension diastolic (3.5–5.7 cm)	5.0	5.3	5.7	5.3		4.9	0.9
IV septum, diastolic (0.6–1.1 cm)	1.1	1.4	1.2	1.3		1.4	1.2
LV posterior wall, diastolic (0.6–1.1)	0.9	1.0	1.2	1.2		1.4	1.2
Pericardial effusion	None	None	None	None		Mild	Moderate

mobilization followed by high dose chemotherapy with melphalan 120 mg/m^2 and autoHCT. His post-transplant course was complicated by severe CHF requiring a prolonged intensive care unit (ICU) stay. Day 100 workup demonstrated decreased 24-hr proteinuria but continued amyloid deposition in the bone marrow. On day + 138, patient was admitted for CHF exacerbation, and alloHCT was offered to the patient because of worsening CHF and proteinuria. However, alloHCT was not authorized by his insurance company, and the patient was risk averse, not wishing to appeal the insurance denial. The patient was being considered for a second autoHCT, but developed refractory CHF and died.

Discussion

Renal and cardiac involvement occur in more than 50% of amyloid light chain (AL) amyloidosis patients with cardiac involvement, the most important factor affecting outcome [1]. The median survival is ~ 6 months in untreated or nonresponsive patients [2]. The optimal management of cardiac AL amyloidosis is controversial. Unlike multiple myeloma, where hematologic response and tumor burden reduction are primary endpoints, cessation and reversal of end organ dysfunction are outcome goals for AL amyloidosis patients. Patients with renal disease will respond to autoHCT with low treatment-related mortality (TRM). However, patients with cardiac amyloid have higher TRM rates because of poor tolerance of the high dose chemotherapy [3]. In a study involving 434 patients with AL amyloidosis undergoing autoHCT, the day 100 mortality was 10.1%, often because of multiorgan failure [4]. A case report from Japan [5] documented improved cardiac function, normalization of serum BNP, and symptom-free survival of over 3 years in a 63-year-old woman after autoHCT. Good organ response and improved survival have been reported in a small number of cardiac AL amyloid patients who underwent cardiac transplant followed by chemotherapy including autoPBSCT [6]. However, cardiac transplantation is limited by organ availability. In addition, amyloid protein deposition in the transplanted heart followed by disease progression post-autoHCT is a major concern. AlloHCT, often with a reduced intensity regimen, can be considered as a reasonable approach in cardiac AL amyloidosis patients. In a report from the european group for blood and marrow transplantation (EBMT), organ responses were observed in 9 of 15 patients undergoing alloHCT for AL amyloidosis [7]. However, the TRM was up to 40% because of the poor performance status of the patients, especially because of cardiac involvement. Among the eight patients who received reduced intensity alloHCT, only two patients (25%) died of TRM. The remaining patients achieved complete response (CR) (2), had progressive disease (1), or died of disease progression (2). Achievement of CR is an important predictor of long-term survival after alloHCT, and the presence of chronic GvHD favored CR [7]. Thus, alloHCT for AL amyloidosis with cardiac involvement, while promising, faces several barriers, including identification of appropriate patients before a decline in performance status, utilization of reduced intensity conditioning regimens and education of insurance company groups regarding the curative potential of alloHCT in an otherwise rare, incurable disease. Further study of reduced intensity alloHCT in young primary amyloidosis patients with good performance status is needed.

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Conflict of interest: Nothing to report.
Published online 14 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22133

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Successful treatment with recombinant soluble thrombomodulin of two cases of sinusoidal obstructive syndrome/hepatic veno-occlusive disease after bone marrow transplantation

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Hepatic sinusoidal obstructive syndrome (SOS), also known as hepatic veno-occlusive disease (VOD), is recognized as one of the most important regimen-related toxicities experienced after hematopoietic stem cell transplantation (SCT). It is a clinical syndrome characterized by painful hepatomegaly, jaundice, ascites, fluid retension, and weight gain [1]. Across 135 studies between 1979 and 2007, the overall incidence was 13.7% in patients undergoing SCT [2], and it usually occurs within the first 3 weeks after high-dose chemotherapy followed by SCT [3]. Severity ranges from mild, reversible disease to a severe syndrome associated with multiorgan failure [4].

Recombinant human soluble thrombomodulin (rTM) is an active, extracellular domain of TM. rTM inactivates coagulation by binding to thrombin and also activates protein C, consequently leading to the inhibition of thrombin formation. A phase III trial has shown that rTM significantly improved disseminated intravascular coagulopathy (DIC) associated with hematological malignancies and infections as compared to unfractionated heparin [5]. Recent studies have shown that rTM also exhibits anti-inflammatory activity by dampening proinflammatory mediators [6]. We here describe two cases of SOS/VOD successfully treated with rTM after bone marrow transplantation (BMT).

