Hydroxyurea in children with sickle cell disease: Practice patterns and barriers to utilization

Amanda M. Brandow,* Danielle L. Jirovec, and Julie A. Panepinto

Hydroxyurea (HU) is underutilized in adults with sickle cell disease (SCD) despite the Multicenter Study of Hydroxyurea (MSH) in sickle cell anemia (1). As little is known about HU utilization in children with SCD we sought to: (1) evaluate patterns of HU utilization; (2) elicit how providers define frequent pain when prescribing HU; and (3) identify barriers to HU use by surveying members of the American Society of Pediatric Hematology/Oncology. Data analysis included descriptive statistics and γ^2. Of the 350 respondents, 63% care was given for SCD patients. Of these providers, only 9% have 50–90% of patients on HU, while 10% have <10% on HU. Criteria used to initiate HU included acute chest syndrome and frequent pain. Approximately half of providers account only for pain requiring hospitalization when prescribing HU. Those accounting for pain managed at home were more likely to have >30% of patients on HU (35.2% vs. 20%; $P = 0.023$; γ^2). Provider-related barriers to prescribing HU included compliance with: HU (86%), laboratory monitoring (85%), and contraception (85%). Our survey suggests substantial variation in HU utilization in children. Providers accounting for pain managed both in and out of the hospital had more patients on HU. Existing barriers to HU utilization should be addressed to optimize care for children with SCD.

Pain is the most common complication of SCD accounting for over 80% of all hospitalizations for children. Home pain diary studies reveal pain is also frequently managed at home and goes underreported [2–4]. Treatment of painful events primarily involves symptomatic care. Preventative measures are limited and HU, an oral drug taken once daily, is the only drug shown to decrease the frequency of SCD painful events [1].

The efficacy of HU was proven in adults in 1995 through a randomized controlled trial, the MSH [1]. The MSH found HU significantly reduced the annual rate of painful events, acute chest syndrome episodes, and transfusions [1]. The 9-year follow up to MSH revealed that HU was associated with significant reduction in mortality, minimal side effects, and was safe [5]. A large trial mimicking the MSH in children was not conducted. However, efficacy studies in children include a randomized, placebo-controlled, cross-over trial with small numbers of children and open-label, single-arm studies [6–8]. These studies demonstrated a significant decrease in painful events in the HU arm [6–8] and led to the introduction of HU into pediatric practice for the prevention of pain. Subsequent studies have proven safety and hematologic efficacy in children [9–12].

As the efficacy of HU was established in a controlled clinical trial environment, its effectiveness is dependent on its utilization in real clinical practice. Despite the impressive findings of the MSH, HU is underutilized in adults limiting its effectiveness [13,14]. The National Institutes of Health (NIH) Consensus Development Conference Statement confirmed this underutilization [15]. The utilization of HU in children has never been studied, thus no data exist to support whether its utilization will be better in pediatrics.

The NIH published recommendations for HU in children and adults in 2002 stating HU should be initiated in patients with "frequent pain episodes" [16]. Currently, there are no national data addressing how pediatric SCD providers define "frequent pain episodes" and how they use this definition to recommend HU. Emerging SCD pain literature reveals majority of pain episodes are managed at home [2-4,17] and it is unknown if providers incorporate these data into their definition of frequent pain.

To assess these important knowledge gaps, we surveyed pediatric hematology/oncology providers and sought to achieve the following objectives: (1) evaluate practice patterns of HU utilization in children with SCD; (2) elicit how providers define frequent pain when prescribing HU; and (3) identify barriers to HU use.

Overall there was a 31% response rate ($n = 350$). Of those that responded, 63% ($n = 220$) take care of SCD patients. The majority of SCD providers were physicians ($n = 213$; 96.8%), about half were female ($n = 103$; 46.8%). The median years in practice was 12 (IQR 5-20). See online Supporting Information for additional provider demographics and characteristics of practice and flow diagram of final study population (Supporting Information Table I and Fig. 1).

Most providers (90%) felt HU was effective or very effective for the prevention of pain.

Only 9% of providers have 50–90% of their patients on HU, 22% have 31–50% on HU, 54% have 10–30% on HU, and 10% have <10% on HU. Table I shows the most common criteria providers use to start HU. About half (54%) indicated they initiated HU in children <4 years of age.

Figure 1 displays how providers define frequent pain in children with SCD. When asked about criteria used to initiate HU for pain, 42% used only pain requiring hospitalization whereas 44% accounted for pain both at home and in the hospital when prescribing HU. Importantly, providers incorporating pain at home into their definition of frequent pain were more likely to have >30% of their patients on HU (35.2 vs. 20%; $P = 0.023$; Pearson χ^2).

The proportions of providers stating a particular factor influenced their decision to not prescribe HU to eligible patients are listed in Table II. The most common factors identified as barriers to prescribing HU revolved around the theme of compliance.

Twenty-six percent of providers reported >20% of their patients/families refused HU when it was offered. The most common reasons for patients' refusal were fear of cancer (51%) and other side effects (62%), do not want to take medication (49%), or do not want required laboratory monitoring (28%). Interestingly, 17% refused HU because they didn't think it would work.

To our knowledge, this is the first study to assess utilization of HU in children with SCD and to identify barriers to its use in children. Our survey suggests substantial variation exists in the use of HU in children with SCD. Very few providers have more than half of their patients on HU and one in 10 rarely use HU and have <10% of patients on HU.

Our survey also found providers use HU for SCD-related complications without data to support its use for these complications, representing clinical drift [18]. Over one-third of providers use HU for secondary stroke prevention and almost half use HU for priapism and pulmonary hypertension; all complications currently lacking HU efficacy data. These data raise the need for continued and future funding of clinical trials to evaluate the unknown efficacy of HU for these complications which will prevent or promote the appropriate use of HU and ultimately avoid clinical drift [18].

Fortunately, majority of providers use frequent pain as criteria for starting HU, however, how providers define frequent pain varied. Our data show almost half of providers use only pain events requiring hospitalization as criteria for starting HU. If this strict definition is used, many children that may benefit from HU will not be considered eligible. Recent pain literature in SCD reveals majority of pain is managed at home [2–4,17], goes underreported and impacts school attendance and children's health-related quality of life [2-4,19,20]. Our survey found providers accounting for pain at home were significantly more likely to have more children on HU, suggesting how providers define frequent pain may be a barrier to using the drug.

The identified barriers to HU use in children at the provider and patient level were similar to previously identified barriers in adults with SCD [13,14,21]. The most common provider-related barriers involved the theme of patient compliance, including compliance with taking HU, required labora-

TABLE I. Criteria Used by Providers to Start Hydroxyurea

Criteria	Proportion (%)
Acute Chest Syndrome	88
≥3 painful episodes/year	86
Requiring hospitalization only	42
At home or requiring hospitalization	44
Chronic pain requiring frequent narcotic use	70
Priapism	48
Pulmonary Hypertension	43
Symptomatic Anemia	40
Stroke	36
Renal Failure	15
Ankle Ulcers	12
Low hemoglobin F levels	9
Elevated white cell count without evidence of infection	6

TABLE II. Provider-Related Barriers to Hydroxyurea Use

Barrier	Proportion (%)
Patient compliance with taking medication	86
Patient compliance with blood tests	85
Lack of contraception in females	85
Patient's anticipation of side effects	75
Patient is too young	68
Concern for male infertility	46
Lack of formal guidelines for use in children	30
Provider discomfort with carcinogenic potential	27
Cost	18
Lack of time/resources to explain risks/benefits	16
Not FDA approved in children	12
Doubt effectiveness of hydroxyurea	11

tory monitoring, and female contraception. Importantly, noncompliance may be a result of poor access to care, a systems-level barrier, or may stem from patients' fears of side effects. In addition, patients' access to HU may be limited if providers are not prescribing HU to eligible patients because of their own biases about the drug.

Other barriers included concerns for toxicities there may or may not be evidence to support, such as concern for carcinogenesis. Long-term follow up data from MSH do not provide evidence supporting this concern in adults taking HU [5]. The Agency for Healthcare Research and Quality systematic review about HU also stated "limited evidence suggests that HU treatment in adults with SCD does not increase the risk for leukemia" [22]. Widespread provider and patient education regarding the limited or nonexistent association between HU and cancer is imperative to eliminate this barrier. Concern for male infertility was a provider-related barrier in almost half. Currently, evidence is lacking to support or disprove this concern. Concern for teratogenesis was a fear for majority of providers and if the provider doubted compliance with female contraception, this was a barrier to the use of HU. Current MSH data reveal harm did not occur to offspring of women taking HU at the time pregnancy occurred [23]. Finally, although some providers place children <4 years on HU, majority of providers reported age (patient too young) was a barrier to prescribing HU. Data from the trial "HU to Prevent Organ Damage in Children with Sickle Cell Anemia" [24] will confirm the safety of HU in young children and potentially reduce the barrier of age in the prescribing of HU to younger children that may benefit.

Ultimately, it is important to remember SCD carries with it significant morbidity and is associated with mortality. On the basis of proven efficacy and safety in children [6-12,25,26], HU provides significant benefit to children suffering from a life-long debilitating disease and likely improves their health-related quality of life; thus, the benefit of HU may outweigh potential risks.

Our study is limited in that survey responses may not reflect true practice. Individual charts would require auditing to verify this information. In addition, we do not have information about non-responders since the survey was anonymous. The overall response rate was 31%; however, this is consistent with other published survey research [27–29].

In conclusion, our survey suggests substantial variation in HU utilization in children with SCD. HU is used for complications other than pain despite insufficient evidence for its efficacy for these complications, representing clinical drift [18]. Studies to determine the efficacy of HU for SCD complications other than pain are urgently needed to prevent or promote the appropriate use of the drug. Although majority of providers report frequent pain as criteria for starting HU, almost half only account for pain bringing a child to the hospital. This strict definition likely misses many children experiencing significant pain at home that would benefit from HU. Finally, provider, patient, and systems-level barriers to HU utilization in children exist and need to be addressed. Future studies should be aimed at evaluating unknown toxicities of HU that influence practice, exploring whether access to care contributes to noncompliance, adherence research, and provider education about the extent of pain experienced by children with SCD.

Methods

An anonymous cross-sectional survey was e-mailed to 1,316 pediatric hematology/oncology providers identified through the published 2008 American Society of Pediatric Hematology/Oncology (ASPHO) membership directory. ASPHO is an international professional society of pediatric hematology/oncology providers who conduct research in and treat children with cancer and other blood diseases. The survey was kept anonymous to encourage providers to be honest with their responses and the anonymity also permitted multiple respondents from the same institution to report their practice patterns as individuals allowing for variability of practice within a large program. This limited biasing responses of individuals towards the views and practices of the institution. The survey was adapted with permission from that done by adult SCD providers [13], pilot-tested among experts in pediatric SCD, modified based on their recommendations, and emailed using a web-based survey program [30]. A brief introductory letter explained the study and stated informed consent was implied with survey completion. The initial email and five reminder e-mails were sent between February 12, 2009 and July 13, 2009 with reminder emails sent only to nonresponders. The survey program allowed for completion of the survey only once by each member emailed. The survey began by identifying providers that care for SCD patients. If the provider did not care for SCD patients, the first question indicated this and the survey was complete. If the provider did care for SCD patients, he/she was directed to complete the remainder of the survey. The response rate includes all respondents, however, all other data includes only those that care for SCD patients. See online Supporting Information for survey details addressing the main manuscript objectives. The study was approved by the Institutional Review Board of the Children's Hospital of Wisconsin, which allowed for completion of the survey to serve as implied consent.

Statistical Analysis

Analyses were conducted with SPSS version 14.0 for Windows (SPSS, Chicago, IL). Respondent survey data was extracted, inputted into SPSS, and descriptive statistics were calculated. We report proportions, medians,

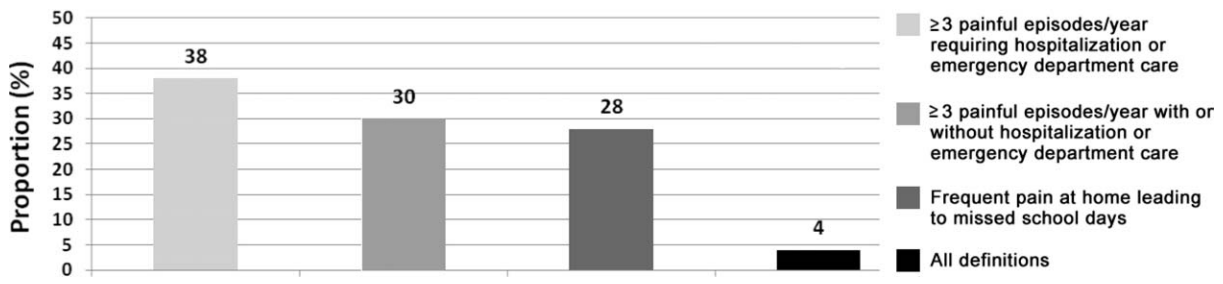

Figure 1. Proportion of providers using various definitions for frequent pain in children with sickle cell disease.

and interquartile ranges as appropriate. χ^2 analysis was used to test differences between proportions. A *P*-value of ≤ 0.05 was considered statistically significant.

Acknowledgments

The authors would like to acknowledge all of the providers who took the time to complete the survey.

Author Contributions

A.M. Brandow designed research, performed research, analyzed data, and wrote manuscript. D.L. Jirovec designed research, performed research, and revised manuscript. J.A. Panepinto designed research and revised manuscript.

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

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Contract grant sponsor: National Institutes of Health National Heart, Lung, and Blood Institute; Contract grant numbers: HL090503, K23 HL80092

Conflict of interest: Nothing to report.

Published online 15 March 2010 in Wiley InterScience (www.interscience.wiley.com).

DOI: 10.1002/ajh.21749

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Utilization of analgesics in the multicenter study of hydroxyurea in sickle cell anemia: Effect of sex, age, and geographical location

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Several factors affect the severity and duration of sickle cell pain and its response to treatment with analgesics [1,2]. Sex has been one of the factors reported to influence the pain experience and the response to therapy [3]. Several chronic pain disorders, such as fibromyalgia, occur more frequently in females than in males [4,5]. Moreover, women seem to be more sensitive to painful stimuli than men [6]. However, whether differences in analgesic use by sex occur in patients with sickle cell anemia (SS) is unknown. Age also has been related to pain experience in many studies [7–9]. Moreover, we and others recently found an effect of geographic location and climatic conditions on frequency and severity of sickle cell pain [10,11]. Studies at single sites and anecdotal reports showed that climatic conditions, especially temperature can precipitate or exacerbate pain in sickle cell disease [12–14]. However, to the best of our knowledge, there are no multicenter, randomized, and placebo-controlled studies that relate all of these factors to pain management in sickle cell disease (SCD). The Multicenter Study of Hydroxyurea (MSH) in SS [15] gave us an opportunity to report on these aspects of sickle cell pain.

TABLE I. Equianalgesic Dosing, by Sex (mg Morphine Equivalent)

	LSM estimates: mean (95% CI)		*P*–value for sex difference in means
	Males	Females	
At home: average daily dose	13.4 (11.38–15.42)	9.8 (8.37–11.23)	0.0029
At home: average 2-week total	72.6 (58.80–87.40)	53.4 (42.83–63.97)	0.034
Acute care crises: average daily parenteral dose	25.3 (22.25–28.35)	20.5 (18.03–22.97)	0.015
Acute care crises: average daily oral dose	21.9 (18.07–25.03)	20.3 (16.16–24.17)	0.56
In-patient crises: average daily parenteral dose	51.8 (42.03–61.57)	34.7 (28.46–40.94)	0.0024
In-patient crises: average daily oral dose	37.3 (30.92–44.22)	31.3 (26.00–36.60)	0.015

LSM, least squares mean; CI, confidence interval.

