Association of anemia and cognitive dysfunction in patients with acute myelogenous leukemia and myelodysplastic syndrome

Susan M. Wood,[1]* Christina A. Meyers,[1] Stefan Faderl,[2] Hagop M. Kantarjian,[2] Sherry A. Pierce,[2] and Guillermo Garcia-Manero[2]

Anemia is a symptom associated with cognitive dysfunction and is diagnosed if the hemoglobin level of a blood sample is too low. The clinical impact of chronically low hemoglobin level may be insufficient brain oxygenation, which may result in a decline in cognitive functioning. Previous studies have provided evidence of decrements in cognitive functioning associated with anemia across various disease processes, but few have investigated the association between cognitive dysfunction and hemoglobin level in patients with acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS). As this population is inherently anemic, studying these patients allowed for an exploration of cognitive changes at mild, moderate, and severe levels of anemia. This investigation explored cutoff points for hemoglobin at which cognitive decline may occur. Findings showed decrements in cognitive functioning occurring at hemoglobin levels of 10 g/dL or below. Performance on measures of word retrieval, attention, and fine motor function was most affected which suggests fronto-temporal lobe dysfunction. Results provided evidence as to a hemoglobin cutoff point below which cognitive function may be affected in patients with AML and MDS. This cutoff value may provide a clinical marker at which cognitive testing and therapeutic interventions could be utilized to improve patients' cognitive function, level of fatigue and overall quality of life.

Anemia is a symptom associated with cognitive dysfunction and reported fatigue across numerous cancers, and as a side effect of various cancer treatments. Other studies have investigated the association between cognitive dysfunction and biomarkers of anemia in various types of disease processes and as associated with chemotherapy treatment, but few have explored the association in untreated patients with AML and MDS whose hemoglobin level often falls in the moderate to severe range.

Prior research has provided evidence of specific differences between cancer patients with hemoglobin values greater than 12 g/dL and patients with values less than or equal to 12 g/dL. Patients reported significantly less fatigue, fewer nonfatigue anemia symptoms, better physical well-being, better functional well-being, and higher general quality of life at higher levels [1]. Jacobsen et al. [2] examined the relationship between changes in hemoglobin levels and changes in reported fatigue and cognitive functioning prior to and after chemotherapy treatment in patients with various types of cancer. They found that in only a smaller subset of patients whose hemoglobin levels dropped to less than or equal to 12 g/dL was cognitive impairment associated with decreased hemoglobin levels. The decline was limited to poorer performances on measures of attention, executive function, and visual memory. Other studies have proposed that difficulty concentrating and slowed processing speed may occur at a hemoglobin level lower than 8 g/dL [3,4]. These findings suggest that below normal hemoglobin levels may impact cognitive functioning.

The current cross-sectional study investigated the association between hemoglobin and cognitive dysfunction in a patient population that is inherently anemic, thus allowing for the examination of a range of lower biomarker levels and their relationship to cognitive impairment in patients with blood cancers. Some studies that have examined the relationship between hemoglobin level and cognitive dysfunction have explored hemoglobin as a continuous variable, which may not allow for elucidation of the true relationship [5]. Various cutoff points have been proposed in the previous literature as discussed above but have not been examined, per se. This study investigated the level at which hemoglobin must decline to contribute to cognitive dysfunction using cutoff values of 12, 10, or 8 g/dL, which were suggested in the previous literature. In particular, the cognitive domains of processing speed, fine motor dexterity, and executive function were hypothesized to be most affected. Another aim was to examine whether patients who were cognitively impaired (as defined by evidencing impaired performance on three or more cognitive measures) would fall within a particular hemoglobin group. As cognitive dysfunction in this population is not generally an imminent concern for physicians, a hemoglobin cutoff level may provide a clinical marker at which cognitive testing and therapeutic interventions may be utilized to improve patients' quality of life.

Our investigation was carried out with 88 adult patients with AML or MDS divided into three groups by hemoglobin level at time of testing. Demographic variables and clinical diagnosis were not significantly different between the groups. Overall, more participants in the study were male, had a diagnosis of MDS, and were predominantly Caucasian and right-handed. The demographic characteristics of the participants are reported in Table I and clinical characteristics are reported in Table II.

Group results of the patients' cognitive testing are provided in Table III. A one-way between-group multivariate analysis of covariance (MANCOVA) was performed to investigate how differences in hemoglobin level were related to performance on measures of cognitive functioning. There was a significant difference between hemoglobin groups and their performance on the collective cognitive measures ($P = 0.026$); however, when considering the cognitive measures separately, there were no significant differences. A discriminant function analysis revealed a subset of measures that was able to explain 69.2% of the variance between the groups ($P = 0.038$). These findings indicate that group differences as shown in the MANCOVA analysis can be explained in terms of one underlying dimension principally consisting of the contributions from the cognitive tests measuring word retrieval, fine motor function, and attention. The findings also revealed that the collective performance on these measures was able to discriminate the moderate and severe hemoglobin groups from the mild hemoglobin group.

Another aim of this study was to investigate not only whether blood groups differed statistically on particular cognitive characteristics, but also whether the degree of clinical cognitive impairment differed between the groups. A final set of analyses was conducted to judge whether not only statistically significant, but clinically meaningful, cognitive impairment was evident in the patient groups. Although there were no significant differences between the groups ($P = 0.746$), the findings revealed a percentage of cognitive impairment in each group (Mild Hg group = 25%, Moderate Hg group = 35%, and Severe Hg group= 33%).

When using hemoglobin level as the indicator of anemia severity, the results showed that anemia severity is associated with differences in cognitive performance. However, differences in cognitive function between the mild, moderate, and severe groups could not be explained in terms of separate performance on cognitive tests. Rather, the results of this study suggest that the group differences are better explained in terms of the combined contributions of the tests. The largest contributors to this combination were those tests which measured immediate and delayed word retrieval (Hopkins Verbal Learning Test–Revised), divided attention and working memory (Trails B and Digit Span), and fine motor dexterity (Grooved Pegboard). These findings are similar to those reported by Jacobsen and associates [2], in that, aspects of fronto-temporal lobe function [6,7] appear to be affected at hemoglobin levels below 12 g/dL.

Cognitive functioning in the moderate and severe hemoglobin groups was more affected than the functioning in the mild hemoglobin group. This finding suggests that a hemoglobin level of 10 g/dL or below (cutoff point for the moderate group) is associated with a change in cognitive functioning. These results help to elucidate the mixed findings in previous studies in which hemoglobin level was not grouped according to severity but investigated as a continuous variable to be compared with performance on cognitive tests. An interesting finding from this study was that only a subset of patients in each group evidenced significant cognitive impairment, per se. In the mild

TABLE I. Participants' Demographic Characteristics ($N = 88$)

Characteristic	No. of patients (%)	Range	Mean (SD)
Age (years)	88	21–84	61 (15)
Education (years)	88	8–19	14 (3)
Sex			
Male	51 (58%)		
Female	37 (42%)		
Race			
White	79 (90%)		
Non-white	9 (10%)		
Handedness			
Right	83 (94%)		
Left	5 (6%)		

TABLE II. Participants' Clinical Characteristics ($N = 88$)

Characteristic	No. of patients	Percentage
Diagnosis		
AML subtype	41	
RAEB-T	5	6
M0	1	1
M1	12	14
M2	12	14
M3	1	1
M4	6	7
M5	2	2
M7	1	1
Mixed lineage	1	1
MDS subtype	47	
RA	6	7
RA-RS	6	7
RCMD-RS	1	1
RAEB	28	32
IPSS High	9	10
IPSS I-2	15	17
IPSS I-1	12	14
IPSS Low	5	6
CMML	6	7
Cytogenetics		
Diploid	23	26
Del 5Q/-5	5	6
Del 7Q/-7	3	3
Both 5 + 7	30	34
CBF	2	2
t(15:17)	1	1
Other	21	24
Insuf. Metaphases	3	3

hemoglobin group, 25% of the patients showed significant cognitive impairment on at least three of the cognitive measures, while in the moderate and severe group a third or more of the patients showed cognitive impairment (33 and 35% respectively). Although these differences were not statistically significant between groups, this finding suggests that between one-fourth and one-third of patients with AML or MDS experience cognitive impairment when hemoglobin level falls below 12 g/dL. It is unclear as to what factors may contribute to this decline in only a subset of this population and further research may help elucidate these findings.

Although IPSS guidelines currently indicate that a hemoglobin level of less than 10 g/dL is indicative of cytopenia, the clinical guideline for the level at which physicians typically transfuse is 8 g/dL [8]. Our finding of the level at which low hemoglobin may affect cognitive functioning may facilitate an improvement in patient care by providing physicians with a higher biologic marker at which intervention strategies for supportive care may be implemented. An earlier intervention for anemia in these patients may improve cognitive function, level of fatigue and overall quality of life. In addition, more cognitive screening may be warranted as part of a current trend involving implementation of more aggressive treatments for this patient population.

Methods

Participants. Eighty-eight outpatient participants were recruited in conjunction with a protocol approved by the institutional review board at the University of Texas M.D. Anderson Cancer Center in Houston, Texas. To be eligible to participate, patients had to be: (1) at least 18 years of age, (2) diagnosed with either AML or MDS, and (3) fluent in spoken and written English. Also they (4) may not have received previous or current chemotherapy treatment, nor (5) have a history of psychiatric illness or head injury with an alteration of consciousness. They must (6) have completed at least 8 years of formal education, and (7) be able to give written informed consent before study entry. Patients who were in contact isolation due to fever or infection were not offered participation in this study. Demographic information was obtained through patient self-report during a brief clinical interview at the time of testing.

TABLE 3. Mean Standardized *z*-Scores on Each Cognitive Measure for Each Level of Hemoglobin Group ($N = 88$)

	Hemoglobin		
Cognitive tests	Mild, M ($n = 16$)	Moderate, M ($n = 51$)	Severe, M ($n = 21$)
Digit span	−0.44	−0.25	−0.24
Digit symbol	−0.04	−0.01	0.38
Trails A	−0.04	−0.62	−0.90
Trails B	−0.96	−0.69	−0.86
COWA	−0.05	−0.52	−0.79
Grooved pegboard			
Dominant hand	−1.19	−1.01	−1.46
Nondominant hand	−0.79	−1.18	−1.46
HVLT-R recall	−0.72	−1.27	−1.13
HVLT-R delayed	−0.52	−1.19	−1.13
HVLT-R discrimination	−0.19	−0.23	0.03

Materials. Patients were evaluated using a battery of neuropsychological tests that were selected based on their sensitivity and suitability for use as a brief assessment. These measures were also chosen for their ability to measure cognitive functions often affected by anemia, including mental processing speed, attention, executive function, verbal memory consolidation, and fine motor speed and dexterity.

The tests administered were (1) Digit Span subtest from either the Wechsler Adult Intelligence Scale-Revised (WAIS-R) or the Wechsler Adult Intelligence Scale-III (WAIS-III) [9] to assess auditory attention span and working memory; (2) Digit Symbol Coding subtest from either the WAIS-R or the WAIS-III as a measure of psychomotor processing speed and sustained attention; (3) HVLT-R to assess verbal learning and memory, including immediate recall, delayed recall, and recognition memory [10]; (4) Trail Making Test Part A to assesses visual scanning ability, simple attention and psychomotor processing speed; (5) Trail Making Test Part B to assess divided attention/executive function [11]; (6) Controlled Oral Word Association Test to assess phonemic verbal fluency [12]; and (7) fine motor speed and dexterity were assessed using the Grooved Pegboard task [13]. Medical records were accessed in order to obtain laboratory values within one day of time of testing for hemoglobin level, as well as information regarding patient diagnosis, type of treatment received, and transfusion dates.

Procedure. A MANCOVA was selected as the statistical method for comparing differences between hemoglobin level and test performance. Participants were divided into a severe group with hemoglobin level less than or equal to 8 g/dL ($n = 21$); a moderate group with levels between 8.1 and 10 g/dL ($n = 51$); and a mild group with levels between 10.1 and 12.5 g/dL ($n = 16$).

Statistical Analyses. Baseline data were collected on 88 participants who met study criteria. The alpha level used for significance was $P \leq 0.05$. A preliminary analyses of variance (ANOVA) was conducted to compare the mean differences for hemoglobin level between groups of participants to ensure that the cutoff points chosen on the basis of the literature produced groups with significantly different levels of hemoglobin and that the sample sizes were reasonably large. In addition, an analysis was conducted to assess whether there was a significant difference between the groups in diagnosis type, race, sex, age, and education using separate ANOVA and Pearson chi-square procedures.

A MANCOVA was conducted using hemoglobin level as the independent variable on the three groups: mild, moderate, and severe. The dependent variables were the standard scores on the administered cognitive measures. Covariates included in this analysis were age and education level, as these variables have been shown to be related to cognitive performance and may not have been completely controlled as different norms were used for different tests. Significant effects were followed by univariate ANOVA tests to further elucidate the findings, and other post-hoc procedures were used as deemed appropriate.

A final analysis was performed to determine if there was a difference between the groups in the number of patients who were cognitive impaired. Impairment was defined as a z-score ≤ 1.50 on three or more of the cognitive measures. A Pearson chi-square analysis was performed on the frequencies of impaired versus nonimpaired patients between hemoglobin groups to assess differences.