The first case (Figure 1A) is a 55-year-old man who was diagnosed with acute myelogeneous leukemia (French-American-British classification, M1) in September 2008. He received two courses of induction chemotherapy before achieving first complete remission (CR). After three courses of consolidation therapy with high-dose cytarabine, he underwent BMT from Human Leukocyte Antigen (HLA)-matched unrelated donor in April 2009. The conditioning regimen consisted of 12 Gy of total body irradiation (TBI) and high dose cyclophosphamide (60 mg/kg/day for 2 days). The graft-ver-

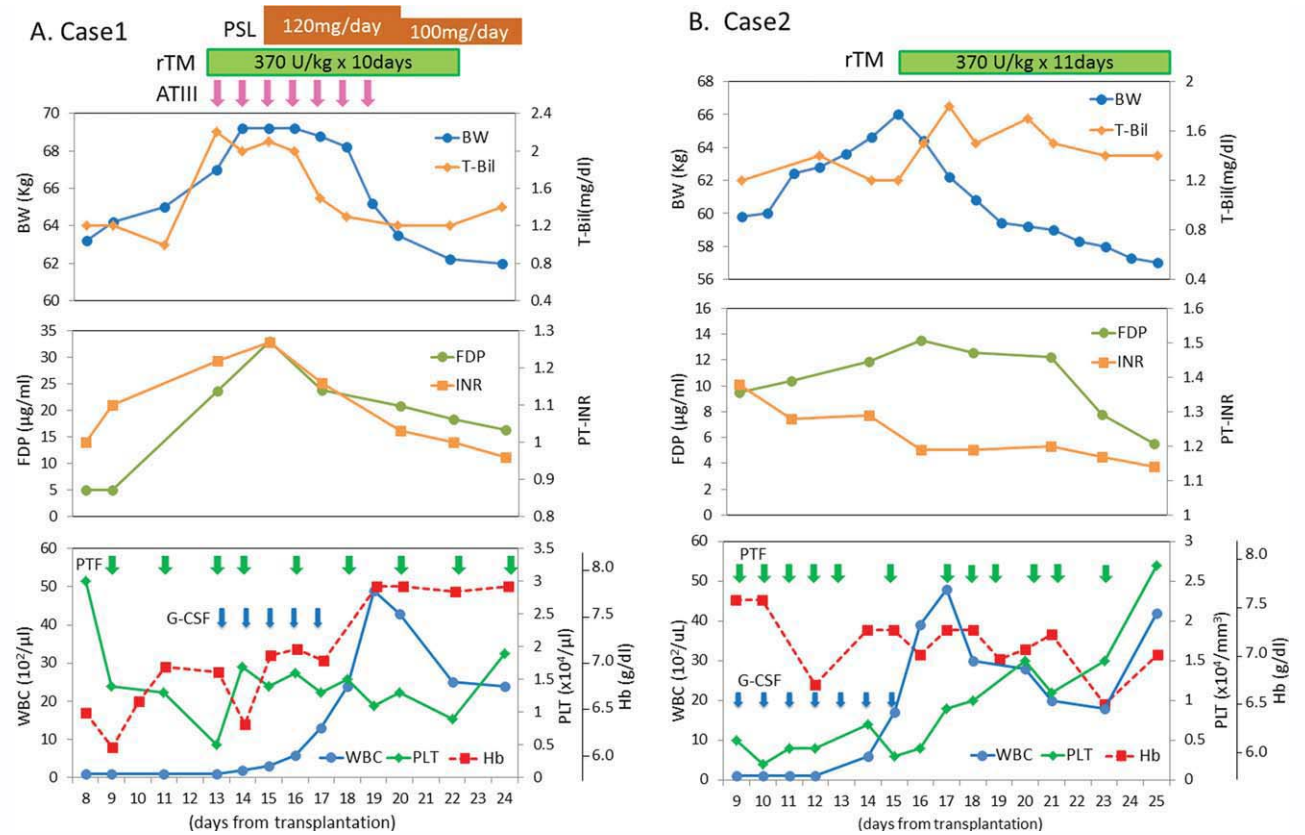

Figure 1. Clinical courses of two cases with SOS/VOD. BW, body weight; T-Bil, total bilirubin; PT-INR, prothrombin time in international normalized ratio; FDP, fibrin degradation products; WBC, white blood cell; PLT, platelet; Hb, hemoglobin; G-CSF, granulocyte colony stimulating factor; PTF, platelet transfusion; PSL, prednisone; ATIII, antithrombin III; rTM, recombinant human soluble thrombomodulin. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

