The number of people with sickle-cell disease in the United States: national and state estimates

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Sickle-cell disease is not a reportable condition, making it difficult to ascertain the number of affected individuals. We estimated the number of people with sickle-cell disease for the United States and each individual state, adjusting for increased mortality. US Census population data for each of the 50 states plus the District of Columbia were obtained. The published prevalence of sickle-cell disease for blacks and Hispanics of either Mexican or non-Mexican ancestry was applied. Analysis revealed 89,079 (95% confidence interval: 88,494–89,664) people with sickle-cell disease in the United States, 80,151 black and 8928 Hispanic. The state with the highest sickle-cell population was New York with 8308, followed by Florida with 7539, and Texas with 6765 people with sickle-cell disease. This study provides important information for researchers and policymakers attempting to better plan for the care of the sickle-cell population.

Sickle-cell disease is the most common inherited blood disorder in the United States. It is responsible for approximately 113,000 hospitalizations and $488 million dollars in hospitalization costs annually in the United States [1]. Although sickle-cell disease is a genetic disease diagnosed in this era by newborn screening, it is not a reportable condition. Therefore, it is difficult to ascertain the number of affected individuals in the United States. Newborn screening is an effective tool for diagnosis, but its use was not widespread until the late 1980s and 1990s [2], and it does not account for immigration. Further complicating population estimates is the increased mortality associated with the disease, both compared to the mortality for the underlying racial/ethnic group and when comparing more severe forms of the disease (hemoglobin SS (HgbSS) and hemoglobin S beta thalessemia ($HgbSB^0$)) to less severe forms of the disease (hemoglobin SC (HgbSC) and $HgbSB^+$) [3–7]. Recent advances such as prophylactic penicillin and vaccines have reduced mortality, but only for those young enough to have received the treatments [8,9]. While no published studies have estimated the prevalence of sickle-cell disease in the United States, one NIH estimate puts the number "between 50,000 and 75,000;" another at 80,000, and the Sickle Cell Disease Association of America estimates the number to be "over 70,000" [10–12]

Methods

Census level-data and prevalence. Census data by age and race/ethnicity were obtained for each individual state and the District of Columbia and summed to equal the United States as a whole [13]. Year 2005 was used as the baseline. The applied prevalence rate of sickle-cell disease for blacks was 289 per 100,000 live births [14]. Prevalence rates for Hispanics were 89.8 Hispanic children of non-Mexican ancestry per 100,000 live births and 3.14 Hispanic children of Mexican ancestry per 100,000 live births [14]. These prevalence rates were applied to the Hispanic population numbers in each state based on the state's ratio of the number of Mexican births to non-Mexican births, assuming birthrate was reflective of the current ethnic population proportions. People identified as black-Hispanic (0.56% of the population) were classified as black. We did not include sickle-cell disease for whites (including Mediterraneans), Asian Indians, or other Asians in our estimate. While the disease occurs in these populations, the prevalence is very low, it is not possible to identify the country of origin from census data, and the mortality data specific to these populations is not known.

Consistent with previous literature, 60% of children at birth were classified as having $HgbSS/HgbSB^0$ and 40% were classified as having $HgbSC/SB^+$ [8,15,16]. Other sickle-cell disease genotypes, because the prevalence of these is very low, were excluded.

Mortality adjustment. We adjusted for mortality based on age and sickle-cell type.

Children (<18 years old): For children with $HgbSC/HgbSB^+$, survival is similar to the underlying population [8]. For children with $HgbSS/HgbSB^0$, Quinn et al. [8] reported a 6.4% sickle-cell disease-related mortality in children by 18 years of age. We used linear interpolation to obtain an adjustment by the year. Although the majority of deaths occur early in childhood, this is countered by the use of the conjugate pneumococcal vaccine, which was started in 2001 and would only affect those $\leq$4 years old in our study.

Young adults (age 18–45 years): For young adults with $HgbSC/HgbSB^+$, there is no increase in sickle-cell-related mortality through 30 years of age [7]. From age 31 years through 45 years, there is a linear decrease in survival to 85% at 45 years of age; for similar ages, the black mortality rate is 5%, leaving an excess mortality of 10% between the ages of 31 and 45 years [7]. For young adults with $HgbSS/HgbSB^0$, published reports show improved survival for those born during or after 1975 [4]. For those born during or after 1975 (18–30 years old), 89% survive to adulthood, and 71% survive to age 30 years [4]. With 75% of the deaths being sickle-cell related, the survival in our population of 18–30 years old was 85.4% of predicted based on population estimates. For those born before 1975 (31–45 years old in our population), 79% survived to age 20 years [4]. Ten-year survival from age 20 years shows that 67% would live to 30 years old, 52% would live to 40 years old, and 43% to 45 years old [4].

Middle-aged adults (age 46–65 years): For those with $HgbSC/HgbSB^+$, approximately 50% will be alive at age 65 years compared to 65% of blacks, but this excess mortality occurred at younger ages [7]. For those with $HgbSS/HgbSB^0$, 43% survived to age 45 years and only 10% will survive to age 65 years [4].

Older adults (age > 65 years): For those with $HgbSS/HgbSB^0$, about 1% fewer each year were alive from age 55 to 65 years and we continued with this rate. For those with $HgbSC/HgbSB^+$, we used linear extrapolation to continue the adjustment to the mortality rate from 45 to 65 years.

States were grouped by geographic census regions. A 95% confidence interval (CI) was included for the national estimate based on a Poisson assumption for the variability of the count data. In addition to the CI, two sensitivity analyses were performed. The first analysis assumed that mortality was 10% lower for each age group than predicted from the literature; the second, based on a small study showing a higher prevalence of severe disease in Hispanics, assumed that 80% of Hispanics had severe disease at birth instead of 60% [16].

Population estimates, with mortality adjusted by age and sickle-cell type, yielded a total year 2005 estimate of 89,079 (95% CI: 88,494–89,664) people with sickle-cell disease in the United States, of which 80,151 were black and 8,928 Hispanic (Fig. 1).

The distribution across age groups revealed 35,726 (40% of the population) children with sickle-cell disease. Regional totals are shown in Fig. 1. The South, with a sickle-cell population of 47,354 people, comprised 53% of the sickle-cell population. The estimated sickle-cell population of New York (8308 people), was more than three-fourths of that of the entire Western region.

The estimated number of people with sickle-cell disease in each state is shown in Supporting Information Table I. The states with the highest estimated number of people with sickle-cell disease were New York with 8308; Florida with 7539; Texas with 6765; California with 6474; and Georgia with 5890. These five states comprised more than 43% of the total sickle-cell population.

Supporting Information Table II shows both the absolute and relative percentages of sickle-cell genotypes by age. The increased mortality for $HgbSS/HgbSB^0$ leads to an alteration in the relative percentages of sickle-

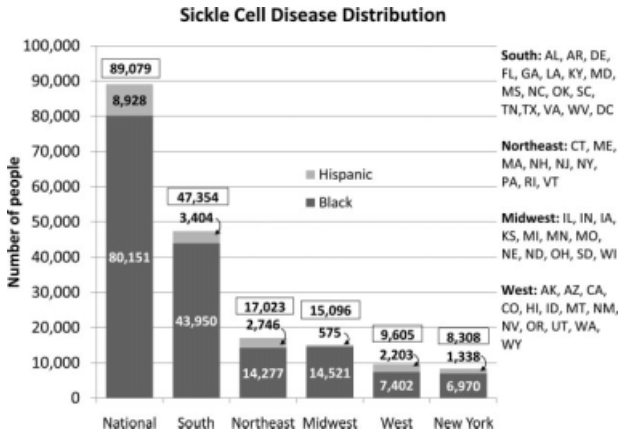

Figure 1. Sickle-cell disease population, by country, region, and state.

cell genotypes, with HgbSS/HgbSB^0 comprising 60% at birth, half at 30 years old, and approximately 25% of the sickle-cell population by 60 years of age.

Our sensitivity analyses show small changes in the estimated sickle-cell population. If our estimated mortality is 10% higher than actual mortality, then the sickle-cell population is 3.1% higher and equals 91,840. If mortality is correct, but Hispanics are more likely to have severe disease (80% HgbSS/HgbSB^0), there would be a decrease in Hispanics from 8928 to 8267, and the total sickle-cell population would be 88,418.

This study shows that the sickle-cell population is significantly higher than previously estimated, with 89,079 people with sickle-cell disease in the Unites States. As of 2005, we estimate that approximately 10% of those with sickle-cell disease are Hispanic. By using census level estimates, we account for immigration in addition to births in the US. With the Hispanic population being one of the largest growing populations in the United States, the percentage of people with sickle-cell disease that are Hispanic will continue to increase.

In addition to the national estimates, we determined the number of people with sickle-cell disease in each state. Previous studies attempting to estimate the sickle-cell population have focused on a single state, the black population only, and/or have not taken into account the increased mortality of sickle-cell disease [3,17]. While these later two omissions would work in opposite directions with regard to over or underestimation of a given state's sickle-cell population, they cast doubt on the accuracy of the estimate and thus the overall study findings.

Another important finding is the relative proportions of sickle-cell genotypes with increasing age. This is the first study to report the fact that above age 40 years, more people with sickle-cell disease will have HgbSC/HgbSB$^+$. It is clear that early intervention into the lives of people with sickle-cell disease is essential in order to maximize the benefit to those with the most severe disease.

The study is limited by the fact that we did not include whites or Asians in our analysis, potentially underestimating the sickle-cell disease population in the Unites States. Despite this exclusion, our estimates are still higher than previous national estimates. We also did not include information on Puerto Rico; race/ethnicity data by age for Puerto Rico is not available on the US Census website.

