Table 2. Comparison between % +12 detected by conventional cytogenetics and by FISH in aCLL series.

Series	Number of aCLL patients analyzed by CC/ISH	Clonal karyotypic aberrations %	+12 detected by conventional cytogenetics %	+12 detected by in situ hybridization %
Criel *et al.*, 1994[2]	21	57 (12/21)	48 (10/21)	57 (12/21)
Matutes *et al.*, 1996[3]	39	72 (28/39)	56 (22/39)	ND
Woessner *et al.*, 1996[4]	13	62 (8/13)	31 (4/13)	54 (7/13)
Criel *et al.*, 1997[3]	67	55 (37/67)	36 (24/67)	ND
Bigoni *et al.*, 1997[6]	43	63 (27/43)	21 (9/43)	33 (14/43)
Hjalmar *et al.*, 1998[7]	37	ND	ND	24 (9/37)
Present series	27	70 (19/27)	22 (6/27)	63 (17/27)

The table only includes those series in which a distinction between aCLL patients and tCLL patients was made. CC: conventional cytogenetics; ISH: in situ *hybridization; aCLL: atypical chronic lymphocytic leukemiat; CLL: typical chronic lymphocytic leukemia.*

Despite the fact that del(13)(q14) is associated with CLL, only one case with this deletion was detected by ISH.[6] Döhner *et al.*[10] observed that abnormalities of chromosome 17 occur at low frequency (9-15%), most of them being structural abnormalities affecting the short arm, where the TP53 tumor suppressor gene is located. However, among our cases analyzed by ISH no monosomy of 17p13 was detected. Summing up, it is accepted that aCLL constitutes a subgroup distinct from typical CLL (tCLL), with different morphologic features, cytogenetic findings and clinical evolution. Despite the fact that large B-CLL series combine cytogenetic and ISH studies, few authors have distinguished between tCLL and aCLL. Further series would be warranted in order to consider both subtypes of B-CLL separately so as to obtain a better characterization of their cytogenetic and molecular behaviors.

Blanca Espinet, Francesc Solé, Soledad Woessner, Lourdes Florensa, Elisabet Lloveras, Carles Besses

Laboratori de Citologia Hematològica. Unitat d'Hematologia 1973; Laboratori de Referència de Catalunya, Hospital de l'Esperança. Institut Municipal d'Assistència Sanitària (IMAS), Barcelona, Spain

Keywords

Atypical B-cell chronic lymphocytic leukaemia (aCLL), cytogenetics, trisomy 12, 13q14, 17p13, in situ hybridization

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Correspondence

Blanca Espinet, Ph.D., Laboratori de Citologia Hematològica, Unitat d'Hematologia 1973, Laboratori de Referència de Catalunya, Hospital de l'Esperança, Av. St Josep de la Muntanya, 12, 1º, Barcelona 08024, Spain. Phone: international +34-93-2850200 ext. 2189-2243 – Fax: international +34-93-2108406 – E-mail: e0037@imas.imim.es

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Massive hemolysis in *Clostridium perfringens* infection

Sir,

Clostridium perfringens infection is a very rare cause of massive intravascular hemolysis, but it should always be kept in mind since early treatment can rescue patients from an otherwise rapidly fatal outcome. We report a case which illustrates the very fast course of this disease, and outline some features that can help to reach a prompt diagnosis.

A 77-year-old woman presented with jaundice and stupor of recent onset. Her temperature was 38ºC and

Table 1. Cases of *C. perfringens* associated with massive hemolysis reported in the English bibliography from 1990.