TABLE II. Frequency of Oral Analgesic Use[a] at Home, by Age, Sex, and Region

	All patients		Females		Males	
Group	At-home diaries (% of all days)[b]	Follow-up visits (% of all two-week periods)	At-home diaries (% of all days)	Follow-up visits (% of all two-week periods)	At-home diaries (% of all days)	Follow-up visits (% of all two-week periods)
18–24 year olds	29.5 (23.3–35.7)[a]	50.4 (42.9–57.9)[a]	28.9 (19.4–38.4)[a]	48.2 (38.5–57.9)[a]	29.9 (21.8–38.1)[b]	52.2 (41.0–63.4)[a]
25–29 year olds	44.9 (38.1–51.6)[b]	62.9 (54.8–71.0)[ab]	42.5 (32.1–52.9)[b]	58.2 45.4–71.0)[ab]	46.7 (37.9–55.6)[a]	66.4 (56.0–76.8)[ab]
30–35 year olds	46.3 (39.8–52.9)[b]	68.7 (60.4–76.9)[b]	42.1 (32.7–51.5)[b]	62.9 (50.1–75.6)[b]	50.8 (41.6–60.0)[a]	74.2 64.8–84.7)[b]
Over 36 years old	40.6 (34.0–47.3)[b]	58.3 (50.4–66.3)[ab]	45.4 (36.9–53.8)[b]	60.5 (50.8–70.2)[ab]	31.7 (20.7–42.8)[b]	53.8 (39.8–67.9)[ab]
	$P = 0.0001$	$P = 0.012$	$P = 0.067$	$P = 0.29$	$P = 0.0018$	$P = 0.014$
Northeast	53.1 (47.0–59.2)[a]	68.4 (67.0–69.8)[a]	50.8 (41.2–60.5)[a]	61.6 (59.4–63.8)[a]	54.9 (47.2–62.7)[a]	73.7 (71.9–75.5)[a]
Midwest	31.0 (25.0–37.1)[ab]	49.1 (47.7–50.5)[c]	36.4 (28.1–44.8)[b]	51.8 (50.0–53.6)[b]	23.8 (15.0–32.6)[c]	45.0 (42.9–47.1)[c]
South	38.8 (33.8–43.8)[ab]	61.9 (60.8–63.0)[b]	37.8 (30.4–45.1)[b]	59.8 (58.1–61.4)[a]	39.8 (33.0–44.6)[b]	64.1 (62.5–65.7)[b]
West	22.8 (6.1–39.5)[b]	41.7 (38.2–45.3)[d]	28.8 (6.9–50.7)[ab]	50.0 (45.5–54.5)[b]	12.2 (0.0–38.5)[c]	26.7 (20.9–32.5)[d]
	$P < 0.0001$	$P < 0.0001$	$P = 0.077$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

[a]Reported means for at-home diaries are least squares means (LSM) estimates from models of frequency of at-home use; [b]Within columns for age and region, LSM estimates with different superscripts are significantly different ($P < .05$) in pairwise comparisons.

The hallmark of SS is the acute painful crisis. About 95% of hospital admissions of adult patients with SCD are for the treatment of the acute painful crisis [16]. The acute painful crisis is often complicated by acute chest syndrome, acute multiorgan failure, and sudden death [17–19]. Prevention of the acute painful crises is a major goal of the management of patients with SCD. This study focused on the effects of sex, age, and geographic location on the painful crisis in patients with SS who were enrolled in the MSH in SS [15].

Several recent studies about pain, in general, showed that there are major differences between males and females in processing painful stimuli and the perception of pain severity [3,6]. Women are at a greater risk for having several types of chronic pain syndromes and they exhibit greater sensitivity to noxious stimuli than men. They also complain of pain more often than men and in more regions of the body. The prevalence of many chronic pain conditions is sex and age dependent. Moreover, women seem to deal with pain better than men do and adopt better coping strategies with pain. This may be due, at least in part, to their frequent encounter with various types of pain during menstruation, dysmenorrhea, pregnancy, labor, and delivery.

Sex differences also exist in responses to opioid medications. These differences seem to pertain to the number of μ-opioid receptors in different areas of the brain. Loyd et al. [20] reported that male rats have more μ-opioid receptor expression in the periaquedutctal gray area of the midbrain compared with female rats. This area of the brain contains a large number of neurons that express μ receptors on their surface. These receptors, in turn, bind morphine and other μ-opioid agonists resulting in inhibition of transmission of the pain signal. This sequence of events suggests that μ-opioid agonists such as morphine are less effective analgesics in females than males. Some but not all clinical studies support this hypothesis [20–22]. κ-Opioid receptors, on the other hand, seem to have more density in the brain of females compared to males [23–25]. Women taking κ-opioid agonists, such as nalbuphine, have significantly greater postoperative analgesia than men [23–27].

Sex differences in response to nonopioid analgesics are equally discrepant with one study showing that only men had significant analgesic effect from ibuprofen in a study of experimental electrically induced pain [28]. Unfortunately, most of these studies were not performed in multicenter, randomized, and controlled trials. Most were single institution studies treating different kinds of pain (dental, thermal, chemical, electrical, etc.) in either ambulatory subjects or in postoperative pain.

Ageing is another important factor that affects pain management especially in the elderly. Aging affects the phararmacokinetics and pharmacodynamics of medications [29]. With ageing body composition (fat/muscle ratio), gastrointestinal motility, hepatic metabolism, renal clearance, and protein binding all decrease, whereas central nervous system sensitivity to medications and their side effects increases [30]. Consequently, older patients require smaller doses of analgesics to achieve adequate pain relief. Although patients with SCD usually do not live as long as the general population, age should be considered in the planning of their pain management. Moreover, organ damage complicates SS, and by the age 40 years, many patients with SCD have evidence of variable degrees of organ damage which, in turn, will affect the metabolism of the medications taken by the patients.

Our data show that the average dose of opioids, expressed in morphine equivalents, required to achieve pain relief is always significantly higher in males irrespective of the place of treatment (Table I). This may be due to a number of possibilities. The pain experienced by males may be more severe in nature or males may use opioids only when their pain is “very” severe. Women, on the other hand, may seek treatment when their pain is relatively milder compared with men. Whether the difference is due to the number of μ-opioid receptors in the brain described above or not is not clear. If males have more receptors one would expect smaller doses of opioids to achieve relief. Binding to receptors, however, is not enough unless it is followed by activation of these receptors. It is the activation of the receptors with consequent membrane hyperpolarizaion that inhibits the transmission of pain signals. Whether this is the situation in males or not awaits further studies.

Another interesting finding of our study is the effect of age on the frequency of opioid utilization and their dosing (Table II). Age was expressed in four quartiles; quartile 1: 18–24 years, quartile 2: 25–29 years, quartile 3: 30–35 years, and quartile 4: more than 36 years. The frequency of utilization of opioids and their dosing at home followed a consistent pattern: the numbers were significantly lowest in quartile 1, reached a significant maximum in quartile 3, and then declined significantly in quartile 4. This pattern was significant in males only and marginally significant in females with regards to the frequency of analgesic use in at-home diaries. This sequence of events suggests that as the patients get older they become tolerant to opioids and, hence, require higher and more frequent dosing to achieve pain relief at home by age 35 years. After age 36 years, the alteration in the pharmacodynamics and

TABLE III. Percent of Acute-Care Painful Crises With Use of Analgesics, by Sex, Region, and Type of Analgesic

	All			Females			Males		
	Parenteral opioids	Oral opioids	NSAIDs[a,b]	Parenteral opioids	Oral opioids	NSAIDs	Parenteral opioids	Oral opioids	NSAIDs
Age group									
18–24 year olds	95.5 (92.1–98.8)	15.5 (6.1–24.9)	21.0 (12.9–29.1)[a]	91.5 (84.4–98.6)	31.9 (15.3–48.5)[a]	23.4 (17.0–29.8)[a]	97.5 (94.4–100.0)	7.2 (3.4–11.0)	19.8 (8.0–31.6)[a]
25–29 year olds	92.9 (87.9–97.9)	11.4 (5.0–17.8)	7.0 (2.5–11.4)[b]	97.6 (94.5–100.0)	13.0 (4.2–21.8)[ab]	17.9 (5.8–29.9)[b]	91.7 (85.4–98.1)	11.0 (3.4–18.6)	4.3 (1.0–7.6)[b]
30–35 year olds	96.8 (95.1–98.6)	8.1 (3.7–12.5)	6.1 (2.4–9.7)[b]	96.1 (93.3–98.8)	9.4 (5.8–13.0)[b]	8.9 (4.4–13.4)[b]	97.3 (95.2–99.5)	7.2 (0.6–13.8)	4.1 (0.0–8.4)[ab]
More than 36 year olds	97.3 (95.0–99.6)	12.0 (5.5–18.5)	7.0 (3.9–10.1)[b]	97.1 (94.4–99.8)	12.3 (4.0–20.6)[ab]	7.6 (4.1–11.1)[b]	97.7 (93.6–100.0)	11.5 (1.2–21.8)	5.7 (0.6–10.9)[ab]
	$P = 0.13$	$P = 0.34$	$P < 0.0001$	$P = 0.15$	$P < 0.0001$	$P = 0.0004$	$P = 0.034$	$P = 0.69$	$P < 0.0001$
Region									
Northeast	95.5 (92.5–98.5)	4.4 (2.1–6.7)[b]	4.7 (0.9–8.5)[b]	97.3 (94.8–99.9)	7.1 (2.3–12.0)	5.8 (1.5–10.1)[b]	95.0 (91.2–98.8)	3.7 (1.3–6.2)[b]	4.4 (0.0–9.0)[b]
Midwest	94.4 (88.5–100.0)	20.6 (12.5–28.7)[a]	9.4 (5.5–13.4)[ab]	95.8 (92.9–99.5)	18.2 (10.3–26.1)	11.2 (5.4–17.0)[ab]	92.2 (78.4–100.0)	24.4 (8.4–40.5)[a]	6.7 (1.7–11.7)[b]
South	95.7 (93.6–97.7)	16.2 (10.6–21.7)[ab]	14.2 (10.4–18.1)[a]	94.6 (91.2–98.0)	17.1 (7.8–26.3)	16.4 (10.7–22.2)[a]	96.7 (94.3–99.1)	15.3 (8.8–21.8)[a]	12.2 (7.1–17.2)[b]
West	100.0	16.7 (9.1–24.3)[ab]	26.7 (0.0–54.3)[ab]	100.0	12.5 (8.4–16.6)	12.5 (3.2–21.8)[ab]	100.0	33.3 [a]	83.3 [a]
	NA	$P < 0.0001$	$P = 0.0002$	NA	$P = 0.036$	$P = 0.0052$	NA	$P < 0.0001$	$P = 0.0002$

[a]NSAIDs, non–steroidal anti–inflammatory drugs; NA, not available (P value not calculated due to 100% use in one or more groups); [b]Within columns for age and region, LSM estimates with different superscripts are significantly different ($P < 0.05$) in pairwise comparisons.

pharmacokinetics described above seems to set in thus increasing the sensitivity to opioids. This sequential pattern of opioid escalation with age, however, was not found when patients were treated in acute care facilities or in the hospital. Thus, there were no significant differences among age quartiles in the frequency of opioid utilization, with the exception of oral opioid use during acute care crises in women where the pattern was reversed, with patients in quartile one using oral opioids more frequently than those in older quartiles ($P < 0.0001$). Similarly, the equianalgesic dosing of opioids during acute care crises and during hospitalization did not significantly differ across the four age quartiles. This suggests that when pain is severe the effect of age diminishes, or even is reversed. The inflammation that accompanies severe painful crises is associated with the release of cytokines, chemokines, acute phase reactants, and other inflammatory mediators that bind opioids and decrease their availability to bind to and activate opioid receptors. Moreover, the psychological impact of severe pain on younger patients with resultant catastrophizing may be a contributory factor. Unlike opioids, the frequency of utilization of non-steroidal anti-inflammatory drugs (NSAIDS) is significantly higher in younger patients of both sexes when treated in acute care facilities but only marginally significant in younger women during hospitalization.

The third goal of this study was to examine the patterns of utilization of analgesics across different regions in the United States. The frequency of utilization of opioids and the dosing of opioids differed considerably among the four regions: Northeast, Midwest, South, and West. There was no simple and consistent pattern across all regions in these parameters of opioid utilization. Notable significant or marginally significant observations included the following. The frequency of utilization of oral opioids at home was significantly highest in the Northeast and lowest in the West with the Midwest and South in between (Table II). The frequency of utilization of parenteral opioids in acute care facilities was highest in the West and lowest in the Midwest whereas the frequency of utilization of oral opioids in acute care facilities was higher in the Midwest than in the Northeast for females but higher in both the Midwest and West when compared to the Northeast for males (Table III). This pattern suggests that the choice of the route of opioid administration is region dependent.

The average daily dose of opioids also differed considerably among geographic regions with a few observations worth noting. For both males and females, the average daily dose of parenteral and oral opioids used in acute care facilities and in the hospital was highest in the Northeast and lowest in the South. However, the average daily oral opioid dose at home was highest in the South. Interestingly, the use of NSAIDS during both acute care and in-patient crises tended to be higher in the South. Thus, it seems that in the South, the trend has been to treat crises at home aggressively with oral opioids and to use NSAIDS more frequently during acute care and in-patient crises, resulting in decreased utilization of parenteral opioids in acute care facilities and in the hospital.

The number and duration of in-patient painful crises were also age, sex and region dependent. The number of crises was highest in the Northeast and lowest in the South. The average duration of a painful crisis was also highest in the Northeast but lowest in the West.

In summary, this study shows that management of acute sickle cell painful episodes at home, in acute care facilities, and in the hospital seems to be sex, age, and geographic region dependent. Further studies are needed to confirm these findings. Recommendations and guidelines to treat acute painful crises should take age, sex, and region into consideration.

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Additional Supporting Information may be found in the online version of this article.
Conflict of interest: Nothing to report.
Received for publication 9 March 2010; Revised 21 April 2010; Accepted 26 April 2010
Published online 30 April 2010 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21750

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Phase I/II study of single-agent bortezomib for the treatment of patients with myelofibrosis. Clinical and biological effects of proteasome inhibition

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A phase I/II trial was undertaken to determine maximum tolerated dose (MTD), toxicity, clinical efficacy, and biological activity of bortezomib in patients with advanced stage primary or postpolycythemia vera/postessential thrombocythemia myelofibrosis (MF). Bortezomib (0.8, 1.0, or 1.3 mg/m^2) was administered on days 1, 4, 8, and 11 by intravenous push to patients previously resistant to at least one line of therapy, or with an intermediate/high-risk score of International Working Group (IWG) [1]. Therapy was repeated every 28 days for six cycles. At 1.3 mg/m^2 dose, one of six patients experienced a dose limiting toxicity, and this was determined to be the MTD. Neither remissions nor clinical improvements were recorded in 16 patients treated at this dose level, fulfilling the early stopping rule in the Simon two-stage study design. Major toxicity was on thrombocytopenia. In 9 of 15 patients bortezomib proved that it is able to reduce bone marrow vessel density. However, the agent was associated with worsening of markers of disease activity, such as enhancement of hematopoietic CD34-positive progenitor cell mobilization, WT-1 gene expression in mononuclear cells, and downregulation of CXCR4 expression on CD34-positive cells. Occurrence of both beneficial and detrimental biological effects claims further investigation on the mechanisms of the drug in MF.

The proteasome inhibitor bortezomib (Velcade®, Millenniun Pharmaceuticals, and Johnson & Johnson Pharmaceutical Research and Development, LLC, Cambridge, MA) induces tumor cell death by inhibiting the degradation of several intracellular proteins involved in cell cycle regulation, and inhibits degradation of IkB blocking the multifunctional transcription factor nuclear factor-kappa B (NFkB) leading to reduced levels of transforming growth factor β-1 (TGF-β1). In addition, bortezomib indirectly inhibits angiogenesis and prevents tumor adaptation to hypoxia by functional inhibition of hypoxia inducible factor 1-alpha (HIF-1α). In MF, several lines of evidence are in favor of a crucial role of the TGF-β1, which is released by clonal proliferation of megakaryocytes or monocytes via activation of NF-kB [2,3]. Moreover, MF shows enhanced bone marrow and spleen angiogenesis that has been documented to be associated with worse prognosis [4,5]. Thus, NF-kB signaling pathway and angiogenesis are candidate targets for bortezomib in MF. Based on these assumptions, in 2007 we initiated a phase I/II trial with the aim to evaluate the safety and efficacy of bortezomib in patients with MF, to evaluate its effect on bone marrow angiogenesis and fibrosis, and on biomarkers of severity and progression of the disease.

