Life threatening steroid-resistant autoimmune anemia successfully treated with rituximab: A case report

To the Editor: A 17-year-old woman was admitted for asthenia and increasing dyspnea. She had had an immune thrombocytopenia treated with intravenous immunoglobulins and corticosteroids but she had no treatment for 5 years.

The examination revealed only pallor and dyspnea to minor exercise. Laboratory investigations showed auto-immune hemolytic anemia, with: hemoglobin 50 g/L, reticulocytes 130 G/L, increased lactate deshydrogenase (1526 U/L) and unconjugated bilirubinemia (195 μmol/L), and low haptoglobin level (0.09 g/L). The direct antiglobulin test was positive (anti-IgG+++, anti-C3d++). Platelets level was 190 G/L, leukocytes 5.5 G/L. C reactive protein was 26 g/L. Bacteriological tests and virus serologies were negative. Antinuclear antibodies were 1/80.

She received three pulses of intravenous methylprednisolone (1 g/day then 1.5 mg/kg/day), and red blood cell (RBC) transfusions, but the hemoglobin level fell to 30 g/L (Fig. 1). Simultaneously, she had several lypothymias, electric signs of myocardial suffering and increased level of troponin I level (0.18 μg/L, $N < 0.05$) which led to transfer in intensive care unit. Despite methylprednisolone increased to 2 mg/kg/day, associated with massive (17) RBC transfusions, hemoglobin level stayed low around 50 G/L. Platelet count fell to 70 G/L, without hemorrhagic syndrome nor consommation coagulopathy. Bone marrow aspirate demonstrated the peripheral origin of the cytopenias.

Splenectomy was proposed but the patient refused. Mycophenolate mofetil (MMF) was added 7 days after hospital admission but because of the long action delay of MMF, rituximab was added, at the dose of 375 mg/m^2 weekly.

The treatment was well tolerated. Hemoglobin level rose up and reached 120 G/L at the fourth course of rituximab, with normalization of platelet count and hemolysis parameters (Fig. 1). No need of RBC was noted after the second perfusion.

Corticosteroids were rapidly tapered, as the AIHA was considered to be corticosteroid-resistant and MMF was maintained 1 year but the patient stopped it deliberately. Two years after the onset, the patient is well with no treatment.

The first-line therapy in AIHA is usually corticosteroids, but relapses are common. Immunosuppressive agents (ciclosporin, MMF, azathioprine), or immunoglobulins have been used in refractory cases but splenectomy remains the most effective alternative therapy for chronic refractory autoimmune cytopenia or emergency situation [1].

Rituximab is a chimeric monoclonal antibody targeting CD20 antigen on the surface of B lymphocytes, successfully used in malignant lymphoproliferative disorders and autoimmune diseases. The main side effects are infusion-related toxicities. Its efficacy in refractory immune thrombocytopenia has been reported [2], but data in warm AIHA are limited [3,4] and focus on chronic refractory AIHA. To our knowledge, life-saving use of anti-CD20 monoclonal antibody has been described in only one case of systemic lupus erythematosus with severe warm AIHA, refractory to various treatments [5].

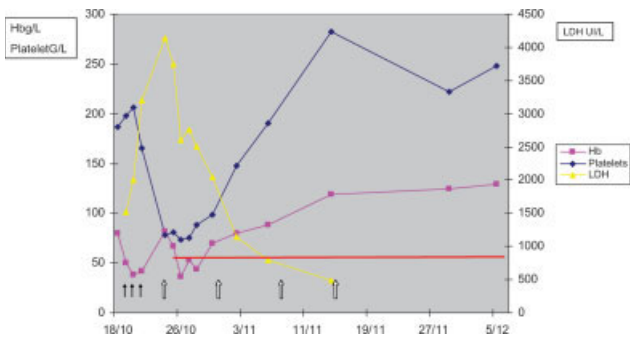

Fig. 1. Evolution of Hb, platelets, and LDH levels with treatment. ↑, methylprednisolone bolus, course (600 mg each, once weekly); ⇧, Rituximab; —, MMF (denoted in red). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

In this case, we used rituximab because of the worsening of the patient's condition despite corticosteroids, the disapproval about splenectomy, the action delay of MMF, and the life threatening situation. Rituximab seemed to be the treatment with the shortest action delay.

Rituximab should be considered as a life saving therapy, in corticosteroid resistant AIHA.