[1]Department of Neuro-Oncology, University of Texas M.D. Anderson Cancer Center, Houston, Texas; [2]Department of Leukemia, University of Texas M.D. Anderson, Cancer Center, Houston, Texas
*Correspondence to: Susan M. Wood, Department of Neuro-Oncology, UT MD Anderson Cancer Center, Unit 431, 1515 Holcombe Blvd., Houston, TX 77030
E-mail: SWood2@mdanderson.org
Conflict of interest: Nothing to report.
Published online 25 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22151

References

1. Cella D. The Functional Assessment of Cancer Therapy-anemia (FACT-An) scale: A new tool for the assessment of outcomes in cancer anemia and fatigue. Semin Hematol 1997;34:9–13.
2. Jacobsen PB, Garland LL, Booth-Jones M, et al. Relationship of hemoglobin levels to fatigue and cognitive functioning among cancer patients receiving chemotherapy. J Pain Symptom Manage 2004;28:7–18.
3. Brown K, Esper P, Kelleher L, O'Neill J, Polovich M, White J. Chemotherapy and biotherapy guidelines and recommendations for practice. Pitssburgh, PA: Oncology Nursing Society, 2001.
4. Brown WS, Marsh JT, Wolcott D, et al. Cognitive function, mood and P3 latency: Effects of the amelioration of anemia in dialysis patients. Neuropsychologia 1991;29:35–45.
5. Meyers CA, Albitar M, Estey E. Cognitive impairment, fatigue, and cytokine levels in patients with acute myelogenous leukemia or myelodysplastic syndrome. Cancer 2005;104:788–793.
6. Cabeza R, Nyberg L. Imaging cognition II: An empirical review of 275 PET and fMRI studies. J Cogn Neurosci 2000;12:1–47.
7. Segalowitz SJ, Unsal A, Dywan J. CNV evidence for the distinctiveness of frontal and posterior enuroal processes in a traumatic brain-injured population. J Clin Exp Neuropsychol 1992;14:545–565.
8. Hast R, Hellstrom E. Therapeutic aspects of myelodysplastic syndromes in chronic phase. Leuk Red 1992;16:95–100.
9. Wechsler D. Wechsler Adult Intelligence Scale,3rd ed. San Antonio: The Psychological Corporation, 1997.
10. Benedict RHB, Schretlen D, Groninger L, Brandt J. Hopkins verbal learning test-revised: Normative data and analysis of inter-from and test-retest reliability. Clin Neuropsychol 1998;12:45–55.
11. Lezak MD, Howieson D, Loring D. Neuropsychological Assessment,4th ed. New York:Oxford University Press, 2004.
12. Benton AL, Hamsher KD. Multilingual Aphasia Examination. Iowa City:AJA Associates, 1989.
13. Heaton RK, Grant I, Matthews CG. Comprehensive norms for an expanded Halstead-Reitan Battery: Demographic Correction, Research Findings, and Clinical Applications. Odessa, FL:Psychological Assessment Resources, 1991.

Non-Hodgkin lymphoma of the prostate

John Steuter,[1] Dennis D. Weisenburger,[2] R. Greg Bociek,[1] Philip Bierman,[1] Julie Vose,[1] Martin Bast,[1] Fausto Loberiza,[1] and James O. Armitage[1]*

Lymphoma presenting in the prostate is rare. We report 10 cases of primary non-Hodgkin lymphoma of the prostate from the Nebraska Lymphoma Study Group. The median age of the patients at time of diagnosis was 68.5 years, with presenting symptoms of urinary obstruction and/or dysuria. Six different types of B-cell lymphoma were identified, including four cases of diffuse large B-cell lymphoma, three cases of extranodal marginal zone lymphoma, and one case each of follicular, mantle cell, and small lymphocytic lymphoma. At the time of presentation only one case had an elevated serum lactate dehydrogenase (LDH) level, and two cases were stage 1, one was stage 2, and seven were stage 4. Three patients expired secondary to prostatic lymphoma, four patients expired due to other causes, and three are still alive with no relapse. The prognosis was poor for patients with systemic symptoms at the time of diagnosis, but survival free from relapse lymphoma was possible. When compared to prior reviews, a higher proportion of patients were treated with chemotherapy, which likely contributed to improved survival.

Lymphoma presenting in the prostate is unusual and accounts for ~0.1% of all newly diagnosed lymphomas [1]. Previous reports have found diffuse large B-cell lymphoma to be the most frequent subtype associated with presentation involving the prostate and suggested that these patients have a poor outlook [2,3]. We report 10 cases of non-Hodgkin lymphoma presenting in the prostate including patient and tumor characteristics at diagnosis and response to treatment.

The clinical and histological findings at time of diagnosis, treatment, and survival are presented in Table I. Ten patients ranged in age from 36 to 84 years of age at the time of diagnosis with a median of 68.5 years. Presenting symptoms were lower urinary tract obstruction, dysuria, and urinary retention. The Karnofsky performance score at the time of diagnosis was 90 in five patients, 80 in four patients, and 70 in one patient.

Only one individual, a patient with diffuse large B-cell lymphoma, had an elevated serum LDH. Three of the 10 patients had systemic symptoms of fevers, chills, night sweats, or weight loss. All of these expired secondary to their lymphoma.

Two patients were stage 1, one was stage 2, and seven had stage 4 disease. Extraprostatic sites included bone marrow in two patients, bladder in two patients, peripheral blood in one patient, and liver in one patient.

Six of 10 patients achieved complete remission lasting an average of 76 months (range 3–146 months). One of these eventually relapsed and expired from their lymphoma. A total of three patients died secondary to prostatic lymphoma and they had a mean survival of 22 months (range 3–60 months). Four patients died from other causes (survival of 103–146 months), and three are still alive (range 3–157 months). Lymphoma-specific survival was 77% at 1 year and 66% at 5 years (Figure 1).

Of the four patients with diffuse large B-cell lymphoma, three had an international prognostic index (IPI) score of 1 and the fourth had a score of 5. The three patients with an IPI score of 1 had an overall survival of 147, 157, and 138 months, respectively, and none of the three expired secondary to their lymphoma. The patient with an IPI score of 5 expired three months after diagnosis. None of the patients with extranodal marginal zone mucosa associated lymphoid tissue (MALT) lymphoma died from lymphoma and all achieved a durable complete remission with therapy. One died after more than 9 years of remission from an unrelated cause.

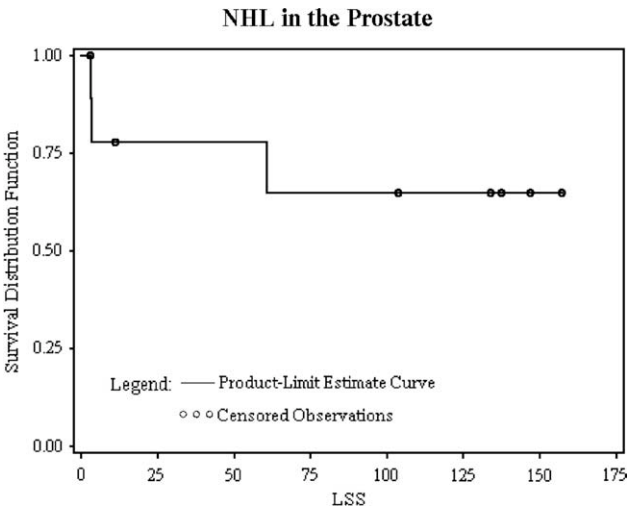

Figure 1. Lymphoma-specific survival.

TABLE I. Clinical Findings

Case	Years	B symptoms	Karnofsky score[a]	Histologic type	LDH	Extraprostatic sites	Stage	Treatment[b]	Response[c]	Lymphoma-specific survival (months)[e]	Overall survival (months)[d]	Current status[f]
1	72	A	80[a]	Diffuse large B-cell	Normal	None	1	CNOP	CR	147.0	147.0	Deceased, other
2	36	A	90[a]	Diffuse large B-cell	Normal	None	4	R-CHOP	PR	157.3	157.3	Alive
3	84	A	80[a]	Diffuse large B-cell	Normal	None	1	CNOP	CR	137.5	137.5	Deceased, other
4	82	B	70[a]	Diffuse large B-cell	High	Bladder	4	R-CHOP	PR	3.2	3.2	Deceased, lymphoma
5	56	A	80	Extranodal marginal zone	Normal	Marrow, peripheral blood	4	R-CHOP	CR	11.2	11.2	Alive
6	69	A	90	Extranodal marginal zone	Normal	Marrow, bladder	4	CHL+ P	CR	103.8	103.8	Deceased, other
7	68	A	90	Extranodal marginal zone	Normal	Liver	4	Rituximab	CR	3.0	3.0	Alive
8	80	A	90	Follicular (grade 2)	Normal	Marrow	4	CAPBOP	PR	134.0	134.0	Deceased, other
9	54	B	80	Mantle cell	Normal	None	2	Cladribine	CR	18.4	60.6	Deceased, lymphoma
10	64	B	90	Small lymphocytic	Normal	None	4	CAPBOP	ED	3.4	3.4	Deceased, lymphoma

[a]Karnosky's score of 80 or higher given a performance scale of 1, for a score of 70 assigned a performance scale or 2, to calculate the IPI score.
[b]Treatment: R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; CNOP: cyclophosphamide, mitoxantrone, vincristine, prednisone; R-CNOP: rituximab, cytoxan, mitoxantrone, vincristine, prednisone; CHL+P: chlorambucil and prednisone; CAPBOP: cyclophosphamide, doxorubicin, procarbazine, bleomycin, vincristine, prednisone.
[c]Response: CR, complete response; PR, partial response.
[d]Overall survival: expressed in months.
[e]Lymphoma survival: number of months from time of diagnosis to relapse of disease or death.
[f]Patient current status: other implies died secondary to reasons not related to lymphoma or treatment of the lymphoma.

TABLE II. Reports with at Least Three Cases of Patients with Primary Non-Hodgkin Lymphoma of the Prostate

Reference	Number of cases	Median age (Range)	Type of NHL	Therapy	Relapse	Overall survival
[3]	22	66 (32–89)	DLBC-12 FL-4 SLL/CLL-4 PTCL-2	NA	NA	24 months median
[4]	18	69 (59–78)	SLL/CLL-13 MZL-3 MCL-1	NA	NA	NA
[2]	7	59(32–78)	DLC/DM-3 DSC-2 CLL/CLL-1 Burkitt-1	Chemo-1 XRT-2 Chemo+XRT-1	NA	8 months median
[5]	5	75 (38–85)	DLBC-3 FL-1 SLL/CLL-1	–	–	1 month, 3 years, 19 years, (2 NA)
[1]	3	18 years, 38 years, 71 years	Burkitt-2 FL-1	Chemo-3	3 CR, No relapse	39 months, 79 months, 15 month
[6]	3	32 years, 41 years, 68 years	DSC-1 DLC/DM-2	Surgery-1 Chemo+XRT-2	NA	5 months, 7 months, 13 years
Present series	10	68.5 years (36–84)	DLBC-4 MZL-3 FL-1 MCL-1 SLL/CLL-1	Chemo-10	6 CR (1 relapse) 3- PR	118 months median

DLBC, diffuse large B-cell lymphoma; FL, follicular lymphoma; SLL/CLL, small lymphocytic lymphoma/chronic lymphocytic leukemia; MZL, marginal zone lymphoma; MCL, mantle cell lymphoma; DLC/DM, diffuse large cell lymphoma; DSC, diffuse small cleaved cell lymphoma.

Lymphoma primarily involving the prostate is a rare condition. However, while most patients presenting with symptoms referable to the prostate will have prostatitis, benign prostatic hypertrophy, or carcinoma of the prostate, a few will have lymphoma. The different therapies required for lymphoma and the excellent results with modern chemoimmunotherapy make the correct diagnosis imperative. Our patients, and those reported by Sarras [1], showed that with modern chemotherapy regimens most patients will achieve a remission and some will be cured.

Making the diagnosis of lymphoma of the prostate will require a high degree of suspicion and biopsy of patients with obstructive symptoms [3]. All the 10 patients in our series were diagnosed at the time of biopsy of the prostate, and the diagnosis of lymphoma was not expected in any case. As our series and those previously reported illustrate [1–16], (see Table II for details of series including at least three patients), a wide variety of histological subtypes can present with prostatic involvement. The most common tumor appears to be diffuse large B-cell lymphoma, but small lymphocytic lymphoma/chronic lymphocytic leukemia, follicular lymphoma, and extra nodal marginal zone lymphoma are all frequently reported. It may be that some of the cases previously described as small lymphocytic lymphoma/chronic lymphocytic leukemia would today be diagnosed as extranodal marginal zone lymphoma.

Table II presents the previously reported series of lymphoma presenting in the prostate that include at least three cases and contrast those to the present series. The median age of patients appears to be in the 60's. A few

young patients had high grade lymphomas (i.e., Burkitt lymphoma). Some older series utilized outdated histological terms, but translating those into the most likely current diagnoses do not suggest any difference in the frequency of the subtypes of lymphoma. It is clear that patients treated with effective chemotherapy regimens have a better outcome. Patients with highly responsive types of lymphoma, when treated with doxorubicin- and rituximab-containing chemotherapy regimen, can be cured.

In summary, non-Hodgkin lymphoma involving the prostate is a rare illness. However, diagnosis is important because treatment choice consistent with the specific histological diagnosis can be effective and lead to extended disease-free survival.