In conclusion, the sickle-cell population is larger than previously reported, with over 89,000 people with sickle-cell disease in the Unites States. The national and statewide estimates will be useful to policy makers at both a state and national level as they attempt to better understand the burden of sickle-cell disease and plan for the management strategies in this population with high rates of healthcare utilization.

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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 21 October 2009 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21570

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Costs and length of stay for patients with and without sickle cell disease after hysterectomy, appendectomy, or knee replacement

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Patients with sickle cell disease (SCD) who undergo surgical procedures experience greater risk for preoperative and postoperative complications than patients without SCD; however, the impact of SCD on inpatient resource use and costs has not been reported. [1–7] **We recently examined inpatient length of stay and total costs for patients with and without SCD who underwent cholecystectomy or hip replacement and found that patients with SCD had longer lengths of stay and incurred higher costs.** [8] **In this study, we extend our previous work to surgical procedures that are less commonly performed in patients with SCD: hysterectomy, appendectomy, and knee replacement. Using a large national database of inpatient stays, we found that patients with SCD had significantly longer lengths of stay and incurred higher costs than patients without SCD who underwent the same procedures, after adjustment for patient, hospital, and procedural characteristics ($P < 0.001$). Higher inpatient costs were largely attributable to longer hospital stays. Future work should investigate the extent to which preoperative and postoperative complications and other factors contribute to longer stays among patients with SCD and how these factors might be addressed.**

SCD is the most commonly inherited hemoglobinopathy, characterized by anemia and unpredictable, acute complications that can rapidly become life threatening [9–14]. SCD affects more than 70,000 people in the United States and is predominant in African Americans, occurring in ~1 out of every 500 African American births [9,15]. Compared to patients without SCD, patients with SCD who undergo surgical procedures are at greater risk for preoperative and postoperative complications, with SCD-related postoperative complications occurring in over 30% of patients, even after preoperative blood transfusion [5–7,16]. In a health care environment increasingly moving toward differentiated payments for lower- and higher-quality care, it is important to understand patient and procedural factors that contribute to higher complication rates, longer stays, and higher costs. We recently performed an analysis comparing inpatient outcomes between patients with and without SCD who underwent the two most commonly performed nonobstetric surgical procedures among patients with SCD in the United States: cholecystectomy and hip replacement. [8] The study revealed that patients with SCD who underwent either procedure incurred longer inpatient stays and higher inpatient costs than patients without SCD.

In this study, we expanded our analysis to other less commonly performed procedures (ie, hysterectomy, appendectomy, and knee replacement) in patients with SCD and found similar results. Using the 2002–2006 Nationwide Inpatient Sample (NIS) from the Healthcare Cost and Utilization Project, we identified 118 SCD and 85,073 non-SCD discharges for hysterectomy, 69 SCD and 24,802 non-SCD discharges for appendectomy, and 62 SCD and 14,517 non-SCD discharges for knee replacement. For each procedure, patients with SCD were younger than patients without SCD (Table I; $P < 0.001$ for each comparison). Hysterectomy and knee replacement were less likely to be recorded as elective surgery for patients with SCD than for patients without SCD [79.7% vs 86.0% ($P = 0.05$) for hysterectomy; 77.4% vs 93.1% ($P < 0.001$) for knee replacement]. Compared to patients without SCD, patients with SCD were significantly more likely to receive blood transfusions during the inpatient stay [28.0% vs 4.3% ($P < 0.001$) for hysterectomy; 18.8% vs 2.5% ($P < 0.001$) for appendectomy; 30.6% vs 17.5% ($P = 0.007$) for knee replacement]. Unadjusted comparisons of length of stay revealed that patients with SCD had mean hospital stays that were approximately two times longer than for patients without SCD who underwent the same procedure ($P < 0.001$ for each comparison). Mean inpatient costs followed a similar pattern, ranging from 32% higher for knee replacement to 91% higher for appendectomy ($P < 0.001$ for each comparison). The average cost among patients with SCD who underwent appendectomy was nearly $10,000 more than for patients without SCD. Percentages of patients with SCD who died after appendectomy or knee replacement were also significantly higher than for patients without SCD who underwent the same procedure [2.9% vs 0.3% ($P = 0.02$) for appendectomy; 3.3% vs 0.1% ($P = 0.001$) for knee replacement], but the absolute numbers of patients with SCD who died were small ($n = 2$ for each procedure).

After adjustment for patient, procedural, and hospital characteristics, length of stay and inpatient cost estimates remained significantly higher for patients with SCD than for patients without SCD ($P < 0.001$ for each comparison). Compared to patients without SCD, the adjusted length of stay for patients with SCD was 35% longer for hysterectomy (length of stay ratio, 1.35; 95% confidence interval [CI], 1.23–1.49), 85% longer for appendectomy (length of stay ratio, 1.85; 95% CI, 1.56–2.20), and 81% longer for knee replacement (length of stay ratio, 1.81; 95% CI, 1.62–2.02). Compared to patients without SCD, the adjusted cost estimates for patients with SCD were 28% higher for hysterectomy (cost ratio, 1.28; 95% CI, 1.18–1.39), 61% higher for appendectomy (cost ratio, 1.61; 95% CI, 1.40–1.85), and 19% higher for knee replacement (cost ratio, 1.19; 95% CI, 1.08–1.32).

After including inpatient length of stay and discharge disposition in the regression model for inpatient costs, we found that higher inpatient costs were largely attributable to longer hospital stays for all three surgical procedures. Inpatient costs for patients with SCD who underwent hysterectomy or appendectomy remained 8% and 22% higher, respectively, than for patients without SCD, after adjustment for inpatient length of stay and discharge disposition (cost ratio for hysterectomy, 1.08; 95% CI, 1.01–1.15; cost ratio for appendectomy, 1.22; 95% CI, 1.11–1.33).

Among patients with SCD, sickle cell crisis occurred in 5.9% (7/118) of patients who underwent hysterectomy, 31.9% (22/69) of patients who underwent appendectomy, and 14.5% (9/62) of patients who underwent knee replacement (unadjusted $P < 0.001$ across the three surgical procedures). Due to the small number of patients with sickle cell crisis, we did not evaluate the impact of this complication on length of stay, costs, or mortality.

In summary, after adjustment for patient, procedural, and hospital characteristics, patients with SCD incurred significantly longer inpatient stays and higher total costs for hysterectomy, appendectomy, and knee replacement than patients without SCD. Patients with SCD who underwent hysterectomy, appendectomy, or knee replacement were also significantly more likely to have a procedure code for blood transfusion during the inpatient stay, although the overall rates may be somewhat low due to undercoding of this procedure [19]. After accounting for inpatient length of stay and discharge disposition, the impact of SCD on inpatient costs was attenuated for all three surgeries, but costs remained significantly higher for appendectomy and hysterectomy. The higher costs for appendectomy and hysterectomy may be associated with higher rates of sickle cell crisis and nonelective admissions for these two procedures. The study results were consistent with previous findings that focused on cholecystectomy and hip replacement [8], indicating that the study results may be generalizable to other procedures.

This study used the 2002–2006 NIS, part of the Healthcare Cost and Utilization Project sponsored by the Agency for Healthcare Research and Quality (AHRQ). The 2006 NIS consisted of complete discharge information for 1045 hospitals in 38 states in the United States [20]. The NIS includes patient demographic characteristics, primary and secondary diagnoses, procedures performed, discharge status, admission and discharge dates, and inpatient charges. Diagnoses and procedures are coded using both International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) and Clinical Classification Software (CCS) codes. Inpatient charges were converted to costs using inpatient cost-to-charge ratios for individual NIS hospitals provided by AHRQ [21,22].

TABLE I. Characteristics and Outcomes of Patients With or Without Sickle Cell Disease Who Underwent Hysterectomy, Appendectomy, or Knee Replacement

Characteristic/ Outcome	Hysterectomy			Appendectomy			Knee replacement		
	SCD (n = 118)	No SCD (n = 85,073)	P Value	SCD (n = 69)	No SCD (n = 24,802)	P Value	SCD (n = 62)	No SCD (n = 14,517)	P Value
Age, mean (SE), y	40.2 (0.7)	44.3 (0.03)	<0.001	32.7 (1.52)	37.8 (0.08)	<0.001	45.8 (1.42)	54.0 (0.07)	<0.001
Male, n (%)	–	–	–	23 (33.3)	11,840 (47.9)	0.02	21 (33.9)	5581 (38.5)	0.45
Elective surgery, n (%)	94 (79.7)	73,127 (86.0)	0.05	7 (10.1)	3903 (15.8)	0.20	48 (77.4)	13,513 (93.1)	<0.001
Blood transfusion, n (%)	33 (28.0)	3680 (4.3)	<0.001	13 (18.8)	610 (2.5)	<0.001	19 (30.6)	2546 (17.5)	0.007
In-hospital outcomes									
Costs, mean (SE), $	10,585 (835)	7201 (23)	<0.001	19,951 (2649)	10,466 (89)	<0.001	20,286 (3209)	15,344 (77)	<0.001
Length of stay, mean (SE), d	4.7 (0.4)	2.8 (0.01)	<0.001	7.4 (0.86)	3.9 (0.04)	<0.001	8.6 (2.10)	3.9 (0.03)	<0.001
Mortality, n (%)	1 (0.9)	65 (0.1)	0.09	2 (2.9)	73 (0.3)	0.02	2 (3.3)	10 (0.1)	0.001
Multivariable regression									
SCD, length of stay, ratio (95% CI)[a]	1.35 (1.23–1.49)		<0.001	1.85 (1.56–2.20)		<0.001	1.81 (1.62–2.02)		<0.001
SCD, inpatient cost, ratio (95% CI)[a]	1.28 (1.18–1.39)		<0.001	1.61 (1.40–1.85)		<0.001	1.19 (1.08–1.32)		<0.001
SCD, inpatient cost, ratio (95% CI)[a,b]	1.08 (1.01–1.15)		0.03	1.22 (1.11–1.33)		<0.001	1.00 (0.91–1.09)		0.99

SCD, sickle cell disease; SE, standard error; CI, confidence interval.
[a]Adjusted for age, sex (for appendectomy and knee replacement), primary expected payer, 29 comorbidities Ref. [17,18], laparoscopic surgery (for hysterectomy and appendectomy), elective surgery, hospital location, hospital teaching status, hospital bed size, hospital ownership, and US geographic region.
[b]Adjusted for inpatient length of stay and discharge disposition.