Twelve patients were enrolled onto phase I of the study. The baseline characteristics of these patients are listed in Table I. Three patients treated at the 0.8 mg/m^2 dose level, and three treated at the 1 mg/m^2 dose level had no dose limiting toxicity (DLT). One of six patients treated at the 1.3 mg/m^2 dose level experienced acute severe pulmonary distress syndrome during the first cycle of treatment and this dose level was defined as MTD. Sixteen patients were enrolled onto the phase II portion of the study. One patient did not complete the first cycle of treatment, 13 patients (81%) completed four cycles of treatment, and nine (56%) patients completed the six cycles of treatment. The primary reason for early withdrawal from the study was unacceptable adverse events (AEs) (three patients), patient's refusal (two patients), and physician's decision (two patients). At intention to treat

TABLE I. Baseline Characteristics of the Study Populations Entering the Phase I and Phase II of the Study

Characteristic	Phase I (N = 12)		Phase II (N = 16)	
	No of patients (%)	Median (range)	No of patients (%)	Median (range)
Age, years		57 (22–69)		58 (46–72)
Sex				
Female	5 (41.7)		6 (37.5)	
Male	7 (58.3)		10 (62.5)	
Type of Myelofibrosis				
Primary	11 (91.6)		10 (62.5)	
Post-PV	1 (8.4)		4 (25)	
Post-ET	0		2 (12.5)	
Prior treatment for myelofibrosis				
Hydroxyurea	8 (66.6)		13 (81.2)	
Splenectomy	1 (8.4)		2 (12.5)	
Danazol	1 (8.4)		2 (12.5)	
Thalidomide	1 (8.4)		2 (12.5)	
Duration of the disease (months)		44.5 (1–228)		35 (1–156)
Transfusion dependent patients	2 (16.6)		3 (18.7)	
Transfusion-independent patients with initial hemoglobin <10 g/dL	5 (41.7)		5 (31.2)	
White blood cell count ($\times 10^9$/L)		7.9 (3.7–71.3)		13.2 (1.7–71.3)
Myeloblasts in peripheral blood (%)		2 (0–7)		1 (0–7)
Immature myeloid cells (nonblasts) in peripheral blood (%)		1 (0–12)		3 (0–15)
Erythroblasts (% leukocytes) in peripheral blood		3 (0–45)		4 (0–45)
Platelet count ($\times 10^9$/L)		302 (106–1066)		285 (70–3405)
Spleen size below the costal margin, cm		15 (2–20)		15 (2–25)
Dupriez prognostic score				
Score 0	4 (33.3)		7 (43.8)	
Score 1	6 (50)		6 (37.5)	
Score 2	2 (16.7)		3 (18.7)	
Serum lactate dehydrogenase (mU/mL)		1486 (358–3024)		1408 (489–2658)
Chromosomal abnormalities[a]				
Not available	5 (41.7)		7 (43.8)	
No chromosomal abnormalities	5 (41.7)		4 (25)	
Chromosomal abnormalities[b]	2 (16.7)[b]		5 (31.2)[c]	

[a]In all patients, analysis of chromosomal abnormalities was performed on peripheral blood; [b]del5, del7; [c]del20, t(x;20), del6/del14, del5, del7.

TABLE II. Toxicity Summary during Treatment with Bortezomib

Event	All adverse events	Grade 3 events
Thrombocytopenia	8	3
Fatigue	4	0
Rash	2	0
Pyrexia	3	0
Dyspnoea with pulmonary distress syndrome	1	1
Dyspnoea with pulmonary hypertension	1	1
Cutaneous vasculitis	1	1
Peripheral neuropathy	1	0
Cutaneous infectious ulcer	1	1

TABLE III. Bone Marrow Vessels Density during Bortezomib Trial in 15 Patients Who Had Serial Bone Marrow Specimens Available for Review

Case	Bortezomib dose mg/m^2	Number of vessels ($\times 10^{-3}\mu^2$)		Change from baseline (%)
		Baseline	Final	
1	0.8	1.41	2.54	80.1
2	0.8	2.33	2.78	19.3
3	0.8	2.66	2.17	−18.4
4	1	3.32	3.02	−9.0
5	1	2.17	2.14	−1.4
6	1.3	1.30	2.49	91.5
7	1.3	2.66	1.40	−47.4
8	1.3	3.65	1.79	−50.9
9	1.3	1.92	2.43	26.5
10	1.3	4.09	3.20	−21.8
11	1.3	2.76	2.77	0.4
12	1.3	4.56	3.15	−30.9
13	1.3	5.93	3.41	−42.5
14	1.3	2.06	2.30	11.6
15	1.3	3.96	2.54	−36.1
Median		2.66	2.53	−9.03

analysis in which all patients who received at least one dose of the drug in the phase II study were evaluable (16 patients), no responses were recorded according the IWG response criteria [6]. At the per protocol analysis in which patients who received at least four cycles of treatment were evaluable, 13 of the 16 patients in the phase II study were evaluable. No patient had clinical improvement. As a matter of fact, no patient had Hb increasing >2 g/dL by the end of the study, and none of the transfusion-dependent patients (n = 4) had decrease in blood transfusion need. One patient with absolute neutrophil count below 1 $\times$ 10^9/L at baseline did not increase neutrophil count by at least 100%. None of the patients had >50% spleen reduction. A patient with 4,448 $\times$ 10^9/L platelet count at baseline decreased the platelet count by 67% by the end of the study, but this response is not included in the criteria for clinical improvement. As depicted in Table II, the most frequent Grade 3 or 4 toxicity was thrombocytopenia. At analysis of individual changes in cellularity, CD34+ cell content, and fibrosis in the 14 patients who completed the six cycles of treatment at any dose and had serial bone marrow specimens available for review, no statistically significant changes in none of the parameters resulted after therapy. At baseline, the patients had a significantly higher level of TGF-β1 than our control normal population (4738 pg/mL vs. 2404 pg/mL; $P = 0.015$). No correlation was evidenced between baseline bone marrow fibrosis grade and plasma TGF-β1 level. Bortezomib treatment did not significantly decrease total TGF-β1 plasma levels (TGF-β1 final, 4959.5 pg/mL) from baseline (Wilcoxon test, P = NS).

In the whole population of patients, bortezomib treatment did not significantly reduce the median vessels density, vessels area, and vessels perimeter. However, a decrease in vessels density was evidenced in 9 of the 15 (60%) patients studied (Table III). The percent decrease in vessels density ranged from 1.4% to 51%. Vessels area and vessels perimeter were reduced in 40% and 66% of cases, respectively. At baseline, the median value of plasma vascular endothelial growth factor (VEGF) in MF patients was 78.9 pg/mL (range, from 15.6 to 236.4 pg/mL), significantly higher than in normal controls (median, 30.16 pg/mL; range, from 15.6 to 130.6 pg/mL; $P = 0.001$). Bortezomib treatment did not significantly decrease VEGF levels from baseline (Wilcoxon test, P = NS).

At analysis of the 17 patients who completed at least four cycles of treatment at any dose, and had serial measurements available, the median baseline hematopoietic CD34+ cell number was 114.1 $\times$ 10^6/L (range, 15.5 to 3026 $\times$ 10^6/L), whereas it was 143.1 $\times$ 10^6/L (range, 17.2 to 3688.3 $\times$ 10^6/L) at the end of the study (Wilcoxon test, $P = 0.05$). Increase in CD34+ cells in peripheral blood at the end of the study was detected in 11 of 17 (64.7%) patients, and the increase at the end of the therapy ranged from 4% to 1125% of basal value.

At analysis of the 14 patients who completed at least four cycles of treatment at any dose, and had serial measurements available, median WT1 expression at baseline was 6,870.68 copies/10^4 ABL copies (range, 221.31 to 67,842.21

copies). After bortezomib, median WT1 expression did not significantly change (Wilcoxon test, NS). However, WT-1 expression increased in 8 of 14 patients (57.1%) with an increase ranging from 10% to 820% of basal value.

At analysis of the 18 patients who completed at least four cycles of treatment at any dose, CXCR4 expression on circulating CD34-positive cells was downmodulated at baseline in patients involved in this study (median, 22%; range, 6.2–92%), as compared with our historical normal controls (median, 76.7%; range 37–97%; $P < 0.001$). By the end of the study, the value of CXCR4 expression was significantly lower than at baseline (median, 15.2%; range, 5.9–90%; Wilcoxon test, $P = 0.05$). Reduction was documented in 10 of the 18 patients analyzed (55.7%). Granulocyte DNA-derived *JAK2* 617F allele burden was measured in 13 patients at baseline and after completion of at least four cycles of treatment. Twelve patients were *JAK2*V617F mutated with a median allele burden of 42.5% (range 4–100%). In none of the patients, the V617F burden variation was >10%. No significant changes in plasma SDF-1, IL-8, IL-6, and TNF were revealed at the end of the study.

In summary, with this phase I/II study, we found that none of the 22 patients either treated with the MTD of 1.3 mg/m^2, or with lower doses in the phase I of the study, achieved a clinical response. Our results are in agreement with the lack of any clinical efficacy described by Mesa et al. who reported the results of a pilot phase II study with bortezomib in nine patients with MF and two with systemic mastocytosis or chronic myelomonocytic leukemia, showing lack of any clinical efficacy of the drug [7].

The results of this trial contrast with the 31–80% response rate in multiple myeloma [8], mantle-cell lymphoma [9], amyloidosis [10], cutaneous T-cell lymphoma [11], Waldestrom macroglobulinemia [12], or mucosa-associated lymphoid tissue (MALT) lymphoma [13] when bortezomib was used as single agent. In an attempt to clarify how bortezomib affects the pathogenetic mechanisms that sustain MF, in this trial we evaluated bone marrow and blood biomarkers variations as secondary endpoints of the study. In a great proportion of patients, the density of bone marrow microvessels was less after treatment than at baseline, reaching up to 51% reduction. The role of proteasome inhibition in angiogenesis has been documented in several preclinical studies [14–17] and one in vivo study in humans [18]. We were not able to document that the effect on angiogenesis could be associated with a decrease of plasma VEGF. This was in accordance with the results in multiple myeloma [19].

In contrast with the potentially beneficial effect on angiogenesis, we documented that the therapy had the potential to exert detrimental effects on biomarkers that mirror disease activity and progression. CXCR4 downregulation seems to represent the most relevant biological consequence of bortezomib therapy in patients with MF. Downregulation of cell surface proteins is a general mechanism of bortezomib [20–22]. However, the decrease of CXCR4 on CD34+ cells of patients with MF seems to be an unique example of chemokine receptor downregulation, because bortezomib has no effect on CXCR4 expression in multiple myeloma cells [23]. Furthermore, because the downregulation of the above-mentioned receptors on the cell surface is potentially beneficial, such as overcoming cell adhesion-mediated drug resistance for VLA-4 downregulation [21], in MF CXCR4 downregulation exacerbates a detrimental disease characteristic that specifically is responsible for hematopoietic cell mobilization and myelopoiesis derangement. We hypothesize that the strong influence of bortezomib on the bone marrow microenvironment may interact with the migration and adhesion mechanisms of hematopoietic stem cells operating in MF, and disrupt a homeostatic equilibrium that is unique and specific for the disease. A better understanding of these mechanisms is necessary for planning a better targeted use of bortezomib in MF.

Methods

Study design

For the Phase I portion of the study, DLT was defined as any Grade 3 or 4 treatment-related nonhematologic toxicity (National Cancer Institute Common Terminology Criteria of Adverse Events, version 3.0); any Grade 4 treatment-related hematologic toxicity; or any Grade 3 treatment-related hematologic toxicity requiring treatment delay of more than 2 weeks. Three patients were to be enrolled at each dose level starting at dose level 1. If no DLT was observed in cycle one, three patients were enrolled at the next dose level. If one DLT was observed, the dose level was expanded to six patients. If two DLTs were observed, the MTD was exceeded and the previous dose level was expanded to six patients. The recommended phase II dose was the highest dose level at which one or less of six patients experienced a DLT. Three dose levels were planned (0.8, 1, and 1.3 mg/m^2). No intra patient dose escalation was allowed.

For the Phase II efficacy analysis, we used an optimum Simon 2 stage design to test the null hypothesis that the complete or major response rate was $\leq$0.05 versus the alternative that this response rate was $\geq$0.20 at an alpha level of 0.05 with 80% power. At the evaluation of response at 18 weeks, if there were no or one responses (complete or major) of first 16 patients, the trial would be terminated for lack of efficacy. If the trial continued to a second stage, a total of 30 patients would be studied.

Bortezomib was administered intravenously on days 1, 4, 8, and 11 of a 21-day cycle. A total of six cycles were planned while on study. Dose reduction was allowed for Grade 3 or 4 thrombocytopenia or any Grade 3 or 4 nonhematologic toxicity.

All patients provided written informed consent. The study protocol was approved by the ethics committee of the IRCCS Policlinico S. Matteo Foundation, Pavia, and of the Florence University Hospital, Florence. The study was conducted in accordance with the policies of the MPD Research Consortium.

Bone marrow histology and microvascular proliferation

Bone marrow samples were obtained before treatment and at the patient's final evaluation. Cellularity and fibrosis were assessed using the EUMNET score [24]. The rate of CD34+ progenitor cells and degree of microvascular proliferation were evaluated on sections stained with antiCD34 (mouse monoclonal Thermo Scientific, Fremont, CA). For microvascular proliferation, sections were evaluated on five randomly selected fields and images digitally acquired using an Olympus BX-60 microscope equipped with the DP-70 camera (Olympus Optical Corporation, Japan). From the total area, the area occupied by bone or eventual art factual spaces was subtracted, and the absolute number, the perimeter, and the area of CD34 positive vascular structures, including small vessels but not arterioles or sinusoids, were measured using CELL^F 2.5 software (Olympus Soft Imaging Solution, Olympus). All the data were parameterized to 10,000 μ^2.

Biomarkers

Blood samples for the measure of biomarkers were obtained on day 0 of treatment cycle one and at the patient's final evaluation. The percentage of circulating CD34-positive hematopoietic progenitor cells was calculated according to the guidelines from the International Society of Hematotherapy and Graft Engineering [25]. For plasma TGF-β1 measurement, human TGF-β1 immunoassay was used (Quantikine kit, R&D Systems). Plasma levels of SDF-1, VEGF, IL-8, IL-6, and TNF were determined with the appropriate human Quantikine kits from R&D Systems according to the instructions of the manufacturer. Samples were assessed in duplicate. Seventeen normal individuals were used as controls for the cytokine level assays. They were 10 men and 7 women, with a median age of 49 years (range 32–65 years). Levels of WT1 mRNA were measured on mononuclear cells according the previously reported method [26]. For CXCR4 expression measurement, cells were stained with specific monoclonal antibodies and analyzed using flow cytometry (Becton Dickson, Oxford, UK) as described earlier [27]. Analysis of *JAK2*V617F mutational status and mutated allele burden was performed as described [28].