Methods

A review of the Nebraska Lymphoma Study Group database was performed to identify cases of primary lymphoma of the prostate gland. Human investigation committee approval and patient consents were obtained. The individual cases were reviewed by a hematopathologist to confirm the histological type. The clinical data was analyzed for age at presentation, stage of lymphoma, presence of systemic symptoms, serum LDH level, number and location of extranodal sites, treatment, initial response to treatment, overall survival, and lymphoma-specific survival. Staging included imaging with computed tomogram (CT) and/or positron emission tomography (PET) and bone marrow biopsy. Survival was estimated using the Kaplan–Meier method. Lymphoma-specific survival was calculated as the percentage of patients who did not expire from lymphoma or secondary to treatment effects.

[1]Department of Internal Medicine, University of Nebraska Medical Center, Omaha, Nebraska; [2]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska
**Correspondence to: J.O. Armitage, MD, Department of Internal Medicine, University of Nebraska Medical Center, 987680 Nebraska Medical Center, Omaha, NE 68198-7680.*
E-mail: joarmita@unmc.edu
Conflict of interest: Nothing to report.
Published online 21 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22150

References

1. Sarris A, Dimopoulos M, Pugh W, Cabanillas F. Primary lymphoma of the prostate: Good outcome with doxorubicin-based combination chemotherapy. J Urol 1995;153:1852–1854.
2. Bostwick DG, Mann RB. Malignant lymphomas involving the prostate. A study of 13 cases. Cancer 1985;56:2932–2938.
3. Bostwick DG, Iczkowski KA, Amin MB, Discigil G, Osborne B. Malignant lymphoma involving the prostate: Report of 62 cases. Cancer 1998;83: 732–738.
4. Chu PG, Huang Q, Weiss LM. Incidental and concurrent malignant lymphomas discovered at the time of prostatectomy and prostate biopsy: A study of 29 cases. Am J Surg Pathol 2005;29:693–699.
5. Schniederjan SD, Osunkoya AO. Lymphoid neoplasms of the urinary tract and male genital organs: A clinicopathological study of 40 cases. Mod Pathol 2009;22:1057–1065.
6. Patel DR, Gomez GA, Henderson ES, Gamarra M. Primary prostatic involvement in non-Hodgkin lymphoma. Urology 1988;32:96–98.
7. Bell CR, Napier MP, Morgan RJ, Dick R, Jarmulowicz M, Jones AL. Primary non-Hodgkin's lymphoma of the prostate gland: case report and review of the literature. Clin Oncol (R Coll Radiol) 1995;7:409–410.
8. Parks RW, Henry PG, Abram WP, Best BG. Primary non-Hodgkin's lymphoma of the prostate mimicking acute prostatitis. Br J Urol 1995;76:409.
9. Ghose A, Baxter-Smith DC, Eeles H, Udeshi U, Priestman TJ. Lymphoma of the prostate treated with radiotherapy. Clin Oncol (R Coll Radiol) 1995; 7:134.
10. Sarlis NJ, Knight RA, Sarlis I, Papadimitriou K, Kehayas P. Primary non-Hodgkin lymphoma of the prostate gland. Int Urol Nephrol 1993;25:163–168.
11. Braslis KG, Lee N, Machet D, Peters J. Primary prostatic lymphoma: A rare prostatic malignancy. Aust N Z J Surg 1994;64:58–59.
12. Suzuki H, Nakada T, Iijima Y, et al. Malignant lymphoma of the prostate. Report of a case. Urol Int 1991;47:172–175.
13. Fell P, O'Connor M, Smith JM. Primary lymphoma of prostate presenting as bladder outflow obstruction. Urology 1987;29:555–556.
14. Kerbl K, Pauer W. Primary non-Hodgkin lymphoma of prostate. Urology 1988; 32:347–349.
15. Morozumi M, Takasu H, Watanabe T, Okada M. Primary non-Hodgkin lymphoma of the prostate: A case report. Nippon Hinyokika Gakkai Zasshi 1993; 84:2023–2026.
16. Mounedji-Boudiaf L, Culine S, Devoldere G, et al. Primary, highly malignant B-cell lymphoma of the prostate. Apropos of a case and review of the literature. Bull Cancer 1994;81:334–337.

High incidence of vitamin D deficiency in patients undergoing allogeneic stem cell transplantation

Richard W. Joseph,[1]* Amin Alousi,[1] Bhavana Konda,[2] Krishna Komanduri,[3] Joyce Neumann,[1] Cindy Trevino,[1] Karen Stolar,[1] Muzaffar Qazilbash,[1] Chitra Hosing,[1] Partow Kebriaei,[1] Daniel R. Couriel,[1] Richard E. Champlin,[1] Rima Saliba,[1] and Uday Popat[1]

Patients undergoing allogeneic stem cell transplants (SCT) are at increased risk for vitamin D deficiency through prolonged hospitalizations and avoidance of sunlight as well as decreased nutritional status and malabsorption related to an allogeneic SCT. Vitamin D deficiency was shown to be as high as 39% in the pretransplant setting [1] and 90% in the postallogeneic transplant setting [2]. The purpose of this study was to confirm the high incidence of vitamin D deficiency in the pre and postallogeneic transplant setting, the demographic factors associated with vitamin D deficiency, and the correlation between vitamin D deficiency and osteopenia and osteoporosis in patients undergoing allogeneic stem cell transplantation. We found that 70% of patients were vitamin D deficient at day 0 of an allogeneic SCT, and 58% of patients were vitamin D deficient at post-transplant day 100. The incidence of osteopenia and osteoporosis in day 100 of the post-transplant setting was 83% and 22%, respectively, however there was no difference in incidence of osteopenia or osteoporosis in patients who were vitamin D deficient or nondeficient. Our study confirms the high incidence of vitamin D deficiency in patients undergoing allogeneic transplant patients.

Vitamin D3 (1,25-dihydroxycholecalciferol) is a steroid hormone that plays a central role in calcium and bone homeostasis as well as various immunologic and antiproliferative functions [3,4]. Vitamin D deficiency can lead to reduced bone density and pathological fractures [5] and is associated with increased risks of cancer and cardiovascular disease [6,7]. In addition, allogeneic SCT patients are at especially high risk of developing osteoporosis and fractures through increased exposure to glucocorticoids to prevent or treat graft versus host disease [8]. Patients undergoing allogeneic SCT are known to have an increased incidence of vitamin D deficiency [1,2]. To confirm the elevated incidence of vitamin D deficiency in patients undergoing allogeneic SCT, we reviewed the vitamin D status in both the pre and postallogeneic SCT setting.

Initially, we examined vitamin D levels in 72 consecutive patients at approximately day 100 postallogeneic SCT (Post-transplant cohort). We found that 42 (58%) patients were vitamin D deficient with a median vitamin D level of 17 ng/mL and a range of 6–51 ng/mL. The baseline demographics of these patients are outlined in Supporting Information (Table 1). The average age of patients was 48 years with a range of 19–71. The majority of patients was male (56%), white (81%), and had a myeloid malignancy (43%). When comparing vitamin D deficient and nondeficient cohorts, there were no statistically significant differences in age, gender, race, and disease status. After recognizing the high incidence of vitamin D deficiency in the post-transplant cohort, we then sought to determine the vitamin D status in patients before allogeneic SCT (pretransplant cohort), and we measured day 0 vitamin D levels in 240 consecutive patients undergoing

TABLE I. Demographic Date of Post-Transplant Cohort

CHARACTERISTIC	ALL PATIENTS *N* = 72	VITAMIN D DEFICIENT *N* = 42 (58%)	VITAMIN D SUFFICIENT *N* = 30 (41%)	*P VALUE*
Vitamin D ng/ml Median (range)	17 (6–51)	14 (6–19)	28 (20–51)	
Age mean (range)	48 (17–71)	49 (19–71)	45 (21–66)	0.38[a]
<50	34 (47)	18 (43)	16 (53)	
>50	38 (53)	24 (57)	14 (47)	
Gender				0.42[a]
Male	40 (56)	25 (60)	15 (50)	
Female	32 (44)	17 (40)	15 (50)	
Race				0.76[b]
White	58 (81)	33 (79)	25 (83)	
Non White	14 (19)	9 (21)	5 (17)	
African American	1 (1)	0 (0)	1 (7)	
Hispanic	10 (14)	8 (19)	2 (7)	
Other	3 (4)	1 (2)	2 (7)	
Disease Dx				0.33[a]
Lymphoid	30 (42)	16 (38)	14 (47)	
Myeloid	31 (43)	20 (48)	11 (37)	
Myeloproliferative	9 (13)	6 (14)	3 (10)	
Other	2 (3)	0 (0)	2 (7)	

[a]indicates *t*-test comparing Vitamin D deficient and sufficient groups.
[b]indicates *t*-test comparing White versus Nonwhite Combine.

TABLE II. Demographics of Pretransplant Cohort

CHARACTERISTIC	ALL PATIENTS *N* = 240	VITAMIN D DEFICIENT *N* = 168 (70%)	VITAMIN D NONDEFICIENT *N* = 72 (30%)	*P VALUE*
Vitamin D median (range)	16 (6–59)	3 (6–19)	25 (20–59)	
Age mean (range)	51 (10–77)	49 (10–76)	54 (18–72)	0.014[a]
<50	88 (37)	70 (42)	18 (25)	
≥50	152 (63)	98 (58)	54 (75)	
Gender				0.931[a]
Male	149 (62)	104 (62)	45 (63)	
Female	91 (38)	64 (38)	27 (38)	
Race				0.02[b]
White	192 (80)	127 (76)	65 (90)	
Nonwhite combined	48 (20)	41 (24)	7 (10)	
African American	15 (6)	13 (8)	2 (3))	
Hispanic	24 (10)	21 (13)	3 (4)	
Other	9 (4)	7 (4)	2 (3)	
Disease Dx				0.177[a]
Myeloid	129 (54)	96 (57)	33 (46)	
Lymphoid	82 (34)	56 (33)	26 (36)	
Myeloproliferative	25 (10)	14 (8)	11 (15)	
Other	4 (2)	2 (1)	2 (3)	

[a]Indicates *t*-test comparing Vitamin D deficient and sufficient groups.
[b]Indicates *t*-test comparing White versus Nonwhite combine.

allogeneic SCT. In the pretransplant cohort, 168 (70%) of the patients were vitamin D deficient, with a median vitamin D level of 16 ng/mL and a range of 6–59 ng/mL. The baseline demographics of the pretransplant cohort are listed in Supporting Information (Table 2). The average age of patients was 51 with a range of 10–77 years. The majority of patients (62%) was male, white (80%), and had a myeloid malignancy (74%). Patients under age 50 were more likely to be vitamin D deficient (80% versus 64%, $P = 0.01$). Nonwhite patients were also more likely to be vitamin D deficient (85% vs. 66%, $P = 0.02$). Gender or disease status did not correlate with vitamin D deficiency.

The high incidence of vitamin D deficiency in both the pre and postallogeneic SCT setting is similar to the incidence of vitamin D deficiency in the general population [9–11] (32–50%), in cancer patients (63–75%) [12], and prior studies involving SCT patients [1,2]. In our study, pretransplant patient had a higher incidence of vitamin D deficiency in comparison to post-transplant patients (70% vs. 58%), however since these were two separate populations we cannot conclude that post-transplants patients are less likely to be vitamin D deficient. There was no demographic variable that was associated with vitamin D deficiency in both the pre and post-transplant cohorts, so in conclusion, we recommend that all patients undergoing SCT should be tested both before and after allogeneic SCT.

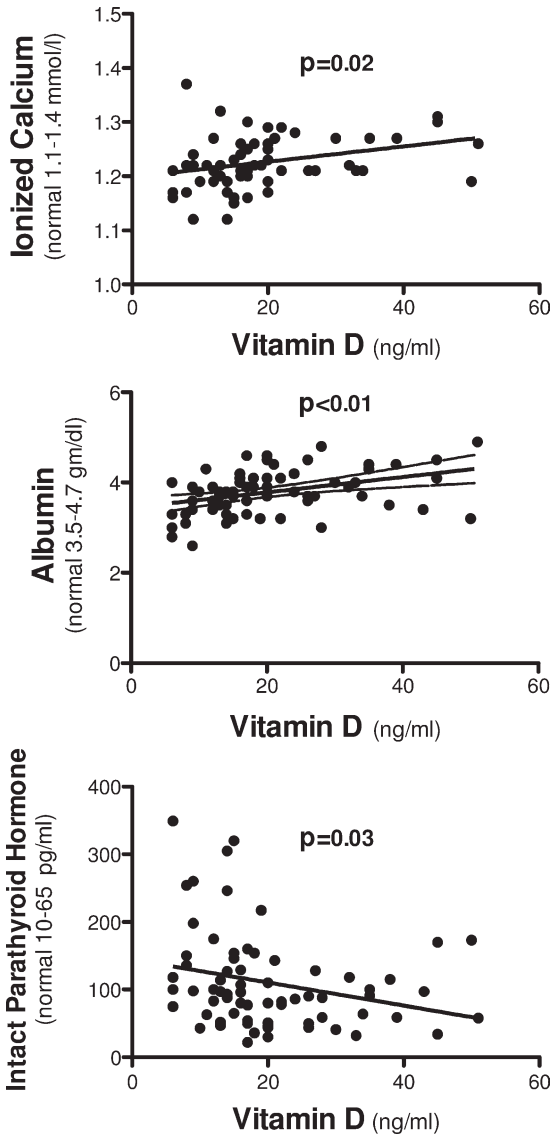

Figure 1. Correlations Between Vitamin D and ionized calcium, parathyroid hormone, and albumin in day +100 SCT patients. Vitamin D was drawn at day +100 after allogeneic stem cell transplant along with other laboratory values. We sought to determine if vitamin D correlated with other laboratory values in a linear fashion using a Pearson correlation coefficient. We found a significant direct relationship between vitamin D and ionized calcium and albumin. We found a significant inverse relationship vitamin D and parathyroid hormone.