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Trisomy 21 in patients with acute leukemia

To the editor: From January 1996 to November 2007, a total of 2,273 patients with newly diagnosed acute leukemia (AL) were registered on Institutional Cytogenetic Laboratory, The First Affiliated Hospital, Zhejiang University College of Medicine. Cytogenetic studies were successfully performed on 2,106 (93%) consecutive patients, including 551 acute lymphoblastic leukemia (ALL) cases, 1,353 acute myeloid leukemia(AML) cases, and 202 others. The cytogenetic results were reviewed. Forty one of the 2,106 patients (1.9%) had acquired trisomy 21 (+21), including 11 with ALL (2.0% of all ALL), 25 with AML (1.8% of all AML), and 5 with others. Twenty one of the 41 patients (51%) had +21 as a sole abnormality, comprising 6 ALL (1.1%), 12 AML (0.9%,) and 3 others. The most frequent additional abnormalities were as follow: high hyperdiploidy (50 chromosomes) in eight (20%), +22 in four (10%), 5 and/or 7 in three (7%), and t(15;17) in two (5%). A trisomy 21 was considered to be acquired if the patient lacked phenotypic characteristics of Down syndrome (DS). The charts of 16 inpatients, including 9 patients with +21 as a sole abnormality and 7 patients with other additional abnormalities, were further reviewed for clinical presentation, immunophenotype, treatment, and clinical outcome. All of the other patients were either treated in local hospitals or gave up treatment and were not available for detailed study.

Sixteen inpatients with acquired +21 were admitted to our hospital from January 1996 to November 2007, including six ALL patients (two with T-ALL and four with pre-B-ALL) and 10 AML patients (two with M1, one with M3, six with M5, and one with M6). The median age was 30 years (range 13–61

TABLE I. Clinical Findings of the 16 Inpatients

Patient no.	Age/sex	Diagnosis	Presentation	WBC (10^9/l)	PB/BM blast cell (%)	Plt (10^9/l)	Hb (g/l)	Karyotype	Outcome	OS (months)
1	60 years/M	T-ALL	Lymphadenectasis	3.5	12/50	175	101	47,XY,+21[11]	ST	8
2	59 years/M	Pre-B-ALL	Gingiva bleeding	59.8	42/89	57	120	47,XY,+21[2]/46,XY[18]	CR	–
3	43 years/M	T-ALL	Pain and fever	1.0	–/58	25	72	47,XX,+21[7]/46,XX[3]	CR	15
4	30 years/M	AML-M1	Fever	92.0	–/95	–	134	47,XY,+21[7]/46,XY[3]	CR	–
5	17 years/F	AML-M5	Fever	88.7	86/87.5	17	84	47,XX,+21[7]/46,XX[4]	ST	12
6	61 years/F	AML-M5	Debilitation and fever	7.7	10/68.5	215	59	47,XX,+21[2]/46,XX[18]	NR	3
7	60 years/M	AML-M5	Debilitation and fever	9.5	13/88.5	13	85	47,XY,+21[10]	CR	12
8	57 years/F	AML-M5	Fever	11.6	12.5/85	148	42	47,XX,+21[9]/46,XX[1]	CR	13
9	14 years/F	AML-M6	Fever and epistaxis	3.2	46/54	103	43	47,XX,+21[9]/46,XX[1]	NR	13 (alive)
10	16 years/M	Pre-B-ALL	Fever	0.9	–/73	82	134	56,XXYY,add(1)(q44),+4, +6,+9,+10,+14,+18,+21, +22[5]/46,XY[15]	CR	–
11	34 years/F	Pre-B-ALL	Debilitation and chest pain	57.1	81/87.5	55	60	48,XX,t(9;22)(q34;q11), add(14)(q32),+21×2[4]/ 48,sl,i(7q)[2]/49,sdl, +22[2]/46,XX[2]	NR	3
12	27 years/F	Pre-B-ALL	Lymphadenectasis	39.3	14/92	11	98	47,XX,+4,der(19)t(1;19) (q32;p13)[2]/48, idem,+21[13]	CR	19
13	29 years/F	AML-M1	Fever	52.7	80/92	–	86	46,XX,del(6)(q22),add(10) (p15),del(11)(q23),16, 19,+21,+22[1]/47,idem,x, +4,+6q-[2]/49,idem,+21, +22[1]/49,idem,+19,22[2]/ 48,idem,+x,8,19[1]/49, idem,+21,+22[1]/49,idem,+ 19,22[2]/48,idem,+x,8, 19[1]/49,idem,+16,+19, 21[1]/48,idem,16[1]/ 47,idem,22[1]	CR	9
14	13 years/M	AML-M3	–	–	–	–	–	46,XX,t(15;17)(q22;q12)[5]/ 47,XX,t(15;17)(q22;q12), +21[6]/45,XX,18[1]/45, XX,t(15;17),18[1]/46, XX,t(15;17),18,+21[1]/ 46,XX,18,+21[1]/46,XX[5]	CR	–
15	56 years/M	AML-M5	Debilitation	31	20/55.5	59	44	46,XY,t(3;3)(der(16)?(q22;q24) [8]/46,idem,8,+21[2]	NR	12
16	14 years/M	AML-M5	Lymphadenectasis	6.5	–/60.5	63	123	47,XY,+22[3]/48,XY,+21,+22[3]/ 46,XY,N[5]	CR	13