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G. Barosi has received a research grant from Janssen Cilag.
This study was supported by the National Cancer Institute grant P01CA108671.
Presented in part at the 49th Annual Meeting of the American Society of Hematology, December 8-11, 2007, Atlanta, GA.
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Conflict of interest: Nothing to report
Published online 5 May 2010 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21754

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Low CD49d expression and long telomere identify a chronic lymphocytic leukemia subset with highly favourable outcome

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CD49d expression and telomere length (TL) represent novel prognostic markers for chronic lymphocytic leukemia (CLL) [1–7]. The prognostic information carried by CD49d expression and TL in CLL has its rationale in the disease biology. CD49d is an adhesion molecule mediating cell-to-cell and cell-to-extracellular matrix interactions [8]. In CLL cells, CD49d transmits prosurvival/antiapoptotic signals from the tumor microenvironment to tumor cells [9]. Telomeres ensure genetic stability and regulate critical cellular functions, including proliferation and senescence [10]. In CLL, short TL associates with a fast proliferative history of the leukemic cells [1,11,12]. Both CD49d expression and short TL have been associated with increased genetic instability of the CLL clone [3,13]. From a clinical standpoint, a peculiar feature shared by CD49d expression and short TL is the association with CLL proliferation markers, including expression of CD38, high lactate dehydrogenase (LDH), high β-2-microglobulin, short time to lymphocyte doubling, and short time to progression to a more advanced stage [1–7]. Here, we tested whether the concomitant presence of high CD49d expression and short TL in the same CLL patient may help refine disease stratification for treatment prediction in patients that presented in early to intermediate Binet stage (Binet A and B) and that are candidate to watch and wait as initial management.

The study is based on a consecutive series of 128 newly diagnosed CLL presenting in Binet stage A or B (Table I). CLL diagnosis and management were based on International Workshop on Chronic Lymphocytic Leukemia (IWCLL) guidelines [14,15].

By flow cytometry, CD49d expression ≥30% was documented in 49 of 128 (38.3%) cases. By Southern blot, median TL was 5955 bp (25th–75th percentiles: 4554–6998 bp). By applying the best cut off point of TL for CLL prognostication [4], 40 of 128 (31.3%) CLL harbored a short TL ≤5000 bp. When treated either as continuous or categorical variables, CD49d expression and TL do not show colinearity (Pearson $R = -0.156$, $P = 0.078$; γ^2 $P = 0.148$, respectively), and therefore do not behave as reciprocal surrogates.

After a median follow-up of 81 months, 64 of 128 patients were treated, accounting for a median treatment-free survival (TFS) of 86.4 months. By univariate analysis, CLL harboring CD49d expression ≥30% at diagnosis were characterized by a significantly higher risk of treatment requirement ($P = 0.002$) compared with cases with CD49d expression <30% ($P = 0.002$; Table II and Fig. 1A). Also, CLL harboring TL ≤5000 bp at diagnosis were characterized by a significantly higher risk of treatment requirement compared with cases with TL >5000 bp ($P = 0.001$; Table II and Fig. 1B).

By multivariate analysis penalized by Firth's bias correction, CD49d expression ≥30% (HR: 2.10; 95% confidence interval (CI): 1.18–3.73; $P = 0.011$) and TL ≤5000 bp (HR: 1.91; 95% CI: 1.09–3.35; $P = 0.023$) were selected as independent predictors of TFS in this CLL cohort, along with

IGHV homology ≥98% ($P = 0.025$), Binet stage B ($P < 0.001$), and absolute lymphocyte count >20 × 10^9/L ($P = 0.005$; Table II).

To test whether the association between CD49d expression and TL has an impact on CLL outcome, patients were grouped into the following categories: (*i*) CD49d^{low}/TL^{long} CLL (CD49d <30%/TL >5000 bp; 58 of 128, 45.3%); (*ii*) CD49d^{high}/TL^{short} CLL (CD49d ≥ 30%/TL ≤5000bp; 30/128, 23.4%); and (*iii*) discordant cases (CD49d^{low}/TL^{short}; 21 of 128, 16.4%; and CD49d^{high}/TL^{long}; 19 of 128, 14.8%).

Bivariate analysis revealed that the combined usage of CD49d expression and TL allowed to segregate CLL cases projected to require treatment from CLL with an indolent course. In fact, CD49d^{high}/TL^{short} CLL and discordant cases were characterized by a TFS significantly shorter than that of CD49d^{low}/TL^{long} CLL ($P < 0.05$ in all pairwise comparisons; Fig. 1C).

Our results document that: (*i*) CD49d expression and TL exert a complementary effect in driving prognosis of early stage CLL; (*ii*) early stage CLL harboring the CD49d^{low}/TL^{long} profile are characterized by a very favorable prognosis. The concomitant assessment of CD49d expression and TL may help refine further the prognostic stratification of early stage CLL and, in particular, may serve the scope of identifying highly stable patients.

The complementary action of CD49d and TL in driving CLL prognosis may stem from the biological effects exerted by these two markers on the CLL clone. In fact, the microenvironmental prosurvival signals provided by CD49d expression, and the genetic instability and proliferating propensity associated with short TL may both confer biological aggressiveness to CLL [1–13]. According to this model, CLL cases with both low CD49d expression and long telomeres (CD49d^{low}/TL^{long} CLL) may be less exposed to deleterious microenvironmental stimuli and genetic instability, and, consequently, at reduced risk of clinical progression and more prone to maintain clinical stability.

TABLE I. Biological and Clinical Characteristics of the CLL Series[a]

Characteristics	
Biological variables	
IGHV homology ≥98%	46/127 (36.2%)
FISH karyotype hierarchical stratification	
Normal	27/128 (21.1%)
del13q14	53/128 (41.4%)
+12	27/128 (21.1%)
del11q22-q23	7/128 (5.5%)
del17p13	14/128 (10.9%)
CD38 ≥30%	25/128 (27.3%)
ZAP70 ≥20%	41/124 (33.1%)
CD49d ≥30%	49/128 (38.3%)
Telomere length (bp)	5955 (4554–6998)
Telomere length ≤5000 bp	40/128 (31.3%)
Clinical variables	
Age (yr)	50 (62–76)
Male:female	76:56
Binet stage	
A	105/128 (82.0%)
B	23/128 (18.0%)
Absolute lymphocytes (×10^9/L)	13.7 (8.4–20.3)
B-lymphocytes (×10^9/L)	11.5 (6.0–20.8)

[a]25th–75th percentiles are reported in parentheses for continuous variables. IGHV, immunoglobulin heavy chain variable gene.

Methods

Patients

The study was based on a consecutive series of 128 previously untreated CLL who presented in Binet stage A or B for initial evaluation at the Division of Hematology of the Amedeo Avogadro University of Eastern Piedmont from June 1996 through June 2008. Median follow-up of alive patients, calculated by inverted censoring method, was 81 months. Patients provided written informed consent in accordance with local institutional review board requirements and Declaration of Helsinki. CLL diagnosis and management were based on IWCLL guidelines [14]. The following biological variables were analyzed on fresh or cryopreserved peripheral blood mononuclear cells collected at CLL diagnosis before treatment: (*i*) IGHV gene homology to germline; (*ii*) FISH karyotype; (*iii*) *TP53* mutations; (*iv*) CD49d, CD38, and ZAP70 expression; and (*v*) TL. In all samples, the fraction of CD19+/CD5+ cells was ≥70% (median: 83%; range: 73–90%).

Flow cytometry

Expression of CD49d was analyzed by three-color immunofluorescence by combining phycoerithrin (PE)-conjugated antiCD49d mAbs with Peridinin-Chlorophyll-Protein-Cyanine-5.5 (PerCP-Cy5.5)-conjugated antiCD19 mAbs and fluorescein isothyocyanate-conjugated antiCD5 mAbs [5,7]. Expression data were reported as percent of $CD5^+CD19^+$ CLL cells displaying specific fluorescence intensity >98–99% of the same cell population stained with control Ig. CD38 and ZAP70 were analyzed as reported [5]. The 30% cut-off value for CD49d expression was selected based on maximally selected rank statistics results and corresponded to a value previously validated in the literature [5]. Cut-off points of 30% and 20% were utilized to define positivity for CD38 and ZAP70, respectively [5].

Determination of TL

Peak TL was determined by Southern blot analysis [16]. Peak TL was preferred to mean TL because this approach allows accurate measurement of TL in the presence of up to 30% contaminating non-neoplastic cells, as documented by extensive cell dilution studies [16]. Briefly, 2 μg of genomic DNA were digested by mixing HinfI and RsaI (Roche Diagnostics, Mannheim, Germany). Telomere restriction fragments were separated by 0.8% agarose gel electrophoresis. Gels were transferred to a positively charged nylon membrane (Roche Diagnostic Mannheim, Germany) and UV cross linked. Hybridization and detection were performed using the TeloTAGGG Telomere Length Assay Kit (Roche Diagnostics, Mannheim, Germany). Membranes were scanned and analyzed with Kodak Digital Science 1D Software (Scientific Imaging Systems, New Haven, CT). The best cut-off point of TL for CLL prognostication has been previously identified as 5000 bp [6].

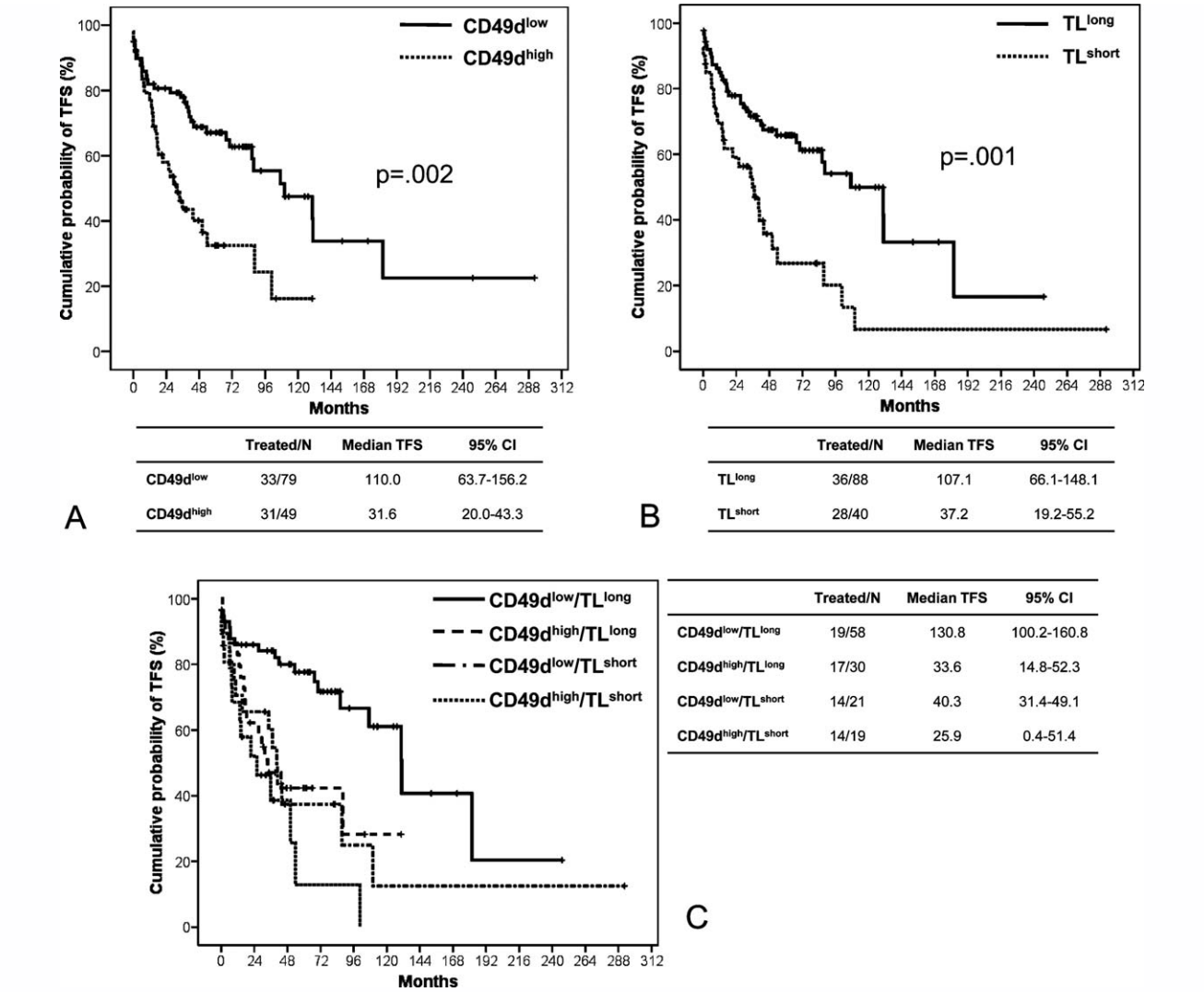
Statistical analysis

TFS was measured from diagnosis to first-line treatment, death, or last follow-up. The accepted indications to initiate treatment were based on IWCLL criteria [14]. Categorical variables were compared by γ^2 test. Correlation between continuous variables was assessed by measuring the Spearman's correlation coefficient. Survival was analyzed by Kaplan-Meier method. Univariate and multivariate Cox models were used to verify the independent prognostic power of each parameter. To account for possible monotone likelihood, the Firth's bias correction method was applied to Cox regression when several highly predictive covariates were introduced in the

TABLE II. Biological and Clinical Characteristics of the CLL Series Predicting TFS by Univariate and Multivariate Cox Analysis

	Univariate				Multivariate			
	HR	95% LCI	95% UCI	*P*	HR	95% LCI	95% UCI	*P*
CD49d ≥30%	2.324	1.372	3.936	0.002	2.102	1.184	3.731	0.011
TL ≤5000 bp	2.420	1.468	3.989	0.001	1.916	1.092	3.359	0.023
IGHV homology ≥98%	3.163	1.881	5.317	<0.001	2.013	1.090	3.718	0.025
CD38 ≥30%	2.324	1.372	3.936	0.002	–	–	–	0.944[a]
ZAP70 ≥20%	2.530	1.490	4.295	0.001	–	–	–	0.215[a]
del11q22-q23/del17p13	2.604	1.445	4.695	0.001	–	–	–	0.128[a]
Age >70 yr	1.408	0.856	2.316	0.178				
Male sex	1.233	0.745	2.042	0.415				
Binet stage B	4.467	2.536	7.867	<0.001	3.693	2.022	6.745	<0.001
PB lymphocytes >20 × 10^9/L	2.359	1.426	3.905	0.001	2.186	1.265	3.776	0.005

[a]*P* value defined by the score statistics of variables left out of the final model after stepwise backward elimination. HR, hazard ratio; LCI, lower confidence interval; UCI, upper confidence interval; IGHV, immunoglobulin heavy chain variable gene; PB, peripheral blood.



	Treated/N	Median TFS	95% CI
CD49d^{low}	33/79	110.0	63.7-156.2
CD49d^{high}	31/49	31.6	20.0-43.3

	Treated/N	Median TFS	95% CI
TL^{long}	36/88	107.1	66.1-148.1
TL^{short}	28/40	37.2	19.2-55.2

	Treated/N	Median TFS	95% CI
CD49d^{low}/TL^{long}	19/58	130.8	100.2-160.8
CD49d^{high}/TL^{long}	17/30	33.6	14.8-52.3
CD49d^{low}/TL^{short}	14/21	40.3	31.4-49.1
CD49d^{high}/TL^{short}	14/19	25.9	0.4-51.4

Figure 1. Treatment-free survival (TFS) according to CD49d expression (Panel A), telomere length (TL) (Panel B), and CD49d expression and TL in combination (Panel C).

analysis. Model minimization was performed by stepwise backward elimination. All statistical tests were two-sided. Statistical significance was defined as $P < 0.05$. The analysis was performed with SPSS software v.17.0 (Chicago, IL).

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

D.R. designed the study, interpreted data, performed statistical analysis, and drafted the manuscript; G.G. contributed to study design, data interpretation, and drafting the manuscript; L.D.P. and M.F. collected biological and clinical data; S.C. performed; S.R. performed FISH analysis and IGHV gene and TP53 mutation analysis; A.Z. and F.M.R. performed CD49d expression analysis; C.L.B. performed telomere length analysis; V.G. and M.L. contributed to data analysis and interpretation.