To determine the impact of vitamin D deficiency on calcium homeostasis, we measured several laboratory correlates in the post-transplant cohort. Patients who were vitamin D deficient had a significantly lower mean albumin level (3.63 vs. 4.0 g/dL, $P = 0.001$), lower mean ionized calcium level (1.21 vs. 1.25 mmol/L, $P = 0.01$), higher intact parathyroid hormone (iPTH) levels (131 vs. 80 pg/mL, $P = 0.005$), and no statistically significant difference phosphate levels. The decrease in ionized calcium with a compensatory increase in serum PTH levels is an expected finding and further emphasizes that vitamin D deficiency in these patients is not an isolated event but a part of larger systemic process. When comparing vitamin D levels versus each laboratory correlate in a linear fashion, there was a positive correlation between vitamin D levels and albumin ($P < 0.001$) and ionized calcium ($P = 0.02$), and negative correlation between vitamin D levels and iPTH levels (P value = 0.03) (Supporting Information; Fig. 1). Interestingly, vitamin D deficiency also correlated with a lower albumin level, potentially signaling that vitamin D deficient patients are also malnourished. In the future we plan to longitudinally assess laboratory values in both the pre and post-transplant setting to determine whether these results hold true.

Post-transplant bone loss due to multiple clinical factors is a well-characterized complication for patients undergoing hematopoietic SCTs [13–15]. Day +100 bone density measurements were available for 46 (64%) of our post-transplant cohort. Of the 46 patients with bone density measurements, 38 (83%) had evidence of osteopenia and 10 (22%) had evidence of osteoporosis. The mean T-score for the right hip, left hip, and spine was −1.30. We did not find any significant differences in the rate of osteopenia or osteoporosis between vitamin D deficient and sufficient patients. This rate of osteopenia and osteoporosis is similar to other reports in patients undergoing allogeneic SCT [14,16]. While our population had a high incidence of osteopenia and osteoporosis, there was no difference in the rates of osteopenia or osteoporosis in vitamin D deficient and sufficient patients. The most likely explanation for the this finding is that other clinical factors including use of glucocorticoids, heparin, lack of exercise, chronic renal disease, gastrointestinal disease, and chemotherapy outweigh vitamin D's impact on bone loss. However, as vitamin D deficiency can be easily corrected at little cost or risk to the patient, we maintain that vitamin D levels should be checked and corrected when deficiency is present.

In conclusion, we confirmed that the incidence of vitamin D deficiency is very high in both the pre (70%) and post (58%) SCT setting, and that the vast majority of patients have decreased bone density (83%) irrespective of their vitamin D levels. Given the increasing epidemiologic and laboratory data implicating the vitamin D axis in inflammation, future studies should be undertaken to assess the impact of vitamin D deficiency on traditional clinical outcomes associated with deficiency (including bone density and fracture rates) as well as immunological endpoints (GVHD and infection incidence).

Methods

Study population and design. All patient and clinical data was collected under an Institutional Board Review protocol. From October 2005 to October 2006, we collected serum vitamin D levels at approximately day 100 in 72 allogeneic SCT patients treated at MD Anderson Cancer Center (post-transplant cohort). As a part of long term follow up care and study of bone health for patients undergoing allogeneic SCT transplants, we also studied the blood levels of calcium, phosphorous, ionized calcium, and bone density. After finding high incidence of vitamin D deficiency in this cohort of post-transplant allogeneic SCT patients, we studied a second cohort of pretransplant allogeneic SCT patients (Pretransplant cohort) to determine if this high incidence of vitamin D deficiency was present before transplantation or resulted from factors in the first 100 days of transplant including lack of sunlight and decreased nutritional intake and absorption. From September 2006 to September 2007, we measured serum vitamin D levels before initiation of conditioning chemotherapy in 240 consecutive patients undergoing allogeneic transplant. Demographic data were obtained from the departmental database. Patients were divided into groups based into four disease categories: lymphoid malignancies including non-Hodgkin lymphoma, Hodgkin lymphoma, and multiple myeloma; myeloid malignancies including acute myelogenous leukemia, myelodysplastic syndrome, and acute lymphoblastic leukemia; myeloproliferative disorders including essential thrombocytosis and CMML; and an "other" category including aplastic anemia, renal cell carcinoma, and breast cancer.

Defining vitamin D deficiency and osteopenia/osteoperosis. While there is no consensus on defining a sufficient level of vitamin D, most clinicians agree that a serum 25-hydoxy vitamin D (calcidiol) of <20 ng/mL as deficient [17,18]. Vitamin D levels were obtained using a CLIA certified assay. For the purpose of our study, we defined vitamin D deficiency as a calcidiol level of <20 ng/mL and Vitamin D nondeficient as a calcidiol level of ≥20 ng/mL. Osteopenia and osteoporosis were defined according NIH consensus [19]. Osteopenia was defined as having a T score < −1.0 standard deviation, and osteoporosis was defined as having T score < −2.5 standard deviations from normal individuals.

Statistical analysis. We analyzed vitamin D deficient and nondeficient patients with respect to basic demographic values such as age, gender, race, and disease status using a Pearson chi squared test or Fisher's exact test. To compare vitamin D levels with respect to lab values, we first compared laboratory values of vitamin D deficient and sufficient patients using an unpaired *t*-test for data that followed normal distribution. For data that did not follow normal distribution even after logarithmic transformation, the Wilcoxin rank-sum test (Mann-Whitney) was used. We also compared vitamin D levels with albumin, ionized calcium, and parathyroid hormone using a Pearson correlation coefficient.

Acknowledgments

The authors thank the patients, the nurses, and the data managers for making this study possible.

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

[1]Department of Stem Cell Transplant and Cellular Therapy MD Anderson Cancer Center, Houston, Texas; [2]Department of Internal Medicine, University of Miami Miller School of Medicine, Regional Campus, Atlantis, Florida; [3]Department of Medicine, Adult Stem Stem Cell Transplant Program University of Miami Sylvester Cancer Center, Miami, Florida
**Correspondence to: Richard W. Joseph, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224.*
E-mail: joseph.richard@mayo.edu

Conflict of interest: Nothing to report.
Published online 21 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22143

References

1. Massenkeil G, Fiene C, Rosen O, et al. Loss of bone mass and vitamin D deficiency after hematopoietic stem cell transplantation: Standard prophylactic measures fail to prevent osteoporosis. Leukemia 2001;15:1701–1705.
2. Sproat L, Bolwell B, Rybicki L, et al. Vitamin D level after allogeneic hematopoietic stem cell transplant. Biol Blood Marrow Transplant 2010;17:1079–1083.
3. Adorini L, Penna G. Control of autoimmune diseases by the vitamin D endocrine system. Nat Clin Pract Rheumatol 2008;4:404–412.
4. Giovannucci E, Liu Y, Rimm EB, et al. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. J Natl Cancer Inst 2006;98:451–459.
5. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266–281.
6. Giovannucci E, Liu Y, Hollis BW, et al. 25-hydroxyvitamin D and risk of myocardial infarction in men: A prospective study. Arch Intern Med 2008;168:1174–1180.
7. Kendrick J, Targher G, Smits G, et al. 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the Third National Health and Nutrition Examination Survey. Atherosclerosis 2009;205:255–260.
8. McClune BL, Polgreen LE, Burmeister LA, et al. Screening, prevention and management of osteoporosis and bone loss in adult and pediatric hematopoietic cell transplant recipients. Bone Marrow Transplant 2011;46:1–9.
9. Gordon CM, DePeter KC, Feldman HA, et al. Prevalence of vitamin D deficiency among healthy adolescents. Arch Pediatr Adolesc Med 2004;158:531–537.
10. Holick MF, Siris ES, Binkley N, et al. Prevalence of Vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. J Clin Endocrinol Metab 2005;90:3215–3224.
11. Hypponen E, Power C. Hypovitaminosis D in British adults at age 45 y: Nationwide cohort study of dietary and lifestyle predictors. Am J Clin Nutr 2007;85:860–868.
12. Everett PC. The prevalence of vitamin D deficiency and insufficiency in a hematology-oncology clinic. Clin J Oncol Nurs 2008;12:33–35.
13. Schulte CM, Beelen DW. Bone loss following hematopoietic stem cell transplantation: A long-term follow-up. Blood 2004;103:3635–3643.
14. Tauchmanova L, Colao A, Lombardi G, et al. Bone loss and its management in long-term survivors from allogeneic stem cell transplantation. J Clin Endocrinol Metab 2007;92:4536–4545.
15. Yao S, McCarthy PL, Dunford LM, et al. High prevalence of early-onset osteopenia/osteoporosis after allogeneic stem cell transplantation and improvement after bisphosphonate therapy. Bone Marrow Transplant 2008;41:393–398.
16. Savani BN, Donohue T, Kozanas E, et al. Increased risk of bone loss without fracture risk in long-term survivors after allogeneic stem cell transplantation. Biol Blood Marrow Transplant 2007;13:517–520.
17. Vieth R. What is the optimal vitamin D status for health? Prog Biophys Mol Biol 2006;92:26–32.
18. Dawson-Hughes B, Heaney RP, Holick MF, et al. Estimates of optimal vitamin D status. Osteoporos Int 2005;16:713–716.
19. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, March 7–29, 2000: Highlights of the conference. South Med J 2001;94:569–573.

Lenalidomide for aggressive B-cell lymphoma involving the central nervous system?

Maria Christina Cox,[1]* Giuseppe Mannino,[2] Luana Lionetto,[3] Virginia Naso,[1] Maurizio Simmaco,[3] and Maria Antonietta Aloe Spiriti[1]

Lymphomas arising or relapsing in the central nervous system (CNS) have a dismal prognosis [1]. Very few active drugs passing through the blood brain barrier are available. Lenalidomide is a novel and extremely active compound in relapsed diffuse-large-B-cell-lymphoma (DLBCL) and mantle cell lymphoma (MCL) [2,3]. The pleiotropic action of Lenalidomide needs to be further exploited in different clinical settings and in combination with other drugs [4,5]. Thus far, patients with aggressive lymphomas and CNS involvement have been excluded from clinical trials and there are no data about Lenalidomide penetration in the CNS. Very recently, it was reported that Lenalidomide induced remission in a case of DLBCL relapsed within the CNS [6]. Here, we refer on a case of blastoid MCL [7] relapsed within the orbit and the CNS. The patient failed chemotherapy but achieved remission on Lenalidomide therapy. Furthermore, the presence of the drug was ascertained in the blood and in the cerebrospinal fluid (CSF) using an LC-MS/MS system consisting of a quadrupole mass spectrometer.

In September 2010, a 74-year-old man came to our observation for the appearance of right exophthalmos and blurred vision. He had been successfully treated 2 years before for blastic MCL with upfront high-dose therapy followed by Z-Beam conditioning regimen and peripheral blood stem cell rescue [8]. Magnetic resonance imaging (MRI) of the brain and the orbits revealed pathological tissue in the right extra cone involving the lachrymal gland and the right superior rectus muscle. The lesion was contiguous to the right eyeball with a maximum anteroposterior diameter of 4 cm. Ophthalmologic assessment comprehensive of fluorine angiography showed a retinal pattern suggestive for lymphoma involvement. The biopsy of the lachrymal gland revealed relapse of blastoid MCL [9]. The complete restaging ruled out other localization but the right orbit and the CSF with 220/μL CD19+/CD5+ cells. He shortly started a 21-day cycle of high dose Ifosfamide, Dexamethasone and Rituximab and weekly intrathecal chemotherapy with Aracytin and Methotrexate. After three cycles of Ifosfamide treatment, chemotherapy was withdrawn, since MRI showed no improvement while the patient complained deterioration of the right eye vision. At this stage, no lymphoma cells were found in the CSF. In December 2010, as a compassionate treatment, he was given Lenalidomide 25 mg/day for a 21-day cycle, combined with 20 mg of weekly Dexamethasone. At the end of the first cycle, he had an impressive recover of the exophthalmos and of the visual acuity of the right eye. Lenalidomide concentration, in the blood and in the CSF was determined using an LC-MS/MS system consisting of a quadrupole mass spectrometer (See Supporting Information in the article). In May 2011, after five cycles of therapy the MRI and the orbit ultrasound revealed a greater than 50% reduction of the lesion and the CT-scan ruled out progression in other sites while the CSF flow cytometry is persistently negative. Presently, he is well in stable partial remission. Aggressive lymphomas with intraocular [10] or orbital [11] localization might be difficult to treat because of the potential damage of chemotherapy and/or radiotherapy to the functional integrity of the eye and the risk, mainly for intraocular lymphoma, of spreading within the brain and the leptomeninges. The case we describe was localized within the ocular adnexa and the CNS since the CSF was contaminated by lymphoma cells [1]. We suppose that the spreading of lymphoma cells in the CSF originated from the orbit due to the direct relation of the orbit with the CSF that flows along the optic nerve. To our knowledge, this is the first report proving that Lenalidomide is detected in the CSF and that it is effective in relapsed blastoid MCL involving the orbit. Currently, the outcome of CNS lymphoma is unsatisfactory, and the search for more effective and less toxic regimes is a big challenge [12] Although the direct or immunomediated and antiangiogenetic modality of action of Lenalidomide on tumor cells is still under debate, the detection of the drug in the CSF further support the experimentation of Lenalidomide in patients with aggressive B-cell CNS lymphomas.

