

LETTERS AND CORRESPONDENCE

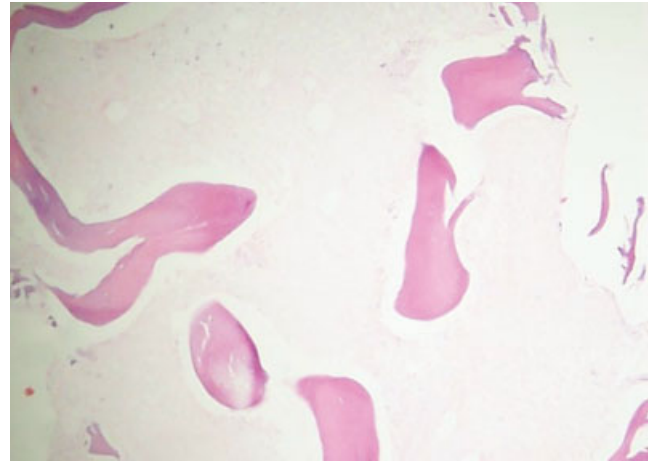
Letters and correspondence submitted for possible publication must be identified as such. Text length must not exceed 500 words and five bibliographic references. A single concise figure or table may be included if it is essential to support the communication. Letters not typed double-spaced will not be considered for publication. Letters not meeting these specifications will not be returned to authors. Letters to the Editor are utilized to communicate a single novel observation or finding. Correspondence is to be used to supplement or constructively comment on the contents of a publication in the journal and cannot exceed the restrictions for Letters to the Editor. The Editor reserves the right to shorten text, delete objectionable comments, and make other changes to comply with the style of the journal. Permission for publication must be appended as a postscript. Submissions must be sent to Jay Umbreit, MD, PhD, Editor of Brief Reports/Letters to Editors, American Journal of Hematology, Winship Cancer Institute, Emory University, 1365-B Clifton Road, Suite B4100, Atlanta, GA 30322 to permit rapid consideration for publication.

**Reversible Bone Marrow Necrosis in a Patient Due to Overdosage of Diclofenac Sodium**

*To the Editor:* Bone marrow necrosis (BMN) is a rare disease, and approximately 270 cases with BMN have been reported in literature up to now [1]. Although malignant hematopoietic diseases are common causes of BMN, benign diseases and drugs including interferon alpha, fludarabine, and paracetamol may be the nonmalignant causes of this pathology [1–3]. There are no reported cases of BMN due to diclofenac in literature. We present a patient with reversible BMN due to overdose consumption of diclofenac sodium because of renal colic.

A 26-year-old male was suffering from symptoms of anemia, fever, and bone pain. He had nephrocalcinosis and was treated with diclofenac because of renal pain. Diclofenac was used intramuscularly for renal colic in a dose of 75 mg repeatedly with 30-min intervals for 12 doses. He experienced fever and bone pain 5 days after diclofenac administration. Physical examination revealed fever and pallor of skin. Remarkable laboratory findings were hemoglobin level (7.7 g/dL), leucopenia ( $1.6 \times 10^9/L$ ), neutropenia ( $0.5 \times 10^9/L$ ), and no thrombocytopenia ( $179 \times 10^9/L$ ). His biochemical profile revealed high levels of alkaline phosphatase (AP) 940 IU/L (normal range, 60–300 IU/L) and lactate dehydrogenase (LDH) 520 IU/L (normal range, 220–450 IU/L). Bone marrow aspiration smears showed striking necrosis and nearly absent intact hematopoietic cells, whereas bone marrow biopsy revealed BMN (100%; Fig. 1), which was graded according to the extent of necrosis in the BM biopsy described by Maisel et al. [4]. Red blood cells were transfused because of symptomatic anemia. Two months later, his physical examination and laboratory results were resolved. Nine months after his initial admission, his physical examination was normal.

Diclofenac is a pain reliever and an anti-inflammatory drug that is usually used as an analgesic in rheumatologic diseases and in general practice. Common adverse effects of diclofenac include gastrointestinal symptoms, peptic ulcer, and bleeding. Although it is usually administered at 75–150 mg daily, diclofenac was given to the patient described herein at a total dose of 900 mg/day. BMN is characterized by necrosis of the medullary stroma and myeloid tissues in large areas of the bone marrow, probably because of failure of the microcirculation. Toxic effects of chemotherapy, microvascular infarction, tumor necrosis factor, and thrombosis were blamed for the pathophysiology of BMN [1]. We believe that BMN in our patient may be due to the inflammation created by the cytokine



**Fig. 1. BM biopsy showing eosinophilic silhouette of bone marrow between bone trabeculae (hematoxylin/eosin, original magnification 100x). [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]**

release because of overdosed diclofenac. Although the prognosis of patients with BMN is usually poor, diseases causing BMN and the age and grade of the necrosis are the main indicators of prognosis [5]. In our case, he was young and had no other diseases. In conclusion, BMN is a rare clinical condition. It should be kept in mind that some patients may have reversible BMN due to drugs and benign diseases.

ISMET AYDOĞDU<sup>1</sup>  
M. ALI ERKURT<sup>1</sup>  
ONUR OZHAN<sup>1</sup>  
EMIN KAYA<sup>1</sup>  
IRFAN KUKU<sup>1</sup>  
ECE YITMEN<sup>1</sup>  
N. ENGIN AYDIN<sup>2</sup>

<sup>1</sup>Inonu University, School of Medicine, Department of Hematology, Malatya, Turkey

<sup>2</sup>Inonu University, School of Medicine, Department Pathology, Malatya, Turkey

Published online in Wiley InterScience ([www.interscience.wiley.com](http://www.interscience.wiley.com)).

DOI: 10.1002/ajh.20536

REFERENCES

- Janssens AM, Offner FC, van Hove WZ. Bone marrow necrosis. *Cancer* 2000;88:1769–1780.
- Argon D, Cetiner M, Adiguzel C, et al. Bone marrow necrosis in a patient with non-Hodgkin lymphoma. *Turk J Haematol* 2004;21:97–100.
- Paydas S, Ergin M, Baslamisli F, et al. Bone marrow necrosis: clinicopathologic analysis of 20 cases and review of the literature. *Am J Hematol* 2002;70:300–305.
- Maisel D, Lim JY, Pollock WJ, Liu PI. Bone marrow necrosis: an entity often overlooked. *Ann Clin Lab Sci* 1998;18:109–115.
- Bashawri L, Satti MB. Bone marrow necrosis: report of five cases and review of the literature. *Ann Saudi Med* 2000;1:78–82.