We selected discharges of adults aged 18 to 64 years who had undergone hysterectomy (CCS code 124), appendectomy (CCS code 80), or knee replacement (CCS code 152). To limit the possibility that differences in inpatient outcomes between patients with and without SCD were attributable to hospital-level factors, we limited the sample of patients without SCD for each procedure to hospitals where at least one patient with SCD was discharged for the same procedure during the study period. Discharges of patients with SCD were identified using CCS code 61 as the primary or any secondary diagnosis. Sickle cell crisis was identified using ICD-9-CM diagnosis code 282.42, 282.62, or 282.64. Laparoscopic hysterectomy was identified using ICD-9-CM procedure code 68.31, 68.41, 68.51, 68.61, or 68.71. Laparoscopic appendectomy was identified using ICD-9-CM procedure code 47.01 or 47.11. We used the presence of at least one of ICD-9-CM procedure codes 99.01 to 99.04 to identify patients who received at least one blood transfusion during the hospitalization. In addition, we used the Elixhauser comorbidity algorithm, developed by AHRQ, to identify relevant comorbid conditions [17,18].

Analyzing each procedure separately, we used descriptive statistics to summarize patient, procedural, and hospital characteristics and patient outcomes. Comparisons of categorical variables were based on chi-square tests or Fisher exact tests; comparisons of continuous variables were based on t tests. A generalized linear regression model was used to evaluate the independent association of SCD with length of stay and costs, after adjustment for potentially confounding variables. For length of stay, we used a negative binomial distribution with a log link. For costs, we specified the model using a gamma distribution with a log link. There were too few inpatient deaths to model mortality. Covariates included in each model for inpatient length of stay and costs were age (as a continuous variable); sex (for appendectomy and knee replacement); primary expected payer (categorized as private, Medicare, Medicaid, self-pay or no charge, or other); Elixhauser comorbid conditions [17,18] (including congestive heart failure, valvular disease, pulmonary circulation disease, peripheral vascular disease, paralysis, other neurological disorders, chronic pulmonary disease, diabetes with or without chronic complications, hypothyroidism, renal failure, liver disease, peptic ulcer disease, acquired immune deficiency syndrome, lymphoma, metastatic cancer, solid tumor without metastasis, rheumatoid arthritis, coagulopathy, obesity, weight loss, fluid and electrolyte disorders, chronic blood loss anemia, deficiency anemias, alcohol abuse, drug abuse, psychoses, and depression); elective vs nonelective surgery; surgery type (ie, laparoscopic vs open surgery for hysterectomy and appendectomy); urban vs rural hospital location; hospital teaching status; hospital bed size; hospital ownership (public, private, not specified); and US geographic location.

To evaluate the impact of SCD on inpatient costs after adjustment for differences in length of stay and discharge disposition, we added length of stay as a continuous variable and discharge disposition as a categorical variable (i.e., routine vs non-routine status, with non-routine status including transfer to short-term hospital, transfer to other, home health care, left against medical advice, died, and alive but destination unknown). In addition, we sought to determine the effect of sickle cell crisis on inpatient length of stay and costs. However, we could not perform multivariable regression analysis because of the small number of patients with SCD who had a diagnosis of sickle cell crisis.

We used SAS software 9.1.3 (SAS Institute Inc, Cary, North Carolina) for all statistical analyses. The institutional review board of the Duke University Health System approved the study.

Acknowledgments

The authors thank Damon Seils of Duke University for assistance with manuscript preparation. Mr Seils did not receive compensation for his assistance apart from his employment at the institution where the study was conducted.

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Contract grant sponsor: National Heart, Lung, and Blood Institute (Sickle Cell Disease Clinical Research Network); Contract grant number: U10HL083698
Conflict of interest: Dr. Reed has made available a detailed listing of financial disclosures at http://www.dcri.duke.edu/research/coi.jsp.
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Published online 27 October 2009 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21576.

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Cytogenetic correlates of TET2 mutations in 199 patients with myeloproliferative neoplasms

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***TET2* is a putative tumor suppressor gene located at chromosome 4q24. *TET2* mutations were recently described in several myeloid neoplasms but correlations with cytogenetic findings have not been studied. Among a recently described cohort of patients with myeloproliferative neoplasms (MPN) who underwent *TET2* mutation analysis, 199 had information on karyotype at diagnosis or time of *TET2* testing: 71 polycythemia vera (PV), 55 primary myelofibrosis (PMF), 43 essential thrombocythemia (ET), 13 post-PV MF, 7 post-ET MF, and 10 blast phase MPN. Forty eight patients (24%) exhibited abnormal karyotype: 15 favorable (sole 20q-, 13q-, or +9), 8 unfavorable (complex karyotype or sole +8), and 25 "other" cytogenetic abnormalities. We found no significant difference either in the incidence or type of cytogenetic abnormalities between *TET2* mutated (*n* = 25) and unmutated (*n* = 174) cases. Seventy nine patients, including 14 with *TET2* mutations, underwent follow-up cytogenetic testing and the findings were again not affected by *TET2* mutational status. We conclude that *TET2* mutated MPN patients are not cytogenetically different than their *TET2* unmutated counterparts.**

The Ten-Eleven Translocation (*TET*) oncogen family members include *TET1*, *TET2,* and *TET3*. The *TET1* oncogene was first identified as a fusion partner to mixed-lineage leukemia (*MLL*) gene during the chromosomal translocation t(10;11)(q22;q23) in acute myelogenous leukemia [1,2]. The *TET1* gene product catalyzes conversion of 5-methylcytosine to 5-hydroxymethylcytosine, suggesting a role in epigenetic regulation [3]. *TET3* is located at chromosome 2p13.1 and its function is unknown. *TET2* is a putative tumor suppressor gene located at chromosome 4q24. Delhommeau et al. first reported the occurrence of *TET2* mutations in patients with *JAK2*V617F-positive MPN [4]. Subsequently, our group reported similar mutations in MPN with and without *JAK2*V617F mutations, systemic mastocytosis, and other myeloid malignancies [5–8]. In this study, we looked for possible correlations between *TET2* mutations and cytogenetic abnormalities in MPN.

This study was approved by the institutional review board. Inclusion criteria included availability of bone marrow cytogenetic results at diagnosis or at the time of blood sample collection for *TET2* mutation analysis. Follow-up cytogenetic data, where available, were also recorded. The specific MPN diagnosis was established on the basis of the 2001 World Health Organization (WHO) criteria [9]. Both direct techniques and unstimulated culture methods were used to harvest metaphases for analysis from bone marrow aspirate [10]. *JAK2*V617F mutation analysis was performed according to previously published methods [11]. Cytogenetic findings were assigned as being favorable (sole 20q-, 13q-, or +9), normal, unfavorable (complex or +8), or "other cytogenetic abnormalities", based on recent prognostic studies in PMF [12,13]. High-throughput DNA sequence analysis was used to screen for *TET2* mutations in bone marrow-derived DNA as described previously [5].

A total of 199 MPN patients had information on both *TET2* mutational status and karyotype: 71 PV, 43 ET, 55 PMF, 13 post-PV MF, 7 post-ET MF, and 10 with blast phase MPN. Overall, 48 (24%) patients exhibited an abnormal karyotype: 15 favorable, 8 unfavorable, and 25 "others". *TET2* mutations were identified in 25 (13%) patients. As reported before, patients who harbored *TET2* mutations were significantly older ($P = 0.001$) than those with wild-type *TET2*. In contrast, the distribution of gender, specific MPN diagnosis, and *JAK2*V617F mutational status was similar between the two groups (Table I). A similar number of patients, 7 (18%) and 41 (24%) with mutated and wild-type *TET2*, respectively, harbored an abnormal karyo-

TABLE I. Clinical and Cytogenetic Features at Diagnosis and During Follow-Up in Patients with Myeloproliferative Neoplasms Who Underwent *TET2* Mutation Analysis