sus-host disease (GVHD) prophylaxis included tacrolimus and short-term methotrexate (MTX). Ursodeoxycholic acid prophylaxis for SOS/VOD was administered from day −14 to day 75 after transplantation. He developed febrile neutropenia on day 6. Administration of broad-spectrum antibiotic was initiated, but his blood culture was negative. On day 13, the patient complained of pain in the right upper quadrant, accompanied by body weight gain, reaching an 11% gain compared with pretransplant baseline, total bilirubin elevation of 2.3 mg/dl, and transfusion-refractory thrombocytopenia. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase were slightly elevated within 2.5 times of normal range. According to McDonald's criteria, the patient was diagnosed as SOS/VOD. At the same time, he also developed DIC, with fibrin degradation products (FDP) elevation, prothrombin time (PT) prolongation, and antithrombin III (ATIII) decrease, which required consecutive administration of ATIII concentrate (1,500 U/day) to maintain ATIII activity at >70%, but no serious bleeding complication was observed. rTM (370 U/kg/day for 10 days) was administered from the same day. At the second day of rTM, pain in the right upper quadrant immediately disappeared and weight gain stopped, which was normalized by day 20 after transplantation. Elevated bilirubin levels and coagulation abnormalities also began to normalize after the initiation of rTM. The patient recovered from DIC on day 22. On day 15, he developed grade II acute GVHD involving the skin (stage 3), along with neutrophil recovery, which was successfully treated by prednisolone (PSL, 2 mg/kg). On day 38, the patient's bone marrow showed CR with 100% donor chimerism, and platelet recovery was complete by day 38. He developed progressive-type extensive chronic GVHD involving the skin, liver, and lungs, 4 months after BMT, which was well controlled by tacrolimus and low-dose PSL. Twenty-four months after transplant, he remains in CR with good performance status.

The second case (Figure 1B) is a 27-year-old male who was diagnosed with T-lymphoblastic lymphoma in December 2009. He achieved CR with alternating regimen of hyper-CVAD (Cyclophosphamide, Vincristine, Doxorubicin, and Dexamethasone) and high-dose MTX plus cytarabine regimen, and underwent BMT from one-locus HLA-mismatched related donor in May 2010. Twelve Gy of TBI and high-dose cyclophosphamide (60 mg/kg/day for 2 days) were given as a conditioning regimen. GVHD prophylaxis consisted of tacrolimus and short-term MTX. The patient received Ursodeoxycholic acid for SOS/VOD prophylaxis from day −14, but he discontinued it due to severe oral mucositis on day 10 after transplantation. He developed transfusion-refractory thrombocytopenia on day 9, and showed weight gain from day 12. His weight showed a 14% increase compared with the pretransplant baseline on day 15. Simultaneously, the total bilirubin level was elevated to 1.8 mg/dl, with massive abdominal effusion and pain in the right upper quadrant. The hepatic ultrasonography showed retrograde flow in the right hepatic vein. In addition, the patient developed DIC with elevated FDP level and PT prolongation without hemorrhagic complications. Laboratory data revealed slightly elevated levels of AST, ALT, and lactate dehydrogenase, within 2.5 times of normal range. The diagnosis of SOS/VOD was made by McDonald's criteria on day 15, and rTM (370 U/kg/day for 11 days) was administered from that day. The right upper quadrant pain improved immediately after the initiation of rTM. On day 25, the body weight normalized to the baseline level and hepatic ultrasonography showed anterograde flow in the hepatic veins. Elevated bilirubin level and coagulation abnormalities were normalized by day 28. Platelet and neutrophil recovery was complete by day 25, and on day 28 the bone marrow showed 100% donor chimerism. CT and positron emission tomography scanning at day 46 showed no evidence of residual tumor. The patient developed grade II acute GVHD involving skin (stage 1) and intestine from day 21, which was well controlled by low dose steroid administration. After 11 months post-transplant, the patient remains in CR with complete donor chimerism and good performance status.

The etiology of SOS/VOD is not yet fully understood, but it is thought to involve sinusoidal endothelial cell injury due to cytotoxic chemotherapy and irradiation. Several markers of endothelial injury, such as plasminogen activator inhibitor-1, tissue plasminogen activator, and protein C have been found to be elevated in patients with SOS/VOD, along with markers that indicate activation of the coagulation system, such as thrombin–antithrombin complexes and prothrombin fragment 1+2 [3,7].

Recently, defibrotide, a single strand polydeoxiribonucleotides, has been shown to be effective in the treatment of SOS/VOD. In a phase II trial, defibrotide showed 100-day survival rate of 35–79% [8–10]. ATIII concentrate has also been reported to be effective, but its efficacy as a single agent against SOS/VOD has not been confirmed [11].

rTM was originally developed as an anticoagulation agent, but it has been recently reported to possess anti-inflammatory potential by binding and decreasing the activity of high-mobility group-B1 protein (HMGB1) [12,13]. HMGB1 is a nuclear architectural chromatin-binding protein released by necrotic cells and has recently identified as a mediator of endotoxin-induced lethality [14]. Its accumulation in the systemic circulation triggers activation of proiflammatory signaling pathways, such as elaboration of reactive oxygen intermediates and nuclear factor-kappa B (NF-κB) activation [6]. It has been shown that plasma HMGB1 levels are increased in patients with DIC [15]. These findings led us to hypothesize that severe damage to the sinusoidal endothelial cells in the transplant recipient might lead to increased systemic circulation of HMGB1, thereby amplifying host inflammatory response and consequently promoting SOS/VOD. rTM could play a protective role by inhibiting such amplification of the host systemic inflammatory response. Recently, published case reports have claimed that rTM is not only effective for SOS/VOD [16] but also for transplantation-associated microangiopathy (TAM) [17]. Both SOS/VOD and TAM are severe post-transplant complications due to endothelial damage induced by chemotherapy and irradiation, further supporting the hypothesis that rTM has a protective activity against endothelial damage.

rTM has also been reported to neutralize lipopolysaccaride [18], a constituent of Gram-negative bacteria, that play a key role in Gram-negative bacterial sepsis. As in case 1, presented above, post-transplant SOS/VOD are usually accompanied by severe infections, so it is reasonable to expect rTM to be effective in such a situation.