**Liposomal Amphotericin B as Antifungal Prophylaxis in Bone Marrow Transplant Patients**

*To the Editor:* Fungal infections are common complications in immunosuppressed patients due to bone marrow transplantation (BMT). To reduce the incidence and severity of these types of infection, several prophylactic regimens with antifungal agents have been used in this population. We note that liposomal amphotericin has been used by a proportion of clinicians as a prophylactic regimen against fungal infections in BMT patients in hospitals in various countries.

However, in our search of the literature regarding this issue, we could not find evidence to support this approach. We searched for randomized controlled trials (RCTs), published in journals indexed in PubMed, Current Contents Connect, and Cochrane Library of Controlled Trials, that examined the incidence and severity of fungal infections, as well as colonization with fungi in BMT patients.

We identified only three RCTs dealing with this issue [1–3]. Table I shows characteristics of the RCTs, such as study design, number of enrolled patients, dosage of liposomal amphotericin B prophylaxis used, as well as several outcomes including mortality, fungal colonization on enrollment and after prophylactic treatment, and proven and suspected fungal infections. In one of the reviewed studies, the majority of the patients were enrolled in a nonrandomized fashion [1]. Subsequently, we combined data from the remaining two studies (that were RCTs) using the methodology of meta-analysis.

Results from the available RCTs do not provide support for the use of liposomal amphotericin B in BMT patients. Specifically, there was no difference in the occurrence of proven fungal infections (OR = 1.03, 95% CI 0.03–37.55), suspected fungal infections (OR = 0.83, 95% CI 0.47–1.45), or mortality (OR = 1.33, 95% CI 0.71–2.52) between patients using liposomal amphotericin B prophylaxis or placebo. Fungal colonization after treatment was less common in the liposomal amphotericin B prophylaxis group compared to placebo (OR = 0.39, 95% CI 0.21–0.72), however.

The results of this limited analysis do not support the practice of providing low-dose liposomal amphotericin B for antifungal prophylaxis in BMT patients. There may be some evidence for the use of azoles in a specific patient population, namely, in allogeneic BMT recipients [4,5]. Subsequently, prophylactic liposomal amphotericin B should probably be avoided in BMT patients, due to the lack of supporting evidence for its use, its high cost, and the common side effects associated with this form of antifungal prophylaxis. However, a large RCT is urgently needed to provide a definitive answer regarding the appropriate antifungal prophylaxis in BMT patients, given the fact that a large proportion of patients undergo allogeneic bone marrow transplantation and the concerns that fluconazole, an azole frequently used in BMT patients as antifungal prophylaxis, is a drug without activity against filamentous fungi, such as *Aspergillus*, a pathogen that causes considerable morbidity and mortality in this population.

**MATTHEW E. FALAGAS<sup>1,2,3</sup>**  
**KONSTANTINOS Z. VARDAKAS<sup>1</sup>**

<sup>1</sup>*Alfa Institute of Biomedical Sciences (AIBS), Athens, Greece*

<sup>2</sup>*Department of Medicine, “Henry Dunant” Hospital, Athens, Greece*

<sup>3</sup>*Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts*

*Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ajh.20535*

**REFERENCES**

- Uhlenbrock S, Zimmermann M, Fegeler W, Jurgens H, Ritter J. Liposomal amphotericin B for prophylaxis of invasive fungal infections in high-risk paediatric patients with chemotherapy-related neutropenia: interim analysis of a prospective study. *Mycoses* 2001;44(11–12):455–463.
- Kelsey SM, Goldman JM, McCann S, et al. Liposomal amphotericin (AmBisome) in the prophylaxis of fungal infections in neutropenic patients: a randomised, double-blind, placebo-controlled study. *Bone Marrow Transplant* 1999;23:163–168.
- Tollemar J, Ringden O, Andersson S, Sundberg B, Ljungman P, Tyden G. Randomized double-blind study of liposomal amphotericin B (Ambisome) prophylaxis of inva-

**TABLE I. Characteristics and Outcomes of the Randomized Controlled Trials (RCTs) Included in Our Analysis\***

First author [ref. no.]	Year of publication	Study type	Population	Intravenous liposomal amphotericin B prophylaxis	ITT patients (total)	Evaluate allogeneic BMT patients	Evaluate autologous BMT patients	Fungal colonization after treatment	Mortality (0%) (fungal related)	Proven fungal infections	Suspected fungal infections
Uhlenbrock [1]	2001	Controlled trial	BMT/ leukemia	1 mg/kg three times/week	29	0 vs. 0	4 vs. 6	ND	0/16 (0%) vs. 0/13 (0%)	ND	ND
Kelsey [2]	1999	DB placebo RCT	BMT	2 mg/kg three times/week	170	42 vs. 43	21 vs. 29	15/74 (20%) vs. 35/87 (40%)	11/74 (15%) vs. 12/87 (14%)	0/74 (0%) vs. 3/87 (4%)	31/74 (42%) vs. 40/87 (46%)
Tollemar [3]	1993	DB placebo RCT	BMT	1 mg/kg/day	84	30 vs. 33	6 vs. 7	8/24 (33%) vs. 18/29 (62%)	17/36 (47%) vs. 14/40 (35%)	1/36 (3%) vs. 3/40 (8%)	5/36 (14%) vs. 7/40 (18%)

\*Outcome related data are presented for the clinically evaluable patients who received prophylactic liposomal amphotericin B and placebo, respectively. ND, not described; ITT, intention to treat; DB, double blind.

sive fungal infections in bone marrow transplant recipients. *Bone Marrow Transplant* 1993;12:577–582.

- Bow EJ, Laverdiere M, Lussier N, Rotstein C, Cheang MS, Ioannou S. Antifungal prophylaxis for severely neutropenic chemotherapy recipients: a meta analysis of randomized-controlled clinical trials. *Cancer* 2002;94:3230–3246.
- Kanda Y, Yamamoto R, Chizuka A, et al. Prophylactic action of oral fluconazole against fungal infection in neutropenic patients. A meta-analysis of 16 randomized, controlled trials. *Cancer* 2000;89:1611–1625.