	Findings at diagnosis or at time of *TET2* testing				Findings at time of follow-up cytogenetic studies			
		TET2				*TET2*		
Variable	All *n* (%)	Mutated *n* (%)	Wild-type *n* (%)	*P* value	All *n* (%)	Mutated *n* (%)	Wild *n* (%)	*P* value
n (%)	199 (100)	25 (13)	174 (87)		79 (100)	14 (18)	65 (82)	
Cytogenetics *n* (%)								
Normal	151 (76)	18 (72)	133 (76)	0.6	48 (61)	10 (71)	38 (58)	0.4
Abnormal	48 (24)	7 (28)	41 (24)		31 (39)	4 (29)	27 (42)	
Cytogenetics *n* (%)								
Favorable	15 (8)	1 (4)	14 (8)	0.6	12 (15)	1 (7)	11 (17)	0.8
Normal	151 (76)	18 (72)	133 (76)		48 (61)	10 (72)	38 (58)	
Others	25 (13)	5 (20)	20 (12)		14 (18)	2 (14)	12 (18)	
Unfavorable	8 (4)	1 (4)	7 (4)		5 (6)	1 (7)	4 (6)	
Gender *n* (%)								
Male	103 (52)	16 (64)	87 (50)	0.2	36 (46)	9 (64)	34 (52)	0.4
Female	96 (48)	9 (36)	87 (50)		43 (54)	5 (36)	31 (48)	
Age in years median (range)	58 (19–93)	66 (51–84)	56 (19–93)	0.001	60 (26–84)	60 (31–78)	58 (26–84)	0.01
Age *n* (%)								
<60	107 (54)	6 (24)	101 (58)	0.001	43 (54)	4 (29)	39 (60)	0.03
≥60	92 (46)	19 (76)	73 (42)		36 (46)	10 (71)	26 (40)	
*JAK2*V617F mutation *n* (%)								
Yes	137 (69)	21 (84)	116 (67)	0.08	55 (70)	12 (86)	43 (66)	0.1
No	62 (31)	4 (16)	58 (33)		24 (30)	2 (14)	22 (34)	
Myeloproliferative neoplasm *n* (%)								
PV	71 (36)	9 (36)	62 (36)	0.4	16 (20)	3 (21)	13 (20)	0.6
ET	43 (22)	1 (4)	42 (24)		12 (15)	0	12 (18)	
PMF	55 (27)	10 (40)	45 (26)		32 (41)	7 (50)	25 (38)	
Post-PV MF	13 (6.5)	2 (8)	11 (6)		10 (13)	2 (14)	8 (12)	
Post-ET MF	7 (3.5)	1 (4)	6 (3)		4 (5)	1 (7)	3 (5)	
Post-MPN AML	10 (5)	2 (8)	8 (5)		5 (6)	1 (7)	4 (6)	

TABLE II. Cytogenetic Findings in 25 *TET2*-Mutated Patients with Myeloproliferative Neoplasms

Specific diagnosis	Age/sex	*TET2* mutation type		Cytogenetic findings
PV	66/M	Nonsense	Normal	
PV	76/M	Nonsense	Normal	
PV	72/M	Nonsense	Normal	
PV	52/F	Nonsense	Normal	
PV	61/F	Nonsense	Normal	
PV	69/F	Frameshift	Normal	
PV	51/M	Frameshift	Normal	
PV	81/M	Frameshift	Normal	
PV	84/F	Frameshift	Normal	
ET	51/F	Frameshift	Normal	
PMF	56/F	Missense	Normal	
PMF	60/F	Missense	Normal	
PMF	69/M	Missense	Normal	
PMF	78/M	Frameshift	Normal	
PMF	74/M	Frameshift	Normal	
PMF	60/F	Frameshift	Normal	
Post-PV MF	69/M	Frameshift	Normal	
Post-ET MF	66/M	Frameshift	Normal	
PMF	58/M	Frameshift	Favorable	46,XY,del(20q)[4]/46,XY[16]
PMF	70/M	Nonsense	Unfavorable	46,XY,t(2;8;17)(p13;q24;q21)[25]
Post-ET AML	68/M	Frameshift	Unfavorable	47,XY,+7[13]/hyperdiploid[3]/46,XY[4]
Post-PV AML	62/F	Frameshift	Unfavorable	Complex[a]
Post-PV MF	74/M	Missense	Others	: 45,XY,der(6)t(6;17),−17[2]/46,XY[18]
PMF	64/M	Frameshift	Others	47,XY,+19
PMF	56/M	Nonsense	Others	46,XY,7q-

PV, polycythemia vera; PMF, primary myelofibrosis; ET, essential thrombocythemia; AML, acute myelogenous leukemia.
[a](43–46, XX, −5, −6, add(7) (p13), del(11) (q13q23),−13, add(13)(p13),−16,−20,−21,−22,+3-7mar[cp20]).

type ($P = 0.6$). The distribution of cytogenetic categories (favorable, normal, unfavorable or "others") was also similar between the 2 groups (Tables I and II). Similar observations were made when the results of follow-up cytogenetic studies that were available in 79 patients were analyzed (Table I). We conclude that *TET2* mutations do not affect initial or subsequent cytogenetic findings in MPN.

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Conflict of interest: Nothing to report.
Published online 9 October 2009 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21562

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Endogenous thrombin potential in the assessment of hypercoagulability in systemic lupus erythematosus

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Endogenous thrombin potential (ETP) is a measurement of thrombin formation capacity of plasma and may be increased in congenital and acquired hypercoagulable states. We assessed whether ETP was associated with antiphospholipid antibodies (aPL) and with thrombosis history in SLE. ETP was performed in 130 SLE patients using a Siemens ETP Assay on BCS Coagulation System analyzer, that is equipped with software to analyze different components of the coagulation wave form, including lag-time (time to initiate thrombin generation), *T*-max (estimate of enzymatic rate), *C*-max (measurement of peak height), and area under the curve (total thrombin formation). Higher *T*-lag values were found with deep venous thrombosis (DVT) (33.6 ± 15.9 vs. 22.9 ± 14.8, $P = 0.0018$), myocardial infarction (MI) (43.6 ± 36.4 vs. 24.6 ± 15.1, $P = 0.0855$) and stroke (27.5 ± 13.5 vs. 24.5 ± 15.8, $P = 0.4883$) than without. *T*-max was also higher in patients with DVT (68.4 ± 21.9 vs. 56.5 ± 24.4, $P = 0.0300$), and MI (123.8 ± 77.0 vs. 57.7 ± 22.1, $P \geq 0.0001$) compared to those without. ETP *T*-lag and *T*-max were higher for patients with aPL. ETP *T*-lag and *T*-max were associated with both venous (DVT) and arterial (stroke, MI) thrombosis in SLE and with aPL. This suggests that ETP measures should now be explored prospectively to determine their predictive value for future thrombosis in SLE.

Systemic lupus erythematosus is a multisystem autoimmune disorder mostly affecting young women [1]. Thrombosis has been reported in 7.2–12% of patients with SLE [2,3]. The proportionate mortality from thrombosis in SLE is 26.7% [2]. Multiple studies in SLE have shown a positive association between antiphospholipid antibodies and thrombosis [3–6]. Because not all patients with antiphospholipid antibodies suffer thrombosis, identification of those with higher risk is important. The mechanism of anti phospholipid antibody associated thrombosis appears to be multifactorial, including complement activation [7], platelet activation [8,9], modulation of endothelial function [10], and interference with the Protein C pathway through the development of acquired protein C resistance and deficiencies of Protein C and Protein S [11,12]. Increased expression of tissue factor in macrophages has also been proposed [13].

The hemostatic system maintains a balance between procoagulant and anticoagulant pathways [14]. The net effect of the hemostatic pathway is to generate thrombin to permit the conversion of fibrinogen to fibrin. A decrease in thrombin generation, either acquired or inherited, can lead to impaired coagulation and bleeding. In contrast, increased thrombin generation can lead to clotting tendency and thrombosis. Hypocoagulation can readily be detected by routine laboratory tests. In contrast, despite remarkable progress in defining new risk factors for thrombosis, a laboratory test that globally measures overall thrombin formation has not, until recently, been available [15].

Measurement of the thrombin generation curve is a well-established tool in coagulation research, reflecting overall function of the blood coagulation system [16]. In platelet poor plasma, thrombin generation measurement reflects all clotting deficiencies except for factor XIII, and is sensitive to oral anticoagulants and heparin. Hyperprothrombinemia, absence of antithrombin, protein C and S as well as activated protein C resistance increase thrombin generation. Despite utilization of thrombin generation in the research laboratory, the clinical application of measuring thrombin generation is only beginning. Recent improvement in reagents, coagulation equipment, and software now permit the study of thrombin potential in many different clinical situations. Recently, this testing has been shown to be promising in assessing the risk of clotting recurrence [17,18]. However, this test has not been approved for clinical use in the United States.

In our study, we hypothesized that the application of thrombin potential might be useful in determining which SLE patients were at risk for thrombosis. We now report the relationship of thrombin generation parameters to clinical thrombosis in a cohort of patients with systemic lupus erythematosus, including those with antiphospholipid antibodies.

Endogenous thrombin potential (ETP) is the amount of thrombin formed in vitro under different conditions in a clotting reaction from the beginning to the end of the reaction. It is thought that the ETP reflects the in vivo capacity of an individual to generate thrombin.

Materials and Methods

Patients: One hundred and thirty patients with SLE were enrolled in this study. These patients were enrolled from the Hopkins Lupus Cohort, a prospective study of disease activity and predictors of morbidity and health status. The study was approved by the Johns Hopkins University School of Medicine Institutional Review Board. All patients gave written informed consent. All patients had been seen quarterly (or more often if warranted) since cohort entry, for assessment of disease activity (by the Physician's Global Assessment on a 0–3 visual analog, and the SELENA SLEDAI) [19,20], laboratory tests (complete blood count, erythrocyte sedimentation rate, serum creatinine, cholesterol, urinalysis, C3, C4, and anti-dsDNA), anticardiolipin (ELISA, INOVA) and dilute Russell viper venom time [20]. Cardiovascular risk factors and thrombotic history were also assessed. Of these 130 patients, 24 had a history of deep venous thrombosis (DVT), 15 had a cerebrovascular accident (CVA) in the past, and two had a myocardial infarction (MI) in the past. The specimens were collected at the time of their routine visit, and there was no relation to occurrence of thrombotic events. 24 patients were on warfarin and one was on heparin at the time of their visit when blood work was done for measurement of ETP. 98 and 60 patients had elevated anticardiolipin antibody and elevated diluted russel viper venom time (dRVVT) respectively, at some point during their work up. The dRVVT test was done according to manufacturer's procedure and employed a confirmatory step with addition of phospholipid for those patients with a prolonged dRVVT (Siemens, Marburg Germany).