This study was supported by Progetto Integrato Oncologia 2006, Ministero della Salute, Rome, Italy; Progetto Giovani Ricercatori 2008, Ministero della Salute, Rome, Italy; Progetto FIRB-Programma "Futuro in Ricerca 2008; MIUR, Rome, Italy; AIRC 5 × 1000, Milan, Italy; PRIN 2008, MIUR, Rome, Italy; Ricerca Sanitaria Finalizzata, Regione Piemonte, Torino, Italy; Novara-Associazione Italiana contro le Leucemie, linfomi e mielomi (AIL) Onlus, Novara, Italy; Associazione Italiana per la Ricerca sul Cancro (Investigator Grant IG-8701), Milan, Italy; Ministero della Salute (Ricerca Finalizzata I.R.C.C.S., "Alleanza Contro il Cancro" and Rete Nazionale Bio-Informatica Oncologica/RN-BIO), Rome, Italy; AIL, Venezia Section, Pramaggiore Group, Pramaggiore (VE), Italy; Ricerca Scientifica Applicata, Regione Friuli-Venezia-Giulia, Trieste ("Linfonet" Project), Italy.

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Conflict of interest: Nothing to report.

Published online 5 May 2010 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21756

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Stability of measurement of the immature platelet fraction

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Immune Thrombocytopenia (ITP) is a heterogeneous clinical entity for which no simple diagnostic test exists. The Immature Platelet Fraction (IPF), a marker of Reticulated Platelets (RP), is a good indicator of thrombopoiesis and can identify accelerated destruction of platelets by demonstrating compensatory increased platelet production. Measuring IPF is not routinely available in clinical practice; it has proven useful in studies of thrombocytopenia and the novel thrombopoietic agents. A multicenter clinical trial would be needed to explore IPF in management of patients with ITP; such a trial would require central testing. Therefore, we sought to assess the reliability of measured platelet counts and IPF in whole-blood filled EDTA tubes when the same tube is run freshly and again 24 hr after venipuncture. Based on analysis of our 103 samples, it is clear that EDTA tubes are stable at room temperature for 24 hr. Therefore, the results can be used to estimate thrombopoiesis when measured within 24 hr after phlebotomy.

Immune thrombocytopenia (ITP) is heterogeneous and affects both children and adults. It is characterized by autoantibody mediated increased platelet destruction, and reduced platelet production, leading to thrombocytopenia and its sequelae [1–3]. No standard diagnostic test, i.e., a platelet antibody test, exists to differentiate thrombocytopenia seen in ITP from other disorders. The diagnosis rests on applying clinical parameters, most of which are by exclusion, as outlined by various professional societies and recently updated [4–6].

In 1969, Ingram and Coopersmith identified certain platelets as having a coarse, punctuated condensation, i.e., reticulum, by using a methylene blue dye. The study suggested those platelets contained an increased amount of cytoplasmic RNA, reflecting active thrombopoiesis [7]. Lee et al., in 1986, reported detection of platelet nucleic acid via flow cytometry using thiazole orange dye [8]. Kienast and Schmitz showed that RNA-rich platelets measured with flow cytometry provided information on the thrombopoietic activity of patients with thrombocytopenic states [9]. Ault et al. espoused that analysis of these reticulated platelets provided a good indication of the rate of platelet production [10]. Furthermore, Rinder et al., in 1993, found that flow cytometric analysis of reticulated platelets was a better discriminant than platelet associated IgG in diagnosing ITP [11].

Subsequently, Sysmex corporation developed a method of measuring an entity called the IPF using a technology similar to thiazole orange staining of platelets; this technology used a proprietary dye and laser imaging to measure its association with platelets. Studies have shown that the IPF is virtually indistinguishable from the RP [12]. Additionally, research has provided evidence on the utility of measuring IPF in the diagnosis of ITP [13–15]. The advantage of the IPF is that it can be routinely obtained as part of the complete blood count using a Sysmex autoanalyzer, such as the XE2100.

Despite the aforementioned studies and others that indicate the utility of measuring RNA-rich platelets (otherwise known as RP or IPF), the IPF has not been widely adopted into clinical practice. One important reason for this lack of utilization is that measurement of IPF requires special technology, which is not routinely available in most hospital laboratories.

Routine measurement of the IPF in patients with ITP would potentially be useful, including in clinical trials. To utilize measurement of reticulated platelets, there would need to be a sufficiently large study to have adequate power. Such a study would ideally be completed at a central location, with samples shipped from various centers to allow for the study itself to be multicentered. The IPF might be particularly useful in exploring the newly licensed thrombopoietic agents [16–18], determining if the IPF parameter discriminates between different causes of thrombocytopenia, and investigating whether it would predict response to various therapies.

The aim of this project was to assess the reliability of measuring platelet counts and IPF in a whole-blood filled EDTA tube when the sample is run on a Sysmex autoanalyzer 24 hr after it was drawn. To determine the stability of the IPF in patients with ITP, samples were run fresh within hours of venipuncture (TO) and compared to the same sample run 24 hr later (T24). No mock or overnight shipping was used.

A total of 103 blood samples were collected by a phlebotomist from 74 subjects with ITP, ranging in age from 4–83 years (mean 41 years) during regularly scheduled clinic visits to the Platelet Disorder Center of New York Presbyterian-Weill Cornell Medical Center between the months of January and March 2007. Each individual subject contributed one or two samples to the database. A total of 45 of the samples were from subjects represented only once in the database; 29 subjects were represented twice. A total of 22 of the 74 total subjects were children, ages 4–21; four children had repeat sampling. For subjects represented twice in the catalogue of samples, the time between obtaining the samples ranged from 1 to 63 days.

The mean IPF% value for all subjects at T0 was 17.64 +/− 15.26 (standard error 1.5); the mean IPF absolute for all subjects at T0 was 7.72 +/− 8.31 (standard error 0.82). The mean IPF% value for all subjects at T24 was 18.38 +/− 13.96 (standard error 1.3); the mean IPF absolute for all subjects at T24 was 8.62 +/− 8.61 (standard error 0.85). Considering all 103 samples, the Pearson correlation for platelet counts at T0 and T24 was 0.995 [r(103) = 0.995] (Fig. 1a). The Pearson correlation for absolute IPF at T0 and T24 was 0.930 [r(103) = 0.930] (Fig. 1b); for IPF%, the correlation at T0 and T24 is 0.941 [r(103) = 0.941] (Fig. 1c). These three correlations are all statistically significant at the level of $P < 0.001$. When considering the 74 samples that do not include repeats (i.e., 45 unique subjects, the first sample for the 29 subjects with repeated sampling), the values for Pearson correlation are essentially unchanged: r(74) = 0.995 for platelets T0 to T24, 0.922 for IPF absolute T0 to T24, 0.995 IPF% for T0 to T24.

For IPF absolute, there seems to be a discrepancy between T0 and T24 of greater than 10 ($\times 10^9$/L) when the IPF values are larger than 30 ($\times 10^9$/L) (Fig. 1b outlier values); for IPF%, the discrepancy between T0 and T24 values

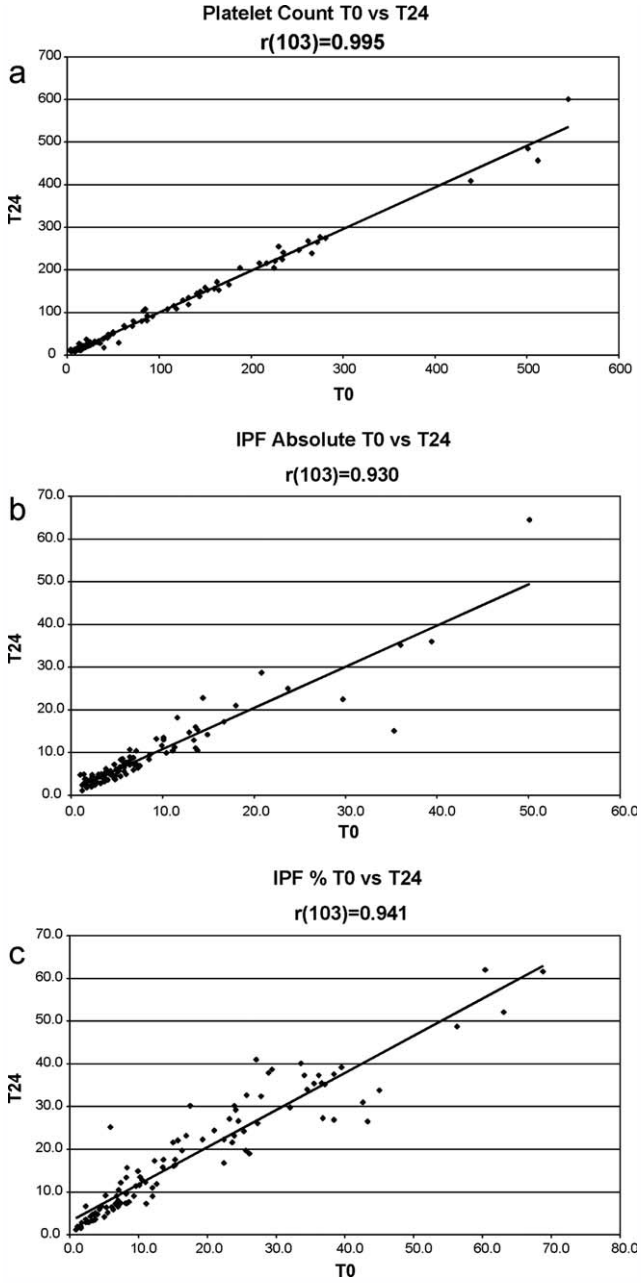

Figure 1. 1(a), 1(b), and 1(c) of T0 versus T24 show near perfect Pearson correlation [$r = 0.995, 0.930, 0.941$, respectively]. Units for both platelet count and A-IPF are $\times 10^9$/liter.

appears approximately 10% when the IPF% values are larger than 40% (Fig.1c, outlier values). Thus, the accuracy of repeated measurements seems to decrease at the very infrequent times when the values increase, with less correlation when A-IPF >30 ($\times 10^9$/L) and IPF% >40%.

Watanabe et al. endorsed the reproducibility and stability of the IPF, but no specific data were provided [19]. Briggs et al. reported that the IPF remained stable over two days when seven blood samples were stored at room temperature; there was no consistent increase or decrease in the IPF values 0.5–48 hr after sampling [12]. The current study is from a larger number of samples ($n = 103$), patients with ITP, and complete data is provided. Furthermore, the estimates for IPF% and A-IPF are similar to that cited elsewhere in the literature, specifically Briggs et al. 2004 article (Table I).

It is worth noting that an investigator can either use percent IPF or absolute values when translating laboratory parameters into clinical practice. Although we have presented data for both, our practice is to preferably utilize absolute values in clinical scenarios. Mathematically, as IPF% is defined at A-IPF/Platelet count, measures of IPF% may seem inordinately elevated in a patient with an extremely low platelet count. Complete considerations of the merits and clinical significance of IPF% versus IPF absolute in patients with ITP is outside the scope of this report since these blood samples were mostly only taken at one time point during a subject's treatment.

TABLE I. Comparison of Mean IPF from Various Studies [For Patients with ITP]

	Mean IPF absolute [$\times 10^9$/L] (range)	Mean IPF% (range)
Briggs et al 2004 article for pts with plt count $>50 \times 10^9$/L[a]	8.4 (1.6–38.6)	16.8 (2.3–52.1)
Briggs et al 2004 article for pts with plt count $<50 \times 10^9$/L[a]	7.8 (1.6–34.3)	22.3 (9.2–33.1)
Current Study	7.7 (1–50.1)	17.6 (0.9–68.8)

[a]Briggs C, et al. 2004. Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. British Journal of Haematology 126, 93–99.

A potential confounder is that 29 subjects were represented twice; thus, trends of platelet and platelet precursor stability in certain individuals may be over represented. Furthermore, those patients who are represented twice may have worse disease, require more frequent visits, and may be more likely to be consented for inclusion in the study. The limit of only two samples per subject, however, makes this unlikely. Moreover, the analysis of the data with each of the 74 samples without repeats yielded essentially identical conclusions to that with all 103 samples. Another potential confounder is that a T24 sample may be run at slightly longer than 24 hr after phlebotomy [there is a 30 hr window if the specimen was drawn at 9 a.m. and then run at 3 p.m. the following day]. However, this potential confounder would likely give worse results for stability of measurement, which was not born out in the data.

Based on the aforementioned results and when performed under conditions used in this study, we conclude that platelet count and absolute immature platelet fraction are reliable when measured at one day later than collection. Power appears to be sufficient in this study, since the "*n*" is 103. These results should allow for further multicenter studies to proceed in which the IPF is thought to be relevant in patients who have ITP, or in discriminating between causes of decreased platelets, by measuring laboratory parameters at a standardized, centralized location. The sample can be shipped overnight at room temperature, and we propose that results would still be valid.

This report lends credence to the reliable determination of the A-IPF and IPF%, despite storage of a sample for 24 hr prior to obtaining the measurement. This finding could be of use in future studies in patients with ITP.

Materials and Methods

Institution Review Board (IRB) approved consent was obtained during the clinic visit prior to venipuncture. It was a prerequisite for subjects to have a diagnosis of ITP, according to the American Society of Hematology, to be included in the study. Those with reduced platelets for other reasons (e.g., malignancy, DIC, aplastic anemia, etc) were excluded. No extra blood was drawn; the testing was performed on the EDTA tube that was used for obtaining routine complete blood count on that day's visit.

A Sysmex XE-2100 machine was used to quantify both platelet count and the immature platelet fraction. The Sysmex machines use fluorescent flow cytometry and advanced cell-counting methods to offer a complete CBC with extended differential [20]. Fluorescent intensity and forward light scatter are measured by flow cytometry, which discriminates reticulated platelets from other blood components, such as mature RBCs, red blood cell reticulocytes, mature platelets, and WBC fragments [21]. Total platelet count, absolute immature platelet fraction (A-IPF), and percent immature platelet fraction (IPF%) were analyzed at the time of collection (T0) and again 24 hr later (T24). Units for both platelet count and A-IPF are $\times 10^9$/liter. Samples were drawn from 9 a.m. to 3 p.m. and usually run (T0) at 3 p.m. The T24 samples were stored at room temperature between measurements, and run at $\sim$3 p.m. the following day.

Results were tabulated using Excel, and statistical analysis was run via SPSS (Version 11.5 for Windows). The Pearson Correlation, a measure of covariance (ranging from -1 to $+1$) was used to determine relationships of variables. IPF could either be analyzed as a percent or as an absolute number. To obtain the absolute IPF, the platelet count is multiplied by the IPF%.

Acknowledgments

Special thanks to Ian Giles of Sysmex Corporation for his expertise on the Sysmex autoanalyzer and to Donna Skerrett, M.D., Director of New York Presbyterian Hospital-Weill Cornell Medical Center Blood Bank.

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Conflict of interests: James B. Bussel—Research Support: Amgen, GlaxoSmithKline, Eisai, Ligand, Sysmex, Calgene, Shionogi. Consultant/Scientific Advisory Board: Amgen, GSK, Ligand, Shionogi, Eisai. Major Stockholder: Amgen, GSK. Speakers Bureau: France Foundation.
The remaining authors declare no competing financial interests.
Group Authorship Information: MR organized and analyzed the data and wrote the manuscript. BP reviewed the data and the manuscript. MW performed the statistical analysis. GV provided technical assistance with the blood samples. JB designed the study, recruited patients from the hematology clinic, and supervised the organization and writing process.
Published online 27 April 2010 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21748

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Association of age, gender, and weight on maintenance dose of intravenous unfractionated heparin

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Unfractionated heparin (UFH) therapy is commonly used for the management of acute coronary syndrome (ACS), atrial fibrillation (AF) or atrial flutter (A-Flutter). Weight based heparin nomogram is widely used to determine the dosage of continuous intravenous infusion. Frequent monitoring is required due to the variable responses to its anticoagulant effects. We investigated factors that may influence the dose of UFH. We performed a retrospective cohort study of all patients who received continuous UFH for ACS, AF, or A-Flutter from January 2008 to December 2008 and reached maintenance dose of UFH within 48 hr. A total of 199 patients were included in this study: 42.7% were females and mean age was 66.6 ± 2.0 years. Univariate analysis revealed that age, gender, weight, serum albumin, and serum protein level were significantly associated with maintenance of UFH dose. After adjustment with multivariate analysis, age ($R = -0.27$, $P \leq 0.001$), gender ($R = -0.14$, $P \leq 0.044$), and weight ($R = 0.54$, $P \leq 0.001$) remained to have significant association with maintenance UFH dose. In conclusion, we have identified not only weight but also age and gender as significant factors that may affect the maintenance of heparin dose in clinical setting.