### Acute Promyelocytic Leukemia in an HIV-Infected Patient: A Case Report

*To the Editor:* A 46-year-old female was HIV-1 positive since 2001. She was treated with nelfinavir and lamivudine, but due to lipodystrophic syndrome, her medication was changed to efavirenz, achieving a CD4<sup>+</sup> count > 500 cell/ $\mu$ L and HIV-RNA < 50 copies/mL. In June 2003, she stopped highly active anti-retroviral therapy (HAART) due to a gradual decrease in hemoglobin and platelets. One week before admission, she had continuous–remittent fever. On admission, her hemoglobin was at 6.7 g/dL, WBC at 5,090/ $\mu$ L (8.4% neutrophils, 55% promyelocytes), and platelets 1,500/ $\mu$ L. Fibrinogen was 167 mg/dL, D-dimer 5,550 ng/mL, and LDH 796 UI/L. Urinalysis, EKG, and chest X ray were normal. Physical examination revealed small laterocervical and inguinal lymphonodes. Bone marrow aspiration showed a massive infiltration of promyelocytes with multiple Auer bodies and strong positivity to peroxidase reaction. These cells expressed CD33 (80%), CD13 (78%), CD 71 (73%), and CD117 (75%). PML/RAR $\alpha$  rearrangement (bc1) was detected by PCR analysis, and cytogenetic analysis was not valuable. Diagnosis of APL was made.

The patient started induction therapy according to the GIMEMA AIDA 2000 protocol, including ATRA and idarubicin with prednisone as prophylaxes for ATRA syndrome. Once a toxic effect of HAART on the bone marrow was excluded, she was started on induction chemotherapy a new anti-retroviral combination, including efavirenz, tenofovir–dipivoxil, and lamivudine, which caused a rapid decline of plasma HIV-1 RNA to below 50 copies/mL. By day 30 after induction chemotherapy, she entered complete remission (CR). Because of her HIV infection, she was consolidated according to the low-risk group in the GIMEMA AIDA 2000 protocol with three courses including ATRA, idarubicin, and mitoxantrone, and started oral maintenance with ATRA for 15 days every 3 months, methotrexate once weekly, and 6-mercaptopurine daily. The patient is now in molecular CR on maintenance therapy 21 months after diagnosis and is on HAART with HIV-RNA < 50 copies/mL, CD4<sup>+</sup> count 125 cells/ $\mu$ L, and a CD4/CD8 ratio of 0.3.

The occurrence of acute myeloid leukemia (AML) has been reported in HIV patients, with predominance of FAB M2, M4, and M5 types [1]. Four cases of promyelocytic leukemia (APL) in HIV infection have been reported. In three of these cases, HAART was not stated; in the fourth case [2], HAART was discontinued for a short period during induction chemotherapy.

In three cases, ATRA was used alone to induce CR, but just two patients achieved CR and they had been consolidated with anthracyclines and/or cytarabine [2,3]. One of the four patients was kept on maintenance without consolidation, as he relapsed and died after 303 days [4]. In one patient [5], “standard” induction therapy was used but failed to achieve CR.

Our patient tolerated chemotherapy and concurrent HAART well despite being the oldest patient reported with HIV and APL.

SERENA DE VITA<sup>1</sup>  
SILVIA DE MATTEIS<sup>1</sup>  
LUCA LAURENTI<sup>1</sup>  
FEDERICA SORA<sup>1</sup>  
MICHELA TARNANI<sup>1</sup>  
ANTONELLA CINGOLANI<sup>2</sup>  
SIMONA SICA<sup>1</sup>

<sup>1</sup>Istituto di Ematologia, Università Cattolica Sacro Cuore, Rome, Italy

<sup>2</sup>Clinica Malattie Infettive, Università Cattolica Sacro Cuore, Rome, Italy

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

DOI: 10.1002/ajh.20553

### REFERENCES

- Aboulafia DM, Meneses M, Ginsberg S, Siegel MS, Howard WW, Dezube BJ. Acute myeloid leukemia in patients infected with HIV-1. *AIDS* 2002;16(6):865–876.
- Kudva GC, Maliekal K, Richart JM, et al. Acute promyelocytic leukemia and HIV-1 infection: case report and review of the literature. *Am J Hematol* 2004;77(3): 287–290.
- Calvo R, Ribera JM, Battle M, et al. Acute promyelocytic leukemia in a HIV seropositive patient. *Leuk Lymphoma* 1997;26(5–6):621–624.
- Sutton L, Guenel P, Tanguy ML, et al. Acute myeloid leukaemia in human immunodeficiency virus-infected adults: epidemiology, treatment feasibility and outcome. *Br J Haematol* 2001;112(4):900–908.
- Gatphoh ED, Zamzachin G, Devi SB, Punyabati P. AIDS related malignant disease at regional institute of medical sciences. *Indian J Pathol Microbiol* 2001;44(1):1–4.