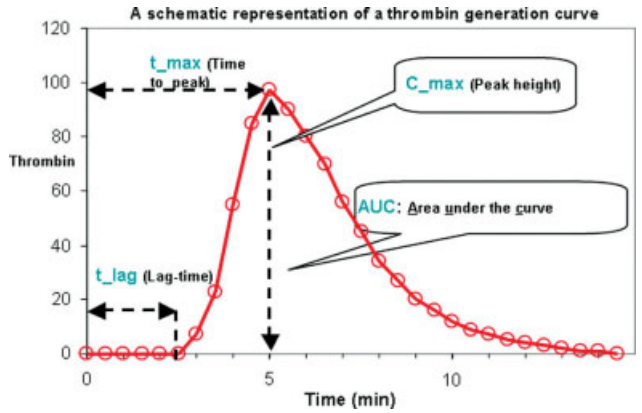

Figure 1. A schematic representation of a thrombin generation curve. Lag-time, time to initial inflection of the curve, *T*-max, time to maximum thrombin generation; *C*-max, maximum amount of thrombin generated; AUC, total thrombin generated, ETP. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ETP was performed using the ETP kit by Siemens (Marburg Germany) and a Siemens BCS analyzer. To determine ETP, the conversion kinetics of a synthetic thrombin substrate were measured by the release of a chromophore in the plasma sample at a wavelength of 405 nm. The thrombin formation was started by the addition of Dade Innovin Reagent and calcium chloride. The thrombin formation curve was obtained by mathematical derivation of the measured formation kinetics, whose integral (described as the "surface area under the curve") corresponds to ETP. In addition to being converted by thrombin, the substrate was also converted by the resulting thrombin-α2-macroglobulin complex. To determine the ETP, the reaction rate of the thrombin-α2-macroglobulin complex was calculated based on the total conversion and then subtracted. The necessary calculations were carried out automatically by the BCS System with the available ETP analysis procedure.

The development of new software by Siemens Diagnostics, Marburg, Germany permits the plotting and analysis of the entire curve of thrombin formation, i.e., the thrombogram. (See Fig. 1) [17]. The parameters that can be derived from the thrombin generation curve are the following: the lag time, endogenous thrombin potential (area under the curve), peak height of thrombin which reflects the maximum amount of thrombin generated from the plasma, and time to peak thrombin formation [21].

Samples were collected in 3.2% citrate. After collection, the samples were spun at 2500*g* on an Ependorf centrifuge to remove any platelets or microparticles. The samples were then frozen at −70°C until testing. At the time of testing the samples were thawed in a 37°C waterbath, and then again spun at 2500*g* before testing.

All results for continuous variables are expressed as means ± SD, unless specified otherwise. Continuous variables were analyzed with a two-sided *t*-test and categorical variables were compared by the Pearson chi-square or Fishers exact test. Oneway ANOVA was performed for normally distributed variables. Statistical analysis was performed using JMP (v5.0.1, SAS Institute, Cary, NC). A *P*-value of 0.05 was taken as statistically significant.

Results and Discussion

Data were obtained from 130 patients with SLE. The patients were 94% female, 6% male, 5% Asian, 2% Hispanic, 38% African-American, 50% Caucasian, and 4% other ethnicities. The mean age was 45 years. The ETP reference ranges in normal patient plasma (20 males and 20 females age 25–60 years), were *T*-lag (12.5 ± 21.3), *T*-max (36.4 ± 60.0), *C*-max (124.5 ± 188.5), and AUC (265.4 ± 637.8). The reference ranges were collected from normal laboratory employees and done on platelet poor plasma. Tables I and II summarize the results of ETP *T*-lag and *T*-max of our patients. SLE patients with deep venous thrombosis (DVT) and myocardial infarction (MI) had greater *T*-lag (the time to initiation of activation of thrombin) and *T*-max (the estimate of enzymatic rate) values than patients without. ETP *T*-lag and *T*-max were also higher in SLE patients with the lupus anticoagulant and anticardiolipin. The presence of lupus anticoagulant and anticardiolipin had no significant association with the *C*-max levels (the highest point, or peak, of the thrombin generation curve). The AUC for SLE patients with DVT, MI and stroke was lower than in patients without DVT, MI, or stroke. The AUC was also lower in patients with the lupus anticoagulant and anticardiolipin. Similarly, it was lower in those patients with venous and arterial thrombosis, than in those without thrombosis.

TABLE I. *T*-lag: Time to Initial Inflection of the Curve—Reference Range in Normal Patient Plasma: 12.5 ± 21.3

Historical variables	Factor negative	Factor positive	*P*-value
All thrombosis ever	21.7 ± 12.9 (95)	33.4 ± 18.6 (35)	0.0001
Deep venous thrombosis ever	22.9 ± 14.8 (106)	33.6 ± 15.9 (24)	0.0018
Myocardial infarction ever	24.6 ± 15.1 (128)	43.6 ± 36.4 (2)	0.0855
Stroke ever	24.5 ± 15.8 (115)	27.5 ± 13.5 (15)	0.4883
Digital Gangrene ever	24.8 ± 15.6 (128)	25.0 ± 17.4 (2)	0.9880
Lupus anticoagulant ever	19.8 ± 7.7 (70)	30.8 ± 19.7 (60)	<0.0001
Anticardiolipin ever	21.8 ± 11.7 (33)	25.8 ± 16.5 (97)	0.1964
Anti-β2-glycoprotein 1 ever	23.3 ± 14.2 (71)	26.8 ± 18.0 (47)	0.2405
Same day visit variables	Rsquare adj	*P*-value	
Thrombocytopenia	0.015121 (neg)	0.0883	
Lupus anticoagulant	0.461029	<0.0001	
Anticardiolipin IgG	0.023364	0.0466	
Anticardiolipin IgM	−0.00289	0.4276	
Anticardiolipin IgA	−0.00527	0.5553	

TABLE II. *T*-max: Time to Maximum Thrombin Generation—Reference Range in Normal Patient Plasma: 36.4 ± 60.0

Historical variables	Factor negative	Factor positive	*P*-value
All thrombosis ever	57.8 ± 24.0 (95)	70.9 ± 26.4 (35)	0.1178
Deep venous thrombosis ever	56.5 ± 24.4 (106)	68.4 ± 21.9 (24)	0.0300
Myocardial infarction ever	57.7 ± 22.1 (128)	123.8 ± 77.0 (2)	<0.0001
Stroke ever	57.7 ± 24.3 (115)	66.3 ± 24.2 (15)	0.1956
Digital Gangrene ever	58.7 ± 24.4 (128)	54.3 ± 27.0 (2)	0.7973
Lupus anticoagulant ever	51.1 ± 19.2 (70)	67.5 ± 26.7 (60)	<0.0001
Anticardiolipin ever	53.4 ± 25.8 (33)	60.5 ± 23.7 (97)	0.1530
Anti-β2-glycoprotein 1 ever	56.6 ± 22.7 (71)	62.4 ± 27.4 (47)	0.2079
Same day visit variables	Rsquare adj	*P*-value	
Thrombocytopenia	0.043193 (neg)	0.0106	
Lupus anticoagulant	0.335316	<0.0001	
Anticardiolipin IgG	0.030387	0.0274	
Anticardiolipin IgM	−0.00597	0.6210	
Anticardiolipin IgA	−0.00811	0.9629	

Anticoagulants like warfarin as well as heparin would produce decreased area under the curve, *C*-max, and prolong the lag time. As the amount of thrombin formed decreases in patients who are adequately anticoagulated, the *T*-max should be lower and not higher.

Past studies have shown increased thrombin generation in women on oral contraceptive pills and with a history of venous thromboembolism [22,23] or with a history of venous thromboembolism only [24]. In these studies [23,24], ETP was measured in the presence of thrombomodulin (TM). However, one of the above studies excluded patients with antiphospholipid syndrome [23] and another study [24] excluded patients with antiphospholipid antibodies/lupus anticoagulants. Our study is the first large study of ETP in SLE, and the first study to report on the different parameters of the thrombin generation curve in SLE.

A recent study [25] has shown that measurement of whole blood thrombin generation assay, peak height, and ETP were higher in patients with a history of venous thromboembolism compared to controls. However, in our study, we used platelet poor plasma. Use of platelet rich plasma in the testing of thrombin generation also reflects the contribution of platelets in the generation thrombin. A major disadvantage of using platelet rich plasma is that only fresh samples can be used to produce reliable results.