ST, supportive treatment; CR, complete remission; NR, no remission.

years). Clinical presentation, hematologic findings, and treatment outcome of the 16 AL patients were tabulated in Table I. The most common presentation was fever, followed by debilitation, lymphadenectasis, and bleeding. Hepatosplenomegaly was not detected in any of these patients. Their median WBC count at diagnosis was 11.6 $\times$ 10^9/l (range 0.9–92.0 $\times$ 10^9/l), median hemoglobin was 85 g/l (42–134 g/l), and median platelet count was 59 $\times$ 10^9/l (11–215 $\times$ 10^9/l). FISH analyses were performed on the 16 inpatients using cells stored in fixative. All of the patients had an extra AML1 signal in more than 0.5% of the cells analyzed, and in all the patients the three copies of AML1 were situated in separate chromosomes suggesting chromosome 21. Immunophenotypic results were available for 14 inpatients. All of the 6 ALL patients expressed HLA-DR (20%), of which the two patients with T-ALL (P1&3) were positive for CD3 and CD7, whereas the other patients with pre-B-ALL were positive for CD10 and CD19. All of the 8 AML patients expressed at least two of the following myeloid-associated markers: CD13, CD14, CD15, CD 33, and MPO. Three of them were positive for one lymphoid-associated marker: P5 for CD10, P6 for CD 19, and P16 for CD7. Other markers characteristic of the lymphoid lineage, including CD2 and CD22, were absent in AML patients. Of the 14 patients given chemotherapy, 10 achieved CR but recurred soon. The median OS was 12 months (range 3–19 months). Two patients (P3&16) received PBSCT after CR, but both developed graft-versus-host disease (GVHD) within 3 months and died.

Trisomy 21 is one of the five most frequent numerical abnormalities occurring in human neoplasms [1]. In AML, +21 is the second commonest trisomy [2]. Chromosome 21 is also frequently involved in trisomies or tetrasomies in hyperdiploid ALL [3]. However, in most cases, the extra chromosome 21 is present together with other numerical and/or structural changes. The incidence of acquired +21 as a sole abnormality was reported to be 0.3% in all patients with AML [4]. In most published reports, AML with sole +21 preferentially showed M2 or M4 phenotypes according to the FAB classification [5,6].

Trisomy 21 was present in 1.9% of our AL patients, but was the sole cytogenetic abnormality in 21 patients, representing 1.1% of all ALL and 0.9% of all AML. In most reports, acquired +21 was associated with poor prognosis [5–8], except for childhood ALL [3,9]. Our study confirmed this conclusion. In general, trisomy 21 may confirm a poor prognosis, either as the sole abnormality or with other additional aberrations.

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Incidence of hepatitis B reactivation following Rituximab therapy

To the Editor: Hepatitis B reactivation and acute hepatitis are increasingly recognized complications following Rituximab therapy [1–3]. Recently, Garcia et al. reported delayed hepatitis B reactivation in two patients with negative serology prior to Rituximab treatment [4]. However, little is known about the incidence of these complications and the influence of hepatitis B serology pattern on the outcomes following Rituximab therapy. To further study the incidence of hepatitis B reactivation with Rituximab, we reviewed the records of 456 consecutive patients treated with Rituximab at Henry Ford Hospital. We identified 32 patients with positive hepatitis B serology who received Rituximab alone (14) or with chemotherapy (18) for NHL (17), ITP (8), Waldenstrom macroglobulinemia (2), CLL (2), or autoimmune diseases (3). The patients were divided into four groups based on their hepatitis B serology prior to therapy as shown in Table I. Acute liver events were defined by acute elevation of liver enzymes, abnormal liver biopsy diagnostic of hepatitis or liver necrosis, hepatic encephalopathy, or demonstration of active viral DNA replication by PCR.