### A Chinese Family With Hemophilia B Leyden Due to T→A Transition at Position +6 of the FIX Gene

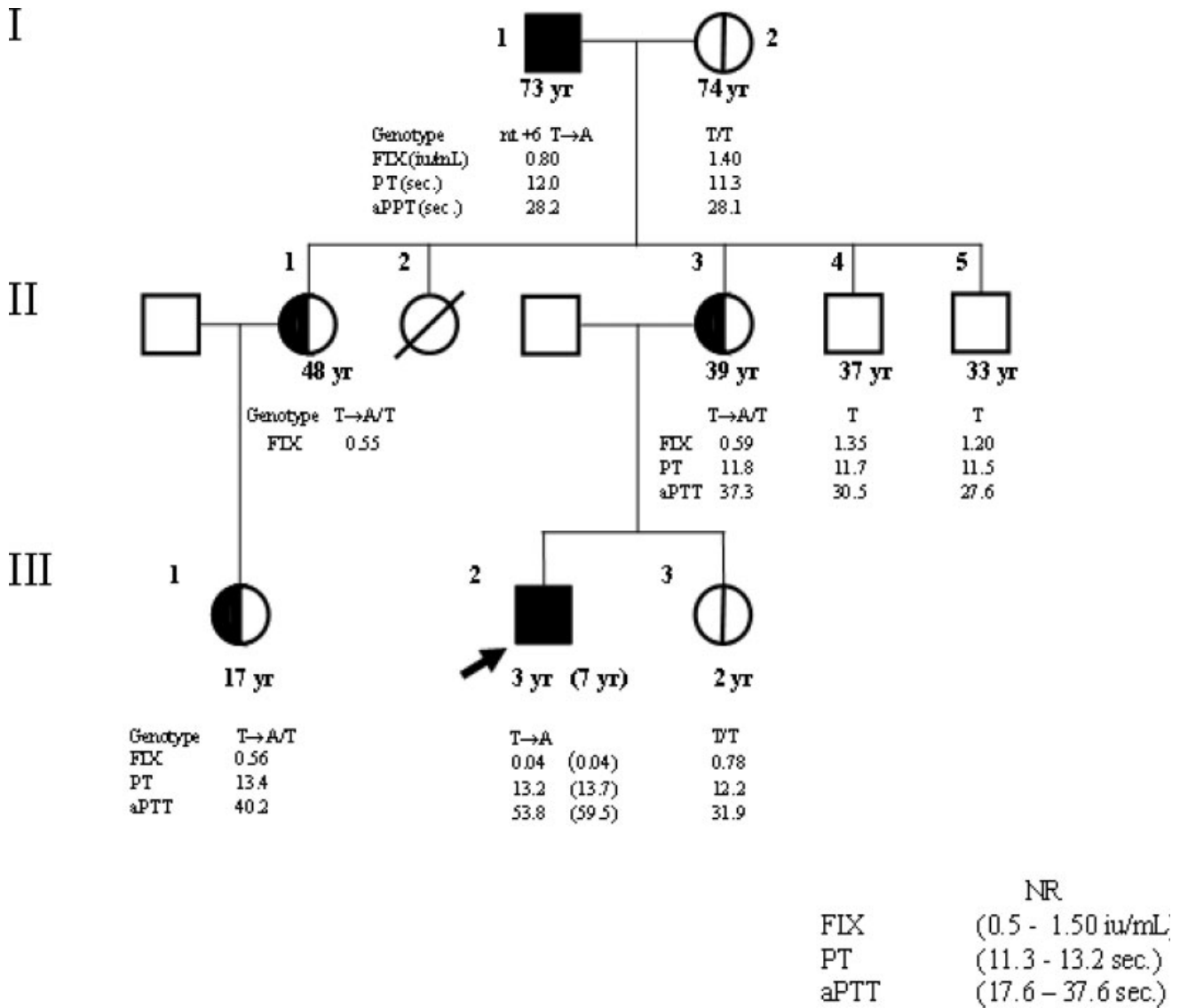
*To the Editor:* A 3-year-old Southern Chinese boy had persistent bleeding for 1 month from a tongue-biting injury. Evacuation of hematoma and repair of the tongue laceration were performed twice, initially under fresh-frozen plasma cover, which failed to stop bleeding, and subsequently with FIX replacement (upon diagnosis of hemophilia B, the FIX level was found to be 0.04 IU/mL). There was no family history of bleeding tendency. His maternal grandfather had no bleeding episodes in recent years.

The family tree, FIX genotype, FIX levels, PT, and aPTT of the patient and family members are shown (Fig. 1). Direct genomic sequencing of the patient's FIX gene revealed a promoter mutation, nt +6 T→A. The boy is currently 7 years old, and his FIX level is still 0.04 IU/mL. His maternal grandfather (73 years old), with FIX at 0.8 IU/mL, has the same gene defect. Sequencing of the FIX promoter confirmed his mother and maternal aunt as obligate carriers. His aunt's daughter had also inherited the defect, while his younger sister is normal.

Hemophilia B Leyden [1] is a group of point mutations within a 40-basepair (bp) region in the FIX promoter [nucleotide (nt) –26 to +13] encompassing the major transcription start sites. Patients have characteristic amelioration of disease phenotype after the onset of puberty, mediated by the action of testosterone on an androgen response element (ARE) in the promoter region [2]. Binding sites for transactivating factors, such as CCAAT/enhancer binding protein (C/EBP), hepatocyte nuclear factor 4 (HNF-4), and albumin D-site binding protein (DBP) [3,4], have also been identified in the region, such that disruption by a mutation would affect gene expression.

The numerous FIX Leyden mutations in Caucasians likely arose from common founder(s) or are recurrent mutations at CpG dinucleotides. To date, there is only a single Asian case from Thailand (nt +8 –T). We report herein the first Chinese patient with hemophilia B Leyden, carrying a nt +6 T→A defect. The clinical picture of him and his maternal grandfather with the same genetic defect conforms to the Leyden phenotype, because the grandfather now has a relatively normal FIX level (0.8 IU/mL) and no recent bleeding episodes.

This mutation at nt +6 affects the first (site 1: nt +1 to +18) of five known cis-acting sites in the FIX promoter [4]. Other C/EBP and DBP binding sites at nt –77 to –99 (site 4) and nt –199 to –219 (site 5) were intact and thought to exhibit the strongest transcriptional activation [5]. At puberty, DBP binding factor is induced and acts synergistically with C/EBP to increase FIX expression [4]. Furthermore, for this nt +6 mutation, testosterone action on the normal ARE site (site 3: nt –36 to nt –22) will have an additive effect, thus raising the



**Fig. 1.** Southern Chinese family with hemophilia B Leyden (nt +6 T→A). The genotype (determined by direct genomic sequencing), FIX level, PT, and aPTT of all the members studied are shown. The husbands of the two daughters did not participate in the study. The proband at 7 years of age has an FIX level of 0.04 IU/mL. Normal ranges are as follows: FIX, 0.5–1.50 IU/mL; PT, 11.3–13.2 sec; aPTT, 17.6–37.6 sec. Squares symbolize male family members, circles symbolize female family members.

FIX level after puberty [3]. These would account for the normal FIX level in the maternal grandfather of this family.