However, one must be aware that the anticoagulant potential of rTM might enhance the bleeding tendency in patients with severe endothelial damage, transfusion-refractory thrombocytopenia, and coagulation abnormalities, which are the manifestations usually recorded in patients with SOS/VOD [19]. Therefore, rTM should be indicated in patients who do not possess critical bleeding complications, and sufficient supportive care and close observation must be given to prevent these. On the other hand, the decision to initiate rTM should be made promptly, before organ damage becomes irreversible. Both of our patients showed rapidly developing manifestations of SOS/VOD, without any signs of bleeding complications. We consider they were the best candidates for rTM administration.

Our experiences show that rTM may serve as an effective treatment option in severe post-transplantation complications induced by endothelial damage. Further prospective studies are required to determine its efficacy, safety, and optimal patient selection.

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Conflict of interest: Nothing to report.
Published online 14 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22137

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Hydroxyurea use in patients with sickle cell disease in a Medicaid population

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The purpose of this study was to examine adoption and utilization of hydroxyurea (HU) in a Medicaid population of adults with sickle cell disease (SCD). A retrospective cohort study of SCD patients aged 16–64 years, enrolled in the Florida Medicaid program during a 5-year period from 2001 to 2005, was performed. HU adoption was determined by the presence of at least one HU pharmacy claim using National Drug Codes. Of 2301 adult patients with SCD, nearly 17% of the cohort (*n* = 384) had at least one pharmacy claim for HU. Furthermore, 33% (*n* = 769) of SCD patients were eligible to receive HU, identified by three or more hospitalizations for crisis in a 12-month period, of whom 38% (292/769) received at least one HU prescription. Finally, for patients who received at least two subsequent HU prescriptions (*n* =

241), 15.4% (n = 37) had a medication possession ratio of ≥ 80% of their cumulative daily dose. The prevalence and the persistence of HU use in this Medicaid population were low. Our results suggest that a small subset of indicated SCD patients received HU prescriptions consistently. Early therapy drop out and low adherence rates were common in patients prescribed HU.

Hydroxyurea (HU) is the only Food and Drug Administration approved oral agent to modify the disease course in sickle cell disease (SCD) [1]. Efficacy data from the Multicenter Study of Hydroxyurea (MSH) in sickle cell anemia supports HU use in symptomatic patients with sickle cell anemia [2]. Follow-up studies of MSH patients also showed cost effectiveness and improved survival for HU users [3–5]. Despite the efficacy and cost-effectiveness evidence obtained from the MSH, there is consensus that HU use is suboptimal in treatment of adults with SCD [6]. A study highlighting underuse of HU was published by Lanzkron et al. [7]. Examination of Maryland hospitalization data for sickle cell anemia between 1995 and 2003 revealed an increase in the number and costs of hospitalizations despite HU receiving an expanded indication for treating symptomatic patients with sickle cell anemia. Examination of pharmacy data from a Medicaid managed care organization in Maryland covering 2001–2005 found that 85.9% of patients with SCD never had a claim for a HU refill [8]. Poor adherence to medication in chronic disease patients may contribute to increased hospitalizations and medical costs [9,10]. A recent publication addressing North Carolina Medicaid recipients with SCD found that only 35% of patients receiving HU were adherent based on a calculated medication possession ratio (MPR) [11]. By examining Medicaid claims high resource utilization has been documented for patients with SCD in Florida [12]. The objective of this study was to describe HU adoption and adherence in a Florida Medicaid population and to identify determinants of HU use in adults SCD patients.

A total of 2301 Medicaid beneficiaries diagnosed with SCD were identified. The majority were female (63.5%), black (85.1%), with a mean age of 25.6 years (10.9 SD, 16.0–63.3 range). Approximately 73% (n = 1673) of the patients in the sample had at least one emergency department (ED) visit, and 88% (n = 2025) had at least one inpatient hospitalization. In addition, 36% (n = 827) received red blood cell transfusions, and only 4% (n = 101) received deferoxamine. Of all SCD patients, 33.4% (n = 769) had at least three inpatient hospitalizations for SCD with pain crisis in any 12-month period, and 17% (n = 393) received a prescription for a long-acting opioid medication. Finally, nearly 17% (n = 384) of the cohort had at least one prescription claim for HU during the study period.