In contrast to a previous study, we did not find higher ETP AUC levels in SLE, nor in patients with known hypercoagulable states, such as antiphospholipid antibodies [26]. Instead, we found that the *T*-lag and *T*-max were

higher in SLE patients with antiphospholipid antibodies. We anticipated that the *T*-max would simply reflect the AUC and we were surprised to observe discordant results between the AUC and *T*-max. We do not have an explanation for this. Because a relatively low amount of phospholipid is used to initiate thrombin formation, it is conceivable that the prolonged lag time reflects the effect of antiphospholipid antibody in blocking the effect of phospholipid. While the increased *T*-max may reflect accelerated formation of thrombin, the prolonged *T*-lag may actually contribute toward the finding. In the calculation of *T*-max both the lag time and slope of the thrombin generation curve determine *T*-max. If indeed there is a higher rate of thrombin formation, the significance of this is unclear. It is conceivable that this may reflect an initial burst of thrombin formation that is dampened by anti-thrombin or other natural thrombin inhibitors. Regardless of this, overall our findings do not indicate that under the conditions we employed could we demonstrate increased thrombin potential. The limitation of our in vitro studies to reflect in vivo phenomenon, is that we are not using whole blood which includes the contribution of platelets and platelet microparticles.

Clearly the pathogenesis of thrombosis associated with antiphospholipid antibodies in SLE is multifactorial, involving not just the procoagulant proteins, the cellular constituents involving hemostasis, the anticoagulant mechanisms and fibrinolytic pathway. We know that the thrombotic complications are ameliorated by anti-thrombin agents including heparin and warfarin. This clearly argues for a key role of thrombin in the pathogenesis of the complications associated with antiphospholipid antibodies.

As we begin to correlate the results of thrombin generation tests with a variety of clotting and bleeding disorders, we are likely to gain a better understanding of the meaning of different parts of the thrombogram. The analysis of wave forms, of which the thrombogram is an example, is a new way to dissect out the complex interactions of coagulation proteins. The challenge is to correlate these parameters with clinical events or other biochemical or functional measurements of coagulation.

Our studies show that there are changes in the thrombogram associated with antiphospholipid antibodies and history of thrombosis in SLE. Future prospective study is now warranted to determine the predictive value of ETP measures for future thrombosis.

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Contract grant sponsor: National Institute of Health; Contract grant number: NIH AR 43727; Contract grant sponsor: General Clinical Research Center; Contract grant number: MO1-RR00052; Contract grant sponsor: Siemens Medical Diagnostics, Marburg, Germany.
Conflict of interest: Nothing to report.
Published online 14 October 2009 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21566

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HIV-negative, HHV-8-unrelated primary effusion lymphoma-like lymphoma: report of two cases

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Primary effusion lymphoma (PEL) is a rare type of lymphoma confined to the body cavities, such as pleural, pericardial, and peritoneal cavities. PEL is usually associated with human herpes virus 8 (HHV-8) and human immunodeficiency virus (HIV) infection, however, there are some reports of HIV-negative and HHV-8-unrelated cases. Recently, these cases are described as HHV-8-unrelated PEL-like lymphoma. Here, we report two

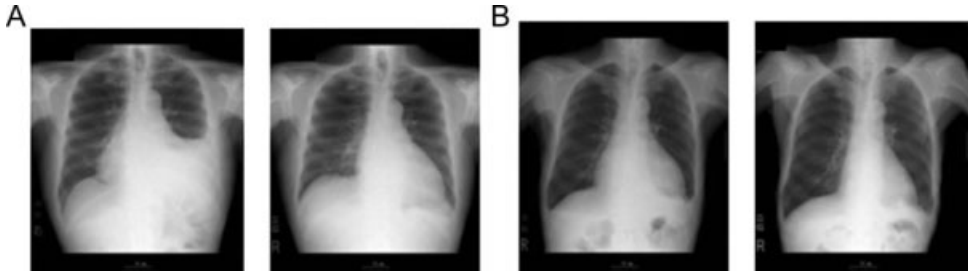

Figure 1. Chest X-rays of the cases. A: Chest X-rays when case 1 was diagnosed (left panel) and now (right panel). B: Chest X-rays when case 2 was diagnosed (left panel) and now (right panel).

such cases. In both cases, no lymphadenopathy or organ involvement with lymphoma was found. Surface marker revealed that they were both CD20 positive lymphoma. Systemic chemotherapy with CHOP regimen with rituximab was effective and gradually led to disappearance of the lymphoma. HHV-8-unrelated PEL-like lymphoma is truly a distinct clinical entity and the prognosis of it seems to be better than PEL.

PEL is a very rare type of non-Hodgkin lymphoma that involves only body cavities [1]. According to the World Health Organization (WHO) classification of hematological malignancies, PEL is classified as a subtype of diffuse large B-cell lymphoma that is closely associated with human herpes virus-8 (HHV-8) and HIV [2]. On the other hand, it has been reported that there are some patients with HHV8-negative and HIV-negative PEL that highly expresses B-cell markers, which are described as HHV-8-unrelated PEL-like lymphoma [2]. The reports of HHV-8-unrelated PEL-like lymphoma are anecdotal and the character of the lymphoma is not well known yet. Here, we report two cases of HHV-8-unrelated PEL-like lymphoma who were successfully treated with R-CHOP and review of the literature.

Case 1

A 82-year-old man went to an outpatient clinic because of edema of his lower extremities in January, 2008. He was found to have massive pericardial effusion, left pleural effusion, and sign of cardiac decompensation. Soon after admission, the patient was treated with drainage of the pericardial and pleural effusion. On cytological examination of the pleural and pericardial effusion, middle to large-sized atypical lymphoid cells were observed. The cells were positive for CD20 and CD79a, but negative for CD3. The immunoglobulin light chain restriction was also observed. He was suspected to have PEL and introduced to our hospital. When he was admitted, he had massive left pleural effusion and moderate pericardial effusion. The serum lactose dehydrogenase (LDH) level was 214 IU/L. Tests for hepatitis C virus (HCV) and HIV antibody were negative. Cytological evaluation of the pleural effusion demonstrated middle to large-sized atypical lymphoid cells with prominent nucleoli. The cell block preparation of pleural effusion revealed that atypical lymphoid cells were negative for HHV-8, but positive for EBER-ISH and EBNA2. The pleural effusion test for HHV-8 using polymerase chain reaction (PCR) method was also negative. No mass or lymphoma cells were detected on whole body CT scan, FDG-PET, and bone marrow biopsy. He was diagnosed as HIV-negative HHV-8-unrelated PEL-like lymphoma. The patient was treated with six courses of chemotherapy consisting of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP). After six courses of R-CHOP, the pleural effusion and pericardial effusion became little left. Now, 12 months passed since the last chemotherapy, and although the slight effusion is still left, disease status has continued to be stable without further treatment. Chest X-rays when the patient was diagnosed and now are shown in Fig. 1A.

Case 2

A 73-year-old man had edema of his lower thighs and he was diagnosed as having pericardial effusion, pleural effusion, and ascites on whole body CT scan. In January 2009, he had shortness of breath and came to the outpatient clinic of our hospital. He was diagnosed as cardiac decompensation with massive pericardial effusion and treated with drainage of it. On the cytological examination of the pericardial effusion, large atypical lymphoid cells with prominent nucleoli were observed. The cell block preparation of the pericardial effusion revealed that the cells were positive for CD20, but negative for CD3, HHV-8, and EBER-ISH. No masses or lymphoma cells were detected on whole body CT scan, FDG-PET, and bone marrow biopsy. Tests for HCV and HIV antibody were negative. He was diagnosed as HIV-negative HHV-8-unrelated PEL-like lymphoma. The patient was treated with six courses of R-CHOP therapy. After repeated courses of R-CHOP, the pericardial effusion and pleural effusion gradually decreased. However, after five courses of R-CHOP, liver dysfunction appeared. The ultrasonographic examination revealed that he had congestion of the liver due to recurred pericardial effusion. Aspiration of fluid from pericardium was performed twice. However, invasion of lymphoma cells were not detected in evaluation of cytology and flow cytometric analysis at this time. After that, liver dysfunction resolved and pericardial effusion was stable with slight pleural effusion. He was performed six round of R-CHOP treatment then discharged. Chest X-rays when the patient was diagnosed and now are shown in Fig. 1B.

PEL was originally described in 1989 as B-cell lymphomatous effusion in a body cavity without detectable tumor masses and associated with HHV-8 and HIV infection, mostly occurs in immunodeficiency status [1–3]. However, this entity has been reported in a small number of cases associated with HIV-negative HHV-8-unrelated PEL-like lymphoma [4–6]. The PEL lymphoma cells are usually negative for pan-B-cell markers, such as CD19, CD20, and CD79a. On the other hand, HIV-negative HHV-8-unrelated PEL-like lymphoma cells highly express B-cell markers. In our cases, the lymphoma cells also expressed CD20 and CD79a. As for the pathogenesis of PEL-like lymphoma, Tanaka et al. reported that some of these were EBV positive [7]. HCV had also been suggested to be an etiological agent [8]. Both of the present cases were HCV negative, although case 1 was EBV positive and case 2 was negative.

As to treatment, there is no standard chemotherapeutic regimen recommended for HIV-negative HHV-8-unrelated PEL-like lymphoma because of small numbers of reports. CHOP-like regimen had been frequently given in these cases. Recently, rituximab, an anti-CD20 monoclonal antibody, has been incorporated into the standard chemotherapy for many B-cell NHLs showing CD20 positivity. In both of our cases, we used rituximab containing regimen because the lymphoma cells were CD20 positive and it was effective in both cases.

The prognosis of PEL is poor and the median survival of PEL is less than 6 months, whereas the prognosis of HIV-negative HHV-8-unrelated PEL-like lymphoma may be better than that [9,10]. In our cases, one is alive for 21 months and another is alive for 9 months after their diagnoses. Thus prognosis of PEL-like lymphoma seems to be better than that of PEL as reported previously. In light of the cases from literature and our present ones, PEL and HIV-negative HHV-8-unrelated PEL-like lymphoma may have different pathogenesis, immunophenotypic features, and prognosis.