Heparin is an indirect thrombin inhibitor that complexes with antithrombin (AT, also known as AT III), converting this circulating cofactor from a slow to a rapid inactivator of particularly thrombin and factor Xa [1,2]. The binding of heparin to the heparin-binding site on AT greatly accelerates the inactivating function of AT [3]. Its main use in medicine is anticoagulation for conditions such as ACS [4–6], AF/A-Flutter [7], deep venous thrombosis (DVT), and pulmonary embolism (PE) [8].

Heparin's effect on anticoagulation can be monitored by obtaining the activated partial thromboplastin time (aPTT). Maintenance of aPTT is generally set between 1.5 and 2.5 times the mean of the control value [1]. A landmark study by Raschke provided the current widely used weight-based heparin nomogram [9]. The formula for the weight-based nomogram starts with an initial starting dose of 80 U/kg body weight bolus, followed by a continuous 18 U/(kg hr) infusion rate with goal aPTT of 60 to 90 sec if patients are having PE or DVT and for ACS, initial dose would be 60 U/kg body weight bolus followed by a continuous 12 U/(kg hr) with goal aPTT 50–70 sec. Monitoring the anticoagulation effect by checking the aPTT is required and it is recommended to check serum aPTT every 6 hr until two stable therapeutic aPTT are achieved [10].

Despite its clinical benefit, continuous UFH infusion is associated with a small but significant incidence of complications such as bleeding. In addition, regardless of the use of weight-based nomogram, UFH requires frequent monitoring to maintain the therapeutic range of aPTT, which can be challenging to control due to the narrow therapeutic window UFH has [11,12].

TABLE I. Baseline Characteristics of Study Patients ($N = 199$)

Demographic characteristics	
Age	66.2 ± 2[a]
Female	85 (43)[b]
Race	
White	48 (24)
Black	22 (11)
Hispanic	48 (24)
Asian	16 (8)
Other	65 (33)
Past medical history	
Hypertension	135 (68)
Diabetes mellitus	61 (31)
Dyslipidemia	53 (27)
Congestive heart failure	13 (7)
Indication of starting intravenous unfractionated heparin	
Acute coronary syndrome	168 (85)
Atrial fibrillation or flutter	31 (15)

[a]Mean ± SD; [b]Number (percent).

TABLE II. Result of Univariate Regression Anlysis Between Maintenance UFH Dose and Confounding Factors

	Coefficient of determination (R^2)	P value
Demographic characteristics		
Age	0.24	<0.001
Gender	0.08	<0.001
Race		
White	0.001	0.64
Black	0.014	0.09
Hispanic	0.007	0.24
Asian	0.014	0.1
Weight	0.43	<0.001
Past medical history		
Hypertension	0.015	0.08
Diabetes mellitus	<0.001	0.94
Dyslipidemia	0.02	0.49
Congestive heart failure	0.001	0.9
Laboratory values		
Serum albumin	0.024	0.03
Serum protein	0.03	0.015

Although the underlying reasons for difficulty controlling heparin infusion rate are not fully understood, there are studies done in the past to explore the factors that may affect the heparin dose. Multiple extensive pharmacokinetic studies in the past, mainly in vitro studies, have indicated heparin nonspecifically binds to plasma proteins that neutralize the effect of heparin by limiting the amount of heparin available to bind to antithrombin [13–17]. Another study showed age-related differences in heparin response between children and adults [18]. There is also an in vitro study that showed that the effect of heparin differs between genders [19].

Currently, there are no dedicated clinical studies examining whether gender, race, various medical conditions, and serum protein level affect the standard heparin based nomogram. We have performed a retrospective cohort study to investigate whether these factors influence the maintenance UFH dose.

One hundred ninety-nine patients met the inclusion and exclusion criteria during the 1-year study period. The mean age was 66.2 years, 42.7% ($N = 85$) were women (Table I), and 84.5% ($N = 168$) received intravenous UFH for ACS. Univariate regression analysis was used to assess the coefficient of determination (R^2) between each confounding factor and maintenance UFH dose. A significant association existed between UFH dose and age and weight (age; R^2: 0.24, $P \leq 0.001$, weight; R^2: 0.43, $P \leq 0.001$; Table II). Furthermore, gender, serum albumin, and serum protein are also shown to have an association with UFH dose (gender; R^2: 0.08, $P \leq 0.001$, serum albumin; R^2: 0.024, $P = 0.03$, serum protein; R^2 : 0.03, $P = 0.015$; Table II). Ethnicity and past medical history were not shown to have correlation with UFH dose (Table II). When these factors were controlled with multivariate regression analysis, statistically significant associations continue to remain in gender, age, and weight. Regression coefficients after multivariate regression analysis in each factor were as follows: age: −4.4 ($P \leq 0.001$), female gender: −60 ($P = 0.044$), and weight: 8.0 ($P \leq 0.001$) with a coefficient of determination (R^2) of 0.49 ($P \leq 0.001$; Table III).

TABLE III. Results of Multivariate Regression Analysis (R^2: 0.49)

	Correlation coefficient (R)	Regression coefficient	P value
Age	−0.27	−4.4	<0.001
Gender (female)	−0.14	−60	0.044
Weight	0.54	8	<0.001
Serum protein	−0.02	−8.5	0.77
Serum albumin	0.05	27.8	0.47

The result from multivariate regression analysis generated following nomogram suggests that female and elderly may require less UFH to maintain therapeutic anticoagulation.

UFH dose (U/hr) for male = 550 + 8 × (Weight: kg) – 4.4 × (Age), (R^2= 0.49).

UFH dose (U/hr) for female = 490 + 8 × (Weight: kg) – 4.4 × (Age), (R^2= 0.49).

UFH is commonly used in the treatment and management of reducing embolic events in patients with ACS, AF, and A- Flutter. While heparin-based nomogram are routinely used to calculate the amount of heparin to administer, effective anticoagulation treatment with UFH remains clinically challenging, mainly due to its narrow therapeutic range and pharmacokinetic characteristics [12]. Limitations of pharmacokinetics in UFH have been studied in the past [20]. An in vitro study by Monte et al. demonstrated the coagulability variations among men and women [19]. However, to our knowledge, no clinical studies exist examining the heparin-based nomogram effects on gender and ethnic.

Our study demonstrated a relationship between gender and heparin dosage rate (R^2: 0.08, $P \leq 0.001$). When controlling other factors using multivariate regression analysis, statistically significant association between UFH dosage and gender remained (Table III).

Although Monte et al. demonstrated heparin resistance in gender, their study was in vitro examination of these differences [19]. The underlying explanation for this result is unclear, but it is possible that different sex hormones produce different types of heparin-binding proteins that may affect the activity of UFH. Hormone-binding protein ratios may play a role in the reduced requirements of heparin in females. Further investigations into hormones, binding protein levels, gender, and heparin interactions should provide more information.

Current study also demonstrated a negative correlation between age and maintenance dose of UFH (R^2: 0.24, $P \leq 0.001$). This difference persisted even after adjusted with multivariate analysis (Table III). This may suggest that elderly requires less UFH to maintain therapeutic aPTT. A recent study by Ignjatovic et al. demonstrated age-related differences in heparin response among pediatric and adult population [18]. This study showed that pediatric plasma samples had higher aPTT compared to adult group when same amount of heparin was administered. Although our study was in vitro, it suggests a potential difference in the adult and geriatrics population. As our study demonstrated heparin requirement decreases with age, further studies to modify nomograms within the geriatrics population may not only demonstrate other potential differences but also decrease morbidity and risks association with excessive anticoagulation.

We formulated a nomogram to demonstrate that females and elderly require lower doses of UFH to maintain therapeutic anticoagulation.

Despite that multiple in vitro studies have showed its nonspecific binding to plasma proteins, which limits the amount of heparin available binding to antithrombin III and decreases anticoagulation effect [13,15–17], nearly no correlation existed between serum protein/albumin level and UFH dosage under multivariate regression analysis (Table III). This can be due to the complex interactions and temporal changes in underlying nutritional status.

Current study has potential limitation. We did not evaluate the effects of renal function on clearance of heparin. Although heparin is cleared mainly by a highly efficient hematological saturation mechanism at low dosages, proper renal functioning may be required, as heparin is cleared predomi-

nantly by renal elimination at higher dosages due to over saturation of cellular binding sites [21,22].

Overall, our study provides a closer investigation and application of UFH. In patients requiring UFH for management of ACS, AF, or A- flutter, factors such as age and gender may affect therapeutic dosage rate, as per the standard nomogram.

Methods

Identification of Study Population and Source of Data

This was a retrospective cohort study of patients admitted to Beth Israel Medical Center from January 2008 to December 2008. All patients of at least 18 years of age and receiving UFH for management of ACS, AF, or A-Flutter were included. Patients were bolused initially with 60 U/kg of UFH, followed by a continuous infusion of 12 U/(kg hr), as per the standard weight-based heparin nomogram set forth by Raschke [10]. This nomogram has been adopted by and recommended by the American College of Cardiology and American Heart Association [10]. Patients receiving heparin for management of DVT or PE were excluded, as heparin requirements are different.

Pharmacy database and laboratory records were used to screen and collect data. Patients were included if they had at least two consecutive therapeutic range of aPTT, defined as aPTT value of 50–70 sec, recorded at least 6-hr apart while on same infusion rate. Laboratory data recorded were weight, serum protein, albumin, baseline international normalized ratio (INR), and aPTT. Inpatient medical records were examined for demographics (age, gender, and ethnicity), past medical history, indication for intravenous UFH, and maintenance dose of UFH. Laboratory and inpatient medical records lacking aforementioned information were excluded from the study. Other exclusion criteria included patients with past medical history of hepatitis, liver cirrhosis, renal insufficiency (defined as serum creatinine 1.3 mg/dl and over), concomitant use of other anticoagulants before reaching two consecutive therapeutic range of aPTT, failure to reach maintenance dose of UFH within 48 hr after starting intravenous UFH, abnormal INR, or aPTT before the administration of UFH.

Statistical Analysis

Univariate regression analysis was used to assess univariate relationships between individual variables and maintenance UFH dose. Multivariate regression was used to adjust for the effect of confounders. *P*-values less than 0.05 were considered statistically significant. The SAS system version 9.1 was used to analyze the data.

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Conflict of interest: Nothing to report.

Published online 30 April 2010 in Wiley InterScience (www.interscience.wiley.com).

DOI: 10.1002/ajh.21751

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Iatrogenic immunodeficiency-associated lymphoproliferative disease of the Hodgkin lymphoma-like variant in a patient treated with mycophenolate mofetil for autoimmune hepatitis

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Immunodeficiency-associated lymphoproliferative disorders (ILDs) are well-described entities that occur in the setting of organ transplant, bone marrow transplant, and congenital or acquired immunodeficiency [1,2]. The latest World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues [3] defines a subset of "other iatrogenic ILDs", composed mostly of lymphoproliferations occurring in the setting of patients receiving immunosuppressive therapy (particularly methotrexate or anti-TNF (tumor necrosis factor) agents) for autoimmune diseases or conditions other than in the transplant setting such as rheumatoid arthritis [4–9] or inflammatory bowel disease [10]. The role of Epstein-Barr virus (EBV) in ILDs is well characterized and the EBV-positive ILDs have a better prognosis than their EBV negative counterparts [9]. Among the morphologic variants of iatrogenic ILDs are classical Hodgkin lymphoma (cHL) and Hodgkin-like lymphoproliferations resembling cHL. These are rare entities with very few cases reported in the medical literature [4,8,11,12]. We report a case of EBV-positive ILD of the Hodgkin-like variant in a patient on long-term mycophenolate mofetil (MMF) for autoimmune hepatitis. Our patient had a complete response after withdrawal of immunosuppression and treatment with rituximab. As many of these cases will resolve without cytotoxic chemotherapy, this case highlights the importance of recognizing this clinical entity [4] and distinguishing it from de novo lymphomas.

A 42-year-old woman with a history of autoimmune hepatitis on immunosuppression since the age of 19 was referred for evaluation of a mandibular growth. The hepatitis had been controlled with prednisone and azathioprine for 20 years. Azathioprine and prednisone were then discontinued and the patient was treated with MMF (1,500 mg twice daily) for the past 3 years. Two months prior to presentation, she developed low-grade fevers, drenching night sweats, 2.5 kg weight loss, and painful, indurated, and ulcerated lesions of the right mandiblular, and maxillary gingivae (Fig. 1). All oral presentations began as small lesions that gradually increased in size and severity. Pertinent tests included hemoglobin of 10 g/dL, normal white blood cell count, normal platelet count, elevated erythrocyte sedimentation rate (ESR) (57 mm/hr), and normal lactic dehydrogenase. The biopsy from the buccal and lingual lesion revealed ulceration of the mucosa and infiltration of the submucosa with mononuclear and rare binucleated large cells with prominent single nucleoli. Figure 2 shows an extensive polymorphic infiltrate consisting of large R-S (Reed-Sternberg)–like cells with prominent nucleoli in a background of small to intermediate sized lymphocytes and histiocytes (Fig. 2A,B). The large atypical R-S–like cells (Fig. 2C) are CD30 positive with membrane and Golgi region–staining and are also dim nuclear PAX-5 and CD20 positive (Fig. 2D,E). R-S–like cells and scattered lymphocytes show positivity for EBV-encoded RNA (EBER) (Fig. 2F). This is consistent with a Hodgkin-like iatrogenic immunodeficiency-associated lymphoproliferative lesion.

Combined PET/CT scan revealed fluorodeoxyglucose-avid lesions in the right maxilla and mandible, hilar and pelvic lymph nodes, lung parenchyma, and gastric wall. In the proper clinical setting, these findings are diagnostic of iatrogenic ILD, Hodgkin-like subtype.

Clinical management consisted of interruption of MMF therapy and administration of rituximab 375 mg/m^2 weekly for 4 weeks. On the second week of therapy, a significant reduction in size of the oral lesions was noted, and on the sixth week, a complete response was documented by physical exam (Fig. 1) and normalization of PET/CT scan. Hepatic enzymes were monitored weekly and no abnormalities were found despite the interruption of immunosuppression.

The term post-transplantation lymphoproliferative disorders (PTLD) indicates the most common clinical setting for ILDs. PTLDs are often an EBV-driven monoclonal or polyclonal proliferation of B cells or less often T cells [13]. Immunosuppressants used to prevent graft rejection also result in relaxation of the immune surveillance provided by T cells over EBV-infected immortalized B cells allowing their uncontrolled and autonomous proliferation.14 The latest WHO classification [3] recognizes the occurrence of ILDs not only in post transplant settings but also in patients undergoing prolonged immunosuppression for autoimmune diseases. Several morphological subtypes occur in this setting such as atypical polymorphous lymphoproliferative disorders, diffuse aggressive non-Hodgkin lymphoma, Hodgkin lymphoma, and Hodgkin lymphoma–like lesions [15]. The latter subtype is one of the rarest morphologies, being EBV+ and CD30+ which is unlike classical Hodgkin lymphoma where only 20–40% of cases are CD20+, the R-S cells show variable stain intensity, and only a subpopulation of cells are CD 20+.