[1]Department of Hematology, A.O. Sant'Andrea, University La Sapienza, Rome, Italy; [2]Department of Ophthalmology, A.O. Sant'Andrea, University La Sapienza, Rome, Italy; [3]Department of Molecular Biology A.O. Sant'Andrea, University La Sapienza, Rome, Italy

**Correspondence to: M. Christina Cox, Hematology, A.O. Sant'Andrea, University La Sapienza, via di Grottarossa 1034, 00189 Rome, Italy*

E-mail: chrisscox@gmail.com

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

Conflict of interest: Nothing to report.

Published online 21 July 2011 in Wiley Online Library (wileyonlinelibrary.com).

DOI: 10.1002/ajh.22148

References

1. Ferreri AJ, Assanelli A, Crocchiolo R, et al. Central nervous system dissemination in immunocompetent patients with aggressive lymphomas: Incidence, risk factors and therapeutic options. Hematol Oncol 2009;27:61–70.
2. Musuraca G, Fattori PP, Ceccolini M. Prolonged 18FDG-PET negative complete remission in a heavily pretreated, elderly patient with diffuse large B cell lymphoma treated with lenalidomide, low dose dexamethasone, and colony stimulating factor (Rd-G). Am J Hematol 2011;86:79–80.
3. Habermann TM, Lossos IS, Justice G. Lenalidomide oral monotherapy produces a high response rate in patients with relapsed or refractory mantle cell lymphoma. Br J Haematol 2009;145:344–349.
4. Zeldis JB, Knight R, Hussein M, et al. A review of the history, properties, and use of the immunomodulatory compound lenalidomide. Ann N Y Acad Sci 2011;1222:76–82.
5. Zhang L, Qian Z, Cai Z, et al. Synergistic antitumor effects of Lenalidomide and rituximab on mantle cell lymphoma in vitro and in vivo†. A m J Hematol 2009;84:553–559.
6. Rubenstein JL, Treseler PA, Stewart PJ. Regression of refractory intraocular large B-cell lymphoma with lenalidomide monotherapy. J Clin Oncol 2011;29:e595–e597.
7. Ott G, Kalla J, Ott MM, Schryen B, Katzenberger T, Müller JG, Müller-Hermelink HK. Blastoid variants of mantle cell lymphoma: Frequent bcl-1 rearrangements at the major translocation cluster region and tetraploid chromosome clones. Blood 1997;89:1421–1429.
8. Zhang MM, Gopal AK. Radioimmunotherapy-based conditioning regimens for stem cell transplantation. Semin Hematol 2008;45:118–1125.
9. Hon C, Au WY, Ma ES, Unusual sites of involvement in non-Hodgkin's lymphoma,: Case 1. Isolated orbit relapse after allogeneic bone marrow transplantation for malignant lymphoma. J Clin Oncol 2002;20:4394–4395.
10. Jahnke K, Thiel E, Abrey LE, et al. Diagnosis and management of primary intraocular lymphoma: An update. Clin Ophthalmol 2007;1:247–258.
11. Precupanu CM, Validire P, Lévy C, Primary high-grade ocular adnexal lymphoma: Clinicopathological characteristics and prognostic factors of a single-centre series. Am J Hematol 2010;85:372–375.
12. Ferreri AJ. How I treat CNS lymphoma. Blood 2011;118:510–522.

Feasibility and benefit of hydroxycarbamide as a long-term treatment for sickle cell disease patients: Results from the North West London Sickle Cell Disease Registry

Annette Gilmore,[1*] Gavin Cho,[1] Jo Howard,[2] Mark Layton,[3] Michele Afif,[1] Robert George Hughes,[4] Nicola J Philpott,[5] Sameer Patankar,[1] and Sally C. Davies;[1,6] the North West London Haemoglobinopathy Registry Group

Despite the benefits of hydroxycarbamide (HU) for sickle cell disease (SCD) patients, it is currently underutilized in routine clinical management because of concerns about effectiveness and safety and ambivalence surrounding optimal HU regimes for heterogeneous SCD clinic populations [1–6]. This UK study followed up 62 SCD patients treated with HU for up to 9 years (IQR 1–6 yrs). Patients benefited from sustained significant increases in mean annual hemoglobin (Hb), fetal hemoglobin (HbF%), mean cell volume (MCV) and reduction in absolute neutrophil count (ANC), mean annual inpatient (IP) days, acute chest syndrome (ACS) and transfusion events compared to pretreatment. Mean daily dose and maximum tolerated dose (MTD) achieved were constrained by patients' concordance with treatment and drug tolerance. All serious adverse events were not prevented during therapy. Side effects did not warrant cessation of HU but deteriorating medical condition did. Long-term treatment warrants close monitoring with multiagency involvement in care.

Most SCD patients suffer intermittent painful episodes and life-threatening events while life expectancy is reduced [7–9]. HU is the only drug which effectively ameliorates the clinical symptoms of the disease [10,11] and increases probability of survival with long term use [12,13]. Findings from small observational studies of children and young adults and from two adult cohorts suggest its effectiveness and safety in the medium to long-term but meagre practical advice and evidence exists to guide clinicians for optimal HU use on an individual patient basis in routine clinical practice [12–17]. The primary objective of this study was to describe the effectiveness and risks of long-term HU treatment in SCD patients cared for in UK National Health Service hospitals. Further we sought to describe which aspects of care and therapy were associated with improved treatment management and outcomes.

Sixty two patients, including 51 adults and 11 children (age $<$ 16 yrs) from four hospitals were included in the cohort. There were 38 males; 55 had HbSS, six HbS/β^0-thalassaemia, and one HbSD-Punjab. Mean age at start of treatment was 28 (range 16–44) years for the adults and 10 (range 1–14) for the children. The majority of patients were started on HU because of recurrent IP painful episodes or IP painful episodes with ACS (see Supporting Information Table I). Thirteen were switched from transfusion programmes to HU therapy. Follow-up ranged from 1 to 9 years, totalling 249 person-years with a median follow-up of 3 years (IQR 1–6).

Tables I and II describe the hematological, biochemical and clinical response to HU treatment. Sustained significant increases in Hb, HbF%, MCV and decrease in ANC were observed from pretreatment levels. Absolute reductions in total bilirubin and mean reticulocyte count were also observed although the reductions were not always significant. There were significant reductions in IP days, ACS events and transfusions administered. The mean annual reduction in painful episodes was not significantly reduced year on year but remained clinically important with a 35% reduction after 1 year, 46% after two, 50% after five and 19% after seven [10,18]. Clinical event rates were 124.5 per 100 patient-years for IP admissions, 1108 for IP days and 50.6 for transfusion events. The incidence of ACS and IP painful episodes was 9.6 and 98 per 100 patient-years, respectively. The death rate was 0.8 and the stroke rate was 1.2 per 100 person-years. Two patients died, due to sickle cell complications, while on HU; one 39-year-old female, who was on HU for 4 years, and a young man of 20 after three and a half years. The female died from a subarachnoid hemorrhage; she had a history of deep venous thrombosis and was on long-term anticoagulation. The male was admitted with an ACS and rapidly deteriorated with multiorgan failure. He did not adhere to his scheduled outpatient department (OPD) appointments or HU regime. Two other patients had infarctive strokes; neither had viscosity symptoms during treatment. No patient developed cancer during the study but there were two occurrences after study closure. One patient, after 11 years of HU with a history of chronic depression, long-term alcohol misuse and severe hepatic iron overload, developed primary hepatocellular carcinoma and subsequently died. The other patient complained of a lump in her thyroid 3 weeks after starting HU which was diagnosed as a "benign colloid module." The lump developed into papillary cell carcinoma after 27 months and was successfully treated with a total thyroidectomy.

There were 175 episodes of transient hematological toxicity experienced by 39 patients, yielding a rate of 70 per 100/patient years. There was no change in the incidence of leg ulcers. Four patients had leg ulcers pretreatment and three of these patients suffered leg ulcer events during therapy. Annual surveys, revealed $\sim$50% of patients experienced mild or moderate melanonychia; two had this before treatment. Skin hyperpigmentation occurred in between 5 and 20% of patients. None stopped HU as a consequence of these side effects. Five pregnancies occurred in four patients while taking HU. In all cases treatment was stopped early in the first trimester. Pregnancy outcomes were two terminations, one miscarriage and two normal live births. One male stopped HU twice and his partner conceived $\sim$4 months later on both occasions and delivered normal healthy babies. Another female conceived 10 weeks after stopping HU and delivered a healthy baby. The questions "date of last menstrual period" (LMP) and "contraception use" were added to the HU follow-up questionnaire, at one site, to help monitor pregnancy risk in adult patients. The question date of LMP was completed on 19.5% of occasions. The contraception question was completed in 33% of the female proformas and in 8.5% of the male proformas.

Thirty six patients achieved MTD, at a median time of 9 months (IQR 5–18 months). Mean MTD was 20 mg/kg/day (SD 4 mg). Reasons for MTD nonattainment were: nonadherence to monitoring/ drug regime (10 patients); insufficient opportunity to achieve MTD as HU was stopped or patients' continued to have frequent IP admissions and blood transfusions (3); dose reduced because Hb increased above 12 g/dl, causing viscosity symptoms (2); patient chose a lower dose (1) and it was not known why MTD was not established in three patients. MTD was not attempted in seven patients for pragmatic reasons: other active diseases which contra indicated high dose HU, difficulties with monitoring (e.g. poor venous access, psychosocial reasons), dose already established by another hospital when care transferred and patient's choice. Thirty patients (83%) were maintained at their MTD. Dose reduction from MTD occurred in six patients because of intolerability over time to original MTD, following an episode of aplastic crisis (parvovirus B19 infection), Hb over 12 g/dl, patients' weight increased but HU dose not increased and to promote healing of leg ulcers.

Twenty patients were on HU and being followed up at study close. Another 19 were on HU but follow-up stopped prematurely due to relocation or care transferred to another hospital (5), did not turn up for appointments (6) and funding for data collection ceased in three hospitals (8). Twenty three patients stopped HU: 11 for medical reasons (summarized in Supporting Information Table II), seven refused and it is not known why five stopped.

Our study is the first to evaluate the use of HU in a UK SCD cohort and results demonstrate its value as a long-term therapy option. Patients accrued significant long-term clinical and hematological benefits with no major side effects, which concur with existing research [13–16]. It did not

TABLE I. Clinical and Biologic Outcomes Achieved with Hydroxycarbamide Treatment

Variable	Year	No.	Year 0 Mean (SD)	Last Year Mean (SD)	Difference Mean (95% CI)	*P*-value
Dose (mg/kg/day)	1	62	15.2 (4.9)	18.1 (8.4)	2.9 (0.4, 5.4)	0.02
	2	46	15.7 (5.2)	19.3 (8.1)	3.5 (0.7, 6.4)	0.02
	5	21	16.5 (4.7)	18.7 (7.5)	2.2 (−1.8, 6.3)	0.26
	7	14	16.7 (4.3)	20.3 (5.6)	3.6 (−1.4, 8.5)	0.14
HB (g/dl)	1	60	9.0 (1.6)	9.7 (1.7)	0.8 (0.4, 1.1)	<0.001
	2	42	9.1 (1.6)	9.7 (1.4)	0.7 (0.3, 1.1)	0.002
	5	20	9.1 (1.6)	9.9 (1.3)	0.7 (0.2, 1.3)	0.01
	7	14	9.1 (1.4)	10.3 (1.3)	1.2 (0.5, 1.8)	0.002
MCV (fl)	1	60	86 (10)	104 (17)	18 (14, 21)	<0.001
	2	41	86 (10)	104 (18)	18 (14, 21)	<0.001
	5	20	91 (8)	113 (13)	23 (18, 27)	<0.001
	7	14	93 (8)	115 (16)	22 (15, 29)	<0.001
HB F%	1	59	5.9 (5.0)	18.7 (11.6)	12.8 (10.2, 15.4)	<0.001
	2	42	6.2 (4.8)	18.3 (11.0)	12.2 (9.3, 15.1)	<0.001
	5	19	5.4 (4.4)	17.8 (9.4)	12.4 (8.7, 16.2)	<0.001
	7	13	5.9 (4.9)	18.8 (9.1)	12.9 (8.7, 17.1)	<0.001
ANC (10^9/L)	1	55	7.4 (3.4)	4.4 (2.2)	−3.0 (−4.1, −1.9)	<0.001
	2	39	7.5 (3.2)	4.5 (2.2)	−3.0 (−4.3, −1.7)	<0.001
	5	20	8.4 (3.5)	4.6 (1.7)	−3.8 (−5.7, −1.9)	<0.001
	7	14	8.5 (3.3)	4.4 (1.9)	−4.1 (−6.6, −1.7)	0.003
Painful episode (IP events)	1	43	2.05 (2.56)	1.33 (1.82)	−0.72 (−1.20, −0.24)	0.004
	2	31	1.90 (2.07)	1.03 (1.72)	−0.87 (−1.60, −0.15)	0.02
	5	17	1.18 (1.38)	0.59 (1.28)	−0.59 (−1.60, 0.42)	0.24
	7	13	1.23 (1.54)	1.00 (1.78)	−0.23 (−1.83, 1.37)	0.76

IP, inpatient; No., number of patients.
The data is paired therefore for a subject to be included in the analysis, values are required at both time points. Consequently there are a different number of subjects in each of the analyses.