Five patients developed hepatic failure, 2/12 in Group A, 1/6 in Group B, 0/8 in Group C, and 2/6 in Group D. However, the patient in Group B had a prior history of allogeneic-related stem cell transplantation for mantle cell lymphoma. The overall incidence of hepatic failure (5/32) was 15.6%.

Four patients developed biochemical hepatitis with elevated liver enzymes, two in Group C, one in Group A, and one in Group B. Active hepatitis B viral replications occurred in two patients in group D with biochemical hepatitis. Hepatitis B viral replication and biochemical hepatitis were observed in (6/32) 19% of the patients. Overall, 11 of 32 patients developed acute liver events as described earlier with an overall incidence of 34%. Acute liver events occurred in 25, 33, 25, and 66% of the patients in Groups A, B, C, and D, respectively. Median duration of onset of acute liver events after Rituximab therapy was 6.2 months except for two patients who developed acute liver events 21 and 36 months later.

TABLE I. Incidence of Acute Liver Events Based on Hepatitis B Serology

Group	Number of patients	HBsAg	HBsAb	HBcAb	Acute liver events (%)
A	12	Negative	Positive	Positive	3 (25)
B	6	Negative	Negative	Positive	2 (33)
C	8	Negative	Not available	Positive	2 (25)
D	6	Positive	Variable[a]	Variable[b]	4 (66)

[a]HBsAb; four patients negative, one patient positive, and one patient not available.
[b]HBcAb; four patients positive and two patients negative.
HBsAg, hepatitis B surface antigen; HBsAb, hepatitis B surface antibody; HBcAb, hepatitis B core antibody.

In this retrospective review of single institution experience, one-third of the patients with positive hepatitis B serology developed acute liver events when treated with Rituximab alone or with chemotherapy. This correlation was more evident in patients with hepatitis B surface antigen (66% of these patients). Surprisingly, acute liver events as well occurred in 25% of patients with negative HBsAg and positive HBcAb. Hepatitis B surface antibody did not provide protection against acute liver events in HBcAb-positive patients with negative HBsAg.

Hepatitis B serology screening is advisable prior to Rituximab treatment. Although positive hepatitis B surface antigen has the strongest correlation with acute liver events in our cohort, Hepatitis B reactivation was as well observed in patients with negative HBsAg and positive HBcAb. Hepatitis B DNA viral load monitoring in addition to standard serology may be helpful in directing the prophylaxis during Rituximab therapy. Prospective controlled trials are needed to confirm these results.

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Intracranial plasmacytoma mimicking meningioma in a patient with Castleman's disease

To the Editor: A 61-year-old woman with a history of stable hyaline-vascular variant Castleman's disease presented for evaluation of an increasingly unstable gait with recurrent falls starting 1 week before presentation. Three months before she had experienced intermittent, bilateral blurry vision with headaches for 1 week that had spontaneously resolved. Physical examination revealed partial left-sided hemiparesis and an unstable gait. Her lymphadenopathy was unchanged since its detection 3 years ago. MRI of the head showed an extra-axial mass anterior and superior to the right frontal lobe extending as a prominent dural thickening to surround the entire right frontal lobe, presumed to be meningioma. CT scan of the chest showed stable enlarged multiple right axillary lymph nodes (see Fig. 1). The patient underwent craniotomy and partial resection of the mass. Histology revealed sheets of plasma cells with mild atypia and occasional mitotic figures. Immunohistochemistry demonstrated the plasma cells to be positive for CD 138 and CD 79a with kappa light chain restriction. Immunophenotyping with flow cytometry showed an abnormal plasma cell population expressing CD 45, CD 38, and partial CD 138 with kappa light chain restriction. Bone marrow biopsy showed 40% cellularity with trilineage maturation and mild plasmacytosis (11%). FISH studies using molecular probes for multiple myeloma were performed on the marrow specimen to detect trisomy of chromosomes