We report a case of ILD, Hodgkin lymphoma–like subtype occurring in a patient after prolonged use of MMF for treatment of autoimmune hepatitis. This case represents a rare variation of an uncommon disease. Only a few cases of MMF-related ILDs have been reported in the literature, predominantly EBV-positive primary central nervous system (CNS) diffuse large B-cell lymphoma (DLBCL) [16]. To our knowledge, this is the first case reported of MMF-related ILD with Hodgkin lymphoma–like histology.

This rare morphologic variant has also been reported following liver transplantation, being characterized by abnormal cells that exhibit features similar

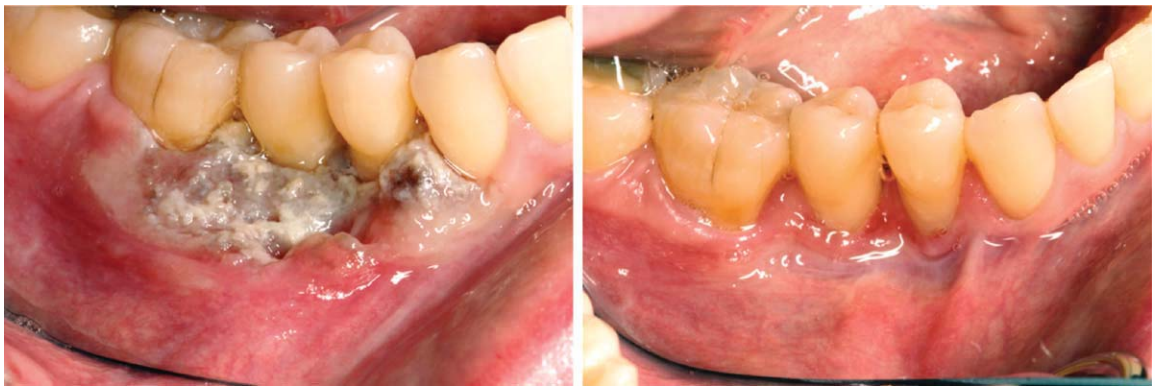

Figure 1. Indurated and ulcerated lesions of the right mandibular and maxillary gingivae. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

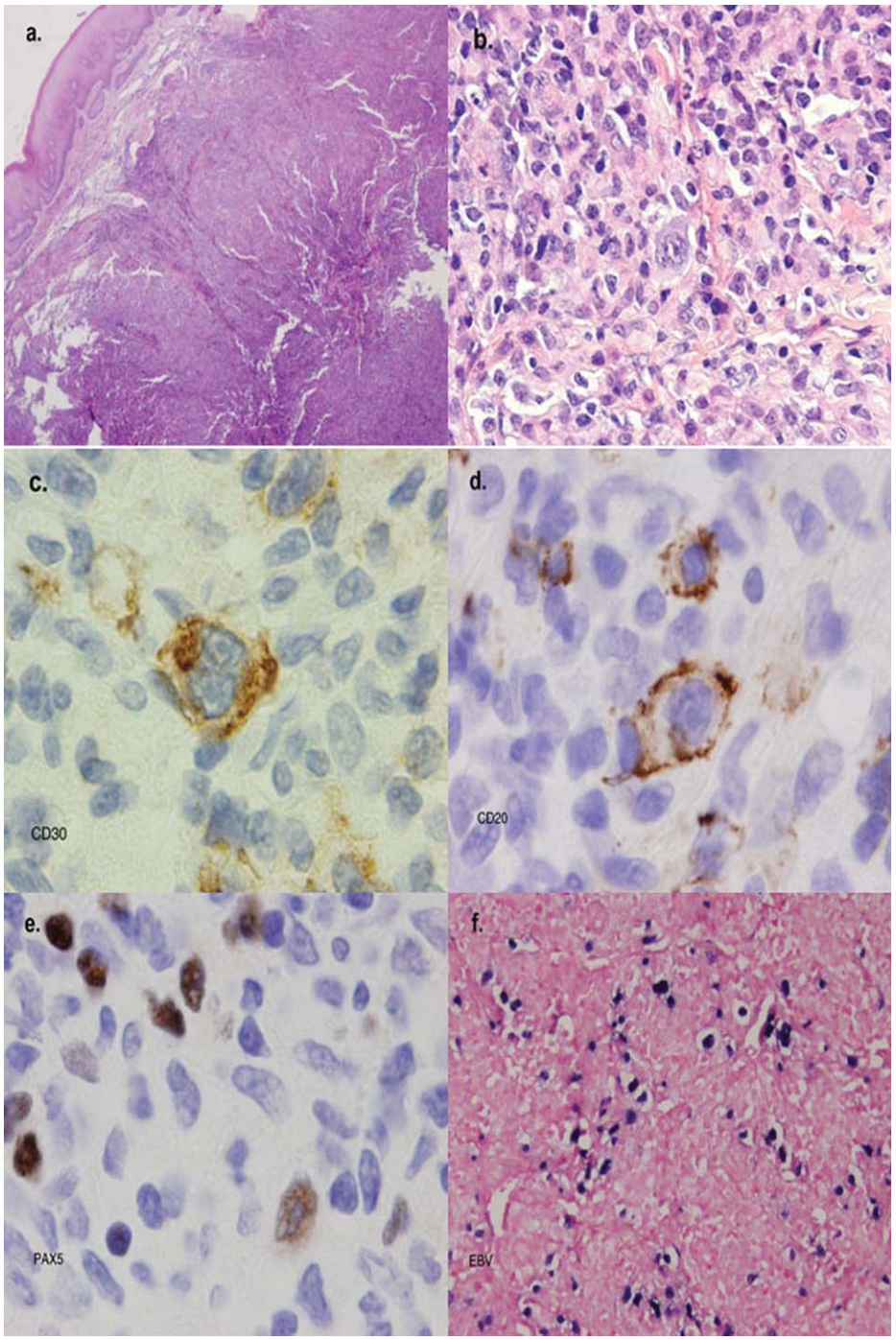

Figure 2. Hodgkin-like iatrogenic limmunodeficiency-associated lymphoproliferative lesion. A, B: Biopsy from the buccal and lingual lesion shows an extensive polymprophic infiltrate consisting of large R-S–like cells with prominent nucleoli in a background of small- to intermediate-sized lymphocytes and histiocytes. C: The large cells R-S–like cells are CD30 positive with membrane and Golgi region staining and (D and E) are also dim nuclear PAX-5 and CD20 positive. F: R-S–like cells and scattered lymphocytes show positivity for EBV-encoded RNA (EBER). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

to classic R-S cells [17]. Kamel et al. reported a series of eight patients with this morphology: four with lymphoproliferative disease that resembled cHD and four cases diagnosed as HD associated with immunodeficiency. The cases developed in patients on methotrexate for rheumatoid arthritis, psoriasis, or polymyositis and all but two resolved when methotrexate was stopped.

Rituximab, a monoclonal antibody directed against the cell surface antigen CD20, is not used to treat classic HL. However, in the setting of umbilical cord blood transplantation, preemptive treatment with rituximab has been shown to control EBV-driven B cell proliferation resulting in decreased incidence of PTLD [18]. In a much more frequent scenario, rituximab can induce durable complete responses in EBV-driven PTLD [19].

This case illustrates how the principles learned from the management of PTLD can be successfully extrapolated to the treatment of the less common iatrogenic ILDs. It also demonstrates how clinical manifestation can potentially present in less common sites. Withdrawal of the immunosuppressant when possible and accurate histological diagnosis, are keystones of successful management without cytotoxic treatment.

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Conflict of interest: Nothing to report.
Published online 3 May 2010 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21753

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What do healthcare providers ask their patients with immune thrombocytopenia?

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Clinical signs suggestive of immune thrombocytopenia (ITP) include bruising, petechiae, nose bleeds, genitourinary (GU) bleeding, gum bleeding, gastrointestinal (GI) bleeding, and gynecologic bleeding [1]. Clinical experience and adverse events in clinical trials reveal other ITP-associated symptoms such as arthritis, abdominal pain, sleep disturbances, headache, and fatigue. Asking patients about such symptoms supplements the platelet count in assessing disease status and impact, and thus helps in the design of a patient-specific management plan. A short practice patterns survey was administered to healthcare providers attending ITP Continuing Medical Education (CME) activities to assess the questions they ask patients in monitoring visits. A high percentage of respondents routinely ask about signs of bleeding and associated symptoms but not as frequently about health-related quality of life (HRQOL). Only 39% of respondents ask about difficulty with sleep. A short standardized questionnaire may be a useful tool to help healthcare providers gather information about their patients with ITP.

Several HRQOL instruments have been used for ITP. McMillan et al. [2] used the SF-36 questionnaire [3] to compare the HRQOL of 73 patients with ITP with a matched control group. The HRQOL of patients with ITP was worse than the general US population and of patients with hypertension, arthritis, or cancer. It was similar to that of patients with diabetes, although better than that of patients with congestive heart failure (CHF) or a missing or paralyzed limb.

The ITP patient assessment questionnaire (ITP-PAQ) probes physical health, emotional health, overall QOL, social activity, women's reproductive health, and work issues [4]. Snyder et al. [5] used a web-based survey to compare the HRQOL of 1,002 patients with ITP with 1,031 controls. Except for bodily pain, all categories of the SF-36 questionnaire were worse for patients with ITP. The large patient base allowed comparison of the platelet count with ITP-PAQ items. Linear regression analysis showed that for 8 of 10 categories, there was a significant correlation between platelet count and scores. Only overall QOL and women's reproductive health did not correlate. The ITP-PAQ scale was used to evaluate patients in a clinical trial of a thrombopoietin agonist [6]. Patients receiving active treatment had significantly higher mean change scores than those receiving placebo in 7 of 10 categories.

A different tailored questionnaire was used to evaluate QOL in patients with ITP who received corticosteroid treatment [7]. The most frequent symptoms in treatment and control groups were fatigue, dry skin, sleep difficulties, and bruises. Of these, sleep difficulties and fatigue caused the most distress for patients. Because of the increasing interest in quantitative QOL in clinical research, we undertook a small study to evaluate physician practices in assessing patients with ITP.

Two hundred fifty-six participants in ITP CME activities answered questionnaires (Appendix 1). The participants practice in California ($n = 56$), New York ($n = 40$), Ohio ($n = 30$), and 30 other states ($n \leq 15$ each). Of the 245 participants who indicated the primary site of their practice, 27% were in academic hospitals, 23% were in community hospitals, 35% were in private practice, 6% were in clinics, and 9% indicated "other." The gender and age of participants were not collected.

Of 245 respondents, 18% are adult hematologists, 16% are adult oncologists, 6% are pediatric hematologists, and 4% are pediatric oncologists. 55% answered "other" but most of these did not specify their specialty. Forty-five participants identified themselves as adult hematologists and 40

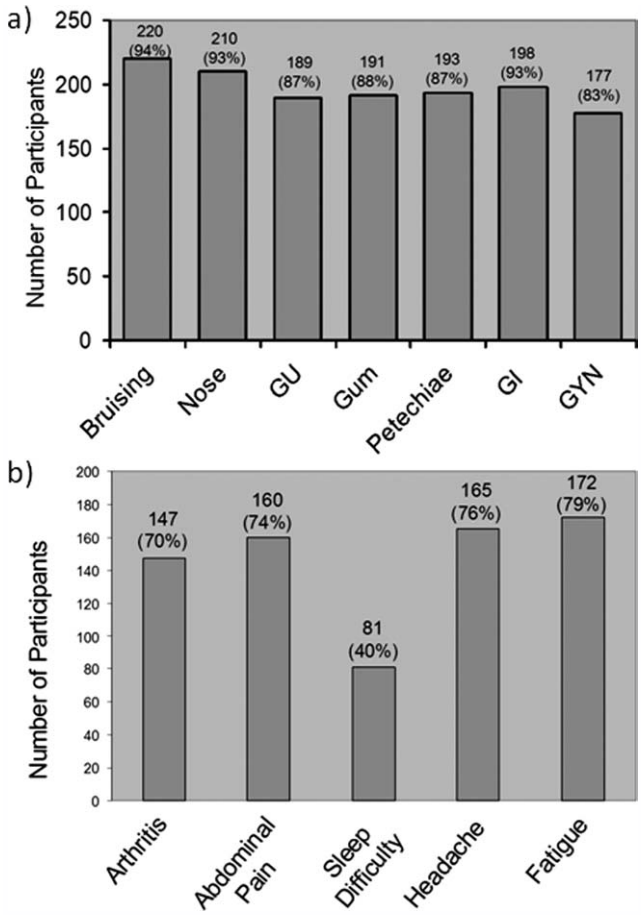

Figure 1. Numbers of respondents who asked their patients about (a) bleeding symptoms or (b) specific ITP-associated symptoms. GU, genitourinary; GI, gastrointestinal; GYN, gynecologic.

as adult oncologists. Almost half the participants were in practice for more than 20 years.

A quarter of respondents saw more than 10 ITP patients a year. The number of ITP patients seen per year was not correlated with the length of time in practice (Pearson $r^2 = 0.00015$). Most participants had low numbers of patients under active treatment (0–5 pts: 82%, 5–10 pts: 12%, 10–15 pts: 3%, and more than 15 pts: 3%). One hundred ninety-six participants reported seeing one or more patients with ITP per year and treating an average of 37% of them. Of 196 participants, 120 reported treating at least one patient with ITP per year.

A high proportion (89 ± 4%) of practitioners reported that they routinely ask patients about each of the bleeding-related symptom: bruises, nosebleeds, gum bleeding and mouth sores, petechiae, GU bleeding, GI bleeding, and gynecologic bleeding (Fig. 1a). The most common question asked was about bruises (94%) and the least common was about gynecologic bleeding (83%). Some participants asked a general question about bleeding signs and proceeded to other issues if the patient answered negatively. Of the 110 participants who answered the question "Do you ask if there is any bleeding and if the answer is no, go on?," 44% indicated that they do ask. At least 90% of the respondents asked about each of the bleeding signs, and all 84 who answered the question about bruising said they did ask about that sign.

Among the associated ITP symptoms (Fig. 1b), most participants reported that they ask patients about arthritis, abdominal pain, headache, and fatigue (70, 74, 76, and 79%, respectively). Only 40% of the 201 respondents reported asking patients about sleep difficulty. A total of 98% of the respondents did not address the open option of "other" in the associated symptoms question. Of the 256 participants, 196 (77%) asked women of childbearing age about pregnancy, while 19 (7%) did not. The remaining 16% did not answer the question. The subgroup of adult hematology and adult oncology participants had similar rates of positive responses as the larger group; their rates were within 6 percentage points of the larger participant pool. Only 34% of the 77 adult hematologist and adult oncologist respondents asked about sleep difficulty.

Most respondents (81 ± 8%) asked their patients about medications. The most often discussed was prednisone (93%) and the least often discussed was thyroid replacement therapy (70%). The number of participants answering the survey in this category was consistent across the various answers (214 ± 4 of the total 256, 84%). Of the 210 participants who answered the question "Do you ask about alternative medications," 78% responded in the affirmative. Participants who identified themselves as adult hematologists or adult oncologists had similar rates of inquiry on medications as the total group of respondents.

The small questionnaire used in this study was designed to probe physician behavior in managing follow-up visits with patients with ITP. The questions cover the common bleeding manifestations of thrombocytopenia, but also associated symptoms such as headache and fatigue, and those associated with medication. The questions addressed symptoms observed as adverse events in 20% or more of the control patients in a 24-week clinical trial [8]. Insomnia, gingival bleeding, and abdominal pain were observed in this cohort at lower rates but were also probed in the current survey.

Most of the questions were asked by 80–90% of the healthcare provider respondents with a few notable exceptions. Although thyroid disease may impact treatment outcome [9], thyroid replacement therapy was probed by only 70% of the respondents. Perhaps this subject was felt to be the purview of other healthcare providers or perhaps the respondents already possessed the relevant information.