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Conflict of interest: Nothing to report.
Published online 16 October 2009 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21568

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Tumor flare reactions and response to lenalidomide in patients with refractory classic Hodgkin lymphoma

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Patients with Hodgkin lymphoma (HL) failing salvage stem cell transplantation are candidates to investigational strategies. Lenalidomide represents an attractive option as it targets several signaling pathways, which regulate survival of HL cells and their microenvironmental interactions. We report the occurrence of Grades 2 and 3 tumor flare reactions in the first three patients entered a lenalidomide-based compassionate program for treatment-refractory HL. Flares occurred in concomitance of the scheduled week-off lenalidomide and upon withdrawal of symptomatic steroid treatment, and were associated with changes in B-cell regulatory cytokines and the concurrent expansion of polyclonal B-cells. Flares mimicked tumor progression but were effectively managed with anti-inflammatory treatment and followed by a clinical response, suggesting that they may mirror the pleiotropic actions of lenalidomide on HL microenvironment.

Patients with Hodgkin's Lymphoma (HL) recurring after stem cell transplantation are incurable and candidates to investigational agents [1]. Lenalidomide, a thalidomide-derivative highly active in myeloma and other B-cell malignancies, represents an attractive option due to its pleiotropic effects on tumor cells and their microenvironment [2]. In this sense, signaling pathways regulating survival of HL cells and their interactions with bystander immune and stromal cells in tumor tissues overlap with those modulating proliferation and cross-talk of malignant plasma cells in the bone marrow (BM) microenvironment. These involve receptors/ligands of the tumor necrosis factor (TNF) superfamily, cytokines, chemokines and adhesion/co-stimulatory and proangiogenic molecules [3]. Lenalidomide targets several of these pathways and might exert a direct effect on tumor cells by inhibiting Akt phosphorylation and/or cyclin-dependent kinases [2,4]. Based on these evidences a lenalidomide-based compassionate program for biopsy-proven treatment refractory HL was launched at our Institution. We herein describe the occurrence of a tumor flare reaction (TFR) in the first three patients entered the program.

The Institutional Ethic Committee approved (April 2008), a program of oral lenalidomide at the dose of 25 mg from day 1 to day 21 of a 28-day cycle until progression. Lenalidomide (Revlimid®) was purchased from Celgene (Summit, NJ), and the patients were treated after written informed consent and the approval of a pregnancy-prevention and risk-management plan. Response evaluation was planned after three courses and scored according to the revised International Criteria integrated by fluorine-18-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography [5]. Changes in superficial nodal sites were monitored by bi-weekly ultrasonography, expressed as the change in sum of the products of the greatest diameter (SDP) from baseline and plotted as bar-graph histogram against time. Serum levels of interleukin (IL)-6 and -7, a proliferation-inducing ligand (APRIL) and the B-lymphocyte stimulator (BAFF/BlyS) were concurrently evaluated as these cytokines are highly expressed in HL microenvironment, by regulating proliferation/activation of normal B-lymphocytes and tumor cells, and correlate with clinical–pathological features and outcomes [3,6]. Serial serum samples were stored frozen at –80°C and assayed in parallel by ELISA kits for IL-6 and IL-7 (DRG, Mountainside, NJ), APRIL (Bender Medsystems, Burlingame, CA), BAFF/BlyS (R&D Systems, Minneapolis, MN) according to manufacturer instructions. Serum free light chains (sFLC) were also assayed to qualify systemic B-cell expansions [7]. Immunonephelometric sFLC testing was performed according to manufacturer instructions (Freelite, The Binding Site, Birmingham, UK).

TABLE I. Patient Characteristics, Toxicity, And Response to Lenalidomide

	Baseline							Treatment-related			
								Response		Toxicity[a]	
N	Age/Sex	Histology	No. of prior treatments	SCT	Stage at entry	Creatinine clearance (mL/min)	ECOG PS	After 3 courses	Days to progression	Hematological	Nonhematological
1	38/M	SN	6	Autologous Allogeneic	IVB	94	1	SD	138	Grade 2 anemia	Grade 2 fatigue
2	34/M	SN	7	Autologous Allogeneic	IVB	110	2	SD	124	Grade 2 anemia Grade 2 thrombopenia	Grade 2 tumor flare Grade 2 fatigue
3	22/M	SN	7	Tandem autologous	IVB	87	1	PR	164	Grade 3 anemia Grade 2 thrombopenia	Grade 3 tumor flare Grade 2 fatigue Grade 2 diarrhea

NS, nodular sclerosis; SCT, stem cell transplantation; PS, performance status; SD, stable disease; PR, partial remission.
[a]According to CTCAE v3.0.