Confirmation of hemophilia B Leyden is important for subsequent genetic counseling of carrier females. The prognosis of hemophilia B Leyden is better than for classical hemophilia B. Even if prenatal diagnosis shows an affected male fetus, mothers need not contemplate termination of the pregnancy.

<sup>3</sup>Department of Pathology, Queen Mary Hospital, Hong Kong Presented at the 29<sup>th</sup> World Congress of the International Society of Hematology, Seoul, Korea, 2002.

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ajh.20534

REFERENCES

1. Veltkamp JJ, Meilof J, Remmelts HG, Van Der Vlerk D, Loeliger EA. Another genetic variant of haemophilia B: haemophilia B Leyden. *Scand J Haematol* 1970;7:82–90.
2. Crossley M, Ludwig M, Stowell KM, De Vos P, Olek K, Brownlee GG. Recovery from hemophilia B Leyden: an androgen-responsive element in the factor IX promoter. *Science* 1992;257:377–379.
3. Crossley M, Brownlee GG. Disruption of a C/EBP binding site in the factor IX promoter is associated with haemophilia B. *Nature* 1990;345:444–446.

VIVIAN CHAN<sup>1</sup>  
SHAU-YIN HA<sup>2</sup>  
PATRICK AU<sup>1</sup>  
CLARENCE LAM<sup>3</sup>  
TAI-KWONG CHAN<sup>1</sup>

<sup>1</sup>University Department of Medicine, Queen Mary Hospital, Hong Kong

<sup>2</sup>Department of Paediatrics, Queen Mary Hospital, Hong Kong



## 302 Letters and Correspondence

- Picketts DJ, Lillicrap DP, Mueller CR. Synergy between transcription factors DBP and C/EBP compensates for a haemophilia B Leyden factor IX mutation. *Nat Genet* 1993;3:175–179.
- Picketts DJ, Mueller CR, Lillicrap D. Transcriptional control of the factor IX gene: analysis of five cis-acting elements and the deleterious effects of naturally occurring hemophilia B Leyden mutations. *Blood* 1994;84:2992–3000.

### Fulminant Hepatitis B Following Rituximab Therapy in a Patient With Evans Syndrome and Large B-Cell Lymphoma

*To the Editor:* Reports of hepatitis B virus (HBV) reactivation following treatment with rituximab have raised concern regarding use of this medication in patients who previously had a non-replicating form of HBV [1,2]. We present a patient with lymphoma who received rituximab therapy and developed fulminant hepatitis B with a fatal course 3 months later.

A 21-year-old man with stage IV large B-cell non-Hodgkin lymphoma (NHL) had been treated with 3 courses of CHOP-R between July and September of 2003. The patient had a history of Evans syndrome since 5 years of age, which had been stable without a need for immunosuppressive treatment for the previous 6 years. His last transfusion was in 1995. The patient's hepatitis B surface antigen (HBsAg), surface antibody, and hepatitis B core antibody (HBcAb) were negative in 1996. His hepatitis B status immediately prior to the institution of chemotherapy is unknown. Four weeks after the last course, on day 91 of the therapy, the patient presented with cough and malaise and was admitted because of pneumonia with *Respiratory syncytial virus* and rapidly rising liver aminotransferase and bilirubin levels. Viral serologic investigations excluded hepatitis A and C. HBsAg was positive, and the hepatitis B core IgM was negative. A high load of HBV ( $>2 \times 10^8$  copies/mL) was detected with real-time PCR. Immunoglobulin levels from September 2003 were within normal limits, and at the time of the admission, only IgM level was slightly low (44 mg/dL). Hepatic function did not recover, and the patient lapsed into coma on the 4<sup>th</sup> day of admission. Treatment with lamivudine was started at this point; however, the patient succumbed to hepatic failure 15 days after admission on day 106 of therapy for lymphoma.

Reactivation of HBV in four patients with lymphoma following treatment with rituximab may prove fatal [2–4]. This information is available at the FDA's Medwatch website [3].

The negative core IgM in the presence of a positive HBsAg excludes a recent exposure to HBV, but it is possible the infection was acquired during therapy, and an IgM response could not be attained because of rituximab-induced B-cell depletion. His fatal outcome underscores the importance of screening for HBV infection prior to chemotherapy and/or rituximab therapy. Patients who are HBsAg-negative and HBcAb-positive, whose aminotransferases are elevated during or after treatment with rituximab, should have screening of qualitative HBV DNA as the HBsAg from mutant viruses may be negative despite active replication. Although no established guidelines yet exist for the optimal management of patients with lymphoma who are HBsAg-positive, recent reports suggest that lamivudine could be used in the prevention of HBV reactivation in lymphoma patients [4,5]. There is at least one case report in which antiviral therapy with lamivudine may have contributed to the successful outcome in a patient [5]. Prophylaxis with lamivudine or other antiviral agents might be considered to prevent the reactivation of HBV in HBsAg-positive patients prior to the initiation of chemotherapy or rituximab therapy.

BÜLENT ÖZGÖNENEL<sup>1</sup>  
DILIP MOONKA<sup>2</sup>  
SÜREYYA SAVAŞAN<sup>1</sup>

<sup>1</sup>Children's Hospital of Michigan, Hematology/Oncology, Wayne State University, Detroit, Michigan

<sup>2</sup>Division of Gastroenterology, Henry Ford Hospital, Detroit, Michigan  
Published online in Wiley InterScience (www.interscience.wiley.com).  
DOI: 10.1002/ajh.20540

*American Journal of Hematology* DOI 10.1002/ajh

## REFERENCES

- Westhoff TH, Jochimsen F, Schmittl A, et al. Fatal hepatitis B virus reactivation by an escape mutant following rituximab therapy. *Blood* 2003;102:1930.
- Hernandez JA, Diloy R, Salat D, del Rio N, Martinez X, Castellvi JM. Fulminant hepatitis subsequent to reactivation of precore mutant hepatitis B virus in a patient with lymphoma treated with chemotherapy and rituximab. *Haematologica* 2003;88: ECR22.
- <http://www.fda.gov/medwatch/SAFETY/2004/safety04.htm#Rituxan>.
- Hamaki T, Kami M, Kusumi E, et al. Prophylaxis of hepatitis B reactivation using lamivudine in a patient receiving rituximab. *Am J Hematol* 2001;68:292–294.
- Tsutsumi Y, Kawamura T, Saitoh S, et al. Hepatitis B virus reactivation in a case of non-Hodgkin's lymphoma treated with chemotherapy and rituximab: necessity of prophylaxis for hepatitis B virus reactivation in rituximab therapy. *Leuk Lymphoma* 2004;45:627–629.