Of those 2301 SCD patients identified by ICD-9-CM codes representing hemoglobin SS and other genotypes, 33% (n = 769) were considered potential candidates for HU based on documentation of at least three inpatient hospitalizations for SCD-related pain crisis within a consecutive 12-month period. Table I summarizes patient demographics and medical resources utilization for potential candidates for HU therapy. Candidates for HU therapy were ∼25 years old (9.7 SD), with 87% (n = 688) blacks and 57% (n = 439) women. In addition, 86% (n = 662) had ED visits, while 64% (n = 495) received red blood cell transfusions, 39% (n = 302) received long-acting opioids, and 7% (n = 52) received deferoxamine. Prevalence of HU use was low; only 38% (n = 292) of patients had at least one prescription claim for HU. Hence, 62% (n = 477) of patients did not have any prescription for HU, indicating potential under use of this therapy.

SCD patients were stratified as HU users and non-HU users. Overall, sufficient evidence existed that race (γ^2 = 4.5, P = 0.034), red blood cell transfusions (γ^2 = 16.3, $P < 0.0001$), iron chelation (γ^2 = 11.1, P = 0.001), long-acting opioids (γ^2 = 39.5, $P < 0.0001$), duration of Medicaid eligibility (t-test = −6.92, $P < 0.0001$) were significantly associated with HU use. A higher proportion of HU users also received red blood cell transfusions (73 vs. 59%), iron-chelation therapy (11 vs. 4%), or long-acting opioids (53 vs. 31%) when compared to non-HU users. Moreover, HU users had longer Medicaid enrollments than non-HU users (54 vs. 44 months). Conversely, the characteristics of HU users and non-HU users were comparably similar by patient age-groups, gender, and use of emergency department services.

Of the 384 SCD patients with at least one HU prescription, 83% (n = 322) received one or more HU prescriptions with 28–30 days supply however, the number of prescriptions dispensed varied. Of these patients, 70% (n = 227) received at least three HU prescriptions during the study period; conversely, at least 30% (n = 95) discontinued HU therapy after two prescriptions.

Of the 384 HU users, 63% (n = 241) received two or more HU prescriptions with a at least 28 days and dispensing lapses less than 90 days. Of these patients, 70% (n = 168) took less than 60% of their cumulative daily dose and had frequent lapses in their prescription refills. Conversely, ∼15% (n = 37) had a high MPR (MPR ≥ 80%), while an additional 15% (n = 36) had a MPR between 60 and 79%. See Figure 1 (Supporting Information).

Adjusted odds ratios summarized in Table II indicated that SCD patients who received red blood cell transfusions (OR = 1.62, 95% CI = [1.15, 2.27]), iron chelation (OR = 2.25, 95% CI = [1.23, 4.12]), long-acting opioids (OR = 2.43, 95% CI = [1.96, 3.03]) had higher odds of using HU. Patient demographics (race, age, and gender), and use of emergency department medical resources did not have a significant effect on the odds of HU use.

Our retrospective study of Florida Medicaid recipients found a low prevalence of HU use, which was consistent with previous studies by Lanzkron et al. [7,8]. In the examination of the Maryland Medicaid data only 38% of patients who averaged 3 or more hospitalizations for every 12 month enrollment period had a HU refill claim [8]. A recent publication by Candrilli et al. using similar methodology as in our study examined 2000–2008 data for North Carolina Medicaid recipients. They reported that 35% of patients under the age of 65 had a HU MPR ≥ 0.80 which is significantly higher than the 15% for the Florida patient population. This difference could possibly be accounted for by better adherence of patients under age 16 who were included in the North Carolina study. In addition, there were two academic centers in North Carolina that participated in MSH which may have influenced the uptake of HU therapy in the state once the results of the study were available [2]. However, both studies indicate adults with SCD who would potentially benefit from HU were not receiving treatment. Patients who started on HU but discontinued therapy after two prescriptions as identified

TABLE I. Description of Sickle Cell Disease Patients Eligible for Hydroxyurea

Characteristics	Total [n(%)] N = 739	HU [n(%)] N = 292	No HU [n(%)] N = 477	χ^2-test (or t-test*)
		Demographics		
Mean age, years (SD, 95% CI)	25 (9.7 SD;24.4–25.7)	24 (9.0 SD;23–25.1)	25.7 (10 SD;24.8–26.6)	
16–20	376 (48.9)	155 (53.1)	221 (46.3)	6.58 (0.087)
21–25	140 (18.2)	49 (16.8)	91 (19.1)	
26–35	144 (18.7)	57 (19.5)	87 (18.2)	
≥36	109 (14.2)	31 (10.6)	78 (16.3)	
African–American/Blacks	668 (86.9)	224 (83.6)	424 (88.9)	**4.5 (0.034)**[a]
Other (Hispanic, Caucasian, unknown)	101 (13.1)	48 (16.4)	53 (11.1)	
Male	330 (42.9)	130 (44.4)	200 (41.9)	0.49 (0.481)
Medicaid eligibility, months (SD, 95% CI)	47.9 (18.2SD,46.6–49.2)	53.6 (12.7SD,52–55)	44.4 (20.2SD,42.6–46.2)	**−6.92 (<0.0001)**[a]
		Resource use		
Mean ED visits (SD, 95% CI)	9.9 (21.6 SD;8.3–11.4)	11.6 (20.8 SD;9.2–14)	8.8 (22 SD;6.8–10.8)	−1.74 (0.082)
Any emergency department (ER) Use	662 (86.1)	258 (88.4)	404 (84.7)	2.02 (0.155)
Any red blood cell transfusions	495 (64.6)	214 (73.3)	281 (58.9)	**16.3 (<0.0001)**[a]
Any iron chelation therapy (deferoxamine)	52 (6.7)	31 (10.6)	21 (4.4)	**11.1 (0.001)**[a]
Any long-acting opioids	302 (39.2)	156 (53.4)	146 (30.6)	**39.5 (<0.0001)**[a]