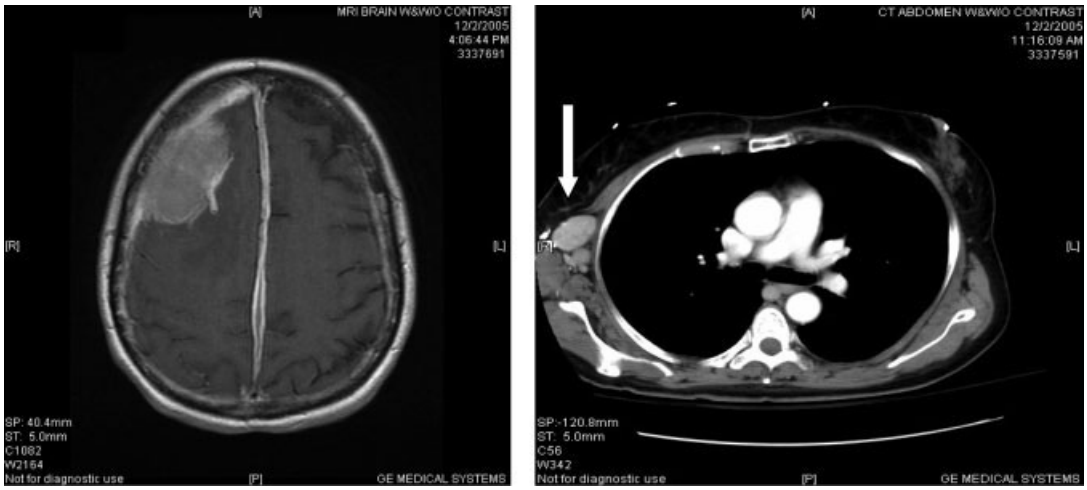

Fig. 1. (a) MRI brain showing an extra-axial dural based mass in the right frontal lobe. (b) CT scan of the chest showing the stable axillary lymphadenapathy of hyaline vascular Castleman's disease.

5, 9, and 15; deletion of *p*53; translocation involving IGH and CCND1 and deletion or monosomy of chromosome 13; all were negative. Cytogenetic analysis of the dural mass and bone marrow yielded normal karyotype. An M-spike of IgG kappa of 1.69 g/dL was detected in the serum. Twenty-four-hour urine contained 225 mg free kappa light chain, which was suspicious of monoclonal band on immunofixation. The serum immunoglobulin levels were IgG 2,252, IgA 523, and IgM 274 mg/dL. The hemoglobin level was 13.7 g/dL, serum creatinine 0.6 mg/dL, and calcium 9 mg/dL. The serum albumin level was 3.1 g/dL and β_2 microglobulin 1.5 mg/L. HIV antibody screen was nonreactive. A bone survey revealed no osteolytic lesions.

The tumor appeared to be a solitary extramedullary plasmacytoma, despite that the extent of marrow plasmacytosis met the criteria for multiple myeloma [1]. Intracranial plasmacytomas are rare and infrequently diagnosed by imaging due to their resemblance to the more common meningioma. On CT, both these lesions appear as a hyperdense, extra-axial mass with broad attachment to the dura that exhibit homogenous enhancement with contrast. In addition, similarities extend to patient demographics and presenting neurologic symptoms.

Our patient also carried a diagnosis of stable hyaline-vascular Castleman's disease. Multicentric Castleman's disease is most commonly seen in patients infected with HIV especially those with concomitant HHV-8 infections [2]. Plasma cells in Castleman's lesions are not uncommonly monoclonal immunophenotypically, suggesting a clonal plasma cell dyscrasia [3]. The possibility that a plasma cell tumor might rarely arise from Castleman's lesions is intriguing. A detailed molecular characterization, such as immunoglobulin gene rearrangement analysis, of the plasmacytoma and Castleman's lesions would be of special interest in delineating the relationship of these two lesions.

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The annexin-V assay reflects susceptibility to in vitro membrane damage in chronic lymphocytic leukemia and may overestimate cell death

To the Editor: Failure of apoptosis is believed to be a feature of chronic lymphocytic leukemia (CLL), but recent studies with nonradioactive stable isotopic labeling suggest apoptotic rates of up to 2% per day in some patients [1]. We aimed to measure apoptosis in freshly isolated CLL cells from 10 patients by flow cytometry using an annexin-V/propidium iodide (PI)-based assay [2]. The study was performed in accordance with the Helsinki Declaration and was approved by the Local Ethics Committee. There were five females and five males, aged 51–79 years. Mononuclear cells (MNC) enriched in CLL cells were isolated by centrifuging whole blood over a Ficoll gradient within 30 min of specimen collection. Annexin-V staining of freshly isolated 1×10^5 MNC was performed using previously described techniques [2]. Two negative controls were used. Control 1 consisted of MNC incubated with binding buffer without annexin-V and treated in an identical manner as the Test. Control 2 comprised of an MNC aliquot not treated with additional washes unlike Test and control 1 cells but mock incubated (i.e., without annexin-V or PI). Fluorescence was measured by flow cytometry using a Becton-Dickinson FACScan immediately after staining.