### Hb D-Punjab [ $\beta$ 121 (GH4) Glu→Gln]/ $\beta^0$ -Thalassemia [IVSII.1(G→A)] in Two Cases From an Iranian Family: First Report

*To the Editor:* Hb D-Punjab [ $\beta$  121 (GH4) Glu→Gln], also known as Hb D-Los Angeles, is a  $\beta$ -chain structural variant that is prevalent in the Punjab of India and has been found in many parts of the world [1,2].

Carriers of Hb D-Punjab and/or even individuals homozygous for Hb D-Punjab are asymptomatic. Compound heterozygous state of Hb D-Punjab with  $\beta^0$ -thalassemia is very rare [3] and is characterized by a mild-to-moderate hemolytic anemia [2].

A family composed of 4 members—the father, a carrier for Hb D-Punjab; the mother, a minor  $\beta$ -thalassemia; and their two sons, both with Hb D-Punjab/ $\beta^0$ -thalassemia—referred to the clinic of Kermanshah University of Medical Sciences were studied. Hematological indices were determined by using an automated Coulter cell Counter (Hialeah, FL), hemoglobin electrophoresis, and alkali denaturation methods.

DNA was extracted from whole blood by the phenol-chloroform extraction method. The presence of Hb D-Punjab in cases was confirmed by polymerase chain reaction (PCR) amplification followed by digestion with *EcoRI* [4]. The presence of IVS II.1(G→A) mutation on  $\beta$ -thalassemia chromosomes was confirmed by amplification refractory mutation system (ARMS) techniques [4]. Eight polymorphic restriction enzyme sites through the  $\beta$ -globin gene cluster in each DNA sample were studied using PCR-RFLP procedures [4]. The polymorphic restriction sites defined were as follows: the *HindII* site 5' to the  $\epsilon$ -globin gene, the *XmnI* site 5' to the  $G\gamma$ -globin gene, the *HindIII* sites within the  $G\gamma$ - and  $A\gamma$ -globin genes, the *HindII* sites within and 3' to the  $\psi\beta$ -globin gene, the *AvaII* site within the  $\beta$ -globin gene, and the *BamHI* site 3' to the  $\beta$ -globin gene. The  $\beta$ -globin haplotypes associated with Hb D-Punjab or  $\beta$ -thalassemia genes were determined by the family linkage analysis.

Hematological and molecular characteristics of studied family are depicted in Table I. The hematological data of two brothers carrying the  $\beta^{D-Punjab}$  gene and the IVSII.1(G→A) mutation indicates significant hypochromia and microcytosis [MCV (56 and 61.0 fL, respectively) and MCH (19.5 and 20.1 pg, respectively)], significant elevation of Hb D-Punjab (76.7% and 78.1%, respectively), and marked increase of Hb F (18.1% and 16.6%, respectively) in the presence of the *XmnI* polymorphic site 5' to the  $G\gamma$  gene as compared to Hb F levels (around 2% and 3–4%) that have been reported for other patients with Hb D-Punjab/ $\beta^0$ -thalassemia [2,3]. Haplotype I, the most common haplotype worldwide [1], was linked to  $\beta^{D-Punjab}$  chromosomes and was associated with the absence of the *XmnI* polymorphic site. The  $\beta$ -thalassemia chromosomes are linked to an atypical haplotype [– + + + + –]. The IVS.II.1(G→A) mutation was in linkage with the presence of an *XmnI* site. The association of an IVS.II.1(G→A) mutation in the presence of an *XmnI* polymorphic site has been suggested [5]. However, the haplotype background modulates the induction of the *XmnI* site on Hb F production [5].

This report demonstrates Hb D-Punjab to be a benign structural variant of Hb, which, in combination with  $\beta^0$ -thalassemia, produces a minor  $\beta$ -thalassemia picture with moderate anemia in the presence of significantly elevated Hb F.

**TABLE I. Hematological and Molecular Data of a Family Affected With Hb D-Punjab/ $\beta^0$ -Thalassemia**

Parameters	Mother	Son 1	Son 2	Father
Age (years)	32	3	10	39
RBC ( $10^{12}/L$ )	5.8	6.9	6.2	5.6
Hb (g/dL)	9	13.4	12.1	16.8
PCV (%)	30.1	38.4	36.5	48.6
MCV (fL)	52.0	56.0	61.0	83.1
MCH (pg)	15.5	19.5	20.1	28.7
MCHC (g/dL)	29.9	34.9	33.0	34.6
Hb D-Punjab (%)	—	76.7	78.1	42.6
Hb A <sub>2</sub> (%)	5.6	5.2	5.3	2.9
Hb F (%)	4.5	18.1	16.6	1.95
$\beta$ -Chain genotype	$\beta^A/\beta^0$ [IVSII.1. (G:A)]	$\beta^{D-punjab}/\beta^0$ [IVSII.1. (G:A)]	$\beta^{D-punjab}/\beta^0$ [IVSII.1. (G:A)]	$\beta^A/\beta^{D-punjab}$
$\beta$ -Chain haplotype <sup>a</sup>	III/[- + + + + -]	I/[- + + + + -]	I/[- + + + + -]	I/[- + + - + + -]
XmnI site 5' to G $\gamma$ gene	+/+	+/-	+/-	-/-

<sup>a</sup>The order of the sites 5' to 3' is as follows: HindII 5' to the  $\epsilon$ -globin gene, HindIII within IVS 2 of the G $\gamma$  and A $\gamma$ -globin genes, HindII within and 3' to pseudo-beta ( $\psi\beta$ )-globin gene, AvaII within IVS 2 of the  $\beta$ -globin gene, and BamHI 3' to the  $\beta$ -globin gene.