TABLE II. Clinical and Biologic Outcomes Achieved with Hydroxycarbamide Treatment

Variable	Year	No.	Year 0 mean (SD)	Last year mean (SD)	Difference ratio (95% CI)	*P*-value
Retics%	1	37	9.8 (6.7)	7.8 (6.5)	0.80 (0.58, 1.10)	0.16
	2	23	9.4 (4.4)	8.2 (3.8)	0.92 (0.72, 1.20)	0.55
	5	10	14.2 (7.1)	8.0 (4.9)	0.53 (0.35, 0.82)	0.009
	7	9	12.8 (8.4)	6.5 (2.0)	0.62 (0.32, 1.19)	0.13
Total bilirubin (umol/L)	1	59	57 (42)	41 (35)	0.71 (0.62, 0.81)	<0.001
	2	43	60 (46)	40 (31)	0.69 (0.59, 0.81)	<0.001
	5	19	67 (47)	50 (31)	0.77 (0.56, 1.04)	0.08
	7	14	67 (46)	47 (28)	0.73 (0.51, 1.04)	0.08
IP days (annual no.)	1	46	29 (34)	18 (29)	0.42 (0.27, 0.66)	<0.001
	2	31	26 (33)	8 (17)	0.22 (0.12, 0.42)	<0.001
	5	17	22 (26)	6 (8)	0.30 (0.12, 0.70)	0.009
	7	13	20 (23)	6 (9)	0.27 (0.13, 0.57)	0.002
Chest syndrome (IP events)	1	43	0.9 (1.0)	0.1 (0.3)	0.66 (0.56, 0.77)	<0.001
	2	31	0.9 (1.1)	0.1 (0.2)	0.66 (0.53, 0.80)	<0.001
	5	17	1.0 (1.2)	0.1 (0.2)	0.61 (0.44, 0.84)	0.005
	7	13	1.1 (1.3)	0.1 (0.3)	0.60 (0.42, 0.84)	0.007
Blood transfusion (events)	1	51	4.0 (4.7)	0.5 (1.1)	0.41 (0.31, 0.54)	<0.001
	2	36	4.6 (5.2)	0.3 (0.7)	0.35 (0.24, 0.51)	<0.001
	5	18	5.7 (5.4)	1.1 (2.0)	0.37 (0.23, 0.60)	<0.001
	7	13	4.8 (5.2)	0.2 (0.6)	0.31 (0.16, 0.60)	0.002

IP, inpatient; No., number of patients; Retics, reticulocytes.
The mean ratio of difference between the times is calculated as value at subsequent time point divided by value at baseline. A ratio of greater than 1 implies an increase in values over time, whilst a ratio below 1 implies a decrease in values over time.

prevent all serious adverse events, including two incidences of ischaemic stroke. Exposure to HU was not considered a contributory factor in the development of thyroid cancer and this patient continued with treatment. Our experience identifies some of the difficulties encountered with maintaining optimum long-term HU therapy in routine practice where SCD patients with an array of complex clinical and psychosocial issues are treated. Mean annual dose and mean MTD were lower than expected and less than half of the cohort was treated at MTD in the long-term [9]. MTD may not be achievable or necessary for every patient however continual careful monitoring of dose efficacy is required as there may be a tendency towards declining treatment intensity over time which could lead to HU being less effective [14,15,19]. It is possible that reduced treatment intensity in later years contributed to HU being less effective at preventing painful episodes in our cohort. Pregnancy surveillance was not totally effective, which is similar to the experience of others [10,13,15,17,20,21]. Patient non adherence with HU and/or the treatment regime was an important contributory factor to MTD non achievement. Attrition rates were substantial; also a finding in other SCD HU studies [14–17,22,23]. Patients who refused to continue HU and those lost to follow–up need further investigation to identify barriers to treatment.

Tailored electronic patient records (EPR) can be used to monitor and engage SCD patients while on HU. Figure 1, in the Supporting Information, shows an example of the treatment specific EPR used in this study. Patient clinical and hematological information was captured on a single Microsoft Excel (Redmond, WA) graph which was used during clinical consultations to aid doctor-patient communication. It enabled the patient and doctor to review progress with and response to HU and plan treatment accordingly. This management tool has the potential to enable doctors, together with their patients, to systematically monitor treatment response which may help improve the quality of communication between care giver and patient and promote concordance with therapy [24]. Additionally it may be beneficial for doctors to involve other health professionals with management of patients treated with HU to facilitate more holistic and creative approaches to patient education, surveillance and monitoring [19,25]. Patients and parent/carers

build up good long-term supportive relationships with SCD nurses/counsellors [26,27] and people with chronic illness have better experienced continuity of care where management is shared between their GP and the hospital clinic compared to hospital clinic alone [28]. As with all observational studies, this one has limitations including the risk of introducing a type I error, although the large treatment effects observed were likely to be genuine [29,30]. Furthermore it was not adequately powered to detect differences for some variables at specific time points, including painful episodes, which should be taken into account when reviewing the results [18].

Methods

Patients and data. Data from all patients, with HbSS, HbS/β^0-thalassamia and HbSD-Punjab, on HU treatment for at least 1 year, in the four hospitals who participated in the North West London Haemoglobinopathy Registry between 2002 and 2007 were included. The Registry is registered annually with the Information Commissioners Office (Registration no. Z5730583) and has multicentre research ethics approval (No. MREC/99/2/4). Patients give written consent for their information to be used for research. Indications for starting HU and monitoring schedule were decided on an individual patient basis, but all centres used similar eligibility criteria which were a history of more than three IP admissions with painful episodes in the previous year, or more than one IP admission with a painful episode and symptomatic in the community, or a history of two or more life-threatening complications of the disease such as ACS. The aim of treatment was to achieve a MTD or a maximum dose of 30 mg/kg/day unless clinically contraindicated [9].

Effectiveness of HU was evaluated using patients as their own control (before/after design). Retrospective and prospective clinical and laboratory data were collected on all patients from start of treatment. Non adherence with monitoring regime was evaluated by counting the proportion of OPD visits the patient attended compared with the number of scheduled OPD visits. Non adherence with the drug was assessed by nonattendance at OPD visits, comments about adherence in the free text portion of the follow-up proforma and medical notes and from the answers to the question about average number of doses missed per week. During the study patients were prescribed sufficient HU to cover the period between OPD visits therefore if they missed scheduled visits they could not maintain their dosing regime. An EPR was generated to assist with patient monitoring and reviewed for evidence of nonadherence, and the trends in MCV and HbF% were inspected to help verify drug usage.

Statistical Analysis. The objective was to compare the changes in variables over time to measure hematological and clinical benefit from treatment. Changes from pretreatment/baseline (year 0), to each of the years 1, 2, 5, and 7 were analysed to identify statistically significant differences associated with HU use. Only eight subjects had 9 years of data which proved too small a sample for robust statistical inferences. Variables were measured on a numerical scale, except for leg ulcers, which was measured on a categorical scale.

The statistical package used was Stata (Version 9.0., StataCorp TX). For changes over time, that were normally distributed numerical variables, the paired *t*-test was used. The mean and standard deviation (SD) was calculated at each time point, together with the mean (95% confidence interval) difference from pretreatment and *P*-value of the difference. Non normally distributed variables were analysed on the log transformed scale, with the paired *t*-test for the analysis. The mean ratio of the difference between the years being measured is reported along with the corresponding 95% confidence interval (CI). The results from the analysis of normally distributed variables are reported in Table I and non normally distributed variables in Table II. The Fishers Exact test for small numbers was used for analysis of the categorical variable. Clinical event rates were calculated for patients treated up to 9 years as follows: (number of occurrences / total follow-up time * 100).

Contribution of Authors

AG, JH and GC wrote and revised the manuscript. AG, SCD and ML conceived and designed the study. AG and SP were responsible for developing the study protocol, managing the data and statistical analyses. All other authors reviewed the manuscript and were involved in patient follow-up.

Acknowledgments

The authors thank the members of the North West London Sickle Cell Disease registry for their support and enrolment of patients: Shubha Allard, Royal London Hospital; Gail Abrahamson, Colin A Michie, and Cathy Rose, Ealing General Hospital; Inderjeet Dokal, Royal London Hospital; Anne E.M. Davies and Mallika Sekhar, West Middlesex University Hospital. They are very grateful to the late Dr. Mabel Alli, Brent Primary Care Trust (PCT), for her support with administering the registry, Pricilla Plocki, Hammersmith Hospital and Sharon Robinson, University College Hospital for data collection. They also thank Paul Bassett, Statsconsultancy Ltd., for his technical advice and assistance with the statistical analysis.

[1]Department of Haematology, Central Middlesex Hospital, London, United Kingdom; [2]Department of Haematology, Guys and St Thomas' Hospital, London, United Kingdom; [3]Department of Haematology, Imperial College, Hammersmith Hospital, London, United Kingdom; [4]Department of Haematology, West Middlesex University Hospital, Middlesex, United Kingdom; [5]Department of Haematology, Wexham Park Hospital, Slough, United Kingdom; [6]Department of Health, London, United Kingdom

Contract grant sponsor: Department of Health (NHS Executive London Regional Office); Contract grant number: RFG 796; Contract grant sponsors: Brent PCT, Ealing PCT, Hammersmith & Fulham PCT, Hounslow PCT, North West London Hospitals General Charitable Fund, Washington University School of Medicine, St. Louis.

**Correspondence to: Annette Gilmore, Department of Haematology, Central Middlesex Hospital, NWLH NHS Trust, Acton Lane, Park Royal, London NW10 7NS, England*
E-mail: annette.gilmore@nhs.net

Additional Supporting Information may be found in the online version of this article.
Conflict of interest: Nothing to report.

Published online 21 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22146

References

1. Ballas SK, Lusardi M. Hospital readmission for adult acute sickle cell painful episodes: Frequency, etiology, and prognostic significance. Am J Hematol 2005;79:17–25.
2. Meyappan JD, Lampl M, Hsu LL. Parents' assessment of risk in sickle cell disease treatment with hydroxyurea. J Pediatr Hematol Oncol 2005;27:644–650.
3. Zumberg MS, Reddy S, Boyette RL, et al. Hydroxyurea therapy for sickle cell disease in community-based practices: A survey of Florida and North Carolina hematologists/oncologists. Am J Hematol 2005;79:107–113.
4. Lanzkron S, Haywood C Jr, Segal JB, Dover GJ. Hospitalization rates and costs of care of patients with sickle-cell anemia in the state of Maryland in the era of hydroxyurea. Am J Hematol 2006;81:927–932.
5. Hankins J, Hinds P, Day S, et al. Therapy preference and decision-making among patients with severe sickle cell anemia and their families. Pediatr Blood Cancer 2007;48:705–710.
6. Lanzkron S, Haywood C Jr, Hassell KL, Rand C. Provider barriers to hydroxyurea use in adults with sickle cell disease: A survey of the sickle cell disease adult provider network. J Natl Med Assoc 2008;100:968–973.
7. Platt OS, Brambilla DJ, Rosse WF, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med 1994;330:1639–1644.
8. Wierenga KJJ, Hambleton IR, Lewis NA. Survival estimates for patients with homozygous sickle-cell disease in Jamaica: A clinic-based population study. Lancet 2001;357:680–683.
9. Davies SC, Gilmore A. The role of hydroxyurea in the management of sickle cell disease. Blood Rev 2003;17:99–109.
10. Charache S, Terrin ML, Moore RD, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. N Engl J Med 1995;332:1317–1322.
11. Ferster A, Vermylen C, Cornu G, et al. Hydroxyurea for treatment of severe sickle cell anemia: A pediatric clinical trial. Blood 1996;88:1960–1964.
12. Steinberg MH, McCarthy WF, Castro O, et al. The risks and benefits of long-term use of hydroxyurea in sickle cell anemia: A 17.5 year follow-up. Am J Hematol 2010;85:403–408.
13. Voskaridou E, Christoulas D, Bilalis A, et al. The effect of prolonged administration of hydroxyurea on morbidity and mortality in adult patients with sickle cell syndromes: Results of a 17-year, single-center trial (LaSHS). Blood 2010; 115:2354–2363.
14. Zimmerman SA, Schultz WH, Davis JS, et al. Sustained long-term hematologic efficacy of hydroxyurea at maximum tolerated dose in children with sickle cell disease. Blood 2004;103:2039–2045.
15. Gulbis B, Haberman D, Dufour D, et al. Hydroxyurea for sickle cell disease in children and for prevention of cerebrovascular events: The Belgian experience. Blood 2005;105:2685–2690.
16. Hankins JS, Ware RE, Rogers ZR, et al. Long-term hydroxyurea therapy for infants with sickle cell anemia: The HUSOFT extension study. Blood 2005; 106:2269–2275.
17. De Montalembert M, Brousse V, Elie C, et al. Long-term hydroxyurea treatment in children with sickle cell disease: Tolerance and clinical outcomes. Haematologica 2006;91:125–128.
18. Altman DG, Bland JM. Statistics notes: Absence of evidence is not evidence of absence. BMJ 1995;311:485.