Ref.	Age	Sex	Underlying diagnosis	Origin of infection	Tº	Hb	Hto	WBC count	PLT count	MCV	LDH	DIC	Blood cultures	Survival
2 (1.5 h)	84	F	None	Intestine (supposed)	37ºC	10.8 (7.6)	32% (12%)	16.5	NR	Normal (68 fL)	1,344 (4,400)	No	*C. perfringens* *E coli*	3 h.
3	47	M	None	Biliary tree	38.1ºC	NR	5%	43.6	20	NR	NR	Yes	*C. perfringens*	22 h.
4	61	M	Diabetes Pancreatic carcinoma (resected)	Biliary tree	41ºC	6.5	16%	38.2	NR	NR	7,600	NR	*C. perfringens*	Recovered
5	54	F	Acute myeloid leukemia Bone marrow transplant	NR	39ºC	2.3	NR	6.5	90	NR	NR	Yes	*C. perfringens*	6 h.
6 (5 h)	66	F	Diabetes Primary sclerosing cholangitis Liver transplant	Liver abscess	39ºC	11.3 (6.2)	NR (3.5%)	11.2 (32.4)	181 (203)	NR	NR	NR	*C. perfringens*	< 12 h.
7 (NR)	58	F	Diabetes Cholecystectomy	Biliary tree (supposed)	39.4ºC	NR	41% (26%)	NR	NR	NR	NR	NR	*C. perfringens*	Recovered
8 (NR)	74	M	None	Liver microabcesses	38.5ºC	13.1	41% (1%)	19.8	NR	NR	1,250	NR	*C. perfringens*	6 h.
9	55	F	Hodgkin disease Chemotherapy		40.2ºC	34	0%	0.2	33	NR	4,503	Yes	*C. perfringens*	4 h.
10	61	M	None	Liver microabcesses	39ºC	NR	NR	NR	NR	NR	NR	NR	*C. perfringens*	4 h.

*Abbreviations: Tº: temperature; Hb: hemoglobin in g/L; WBC: white blood cell $\times 10^9$/L; PLT: platelets $\times 10^9$/L; Htc: hematocrit in %; MCV: mean corpuscular volume; LDH: lactate dehydrogenase in international units/L; DIC: disseminated intravascular coagulation; h: hours; NR: not reported; F: female; M: male. *time between first and second blood samples.*

her abdomen was distended and tender. Bilirubin concentration was 43.1 mg/dL, and LDH 14,255 IU/L. In an automated blood count, her Hb was 48 g/L, Htc 0.07 L/L, MCV 54 fL, MCH 38 pg, WBC 25.89×10^9/L, platelets 50×10^9/L, and reticulocytes 14%. The blood smear showed a remarkable scarcity of RBCs of normal size. A direct antiglobulin test was negative. Centrifugation of a second blood sample, taken half an hour later, yielded no RBCs. An abdominal ultrasound showed abundant intra-abdominal gas. Despite treatment with imipenem and transfusion of four packed RBCs units, the patient died four hours after admission. Two days later *C. perfringens* was identified in the blood cultures.

Only 19 cases of *C. perfringens*-associated massive hemolysis have been previously published. Chaplin *et al*.[1] reported on six patients seen before 1990, and we found nine cases in the English bibliography from 1990 (Table 2).[2-10] Four additional patients have been reported in non-English journals. From our case and previously reported ones, *C. perfringens*-associated hemolysis emerges as an extremely rapid illness that usually leads to patient's death in a few hours (Table 1). Most patients are middle-aged or elderly, and have underlying malignancies, diabetes or recent abdominal surgery. Intra-abdominal organs, mainly the biliary tract, are the usual foci of infection, which may be suspected if extraluminal gas is seen on radiographs.[1,3] Although most patients are febrile at presentation, fever and other signs of infection may be inconspicuous or absent.

Hematologists are often the first to appreciate the severity of the hemolysis, when they receive blood samples without enough RBCs to perform a blood grouping, a Coombs' test or a reliable hemocytometric profile.[1] Photometric assays, such as bilirubin or LDH assay are affected by the free hemoglobin and their results are unreliable.[1] Automated blood counts are also source of several inaccuracies. Hemoglobin levels are disproportionately high in comparison with hematocrit, and MCH is artefactually increased. A typical finding is a very low MCV without microcytes in the blood smear. This is due to fragmented RBCs, and increases as the hemolysis becomes more severe.[2] T-activation on patients RBCs, as assessed by the *Arachis hypogea* lectin, may also be of diagnostic help.[4]

Penicillin and surgical debridement of the infectious focus is the treatment of choice. As can be seen in the table, the few patients who survived were just those in whom treatment had been instituted before the anemia became life-threatening. The paramount importance of prompt diagnosis and treatment is further emphasized by the fact that some patients had only mild anemia at presentation, but were already severely ill when treatment was instituted a few hours later.