[a]Significant at α = 0.05.

TABLE II. Odds of Receiving Hydroxyurea Therapy (*N* = 769)

Characteristics	HU Use (OR, 95% CI)	
	Unadjusted	Adjusted
Demographics		
16–20	1.30 (0.87–1.94)	1.44 (0.93–2.23)
21–25	(Reference)	(Reference)
26–35	1.21 (0.75–1.96)	1.03 (0.61–1.71)
≥36	0.74 (0.42–1.26)	0.60 (0.34–1.08)
African–American/Blacks	0.64 (0.42–0.97)[a]	0.64 (0.41–1.00)
Other (Hispanic, Caucasian, unknown)	(Reference)	(Reference)
Male (vs female)	1.11 (0.83–1.49)	1.10 (0.81–1.51)
Resource use		
ER Use (vs no ER use)	1.37 (0.86–2.12)	1.03 (0.65–1.66)
Long-acting opioids (vs no LA opioids)	**2.60 (1.92–3.51)**[a]	**2.43 (1.96–3.03)**[a]
Transfusion (vs no transfusion)	**1.91 (1.39–2.62)**[a]	**1.62 (1.15–2.27)**[a]
Iron chelation (DFO vs no DFO)[b]	**2.57 (1.45–4.58)**[a]	**2.25 (1.23–4.12)**[a]
Medicaid eligibility gap >90 days (vs < 90 days)	**0.42 (0.27–0.65)**[a]	**0.56 (0.35–0.88)**[a]

[a]Significant if 95% Confidence Interval does not contain 1.
[b]Deferoxamine.

in this study would not accrue the potential benefits of treatment. Furthermore, only a small subset of the Florida patients received HU prescriptions consistently and had few lapses in their prescription refills.

This observational study used Medicaid administrative claims data. Our results may not be generalizable to non-Medicaid SCD patients. Also, there are limitations in discerning disease severity from analysis of administrative claims data without verification of findings such as radiologic evidence of lung infiltrates in the diagnosis of acute chest syndrome. Thus, the criterion for HU therapy was hospitalizations for crisis. Finally, in the absence of actual information on patient compliance with their medications, using the medication possession rate may actually over-estimate patient compliance with HU.

In conclusion, our observational study provides insights into HU use in adults diagnosed with SCD in a non-managed care Medicaid population 6–10 years after publication of the MSH trial. HU appeared to be underused in this population, as a substantial number of patients who had frequent painful episodes did not receive treatment during the study period. Furthermore, only a small subset of patients received prescriptions consistently, which agrees with the findings of Candrilli et al. [11]. It also emphasizes the importance of improving translation of evidence-based treatment guidelines into clinical practice and disseminating patient education in order to increase adoption and promote adherence to HU therapy in SCD patients.

Methods

This retrospective cohort study used claims data from Florida Medicaid fee-for-service program from January 1, 2001 to December 31, 2005. The dataset includes information on monthly eligibility as well as any medical and pharmacy claims for medically-needy or low-income beneficiaries submitted to Medicaid for reimbursement. The study was approved by the University of Florida Institutional Review Board. Please see Supporting Information for methodology pertaining to subject identification and statistical analysis.

Measures of medication persistency were defined as follows. Therapy adoption was defined as having at least one or more HU prescriptions dispensed with a drug supply of 28 days or longer. Therapy adherence was determined by calculating the MPR, defined as the cumulative daily dose dispensed (not including the last prescription), divided by the time period between first and last prescription refills dispensed for patients with at least two prescriptions for 28 days supply or more [13]. Because any treatment provided during inpatient hospital stays was not captured in the outpatient pharmacy claims data, length of hospitalization (days) was subtracted from the denominator (duration of treatment), MPR was categorized by quintile interval. A MPR of 100% indicated that the patient had the drug dispensed to complete the course. Additional details of methods are presented in Supporting Information.

Acknowledgments

The authors would like to acknowledge the assistance of Dionne Mayhew PhD. No external funding or financial support was received for the study. Intangible support for the study was provided by the University of Florida Center for Medicaid and the Uninsured.