19. Thornburg CD, Rogers ZR, Jeng MR, et al. Adherence to study medication and visits: Data from the BABY HUG trial. Pediatr Blood Cancer 2010;54:260–264.
20. Thauvin-Robinet C, Maingueneau C, Robert E, et al. Exposure to hydroxyurea during pregnancy: A case series. Leukemia 2001;15:1309–1311.
21. Ballas SK, McCarthy WF, Guo N, et al. Exposure to hydroxyurea and pregnancy outcomes in patients with sickle cell anemia. J Natl Med Assoc 2009; 101:1046–1051.
22. Kinney TR, Helms RW, O'Branski EE, et al. Safety of hydroxyurea in children with sickle cell anemia: Results of the HUG-KIDS study, a phase I/II trial. Blood 1999;94:1550–1554.
23. Lanzkron S, Strouse JJ, Wilson R, et al. Systematic review: Hydroxyurea for the treatment of adults with sickle cell disease. Ann Intern Med 2008;148:939–955.
24. Vermeire E, Hearnshaw H, Van Royen P, Denekens J. Patient adherence to treatment: Three decades of research. A comprehensive review. J Clin Pharm Ther 2001;26:331–342.
25. Brawley OW, Cornelius LJ, Edwards LR, et al. National Institutes of Health Consensus Development Conference statement: Hydroxyurea treatment for sickle cell disease. Ann Intern Med 2008;148:932–938.
26. Gould D, Thomas V, Darlison M. The role of the haemoglobinopathy nurse counsellor: An exploratory study. J Adv Nurs 2000;31:157–164.
27. De D. Sickle cell anemia 2: Management approaches of painful episodes. Br J Nurs 2005;14:484–489.
28. Gulliford MC, Naithani S, Morgan M. Measuring continuity of care in diabetes mellitus: An experience-based measure. Ann Fam Med 2006;4:548–555.
29. Pocock SJ, Hughes MD, Lee RJ. Statistical problems in the reporting of clinical trials. A survey of three medical journals. N Engl J Med 1987;317:426–432.
30. Grimes DA, Schulz KF. Bias and causal associations in observational research. Lancet 2002;359:248–252.

Acute gout at engraftment following hematopoietic transplantation

Rammurti T Kamble,* Shan Guo, Carlos A. Ramos and George Carrum

Acute exacerbation of gout or pseudogout has been reported following administration of granulocyte colony stimulating factor (G-CSF) in a healthy donor undergoing stem cell mobilization [1] and in drug-induced cytopenia [2,3]. Release of proinflammatory cytokines along with rapid cell turnover have been implicated [3–5]. Acute gout exacerbations at engraftment following autologous or allogeneic hematopoietic stem cell transplantation have not been reported; we herein report five consecutive such patients.

Between January 2005 and April 2011, a total of 292 hematopoietic transplants (allogeneic = 120, autologous = 172) were performed in adults. Following approval from the Institutional Review Board, their medical records were examined. Twenty-three (7.8%) patients had a history of gout. Of these, five (21.7%) patients developed acute gout during the course of transplant. All five patients had prior established diagnosis of symptomatic gout and were receiving allopurinol prophylaxis. Patients, disease, and transplant characteristics are summarized in Table I. All but one patient were males with median age of 57 years (range = 47–69) and received peripheral stem cell graft with median of 5.45×10^6 $CD34^+$ cells/kg (range = 3.2–7.32).

The median time to engraftment for neutrophils [absolute neutrophil count (ANC) of 500 for 3 consecutive days] and platelets (untransfused platelets of more than 20,000 × 3 days) was 15 days (range = 12–17) and 11 days (range = 10–16), respectively. Acute gout preceded engraftment by a median of 2 days (range = 1–3 days) in three patients; two patients had acute gout 1 to 2 days following engraftment (patients #4, 5). The median white blood cell count, ANC and platelet count at the time of acute gout were $2.51 \times 10^3/\mu L$ (range = 1.64–5.47), 917/μL (range = 721–3938) and $25 \times 10^3/\mu L$ (range = 20–37), respectively. The median serum uric acid level at the onset of acute gout was 5.1 mg/dl (range = 3.1–9.1). In all patients, the clinical presentation of gout was typical of their previous attacks, and in view of their thrombocytopenia, we did not perform diagnostic joint aspiration, using established diagnostic criteria instead [6,7]. Of the five patients, two were receiving G-CSF at the time of their exacerbation (patients #4, 5). One patient received G-CSF after the acute gout attack on Day 16 (patient #3) and two patients received no G-CSF. Neither of the two patients who received autologous transplant had acute gout during stem cell mobilization. There were no characteristics of the patients, their disease or transplant type that were consistently related to gout exacerbation in this small series.

Treatment of acute gout included a short course of systemic steroids in all five patients; three patients also received colchicin. The decision to use colchicin in our patients was based on their quick response to treatment with this agent in previous episodes. Given its myelosuppressive potential, colchicin should generally be reserved for patients who are refractory to steroids.

Acute gout can manifest in context of normal uric acid levels, precipitated by trauma, surgery, dehydration, starvation, dietary alcohol, and drugs. Several of the hematopoietic transplant supportive drugs (that can either increase or decrease uric acid levels) including allopurinol, cyclosporine, tacrolimus, low dose aspirin, and loop diuretics among others can promote acute gout. All five patients received allopurinol and variable doses of intravenous furosemide.

We do not know why in all five patients gout was precipitated by hemopoietic engraftment. Possible explanations include release of proinflammatory cytokines such as interleukin-1(IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha [5,8,9]. These cytokines may peak at Day 12 of hematopoietic transplant [9]. In addition, monosodium urate monohydrate crystals induced release of proinflammatory cytokines from neutrophils have been demonstrated [4].

Thus, gout exacerbation may occur at engraftment even without G-CSF administration and must be considered in the differential diagnosis of joint pain in these patients.

TABLE I. Patient, Disease and Transplant Characteristics

No.	Age/Sex	Diagnosis	Conditioning	Donor	G-CSF	Acute gout	Engraftment
1.	54/M	MDS-RAEB	BU/CY	MRD	None	Day 13	Day 16
2.	65/M	AML	FL/Mel	MMUD	None	Day 12	Day 15
3.	57/M	MM	Mel	Auto	Day 16	Day 16	Day 17
4.	68/M	MM	Mel	Auto	Day 7	Day 13	Day 12
5.	47 F	MF	FL/TBI/Al	MRD	Day 7	Day 14	Day 12

Received dose in the evening after acute gout.No., number; M, male; F, female; G-CSF, Granulocyte colony stimulating factor; MDS, myelodysplastic syndrome; RAEB, refractory anemia with excess of blast; BU, busulfan; CY, cyclophosphamide; MRD, matched related donor; AML, acute myelogenous leukemia; FL, fludarabine; Mel, melphalan; MMUD, mismatched unrelated donor; MUD, matched unrelated donor; MF, myelofibrosis; TBI, total body irradiation; Al, alemtuzumab; MM, multiple myeloma; Auto, autologous.

Center of Cell and Gene Therapy, Baylor College of Medicine, The Methodist Hospital, Houston, TX
**Correspondence to: Rammurti T Kamble, Associate Professor Medicine, Center for Cell and Gene Therapy, Baylor College of Medicine, Methodist Hospital, M970, 6565, Fannin Street, Houston, TX 77030*
E-mail: Kamble@sbcglobal.net
Conflict of interest: Nothing to report.
Published online 21 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22149

References

1. Spitzer T, McAfee S, Poliquin C, Colby C. Acute gouty arthritis following recombinant human granulocyte colony-stimulating factor therapy in an allogeneic blood stem cell donor. Bone Marrow Transplant 1998;21:966–967.

2. Yamamoto H, Teramoto S, Matsui H, Matsuse T, Toba K. An elderly case with pseudogout exacerbated by the administration of granulocyte-colony stimulating factor during drug-induced granulocytopenia]. Nippon Ronen Igakkai Zasshi 1999;36:572–575.
3. Ames PR, Rainey MG. Consecutive psuedogout attacks after repetitive granulocyte colony stimulating factor administration for neutropenia. Mod Rheumatol 2007;17:445–446.
4. Ryckman C, Gilbert C, de Médicis R, Lussier A, Vandal K, Tessier PA. Monosodium urate monohydrate crystals induce the release of the proinflammatory protein S100A8/A9 from neutrophils. J Leukoc Biol 2004;76:433–440.
5. Chen CJ, Shi Y, Hearn A et al. MyD88-dependent IL-1 receptor signaling is essential for gouty inflammation stimulated by monosodium urate crystals. J Clin Invest 2006;116:2262–2271.
6. Wallace SL, Robinson H, Masi AT, Decker JL, McCarty DJ, Yü TF. Preliminary criteria for the classification of the acute arthritis of primary gout. Arthritis Rheum 1977;20:895–900.
7. Janssens HJ, Fransen J, van de Lisdonk EH, van Riel PL, van Weel C, Janssen M. A diagnostic rule for acute gouty arthritis in primary care without joint fluid analysis. Arch Intern Med 2010;170:1120–1126.
8. Schawabe M, Hartert A.-M, Bertz H, Finke J. Treatment with granulocyte colony-stimulating factor increases interleukin-1 receptor antagonist levels during engraftment following allogeneic stem-cell transplantation. Eur J Clin Invest 2004;34:759–765.
9. Rabinowitz J, Petros WP, Stuart AR, et al. Characterization of endogenous cytokine concentrations after high-dose chemotherapy with autologous bone marrow support. Blood 1993;81:2452–2459.

A nonsynonymous *LNK* polymorphism associated with idiopathic erythrocytosis

Mary Frances McMullin,[1,2]* Chao Wu,[3] Melanie J Percy,[2] and Wei Tong[3]*

Idiopathic erythrocytosis (IE) comprises a heterogeneous group of disorders characterized by hyperplasia of the erythroid lineage; however, in many cases, the molecular basis remains undetermined. Serum erythropoietin (EPO) levels can be raised, normal, or reduced, suggesting that there are at least two underlying etiologies involving either the control of EPO production or modulation of EPO-induced signaling. EPO production is regulated by the oxygen-sensing pathway via the hypoxia inducible transcription factor (HIF) complex. Proteasomal turnover of HIF is controlled by interactions with the von Hippel Lindau (VHL) and prolyl hydroxylase domain 2 (PHD2) proteins. Erythrocytosis-associated mutations have been detected in the oxygen sensing pathway indicating that EPO is regulated by the HIF-2alpha-PHD2-VHL axis (reviewed by McMullin [1]). Aberrant EPO-induced signaling in IE patients with subnormal serum EPO levels can arise from mutations in the EPO receptor (EpoR) gene which result in the receptor being hypersensitive to EPO with prolonged activation of the EPO-dependent signaling pathways (reviewed by Percy [2]).

Recent reports have uncovered several mutations in the lymphocyte-specific adaptor protein (LNK) in a variety of myeloproliferative neoplasm (MPNs), including *JAK*2V617F-negative erythrocytosis, primary myelofibrosis (PMF), and chronic or blast-phase MPNs [3,4].

LNK is a plasma membrane-bound protein whose functions include inhibition of both wildtype and mutant JAK2 activity [5]. LNK is mainly expressed in hematopoietic tissues and contains pleckstrain homology (PH) and SH2 interaction domains. It modulates thrombopoietin and EPO signaling by interacting with JAK2, inhibiting downstream Signal transducer and activators of transcription (STAT) activation [6–8]. Thus, it is plausible that aberrant LNK function may allow dysregulation of EPO-induced signaling resulting in hypersensitivity to EPO and consequently erythrocytosis.

The first known disease-associated *LNK* mutations were somatically acquired. These include a small internal deletion that leads to a premature termination of protein translation and a functionally relevant mis-sense mutation in the PH domain (p.Glu208Gln) in patients with PMF and ET, respectively [4]. In a study of 341 MPN patients, 11 *LNK* mutations were found in cases of ET, PMF, and chronic myelomonocytic leukemia but not erythrocytosis [9]. Notably, in two instances the mutations were found in the germline of the patients. In an independent study of a cohort of 172 patients with chronic phase and blast-phase MPN, eight additional mutations in the LNK PH domain were described. These reports suggest that LNK mutations target an exon 2 "hot spot" in the PH domain spanning residues Glu208-Asp234 [10,11].

Finally, in a group of eight patients of IE with subnormal serum EPO levels and no alterations in *JAK*2, two cases were found to harbor mutations in the PH domain of LNK [3]. The above results support *LNK* mutations a possible cause of MPNs. This includes otherwise unexplained cases of erythrocytosis such as those with normal or low EPO levels. To understand the potential role of LNK in the development of IE, we investigated *LNK* in a group of 23 patients with subnormal serum EPO levels, with wild-type *EPOR*, and *JAK*2. The clinical parameters of the patient cohort are displayed in Table I.

Our analysis of *LNK* coding regions did not detect any LNK PH domain mutations in any of the 23 erythrocytosis patients. Instead, we identified, in two cases in the heterozygous state, a nonsynonymous change in the SH2 domain of LNK, p.Glu400Lys (or p.E400K; rs72650673; Fig. 1A). This alteration corresponds to a known single nucleotide polymorphism (SNP) but its frequency in the general population is unknown based on searches of public SNP database (NCBI dbSNP data base located at website address: http://www.ncbi.nlm.nih.gov/snp?term=rs72650673). However, a group of 200 normal control samples were screened by allele refractory mutation specific (ARMS)-PCR but none were positive for the p.Glu400Lys SNP (Table II). In contrast, we found that three additional patients with variable EPO levels were found to be heterozygous for this SNP (Table II). Thus, five out of 96 (5%) of the total IE patients possess this SNP, in comparison to zero out of 200 (0%) control subjects ($P = 0.0033$, two-tailed Fisher's exact test, Table II). Taken together, our data suggest an association of the LNK p.Glu400Lys polymorphism with erythrocytosis.