ZOHREH RAHIMI<sup>1</sup>  
 REZA AKRAMIPOUR<sup>2</sup>  
 SHAHLA KORANI<sup>1</sup>  
 RONALD L. NAGEL<sup>3</sup>

<sup>1</sup>Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

<sup>2</sup>Department of Pediatrics, Division of Hematology, Kermanshah University of Medical Sciences, Kermanshah, Iran

<sup>3</sup>Department of Medicine, Division of Hematology; Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ajh.20537

REFERENCES

- Fioretti G, De Angioletti M, Pagano G, et al. DNA polymorphisms associated with Hb D-Los Angeles [ $\beta$ 121 (GH4) Glu→Gln] in Southern Italy. Hemoglobin 1993;17:9–17.
- Perea FJ, Casas-Castaneda M, Villalobos-Arambula AR, et al. Hb D-Los Angeles associated with Hb S or  $\beta$ -thalassemia in four Mexican Mestizo families. Hemoglobin 1999;23:231–237.
- Adekile AD, Kazanetz EG, Leonova JY, Marouf R, Khmis A, Huisman TH. Co-inheritance of Hb D-Punjab (codon 121; GAA→CAA) and beta (0) thalassemia (IVS-II-1; G→A). J Pediatr Hematol Oncol 1996;18:151–153.
- Old JM. Hemoglobinopathies. In: Elles R, editor. Methods in molecular medicine: molecular diagnosis of genetic disease. Totowa, NJ: Humana Press, Inc.; 1996. p 169–183.
- Dedoussis GV, Mandilara GD, Boussiu M, Loutradis A. HbF production in beta thalassemia heterozygotes for the IVS-II-1 G→A beta (0)-globin mutation. Implication of the haplotype and the (G) gamma - 158 C→T mutation on the HbF level. Am J Hematol 2000;64:151–155.

**Near-Total Resolution of Multicentric Castleman Disease by Prolonged Treatment With Thalidomide**

To the Editor: We previously reported successful treatment of a patient with multicentric Castleman disease (MCD) with thalidomide [1]. At the time of initial diagnosis, the patient had extensive lymphadenopathy, ascites, a pericardial effusion, profound anemia, and thrombocytopenia. A biopsy of a lymph node was consistent with a diagnosis of Castleman disease. The patient

was initially treated with steroids alone, but her condition continued to deteriorate rapidly. Because systemic manifestations of Castleman disease are believed to be potentiated by cytokines, in particular IL-6, thalidomide treatment was initiated approximately 1 month after diagnosis. The patient showed dramatic improvement, with complete resolution of the cytopenias, ascites, and pericardial effusion after 2 months of treatment. The initial dose of 300 mg of thalidomide per day was decreased to 200 mg per day due to the development of mild peripheral neuropathy. After 40 months of continuous thalidomide therapy, the patient is asymptomatic and has returned to work full-time, and has no impairment of activity due to her residual mild neuropathy. The patient does, however, have persistent lymphadenopathy that has shown no substantial changes by CT scan, compared to previous studies.

Because of her excellent performance status, a second lymph node biopsy was performed to assess the need for continued thalidomide therapy. The biopsy showed nonspecific changes, however, and focal Castleman-like features, including minimal “onion skinning” and occasional atretic/sclerotic germinal centers (Fig. 1) were also observed. Because this second lymph node biopsy had focal features characteristic for at least minimal persistence of Castleman disease, the patient was advised to continue thalidomide therapy indefinitely.

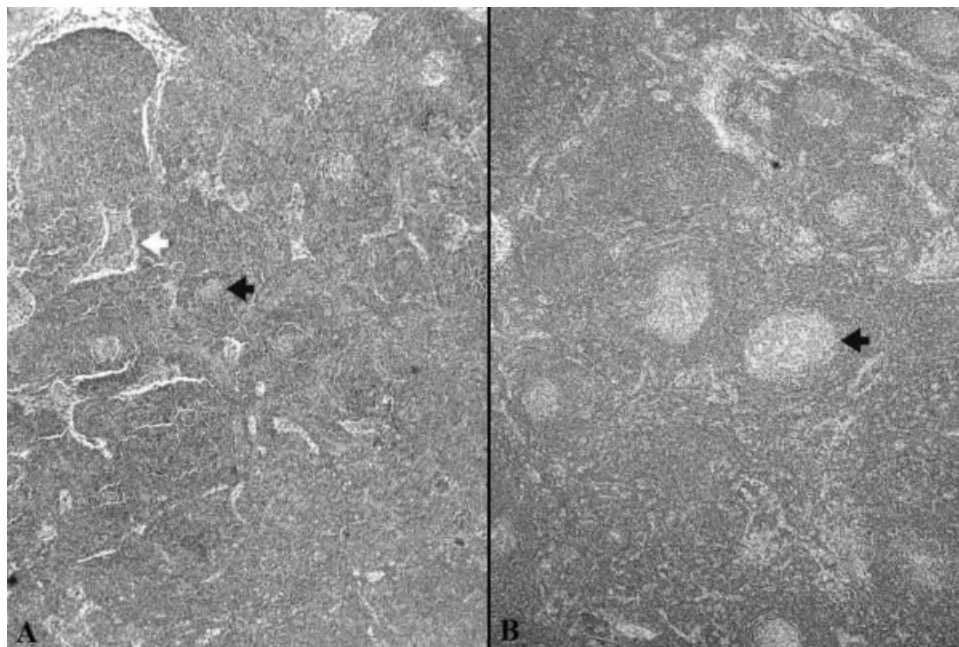
The data regarding potential side effects of long-term use of thalidomide is very scarce. To our knowledge, thalidomide does not carry many of the long-term side effects commonly associated with steroid or immunosuppressant therapy, such as osteoporosis, cataract formation, bone marrow toxicity, or increased susceptibility to infections. Fishman et al. reported long-term thalidomide treatment in a patient with Crohn disease, who tolerated 5 years of thalidomide treatment with good control of disease activity [2]. Similarly, our patient has received more than 40 months of continuous thalidomide therapy and has good control of her disease, with only minimal peripheral neuropathy and without impairment of her daily activity. Thus, for now, the risk-to-benefit ratio is in favor of continued thalidomide therapy in our patient.