An area that was addressed with patients by a minority of respondents (40%) was "difficulty sleeping at night." Insomnia was reported in 7% of the ITP patients in the control group of a phase 3 romiplostim trial [8]. It also emerged as one of the most common symptoms uncovered in a recent study [7] and can occur as a side effect of corticosteroid therapy [7,10]. The physiologic relationship between ITP and insomnia is not clear, although sleep quality and quantity certainly contribute to QOL. Since patients with ITP suffer a diminished QOL and our survey reveals uneven exploration of the components of QOL by healthcare practitioners, a short standardized QOL instrument should be incorporated into routine monitoring of patients with ITP.

Methods

During 2008–2009, The France Foundation sponsored the ITP Community Case Exchange series of CME activities in the United States. These were designed for hematologists and oncologists looking to obtain practical information about adult ITP disease mechanisms, diagnosis, and management. A brief practice patterns survey (available on line) was administered after the activity. In 2008, the survey was conducted at small dinner meeting CME activities with an average attendance of 6.7 participants, but in 2009, the survey was distributed at various meetings such as regional conferences and grand rounds with a typical attendance of 20–50 individuals. Some participants did not answer all the questions; individual questions without responses were excluded from the analysis.

Acknowledgments

The authors thank Elizabeth Facchini, MBA, and Thomas Medlin for assembling the survey data.

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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 23 May 2010 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21764

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Testicular lymphoma, intraocular (vitreoretinal) lymphoma, and brain lymphoma: Involvement of three immunoprivileged sites in one patient

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The brain, the testicles, and parts of the eye, including, inter alia, the vitreous, and the retina, are immune-privileged organs with relatively sealed blood-tissue barriers. These three organs may, rarely, develop aggressive primary B-cell lymphoma. Vitreoretinal lymphoma is commonly associated with primary central nervous system lymphoma (PCNSL) and with testicular lymphoma, which has a high incidence of relapse, one of the common sites of relapse is the brain. The association of testicular lymphoma with vitreoretinal lymphoma is extremely rare. In these three organs, in treating the lymphoma, the blood-tissue barrier must be overcome. We present a unique case of a patient with testicular lymphoma who 3 years later was diagnosed with monocular vitreoretinal lymphoma, and while being treated for the ocular lymphoma developed PCNSL. The lymphomas in the three organs were treated successfully: the testicular lymphoma by chemotherapy, rituximab, and radiation therapy, with intrathecal methotrexate (MTX) as a preventative measure; the vitreoretinal lymphoma by intravitreal injections of MTX alone; and the PCNSL by intravenous high-dose MTX, rituximab, dexamethasone, procarbazine, and intrathecal MTX. Ten months after completion of the last treatment, there were no signs of systemic, CNS or ocular recurrence.

A 51-yr-old man presented to the Department of Hematology at the Rambam Health Care Campus (Haifa, Israel) with a testicular mass in February 2005. Following orchiectomy, a diagnosis of testicular B-lineage diffuse large-cell lymphoma was made. Immunophenotypic characterization of the testicular lymphoma revealed this to be CD20+ with a proliferative index of 90%. Physical examination and laboratory tests, including lactic dehydrogenase, were otherwise unremarkable. Complete systemic staging, including FDG-18-PET/CT, lumbar puncture and bone marrow examination, were all negative.

After orchiectomy, the patient was treated with six cycles of rituximab, cyclophosphamide, adriamycin, vincristine, and prednisone (R-CHOP). With each cycle, he received intrathecal methotraxate. After the chemotherapy, he received local radiation therapy of 3000 cGy to the contralateral testis and remained in complete remission for 3 years.

Because of a complaint of blurred vision in his right eye, he was examined in June 2008 by an ophthalmologist at the Hadassah-Hebrew University Medical Center in Jerusalem, Israel. An elevated white-yellow subretinal lesion was found in the superiotemporal quadrant of the fundus of his right eye (Fig. 1). Only a few cells were seen in the vitreous body. At that time, the visual acuity in his right eye was 1.0 (6/6) and in his left amblyopic eye 0.1 (6/60). Because of the suspicion of vitreoretinal lymphoma, the patient was referred to Hadassah's ocular oncology service for diagnosis and treatment. He underwent diagnostic vitrectomy. The cytopathological examination revealed atypical B-cell lymphoma cells. The IL-10:IL-6 ratio was >1. Molecular evaluation did not demonstrate B-cell rearrangement. Within 2 days of the diagnostic vitrectomy, there was significant progression of the disease in the retina, and with the diagnosis of vitreoretinal lymphoma, he immediately started treatment with intravitreal injections of 400 μg/0.1 ml methotrexate (MTX).

The patient was treated according to the Hadassah protocol [1] with 25 injections of intravitreal MTX—during the induction phase, two weekly injections in the first month, in the consolidation phase, a weekly injection for 8 weeks, and in the maintenance phase a monthly injection for an additional 9 months. To prevent keratopathy, which is a common side effect of this therapy, the patient was treated with lubricants, using artificial tears and ointment. The subretinal lesion in the right eye shrank gradually, until its disappearance after 16 injections (Fig. 1). The entire course of 25 injections took almost a year; in the 9-month follow-up after the last injection, there were no signs of intraocular recurrence, and only a gray scar was seen at the site of the lymphoma.

During local treatment of the vitreoretinal lymphoma, the patient was also given intravenous high-dose MTX at the Rambam Medical Center to prevent systemic/central nervous system (CNS) recurrence at a dose of 3 g/m^2. However, he developed hepatitis and elevation of hepatocellular enzymes to twice the normal limit, and gamma glutamyl transferase to 5 times the upper normal limit. Moreover, after the 18th intravitreal injection and about 4 months from the beginning of the intravitreal MTX injections, the patient developed neurological symptoms. FDG-PET/CT and MRI of the brain revealed involvement of the right temporal lobe of the brain by lymphoma. Lumbar puncture showed lymphoma cells in the CSF. The patient was treated using the Memorial Sloan-Kettering Cancer Center protocol for primary CNS lymphoma [2] that included intravenous high-dose methotraxate, rituximab, dexamethasone, procarbazine, intrathecal MTX together with rituximab. PET/CT MRI and repeated CSF samples demonstrated complete remission. Because of changes in the brain tissue demonstrated on MRI, radiation therapy was omitted, and the patient received another two cycles of high-dose ARA-C according to the protocol. Currently, 8 months after completion of the chemotherapy, there are no signs of systemic or CNS recurrence.

Testicular lymphomas are rare tumors that account for ~5% of testicular neoplasms and are the most common testicular malignancies in men during the age of 60 years [3]. It comprises ~0.6% of all patients with non-Hodgkin lymphoma in the USA [4]. Testicular lymphoma is associated with a high incidence of relapses (52%) and up to 72% of the relapses were extranodal [5]. The CNS, both parenchymal brain tissue and leptomeningis, are common sites for such an involvement [5,6]. In most cases, testicular lymphoma is a B-cell non-Hodgkin lymphoma, although cases of testicular T-cell lymphoma have been reported as well [7].

Primary central nervous system lymphoma (PCNSL) is also a rare disease, mostly of the B-cell type that, like testicular lymphoma, affects mainly the elderly population [8]. It is an aggressive disease, with survival of a few months, if not treated.

Intraocular lymphoma may arise in different parts of the eye, expressing various clinical manifestations [9]. Vitreoretinal lymphoma, which is mostly a high-grade B-cell malignancy, is the most common and most aggressive one and predicts for a poor prognosis. It is usually associated with PCNSL and occurs in ~15 to 25% of the patients with PCNSL [8,10]. On the other hand, over 50% of vitreoretinal lymphomas appear in association with PCNSL [11,12] and may be considered as a subset of PCNSL [10].

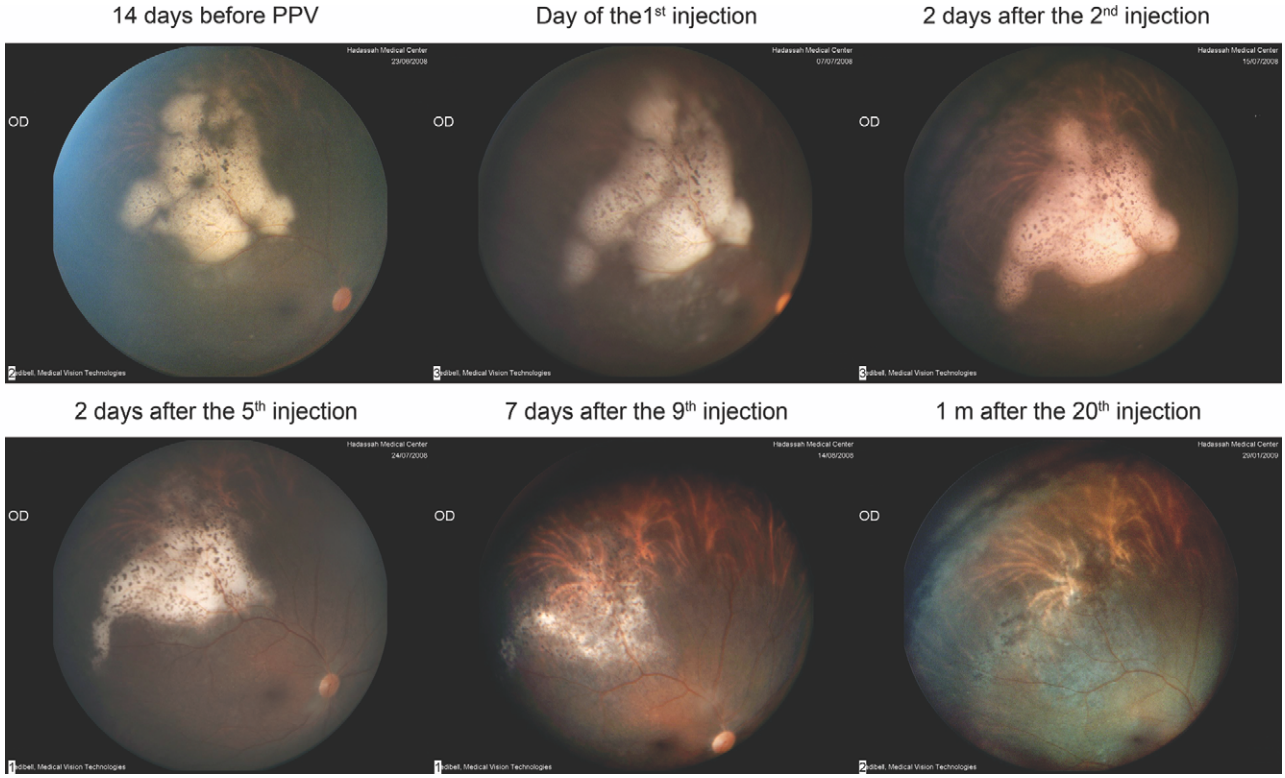

Figure 1. Fundus pictures of the patient. The upper left picture shows the retinal lymphoma infiltrate at the time of diagnosis, before the pars plana vitrectomy (PPV). The upper central picture shows further retinal involvement just before beginning the treatment with intravitreal methotraxate. The four remaining pictures show the response to treatment, until complete disappearance of the lymphoma in the retina. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I. Testicular Lymphoma Cohorts: Outcome and Incidence of CNS Involvement

Cohorts of patients with testicular lymphoma	Therapy	Progression (%)	Extranodal site of progression	Reference
27	Three cycles of adriamycin containing regimen + regional radiation	12 (44%)	Brain parenchyma, 4	Shenkier et al. [18]
16	Three cycles with augmented anthracycline therapy + IT[a] + brain radiotherapy	7y DFS, 70%; OS, 65%	Brain, 1; testicular, 1	Linassier et al. [19]
64	Radiation only, 11; chemotherapy, 15; chemo + radiotherapy, 12	4y DFS, 36%	23/40 (57%) relapses: extranodal, 19; brain, 4; ocular, 2	Ferry et al. [14]
373	Anthracycline based therapy (68%), IT (18%), RT[b] (36%)	5y OS, 48%; 10y OS, 27%; relapse at median f/u 7y, 52%	140/195 (72%) extranodal relapses: CNS, 56; brain, 30; meninges, 19	Zucca et al [3]

[a]IT, intrathecal therapy; [b]RT, radiation therapy.

The association of testicular and vitreoretinal lymphomas is extremely rare, and only seven cases have been reported to date at 2 months to 10 years from diagnosis [7,13–17]. We reported a case of a patient with testicular lymphoma who, after remission of 3 years, developed vitreoretinal lymphoma with subsequent brain lymphoma.

Testicular lymphoma was reported as an entity with inferior prognosis compared with other early-stage lymphomas (Table I). It is treated with combination therapy and local radiotherapy [18]. In the cohort of patients treated in British Columbia during 1980–1998, there were 27 patients with testicular lymphoma and 12 of them had disease progression after brief chemotherapy and radiotherapy to the contralateral testis. Four of these relapses were to the brain parenchyma. The International Extranodal Lymphoma Group reported 10-year progression-free survival (PFS) rate of only 33%, and continuous relapses occurring up to 10 years from diagnosis in 15% of patients [5]. However, it should be noted that in this study, only 20% of 373 patients received prophylactic intrathecal therapy and only 8% were given high-dose MTX as part of their therapy. Because of the relatively frequent relapse of testicular lymphoma in the CNS [14] in patients treated with orchiectomy and local radiation, intrathecal prophylaxis was added to various treatment protocols. However, there are debates about the effectiveness of prophylactic intrathecal chemotherapy to the brain, because in the majority of cases, CNS recurrences involve the brain parenchyma [6]. Several study groups added prophylactic cranial irradiation and in this cohort of patients CNS relapse was reduced [19]. Although addition of rituximab, an anti-CD20 monoclonal antibody, improved the PFS of elderly patients with aggressive CD20 positive lymphoma, such an effect was not observed in patients with testicular lymphoma [4]. The brain, testicles, and parts of the eye including, inter alia, the vitreous humor, and the retina, are immune privileged or sanctuary organs with strong blood-tissue barriers: the blood-brain barrier, the blood-retinal barrier, and the blood-tubular barrier [20,21]. These barriers impair drug entrance and reduce effectiveness of monoclonal antibodies. An altered immune response allows cells, including certain malignant cells expressing nonself antigen, to escape destruction by the immune system [20–22]. The uniqueness of these three organs may partially explain the development of primary B-cell lymphoma in multiple immunoprivileged sites at different times or parallel to each other and may hint at specific features of this type of lymphoma. Batchelor et al. [23] showed that measurement of intravitreous levels of MTX after the administration of 8 g/m^2 of MTX demon-

strated a therapeutic dose at 4 hours after end of therapy; however, in their study, only four of nine patients had a sustained response. However, such high dose may not be tolerated by elderly patients. A therapeutic dose of >1 μM was obtained for 48 to 72 hours following intravitreal MTX [24].

The use of high dose of MTX (3 g/m^2) should be evaluated in a randomized controlled study even for patients with Stage I testicular lymphoma. A high index of suspicion for intraocular lymphoma (IOL) must be maintained because early visual symptoms are not specific. Because eye examination is abnormal in $>$90% of patients with IOL revealing vitreous cells and retinal or subretinal infiltrate, it should be added to routine follow-up of testicular lymphoma patients.

In summary, patients with testicular lymphoma are at high risk for extranodal disease progression, especially for CNS involvement. Any visual disturbance or uveitis should raise the suspicion of IOL. Routine eye examination should be considered as part of the follow-up because of reported IOL up to 10 years from the diagnosis of testicular lymphoma.

Methods

This is an observational case study. The patient has been followed routinely for the three sites of involvement, including imaging studies. The data on the patient were collected from the patient's files in the Department of Hematology and Bone Marrow Transplantation of the Rambam Medical Center in Haifa, Israel, and at the Department of Ophthalmology of the Hadassah-Hebrew University Medical Center in Jerusalem, Israel.

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Conflict of interest: Nothing to report
Published online 23 May 2010 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21767

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