Alberto Alvarez, Susana Rives, Benet Nomdedeu, Arturo Pereira*

*Servicio de Hemoterapia y Hemostasia and *Servicio de Hematología, Hospital Clínic, Barcelona, Spain*

Key words

Massive, hemolysis, Clostridium perfringens.

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Correspondence

Dr. A. Pereira, M.D., Servicio de Hemoterapia y Hemostasia Hospital Clínic de Barcelona, Villaroel 170, 08036 Barcelona, Spain. Fax: international +34.93.2275448 – E-mail: apereira@medicina.ub.es

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An atypical (b3/a3) junction of the *bcr/abl* gene lacking *abl* exon a2 in a patient with chronic myeloid leukemia

Sir,

We report the case of a patient with Philadelphia+ chronic myeloid leukemia expressing a rare type of BCR/ABL mRNA lacking ABL exon a2 sequences (BCR/ABL junction b3/a3). Clinical outcome showed brief hematologic remission. The role of the diversity of BCR/ABL fusion proteins and their relationship to leukemia phenotype are discussed.

A small proportion of Philadelphia chromosome + (Ph+) chronic myeloid leukemia (CML) patients fail to express a b2/a2 or b3/a2 transcript[1-8] (Figure 1a). At present 8 Ph+ cases have been reported (3 CML, 4 ALL, 1 unknown) with a variant BCR/ABL mRNA expression lacking ABL exon a2 sequences[5,6] (Table 1). Of the three CML patients only two had a b3/a3 junction. Herein, we report a further b3/a3 type of BCR/ABL transcript detected by RT-PCR in a patient with Ph+ CML. Conditions for blood cell lysis, RNA extraction, RT and PCR for BCR/ABL have been described.[7,8] Specific PCR for BCR/ABL transcripts was performed using primers R110 (BCR exon e1) and AZ (ABL exon a3).

A 23-year-old male patient (M.R.) was diagnosed with chronic-phase CML in May 1994. His peripheral blood showed leukocytosis: WBC: 95.8×10^9/L; 1% basophils; 1% promyelocytes; 78% neutrophils; 13% lymphocytes; 7% monocytes; platelet count: 485×10^9/L; hemoglobin level: 12.7 g/dL. The spleen was not palpable. Cytogenetic analysis showed Ph chr. Therapy with Ara-C (40 mg/die/i.m. for ten days) plus α-interferon (α-IFN)[9] (4 million μ/day/m^2) (18 monthly course) was started: the WBC fluctuated between 2 and 5.5×10^9/L during the following 18 months (hematologic remission). Then, due to progressive loss of hematologic remission and increased WBC, α-IFN was withdrawn and hydroxyurea therapy started. The patient is currently alive and well in first chronic phase. In initial screening by PCR, no expected amplification product was found with EA500 (ABL exon a3) and EA122 (BCR exon e12) primers (for e13/a2 or e14/a2 junctions), which usually amplify 99% of CML associated transcripts. A lower weight amplification product was detected 173 base pairs (bp) smaller than that expected for b3/a2 junction: 215 bp rather than the expected 388 bp (Figure 1b). Directly sequenced BCR/ABL cDNA PCR products revealed a b3/a3 junction and absence of c-ABL exon 2 derived sequences. To our knowledge, this

Table 1. Summary of leukemia types and molecular features of eight reported cases including the case presented in this letter.

Patient (age at onset)	*Type of fusion products*	*Type of leukemia*
1* (3 years)	b3/a3	cALL
2* (1 year)	e1/a3	cALL
3* (39 years)	e1/a3	ALL (pre-pre-B)
4*(61 years)	b2/a3	ALL (pre-B)
5*(unknown)	b2/a3	unknown
6*(59 years)	b2/a3	CML
7# (19 years)	b3/a3+b2/a3	CML
8* (39 years)	b3/a3	CML
9° (23 years)	b3/a3	CML

*cALL, common ALL; pre-B ALL, precursor-B ALL; *cases reviewed in ref. 5; # case in ref. 6.; °present case.*