Two populations of cells were evident, based on forward-scatter (FSC) in the Test and Control 1 samples (Fig. 1A). In all, 26% $\pm$ 8% of freshly isolated cells bound annexin-V (Fig. 1B), restricted to the subpopulation of cells with smaller FSC (98% of cells within this population were annexin-V positive). Cells binding annexin-V were also permeable to PI, suggesting that an unexpectedly high proportion of circulating CLL cells are membrane-damaged or necrotic. There was no correlation between the proportion of annexin-V/PI positive cells and the total white cell count (mean 156×10^9/l, range 15–633) ($r^2 = 0.2$; $P = 0.17$) or CLL stage ($P = 0.6$). Additional assays for apoptosis, including activated caspase-3 by immunocytochemistry and morphological assessment of stained cytocentrifuge preparations further confirmed absence of apoptosis.

Interestingly, when Control 2 cells (not exposed to additional washes) were analyzed by flow cytometry, a single population of cells was observed by FSC, suggesting that the subpopulation of cells in the "Control 1" or "Test"

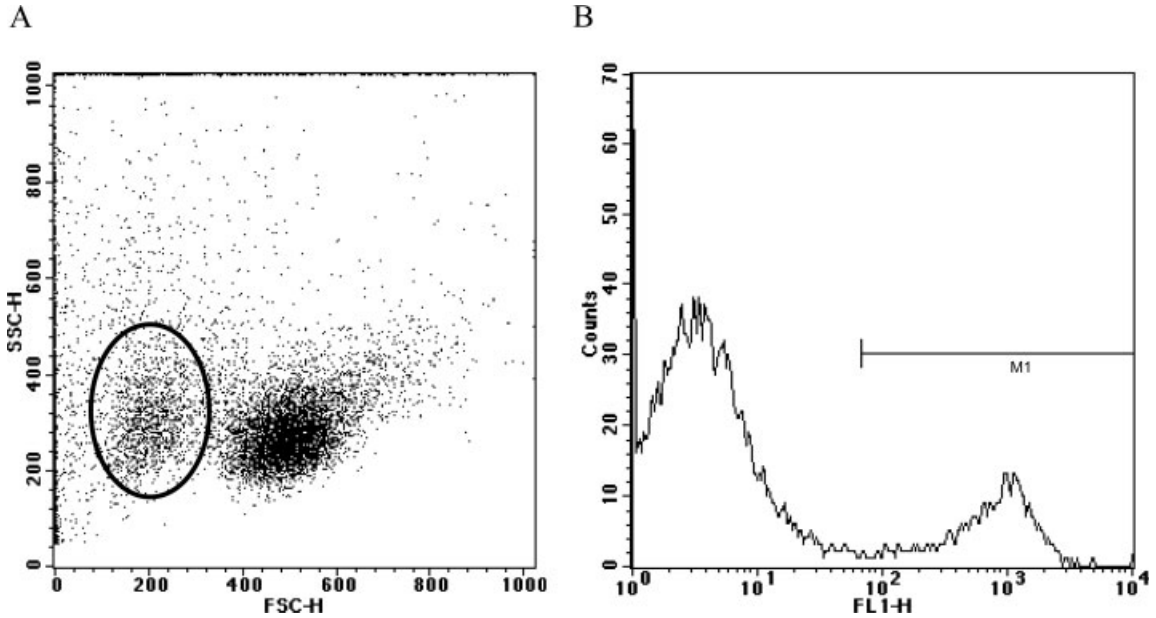

Fig. 1. Flow cytometric assessment of cell size (A) and annexin-V positivity (B) in freshly isolated blood CLL cells. The subpopulation of cells (20%) with smaller FSC is gated in Figure 1A. This subpopulation comprises all events selected by marker M1 in the fluorescence histogram (Fig. 1B), which indicates annexin-V positive cells.

had appeared during MNC processing. Annexin-V/PI binding therefore reflects the propensity to in vitro damage in CLL cells. To investigate whether this tendency results from changes in cytoskeletal vimentin, we measured changes in vimentin content by flow cytometry [3] and compared it to annexin-V positivity. The fluorescence for membrane vimentin in MNC measured immediately following Ficoll enrichment (7.2 $\pm$ 8.2) was unchanged compared with cells analyzed after additional washes (9.9 $\pm$ 3.5) (*P* 0.13, *n* 3), suggesting that annexin-V binding may be independent of vimentin defects.