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Conflict of interest: Nothing to report.
This article was published online on 22 August 2011. An error was subsequently identified. This notice is included in the online and print versions to indicate that both have been corrected 29 August 2011.
Published online 14 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22134

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Basophils and plasmacytoid dendritic cells are potential sources for error in flow cytometric monitoring of patients receiving anti-CD22 therapies. AKA not all anti-CD22 antibodies are created equal

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We would like to share our laboratory's experience with flow cytometric monitoring of patients receiving anti-CD22 targeted therapies, to describe a potential pitfall and propose a simple solution for this type of analysis. This experience also highlights the importance of strengthening interactions between treating physicians and laboratory physicians in the rapidly evolving era of targeted and/or personalized medicine.

Several pharmacological agents based on anti-CD22 antibodies are currently available in the clinic at various stages of development. These specifically target B lymphocytes that uniformly express high levels of CD22 molecule on their surface, including both benign conditions associated with aberrant B-cell function (e.g., systemic lupus erythematosus) and malignant B-cell non-Hodgkin lymphomas (B-NHL). Flow cytometry may be used to assess the effect of anti-CD22 therapeutic agents by following the level of CD22-positive B lymphocytes in the blood and/or bone marrow. However, we have observed potentially misleading flow cytometric results while monitoring "CD22-positive cell counts" ordered on patients receiving anti-CD22 therapies. Specifically, we have observed persistence of low levels of CD22-positive events in patients treated for B-NHL with inotuzumab ozogamicin (IO)—a targeted cytotoxic immunoconjugate composed of a humanized IgG4 anti-CD22 antibody covalently linked to *N*-acetyl-gamma-calicheamicin dimethyl hydrazide—who otherwise achieved a complete clinical and morphological remission, suggesting that there may be minimal residual disease. These events are not targeted neoplastic B lymphocytes, rather basophils that are also known to express CD22. The basophils in these patients were unaffected by the therapy, even in patients who achieved complete suppression of CD22-positive lymphocytes (Fig. 1). Thus, reporting only a "total CD22-postive cell count" as requested by the treating physicians would have led to a false-positive minimal residual disease results and possibly inappropriate treatment decisions. CD22 expression, as determined by reactivity to the S-HCL-1 antibody, has also been reported recently on normal plasmacytoid dendritic cells (PDCs) in the blood [1], leading to another source of false positive flow cytometry results.

Closer inspection of the biochemistry of the CD22 molecule and specific antibodies used in therapeutics and diagnostics explains the reactivity of IO with B lymphocytes but not with basophils. The antibody used in IO is humanized antibody derived from clone g5/44, which binds to the N-terminus of the CD22 molecule. In contrast, the most commonly used diagnostic anti-CD22 antibody, and the one used in our laboratory, is derived from the S-HCL-1 clone, a murine antibody that binds to the ligand binding interface between IgLD 1 and IgLD 2. Previous work has shown that several of the commercially available anti-CD22 monoclonal antibodies show differential affinities for the CD22 molecules on normal B lymphocytes versus basophils [2,3]. Specifically, S-HCL-1-derived antibody binds well to CD22 on the surface of B lymphocytes, basophils, and PDCs; whereas g5/44-derived antibody binds CD22 on B lymphocytes but not on basophils or PDCs. The mechanism of this differential binding of anti-CD22 antibodies on B lymphocytes and basophils appears to be due to three-dimensional conformational differences in the CD22 molecule rather than alternative splicing, as the mRNA from B lymphocytes and basophils is identical. With this background, it is quite clear why IO effectively eliminates B lymphocytes but not basophils from the peripheral blood.

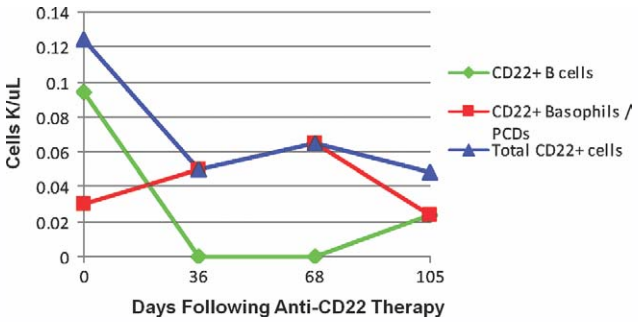

Figure 1. Example of serial CD22-positive cell counts in a patient treated with inotuzumab ozogamicin. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Based on these observations and on the protocols for analyzing basophils suggested by Han et al. [4], Toba et al. [5], and Han [6], our laboratory has adopted a simple, cost efficient and effective four-color flow cytometric assay that readily separates basophils and PDCs from the targeted CD22-positive B lymphocytes for monitoring patients receiving anti-CD22 antibody therapies. We use the following combinations of antibodies in a single tube: CD45 PerCP/CD13+CD33 PE/CD22 FITC/CD19 APC (all from BD Biosciences, San Jose, CA). With this simple methodology, standard gating using CD45 versus side-scatter will provide fairly good discrimination between basophils (R1) and the mature lymphocytes (R2). Confirmatory analysis will separate basophils as CD22-positive, CD13+CD33-positive, and CD19-negative, and PDCs as CD22-positive, CD13+CD33-positive/negative [7], and CD19-negative; whereas B cells will be CD22-positive, CD13+CD33-negative, and CD19-positive (Fig. 2).