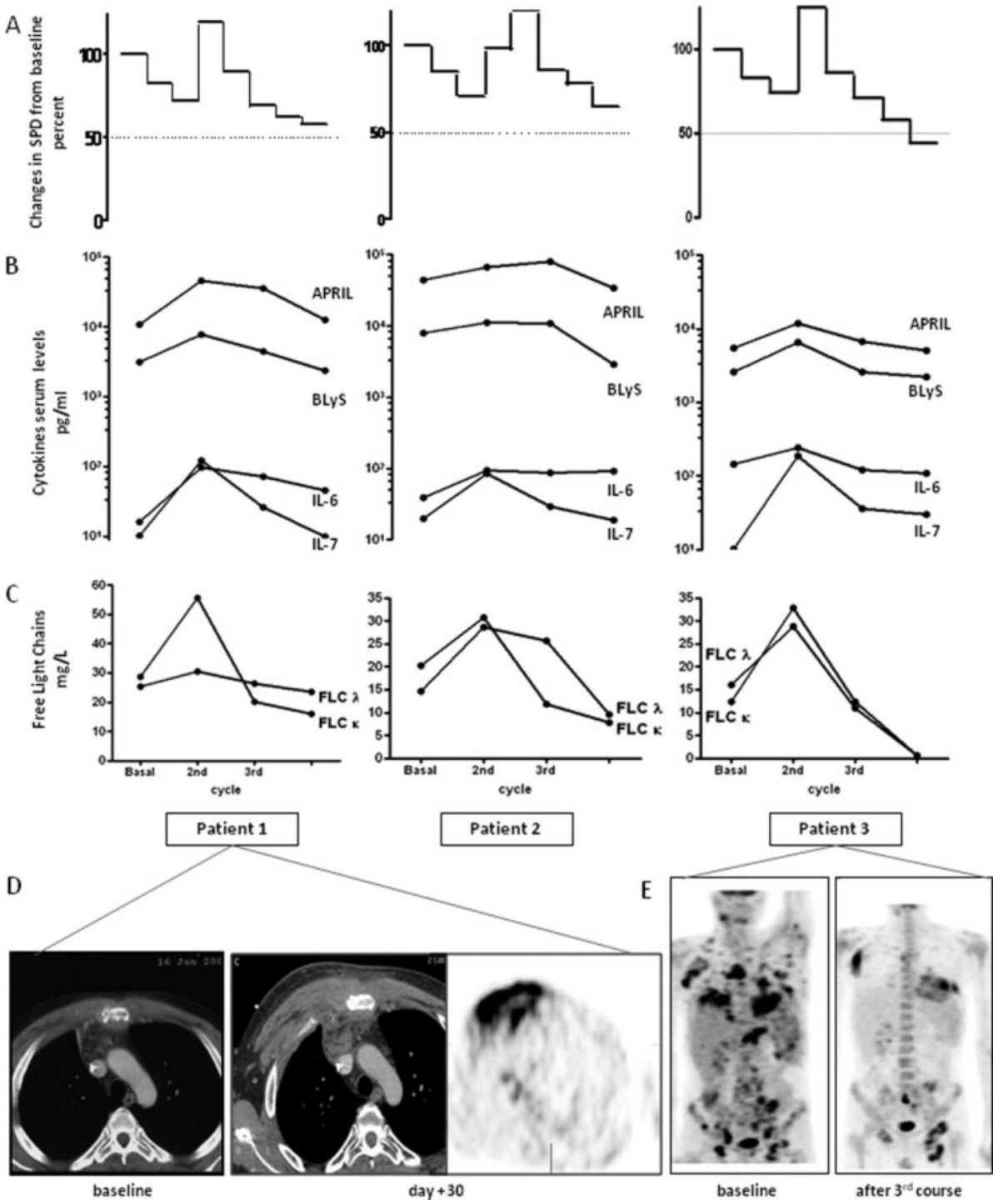

Figure 1. Lenalidomide-related development of tumor flares and responses in patients with Hodgkin lymphoma. (A) Dimensional changes in disease-involved target sites during lenalidomide therapy. Data is presented as percentage variation in the sum of products of the greatest diameter (SDP) of measurable lesions from baseline and plotted as bar-graph histogram against time. Target lesions were evaluated by biweekly ultrasonography as follows: patient 1, bilateral supraclaevear lymph nodes (left panel); patient 2, left laterocervical, lumboaortic and bilateral iliac and inguinal lymph nodes and spleen (middle panel); patient 3, bilateral retroclavicular, right axillary, lumboaortic, bilateral iliac and inguinal lymph nodes and spleen (right panel). (B) Changes in circulating B-cell stimulatory cytokines during therapy with lenalidomide. Serum samples were collected at baseline (before the first course), before starting the second and third courses of lenalidomide and at one month after the third course. Data is presented as serum concentrations in picogram per milliliter, for each patient: patient 1, left panel; patient 2, middle panel; patient 3, right panel. (C) Changes in serum free light chains (FLC) levels (normal ranges, κ: 3.3–19.4 mg/L; λ: 5.71–26.3 mg/L) during therapy with lenalidomide. Data is presented as serum concentrations in milligram per liter, for each patient: patient 1, left panel; patient 2, middle panel; patient 3, right panel. In all instances, the serum FLC ratio, calculated as κ/λ (free κ concentration divided by free λ) remained within the normal reference range (0.26–1.65), except for the second time point of patient 1, where a free κ/λ ratio of 1.82 was calculated. (D) Patient 1, computed tomography scan of the thorax at the start of lenalidomide therapy (left panel). Computed tomography (middle panel) and ^{18}F-FDG-PET (right panel) scans of the same thorax area during tumor flare reaction (TFR) occurrence (i.e., day +30 from starting of treatment). The infiltrative tissue at the right sternocostal–osteochondral junction, with lytic microlacunar erosion of the bone margin associated with small retrosternal confluent nodulations (left panel), evolved into a markedly edematous inflammatory area of hypodensity, which deformed the anterior chest wall (middle panel) and strongly retained ^{18}F-FDG (right panel), so mimicking tumor progression. The TFR extensively involved the muscoloaponeurotic planes of the pectoral region with inflammatory edema spreading to pleurocostal planes and mediastinum. (E) Patient 3, ^{18}F-FDG-PET-documented partial response to lenalidomide after three complete courses. Note that the marked reduction in number, size, and intensity of ^{18}F-FDG uptake of disease sites, including spleen and extranodal lesions.

Patients' characteristics, response outcomes and toxicity to lenalidomide (NCI Common Toxicity Criteria v3.0), are detailed in Table I.

Patient 1 had baseline bilateral supraclavicular nodal involvement and a retrosternal confluent adenopathy infiltrating the right sternocostal–osteochondral junction. He was under continuous methylprednisone (16 mg/day) for persistent fever and sweats. Two weeks after starting lenalidomide, a 30% decrease in SDP of superficial lymph nodes was observed (Fig. 1) along with B symptoms resolution, leading to complete steroid taper at day +19. Shortly before the second course (day +26) the patient presented with a re-enlargement of the superficial nodes and a massive painful swelling which deformed the right anterior chest wall. Imaging documented a markedly edematous hypodensity area extensively involving pectoral muscoloaponeurotic planes and spreading to pleurocostal planes and mediastinum, which avidly retained ^{18}F-FDG (Fig. 1). A Grade 2 TFR was diagnosed, and tramadol, naproxen and methylprednisone (8 mg/day) were administered while continuing on full-dose lenalidomide. Ten days later, pain and swelling resolved and two further courses were given leading to a stepwise decrease of nodal sizes down to 58% of pretreatment values (Fig. 1), consistent with disease stabilization.

In patient 2, disease involved multiple nodal (laterocervical, internal mammary, bilateral lung hilar, lumboaortic, iliac, and bilateral inguinal) and extranodal (dorsal and lumbar spine, femurs) sites. Despite steroid-related diabetes, the patient was under methylprednisone (24 mg/day), due to profuse sweating, fever and analgesic-requiring generalized bone pain. He had thrombocytopenia (68.000 μL^{-1}), a hypocellular BM and a massively enlarged spleen (158 × 153 × 58 mm) with miliary/micronodular lesions. After 2 weeks of lenalidomide, B symptoms subsided, nodal lesions shrank by 30% (Fig. 1) and steroids were withdrawn by day +19, also to reduce insulin. At day +27, the patient was admitted with a painful nodal re-enlargement (SDP 120%), a gross right groin lesion (maximum diameter, 69 mm) and severe back and pelvic pain. A Grade 2 TFR was diagnosed and treatment with oxycodone and ketoprofen led to complete symptoms resolution, including bone and groin pain disappearance at analgesics weaning. Following courses were delivered at full doses, yielding to disease stabilization (39% reduction in overall SDP) and normalization of platelets to 194.000 μL^{-1} (Fig. 1).

In patient 3, disease involved nodal (bilateral retroclavicular, axillary, lumboaortic-iliac, and inguinal) and extranodal (lungs, sternal ribs) sites (Fig. 1). Spleen was enlarged (169 × 149 × 63 mm) with multiple nodulations. The patient displayed thrombocytopenia (77.000 μL^{-1}), anemia (Hgb 7.7 g/dL) and suffered from cough, exertion dyspnea and persistent fever requiring methylprednisone (16 mg/day). Lenalidomide was withheld on day +18 due to afebrile microbiological-negative Grade 2 diarrhea, controlled by octreotide acetate. Meanwhile, a 26% reduction of nodal sites was recorded along with cough resolution and improvement of dyspnea, allowing steroid withdrawal by day +24. The second course was started at 25 mg/day, but 5 days later the patient developed a disabling chest bone pain exacerbated by finger pressure and breathing, and crippling gait impairment due to a wide and painful inflammatory swelling of left inguinal lymph nodes involving the whole groin and the upper thigh. A Grade 3 TFR was diagnosed and lenalidomide lowered to 15 mg/day. Tramadol, naproxen and methylprednisone (8 mg/day) were administered, leading to complete clinical resolution after 7 days. The third course was delivered at 15 mg/day, leading to a partial remission with reduction in number, size and ^{18}F-FDG uptake of all disease sites including spleen (Fig. 1), and a stable recovery of platelets (>130.000 μL^{-1}) and hemoglobin (>8.5 g/dL).

In none of the patients lenalidomide was discontinued. Best responses were recorded after three courses, and the progressions were documented on days +138, +124, and +164 (Table I). As show in Fig. 1, development of TFRs displayed a close temporal association with rise in serum levels of cytokines stimulating B-cell proliferation and activation, such as IL-6, IL-7, BAFF/BLyS, and APRIL. Concurrently, sFLC testing disclosed the elevation of both κ and λ sFLC levels with normal κ/λ ratios, consistent with an inflammatory-like generalized B-cell activation and polyclonal expansion [7]. Upon lenalidomide continuation, serum levels of cytokines and of polyclonal sFCL stepwise decreased to normal limits. The compassionate program was stopped after enrolment of these first three patients to be replaced by an ongoing phase I/II study.

We have described three cases of very likely TFR in HL patients given lenalidomide. Following early shrinkage of nodal lesions, patients displayed distinctive signs of TFR syndrome with a sudden-onset painful re-enlargement of tumor-involved sites (up to 50%), accompanied by inflammatory edema of overlaying skin, and disabling bone pain at disease-bearing skeletal sites. This occurred at days +26 (patient 1), +27 (patient 2), and +33 (patient 3), in concomitance of the scheduled week-off lenalidomide and after steroids had been withdrawn to verify for response on systemic symptoms or reduce insulin. Symptoms and signs resolved upon anti-inflammatory/analgesic treatment and never recurred. Although disease progression could have been deemed, lenalidomide was continued and all patients attained an objective response. It would have been otherwise surprising for a tumor progression of such magnitude to display a so prominent and painful inflammatory picture and promptly reverse with anti-inflammatory agents only.

The occurrence of a lenalidomide-related TFR syndrome has been reported in about 50–60% of patients with chronic lymphocytic leukemia (CLL), reaching a Grades 3 and 4 severity in 8–9% of the cases [8–10]. In CLL patients, development of lenalidomide-induced TFRs was frequently associated with steroid taper/suspension [9,10] and the presence of large tumor volumes [8], while low-dose prednisone prophylaxis was shown to reduce severity of flares [2,9,11]. In our cases, a straightforward association with steroid taper/withdrawal and the presence of large tumor masses was also evident. Given the nature of our study, we are unable to provide conclusive figures on the incidence rate of TFRs in HL or document whether TFRs may also occur in HL patients receiving lenalidomide without steroids. As patients with progressive HL may typically receive steroids as part of their treatment, final results of ongoing trials will be needed to unravel these issues [12–14].

Development of TFRs has been related to lenalidomide-induced upregulation of immune-stimulatory molecules on tumor cells, triggering a prominent inflammatory cytokine release syndrome, and activation of bystander immune cells [2,8,9]. We documented that serum levels of B-cell-activating cytokines were abnormally elevated concurrently with TFR development and displayed a stepwise decrease at resolution. Flares were also associated to the transient elevation of sFLC levels with a normal free κ/λ ratio, consistent with a generalized expansion of polyclonal B-cells such as occurring in systemic inflammatory disorders [7,15]. This may reflect the activity of elevated B-cell-activating cytokines and/or a direct effect on bystander B-cells. As lenalidomide promotes survival of normal, but not neoplastic, B-cells [4], it may have contributed to the expansion of reactive B-lymphocytes primed by the proinflammatory 'milieu' of HL [3]. While the contribution of other immune effectors was not explored in our study, the association of flares with steroid suspension further supports an underlying immune-modulatory mechanism.

Lenalidomide-induced TFRs may also occur in HL patients. They mimic early tumor progression but are manageable and followed by clinical responses upon drug continuation. While conclusive data in HL are expected from ongoing studies, our observations suggest that TFRs may reflect the pleiotropic actions of lenalidomide on HL microenvironment.

Acknowledgments

This work was supported in part by Ministero della Salute, Ricerca Finalizzata FSN, IRCCS, Rome, Italy. We thank Dr. Alessandro Marchei for help in correlative studies.

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Conflict of interest: B.A. acts as a consultant to the Binding Site. All other authors declare no competing financial interests.
Published online 21 October 2009 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21571

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