CINDI R. STARKEY<sup>1</sup>  
 NANCY E. JOSTE<sup>1</sup>  
 FA-CHYI LEE<sup>2</sup>

<sup>1</sup>Department of Pathology, University of New Mexico, Albuquerque, New Mexico

<sup>2</sup>Department of Internal Medicine, Division of Hematology/Oncology, University of New Mexico, Albuquerque, New Mexico

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ajh.20538



**Fig. 1. (A) Initial lymph node biopsy demonstrates florid Castleman disease with atretic follicles (dark arrow) with hyalinized vessels (light arrow) and “onion skin” layering of lymphocytes. (B) Lymph node biopsy after prolonged thalidomide with near-total resolution of previous changes, now with predominantly normal follicles (dark arrow).**

#### REFERENCES

1. Lee FC, Merchant SH. Alleviation of systemic manifestation of multicentric Castleman's disease by thalidomide. *Am J Hematol* 2003;73:48–53.
2. Fishman SJ, Feins NR, D'Amato RJ, Folkman J. Thalidomide for Crohn's disease. *Gastroenterology* 2000;119:596–602 (Letter).

#### Response to Peginterferon Treatment in Hepatitis C Virus-Associated Splenic Lymphoma With Villous Lymphocytes

*To the Editor:* The prevalence of HCV infection is high patients with splenic lymphoma with villous lymphocytes (SLVL) [1,2]. There are studies showing the benefit of antiviral treatment with interferon and ribavirin in HCV-infected SLVL [3]. Herein we report SLVL in a patient with HCV-associated active viral hepatitis who recovered after antiviral treatment.

A 61-year-old male was admitted with complaints of abdominal pain and distention. His medical history was unremarkable. On physical examination, the spleen was palpable 18 cm below the costal margin. Complete blood cell count showed mild anemia (hemoglobin 11 g/dL) and thrombocytopenia (84,000/mm<sup>3</sup>). Peripheral blood smear showed a differential count of 85% lymphocytes, most of which had villous cytoplasmic projections. Liver and renal function tests were normal. Abdominal computed tomography revealed splenomegaly (265 mm), multiple intra-abdominal lymphadenopathies, heterogeneity of hepatic parenchyme, and enlargement of the portal vein. Anti-HCV antibody was positive as well as HCV RNA with 500,000 copies. Serum cryoglobulin was negative. Bone marrow biopsy was performed because of marked lymphocytosis and revealed 37% mature lymphocytes, some having villous projections. CD23, CD22, CD20, CD19, and CD45 were positive and CD5, CD103 and CD11c were negative on immunophenotyping of bone marrow. TRAP (tartrate-resistant phosphatase) stain for differential diagnosis with hairy-cell leukemia showed negative staining. With all of these data, we diagnosed the patient with SLVL. Treatment with pegylated interferon and ribavirin was initiated. He tolerated peginterferon and

ribavirin without difficulty. Six months after starting treatment, his serum HCV RNA was negative, the size of spleen had decreased to 130 mm, and the size of the lymphadenopathies had also decreased. Control bone marrow biopsy and peripheral blood smear were normal.

SLVL is a B-cell neoplasm with an indolent course. Patients usually present with moderate to massive splenomegaly and hepatomegaly. The frequent HCV positivity seen with SLVL supports the etiological linkage between HCV infection and lymphomagenesis. Pathophysiological studies suggest that the immunological response to HCV antigens induces clonal B-cell proliferation [2,3]. To our knowledge, the effects of antiviral treatment on SLVL is limited, but there are promising results; Hermine et al. treated HCV-positive SLVL patients with interferon, with and without ribavirin, and complete remission was achieved in eight of 9 patients [3,4]. Arcaini et al found that treatment with interferon can lead to regression of the lymphoma [5]. Prolonged treatment over 12 months may be needed because discontinuation of antiviral therapy can cause relapses. Antiviral therapy alone is not suggested for clinically aggressive disease.

It is well known that peginterferon is well-tolerated and has fewer side-effects than interferon. Moreover, in HCV-positive patients the response rate with peginterferon is higher than interferon. To our knowledge, this is the first case of SLVL in the literature treated with peginterferon, and a good response was achieved without significant side effects. In conclusion, we emphasize that, in patients with SLVL secondary to HCV infection, peginterferon may be a better alternative to interferon, considering its higher activity against HCV, better tolerability, and side-effect profile.

MEHMET KANBAY<sup>1</sup>  
HALDUN SELCUK<sup>2</sup>  
GURDEN GUR<sup>2</sup>  
NESLIHAN DAGLI<sup>3</sup>  
SEMA KARAKUS<sup>3</sup>  
UGUR YILMAZ<sup>2</sup>

<sup>1</sup>Baskent University School of Medicine Department of Internal Medicine, Ankara, Turkey

<sup>2</sup>Baskent University School of Medicine Department of Gastroenterology, Ankara, Turkey

<sup>3</sup>Baskent University School of Medicine Department of Hematology,  
Ankara, Turkey  
Published online in Wiley InterScience (www.interscience.wiley.com).  
DOI: 10.1002/ajh.20552

REFERENCES

1. Gisbert JP, Garcia-Buey L, Pajares JM, Moreno-Otero R. Prevalence of hepatitis C virus infection in B-cell non-Hodgkin's lymphoma: systematic review and meta-analysis. *Gastroenterology* 2003;125:1723–1732.
  2. Saadoun D, Suarez F, Lefrere F, et al. Splenic lymphoma with villous lymphocytes, associated with type II cryoglobulinemia and HCV infection: a new entity? *Blood* 2005;105: 74–76.
  3. Hermine O, Lefrere F, Bronowicki JP, et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. *N Engl J Med* 2002;347:89–94.
  4. Troussard X, Valensi F, Duchayne E, et al. Splenic lymphoma with villous lymphocytes: clinical presentation, biology and prognostic factors in a series of 100 patients. Groupe Français d'Hematologie Cellulaire (GFHC). *Br J Haematol* 1996;93:731–736.
  5. Arcaini L, Pauli M, Boveri E, et al. Splenic and nodal marginal zone lymphomas are indolent disorders at high hepatitis C virus seroprevalence with distinct presenting features but similar morphologic and phenotypic profiles. *Cancer* 2004;100(1):114–121.
-