We failed to detect another previously reported polymorphism in the LNK PH domain, p.Trp262Arg (or p.W262R; rs3184504), which is associated with increased proliferation of peripheral blood monocytes in diabetic patients [12]. However, Trp262 is not conserved between human and mouse, raising questions as to its functional significance. Nevertheless, because of previous work suggesting a role of this residue in altered LNK function in diverse human diseases [13–17], we examined its function in this context.

To examine the functional consequences of this polymorphism, we stably expressed E400K and, as controls, a previously established SH2-null mutant, R392E, and wild-type LNK in hematopoietic 32D/EpoR cells (a hematopoietic progenitor cell line stably expressing EpoR). In parallel, we included in our analysis the W262R version. As previously reported, wild-type LNK impaired cell growth while the R392E version was inactive [7,18,19]. Neither the E400K nor the W262R mutation measurably impaired LNK function in this assay (Fig. 1B). We also examined a W262P conversion

TABLE I. Clinical Parameters of 23 Idiopathic Erythrocytosis Patients with Subnormal Serum EPO Levels

	Male	Female	Overall
Sex	15	8	23
Age at presentation	39 (20–60)	35 (19–57)	36 (19–60)
Hb (g/dl)	20.8 (18.1–23.2)	18.6 (17.3–19.9)	19.5 (17.3–23.2
WCC ($\times 10^9$/L)	8.1 (2.4–14.0)	9.1 (6.3–12.2)	8.5 (2.4–13)
Platelets ($\times 10^9$/L)	199 (166–312)	256 (160–400)	221 (160–400)
Erythropoietin (mU/L)	3.1 (0–5)	4.4 (0–9.3)	3.4 (0–9.3)

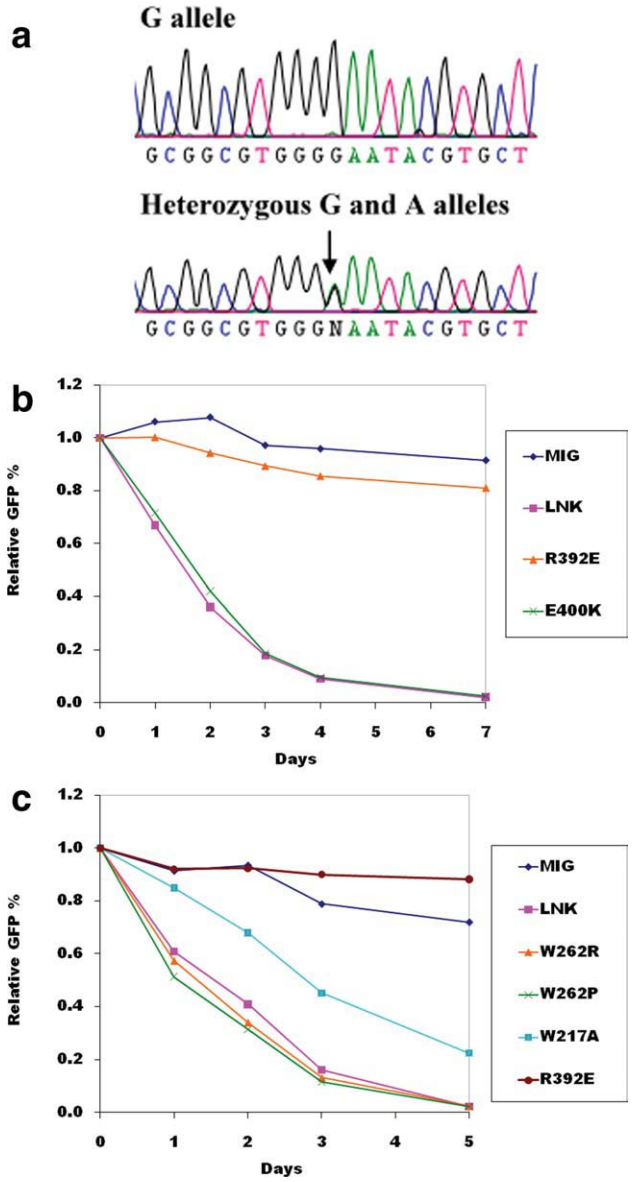

Figure 1. Identification and functional analysis of *LNK* p.Glu400Lys mutation. (a) LNK p.Glu400Lys (or p.E400K) mutation in patients with idiopathic erythrocytosis. G to A change at base g.41,559 (genomic sequence NT_009775, NCBI) in exon 5 of *LNK*, which results in exchange of glutamic acid at amino acid 400 with lysine (p.E400K). (b) The p.E400K mutant does not compromise LNK growth inhibitory function. 32D/EpoR cells were infected with retroviruses encoding either wild-type or mutant human LNK in MSCV-IRES-GFP (MIG) bicistronic vectors. The percentage of GFP^+ cells relative to the initial infection rate is plotted as a measurement of effects in cell growth (details in Design and Methods). Representative of three independent experiments are shown. (c) The W262R mutant does not compromise LNK growth inhibitory function. 32D/EpoR cells were infected with retroviruses encoding either wildtype or mutant human LNK in MIG vectors. The growth inhibition was measures as percentage of GFP^+ populations decline. W262 was mutated to R, as well as to P that is the equivalent residue in mouse Lnk. As a control, the W217A mutation in LNK, which is equivalent to the PH domain null mutation in mouse Lnk W191A, was generated and tested. Representative of two independent experiments are shown.

as this corresponds to the equivalent residue in mouse Lnk. As an additional control, we generated the W217A mutation, which is equivalent to the PH domain null mutation previously reported in mouse Lnk [6,7]. While the W262P version displayed growth inhibitory activity similar to wild-type LNK, the W217A mutation significantly impaired LNK function as expected (Fig. 1C). Together, our data suggest that neither p.Glu400Lys nor p.Trp262Arg SNPs produce any obvious defects in LNK based on this cell system. One caveat of this study is that although the 32D cell assay has been a reliable tool in measuring LNK function, it remains possible that subtle defects are only revealed in the context of whole animal studies.

TABLE II. Frequency of Glu400Lys SNP Detected by ARMS-PCR in IE Patients and Normal Control Subjects

Sample	EPO level	Number screened	Number (percentage) positive for Glu400Lys	Overall percentage	*P* Value
Normal control	N/A	200	0 (0%)	0 %	0.0033*
IE	Low	23	2 (8.6%)	5.2 %	
IE	Normal	46	2 (4.3%)		
IE	Elevated	27	1 (3.7%)		

[a]Two-tailed *P* value calculated with Fisher's exact test.

In summary, we have identified a nonsynonymous polymorphism in LNK (p.Glu400Lys) which is associated with IE. Although functional assays using a cell line model do not indicate that p.Glu400Lys impairs the ability of LNK to inhibit JAK2 activation of STAT5, it does not negate the possibility of a subtle loss of function. Consequently, impaired negative regulation of EPO-induced signaling, albeit minor, may support the erythrocytosis phenotype independent of the patient's serum EPO level.

Design and Methods

Patients. A group of 181 patients with a raised red cell mass and who did not fulfil the polycythemia vera diagnostic criteria proposed by the British Committee for Standards in Haematology [20] have been referred to Belfast City Hospital from clinics throughout the United Kingdom and Ireland. All patients gave informed written consent on entering the study. The study was approved by the office for Research Ethics Committee, Northern Ireland (06/NIRO1/57).

Mutation screening. Polymerase chain reaction (PCR)-direct sequencing of exons 2–6 of *LNK* was performed using standard protocols. A group of 200 normal control samples (Human Random Control DNA panels, ECACC, Salisbury, UK) was screened for the p.Glu400Lys base change by ARMS-PCR. Primers were designed using the primer design program devised by Ye et al. [21].

Functional assays. Interleukin (IL)-3-dependent 32D hematopoietic cells were used to establish a stable cell line expressing the EpoR, designated as 32D/EpoR. While the parental cells did not respond to EPO, 32D/EpoR cells proliferated in a dose-dependent manner [7]. The effect of LNK in EPO-dependent 32D cell growth was examined by overexpression of wild-type LNK using the Murine Stem Cell Virus (MSCV)-internal ribosomal entry site (IRES)-green fluorescent protein (GFP) (MIG) vector. MIG is a bicistronic vector containing GFP downstream of an IRES. As GFP expression is tightly correlated with the expression of the gene cloned upstream of the IRES, we were able to identify cells expressing LNK by analyzing GFP fluorescence. We introduced either MIG vector alone or LNK into 32D/EpoR cells and determined the fraction of GFP^+ infected cells 2 days later. We then measured the GFP^+ fraction every 3 days, as the cells divide, relative to the level 2 days after infection.

Acknowledgments

We would like to thank all the clinicians and scientists who provided blood samples and clinical information for the erythrocytosis study.

[1]*Department of Haematology, Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, Northern Ireland, United Kingdom*
[2]*Department of Haematology, Belfast City Hospital, Belfast, Northern Ireland, United Kingdom*
[3]*Children's Hospital of Philadelphia and Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania*
Contract grant sponsor: NIH; Contract grant numbers: R01HL095675, R21HL102688 (to W.T.); Contract grant sponsors: Myeloproliferative Neoplasm (MPN) Foundation (New Investigator Award) and Bingham Trust (Pilot Award)
**Correspondence to: Mary Frances McMullin, Department of Haematology, Queen's University Belfast, Lisburn Road, Belfast BT9 7AB, Northern Ireland, United Kingdom. E-mail: m.mcmullin@qub.ac.uk (or) Wei Tong, Abramson Bldg, Suite 316A, Children's Hospital of Philadelphia, 3615 Civic Center Boulevard, Philadelphia, PA 19104-4318. E-mail: tongw@email.chop.edu*
Conflict of interest: All authors declare there are no conflict of interests.
Published online 27 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.22154

References

1. McMullin MF. HIF pathway mutations and erythrocytosis. Expert Rev Hematol 2010;3:93–101.
2. Percy MJ. Genetically heterogeneous origins of idiopathic erythrocytosis. Hematology 2007;12:131–139.
3. Lasho TL, Pardanani A, Tefferi A. LNK mutations in JAK2 mutation-negative erythrocytosis. N Engl J Med 2010;363:1189–1190.
4. Oh ST, Simonds EF, Jones C, et al. Novel mutations in the inhibitory adaptor protein LNK drive JAK-STAT signalling in patients with myeloprolifetrative neoplasms. Blood 2010;116:988–992.
5. Bersenev A, Wu C, Balcerek J, Tong W. Lnk controls mouse hematopoeitic stem cell self-renewal and quiescence through direct interactions with myeloproliferative disorders. J Clin Invest 2008;118:2832–2844.
6. Tong W, Lodish HF. Lnk inhibits Tpo-mpl signaling and Tpo-mediated megakaryocytopoiesis. J Exp Med 2004;200:569–580.
7. Tong W, Zhang J, Lodish HF. Lnk inhibits erythropoiesis and Epo-dependent JAK2 activation and downstream signalling pathways. Blood 2005;105:4604–4612.
8. Bersenev A, Wu C, Balcerek J, et al. Lnk constrains myeloproliferative diseases in mice. J Clin Invest 2010;120:2058–2069.
9. Oh ST, Zahn JM, Jones CD, et al. Identification of novel LNK mutations in patients with chronic myeloproliferative neoplasms and related disorders. Blood 2010;116:315.
10. Lasho T, Tefferi A, Vannucchi AM, et al. LNK mutation studies in chronic and blast-phase myeloproliferative neoplasms and JAK2 mutation-negative erythrocytosis. Blood 2010;116:4105.
11. Pardanani A, Lasho T, Finke C, et al. LNK mutation studies in blast-phase myeloproliferative neoplasms, and in chronic-phase disease with TET2, IDH, JAK2 or MPL mutations. Leukemia 2010;24:1713–1718.
12. Lavrikova EY, Nikitin AG, Kuraeva TL, et al. The carriage of the type I diabetes associated R262W variant of human LNK correlates with increased proliferation of peripheral blood monocytes in diabetic patients. Pediatr Diabetes 2011;12:127–132.
13. Hunt KA, Zhernakova A, Turner G, et al. Newly identified genetic risk variants for celiac disease related to the immune response. Nat Genet 2008;40:395–402.
14. Smyth DJ, Plagnol V, Walker NM, et al. Shared and distinct genetic variants in type 1 diabetes and celiac disease. N Engl J Med 2008;359:2767–2777.
15. Newton-Cheh C, Johnson T, Gateva V, et al. Genome-wide association study identifies eight loci associated with blood pressure. Nat Genet 2009;41:666–676.
16. Levy D, Ehret GB, Rice K, et al. Genome-wide association study of blood pressure and hypertension. Nat Genet 2009;41:677–687.
17. Gudbjartsson DF, Bjornsdottir US, Halapi E, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. Nat Genet 2009;41:342–347.
18. Gery S, Cao Q, Gueller S, et al. Lnk inhibits myeloproliferative disorder-associated JAK2 mutant, JAK2V617F. J Leukoc Biol 2009;85:957–965.
19. Baran-Marszak F, Magdoud H, Desterke C, et al. Expression level and differential JAK2-V617F-binding of the adaptor protein Lnk regulates JAK2-mediated signals in myeloproliferative neoplasms. Blood 2010;116:5961–5971.
20. McMullin MF, Bareford D, Campbell P, et al. Guidelines for the diagnosis, investigation and management of polycythaemia/erythro-cytosis. Br J Haematol 2005;130:174–195.
21. Ye S, Dhillon S, Ke X, Collins AR, Day IN. An efficient procedure for genotyping single nucleotide polymorphisms. Nucleic Acids Res 2001;29:E88–8.