As this example indicates, in the age of rapidly blossoming targeted therapies, disease monitoring in the clinical laboratory will need to expand to provide accurate results to guide therapies. The pivotal role of a professional laboratory physician to develop and interpret increasingly complex tests must expand to meet future needs of patients and their treating physicians.

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Received for publication 27 June 2011; Revised 1 July 2011; Accepted 6 July 2011
Conflict of interest: Nothing to report.
Published online 14 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22130

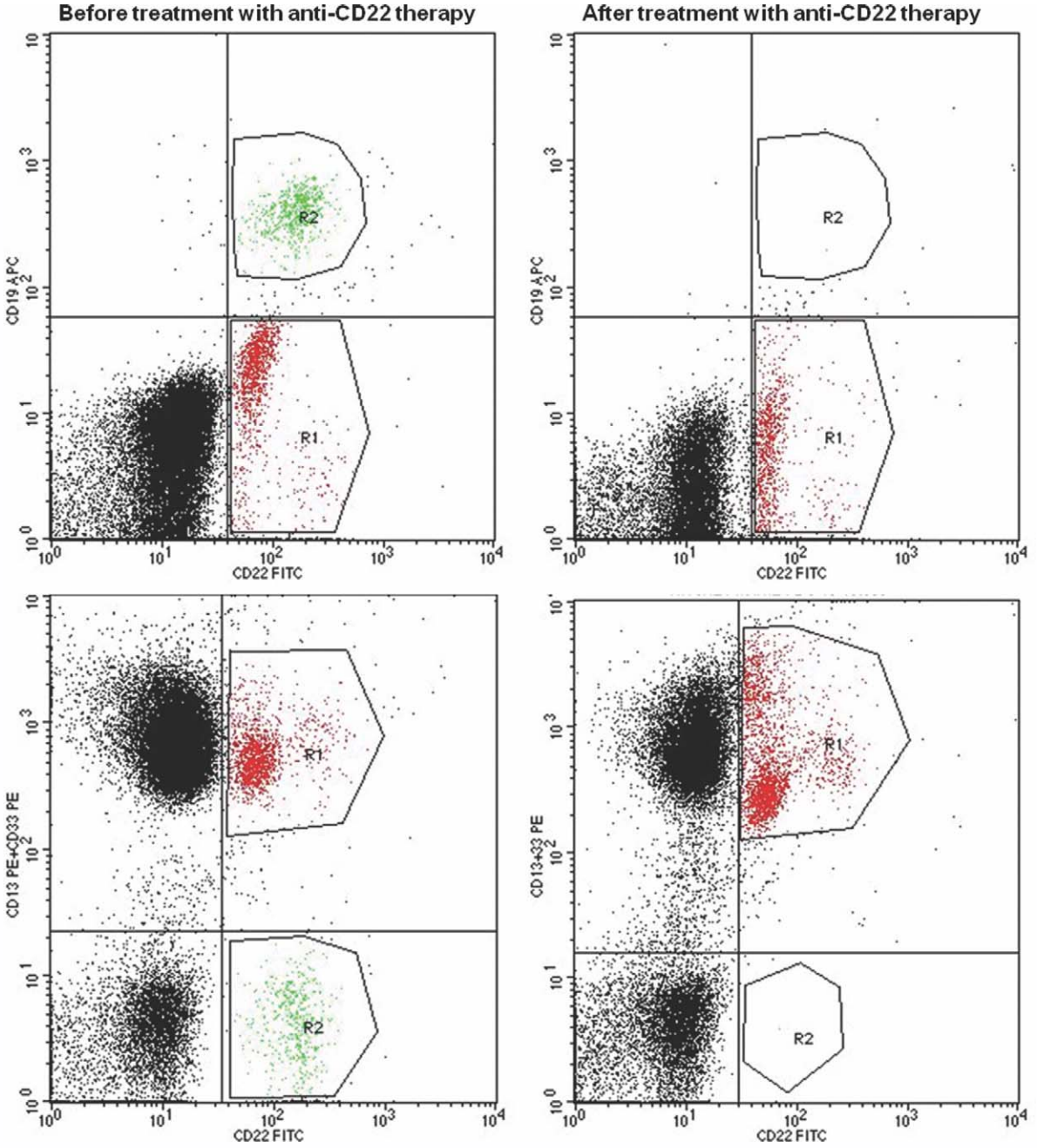

Figure 2. Discrimination of CD22-positive basophils and PDCs from mature lymphocytes by flow cytometric analysis before and after treatment with anti-CD22 therapy. Basophils and PDCs (R1), which express CD13+CD33 and CD22, are contrasted with B lymphocytes (R2) expressing CD19 and CD22. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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