Our studies highlight limitations of widely used techniques to separate and measure cell death in CLL cells because of cellular propensity for mechanical damage. The fragility of CLL cells highlighted by this study may result in cell loss during cell processing and potentially introduce bias in ex vivo studies.

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Novel 27.9 kb α^0-thalassemia deletion in a Filipino woman

To the Editor: α-Thalassemia is a common hereditary disorder due to deficiency or complete absence of α-globin chains required for fetal hemoglobin (Hb F, $\alpha_2\gamma_2$) in the developing fetus and newborn, and later for adult hemoglobin (Hb A, $\alpha_2\beta_2$). The α-globin gene cluster consists of seven genes and pseudogenes arranged in the linear order: $\zeta - \psi\zeta - \psi\alpha2 - \psi\alpha1 - \alpha2 - \alpha1 - \theta1$. α^0-Thalassemia deletions remove both functional α2- and α1-globin genes in *cis* and are associated with the most severe α-thalassemia syndromes [1,2]. Homozygotes for α^0-thalassemia deletions have Hb Bart's hydrops fetalis syndrome, a condition that usually results in death late in third trimester or shortly after birth [2].

Herein, we report the case of a Filipino couple referred for thalassemia testing during the first trimester of their first pregnancy. Both individuals had hematological indices consistent with α^0-thalassemia trait (Table I). DNA samples were screened by deletion-specific PCR for the two common α^0-thalassemia deletions found in the Filipino population ($--^{SEA}$ and $--^{FIL}$) [3]. The father was shown to be heterozygous for the $--^{FIL}$ deletion, but the mother was negative for both deletions. Southern hybridization with probes specific for the ζ-globin gene and 3′αHVR indicated that the mother carried a large deletion beginning downstream of the ζ-globin gene and extending to the region between the θ1-globin gene and 3′αHVR (data not shown).

The paternal $--^{FIL}$ deletion removes the ζ- and α-globin genes [1], whereas the maternal deletion removes only the α-globin genes and spares the embryonic ζ-globin gene. This is an important distinction because at least one active ζ-globin gene is necessary for affected pregnancies to proceed beyond the early embryonic stage [2]. The couple was counseled that the

TABLE I. Hematological Indices of the Parents

	Mother	Father
Age (years)	29	29
Hb (g/dL)	110	144
MCV (fl)	67.0	67.0
MCH (pg)	20.9	21.4
MCHC (g/L)	314	320
RBC (10^{12}/L)	5.25	6.71
Hb pattern	Normal	Normal
Hb A_2 (%)	2.5	2.4
Hb H inclusion body test (brilliant cresyl blue test)	Positive	Positive

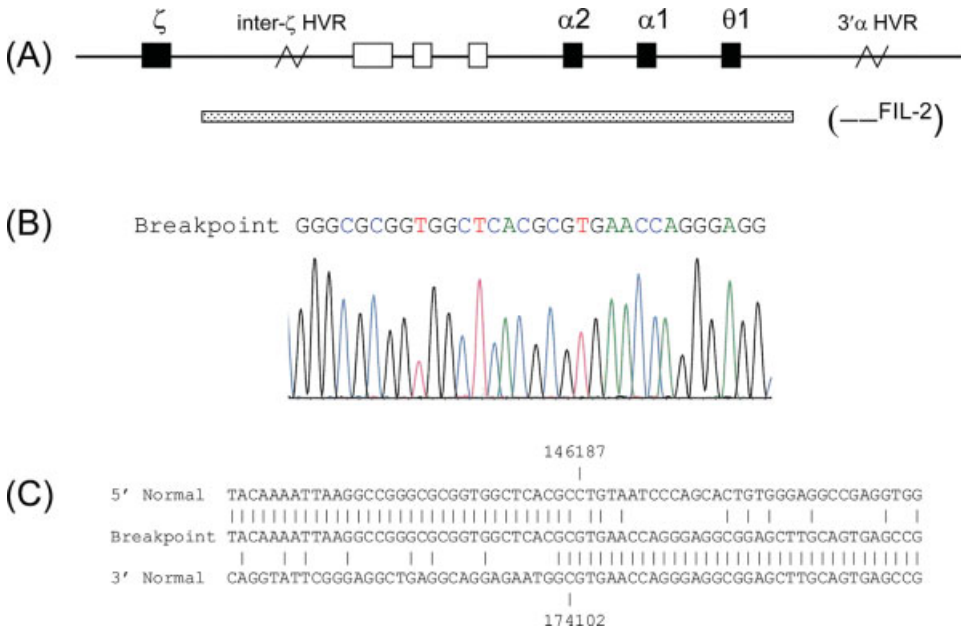

Fig. 1. Characterization of the Filipino-2 α^0-thalassemia deletion. (A) Gene map of the ζ-α globin gene cluster showing the extent of the deleted region (stippled box). The deletion breakpoint was amplified as a ~1.6 kb fragment using a forward primer located 881 bp downstream of the ζ-globin gene (5′-GGTG TCCA TCAT CAGG ACTA ACTG-3′) and a reverse primer located 3,641 bp downstream of the θ1-globin (5′-AGTG TTGT AGTC ATGG CTTA CTGC-3′). The PCR mixture contained 10 mM Tris·HCl pH 8.3, 50 mM KCl, 3.0 mM $MgCl_2$, 200 μM each dNTP, 750 mM Betaine, 5% (v/v) DMSO, 0.32 μM forward and reverse primers, 2.5 U AmpliTaq Gold® DNA Polymerase, and 500 ng genomic DNA. PCR cycling conditions were as follows: initial denaturation for 12 min at 94°C; 40 cycles of 40 sec 94°C, 20 sec at 60°C, 4 min at 72°C; final extension for 7 min at 72°C. (B) Nucleotide sequence of the breakpoint fragment. (C) Comparison of the breakpoint sequence with the normal 5′ and 3′ sequences. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

pregnancy had a one in four risk for Hb Bart's hydrops fetalis syndrome, and they chose to have prenatal testing by amniocentesis. DNA from cultured amniocytes was tested by deletion-specific PCR and Southern hybridization, the results of which established that the fetus inherited the maternal deletion and the normal paternal allele (data not shown).

Subsequent investigations were undertaken to define the precise endpoints of the maternal deletion. A fragment spanning the deletion breakpoint was amplified by PCR and sequenced (see Fig. 1). The deletion extends from nucleotide positions 146187–174102 relative to the chromosome 16 reference sequence (GenBank Accession NC_000016), spanning a total of 27,916 bp. The 5′ deletion endpoint is located 1,787 bp downstream of the translation termination signal of the ζ-globin gene, and the 3′ deletion endpoint is located 2,995 bp downstream of the translation termination signal of the θ1-globin gene. There is no appreciable homology between the 5′ and 3′ flanking sequences, indicating that the deletion resulted from nonhomologous (illegitimate) recombination.

To date, more than 20 different α^0-thalassemia deletions have been reported in the literature; 14 of which remove both the α2- and α1-globin genes while leaving the ζ-globin gene intact. Of this subset, the precise endpoints have been established for the following deletions: $--^{SEA}$, $--^{MED}$, $-(\alpha)^{20.5}$, $-(\alpha)^{5.2}$ [1], $--^{SA}$ [4], $--^{11.1}$ [5], $--^{BRIT}$ (also known as $--^{BLACK}$) [Waye et al., unpublished], and $--^{PP}$ [Rugless et al., unpublished]. Approximate endpoints, as defined by Southern hybridization, have been established for six other deletions: $--^{MA}$, $--^{CANT}$, $--^{SPAN}$, $--^{YEM}$, $--^{CL}$, and $--^{GEO}$ [1]. The endpoints of the deletion described in this report are different from all of the previously reported deletions; hence, we have designated this deletion as the Filipino-2 α^0-thalassemia deletion ($--^{FIL-2}$).

Among Filipinos, ~3.0% of the population is heterozygous for the southeast Asian deletion ($--^{SEA}/\alpha\alpha$) and 2.0% is heterozygous for the Filipino deletion ($--^{FIL}/\alpha\alpha$) [3]. The frequency of the $--^{FIL-2}$ deletion in the Filipino population remains to be determined, but is unlikely to be a common thalassemia allele [3].

The present case illustrates the importance of molecular genetic diagnosis of α-thalassemia for accurate assessment of reproductive risks. Diagnosis of α-thalassemia trait is also clinically important to avoid potentially harmful iron supplementation therapy or invasive procedures. The existence of rare or novel α^0-thalassemia deletions, such as the Filipino-2 deletion, is potentially problematic for laboratories in which testing is restricted to a panel of common deletions. This underscores the necessity of investigative techniques such as Southern hybridization mapping or the multiplex ligation-dependent probe amplification (MLPA) [6].

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