encapsulated yeast isolated worldwide from soil, usually in association with bird droppings, primarily of pigeons and parrots. These elective and asymptomatic hosts are vectors of infections especially if they closely share habitat with men as in the case of lovebirds (*Agapornis*). Inhalation of fungal spores is usually the initial event of cryptococcosis which is primarily an asymptomatic, pulmonary infection, remaining localized to that site in immunocompetent subjects. In immunocompromised hosts, however, the infection may disseminate and meningitis represents the most typical manifestation.[1]

We report the case of a 13-year old child who owned a lovebird, and who developed cryptococcal meningitis during the induction phase of AIEOP (*Associazione Italiana Ematologia Oncologia Pediatrica*) ALL-9502 protocol for treatment of intermediate risk acute lymphocytic leukemia (ALL) (prednisone, vincristine, daunomycin, L-asparaginase and triple intrathecal therapy with methotrexate, prednisone and cytosine arabinoside, after 7 days of prednisone at increasing dose).[3]

Thirty-five days after beginning therapy, in a condition of bone marrow hypoplasia, but not of neutropenia (WBC 3,300/m^3, ANC 1,120/m^3), the patient developed persistent hyperpyrexia associated with bronchopneumonia. Despite 18 days of empirical antibiotic therapy with ceftriaxone (100 mg/kg/day) alone, followed by ceftriaxone plus amikacin (20 mg/kg/day), teicoplanin (10 mg/kg/day) plus amikacin, and clarithromycin (10 mg/kg/day) plus imipenem (60 mg/kg/day), the fever persisted and patient's clinical status worsened with onset of headache, nausea, vomiting and psychomotor disturbances up to the appearance of seizures, syndrome of inappropriate secretion of ADH, and isolated deficit of the VI cranial nerve.

On the basis of lumbar puncture results (protein 0.6 g/L, glucose 0.42 g/L and 50 lymphocytes/m^3), the ineffectiveness of the antibiotic therapy, and the history of cohabitation with a lovebird, a search for fungal infections was instigated (India ink smear, serum antigen titer assessment, cerebrospinal fluid (CSF) antigen titer assessment and cultures) and was positive for *Cryptococcus neoformans*. Therapy with liposomal amphotericin B (1 up to 3 mg/kg/day) plus flucytosine (150 mg/kg/day) was promptly initiated and maintained for 7 weeks until complete clinical resolution.[4] At that time reinforced consolidation chemotherapy was restarted, and contemporaneously the patient received fluconazole 10 mg/kg/day. Serum and CSF antigenic titers remained high (1:1024 and 1:32, respectively) during the first 5 months of treatment with fluconazole, then subsequently decreased progressively and after 12 months became negative (1:2 and 1:4, respectively). After 19 months of treatment, fluconazole was stopped.

After 2 years of follow-up, the patient is alive and well in first complete remission and serum and CSF antigenic titers remain negative.

A front-line chemotherapy for leukemia based on long-term steroid treatment may damage cell-mediated immunity, which represents the most important host defence against fungi.[5] For this reason, cryptococcosis should be suspected in every case of hyperpyrexia and persistent headache, especially when associated with bronchopneumonia, in all leukemic patients receiving front-line chemotherapy. Careful enquiries should be made about exposure to birds.

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Chromosomal instability in chronic myeloid leukemia: Philadelphia breakpoints are independent of spontaneous breakage and fragile sites

Increased frequencies of spontaneous and bromodeoxyuridine induced breakages with a non-random distribution of breakpoints were found in patients with chronic myeloid

leukemia, suggesting the presence of chromosome instability. Bands 9q34 or 22q11 were not damaged in these patients, demonstrating that the origin of the Philadelphia chromosome is independent of spontaneous breakage or fragile site expression.

Sir,

Genomic instability, which has been implicated in the formation of cancer rearrangements, could have different end-points such as karyotypic abnormalities, chromosome instability, fragile sites expression or gene mutations.[1,2] The frequency and distribution of spontaneous chromosome breakages and fluorodeoxyuridine (FUdR) or bromodeoxyuridine (BrdU)-induced fragile sites were studied in 8 Philadelphia chromosome (Ph[1]) positive patients with chronic myeloid leukemia (CML) and 8 controls in order to clarify the potential relationship between chromosomal instability and the origin of this marker. Karyotypes of bone marrow cells from patients were established on 24-48 hr cultures without mitogens. Constitutional karyotypes and chromosome instability were studied on PHA-stimulated peripheral blood lymphocytes (PBL) cultures from patients and controls, with and without the addition of FUdR (10 μg/mL) or BrdU (50 μg/mL).[3] CML patients showed the Ph[1] chromosomes in bone marrow cultures, and 3 cases also had additional Ph[1], +8 or iso(17q). No chromosome changes were observed in cultured PHA-stimulated PBL from patients or controls. Significantly higher frequencies of spontaneous and BrdU-induced chromosome aberrations were found in patients than in controls (Table 1). The distribution of spontaneous breakpoints analyzed with Brøgger's test identified a random pattern among controls and 6 non-random bands - 3p14, 4q27, 5q31, 11q13, 13q21 and 15q22 - significantly affected in patients (p <0.0012). In controls, χ^2 analysis identified 23 fragile sites induced by FUdR and 13 by BrdU (p <0.001). The patients with CML had 24 FUdR- and 17 BrdU-induced fragile sites (p <0.005). The low frequencies of expression confirmed that all sites were common (c-fra) occurring at different frequencies among leukemic and healthy individuals. No c-fra were identified at or near breakpoints 9q34 and 22q11, but 2 cases expressed a BrdU c-fra 22q12 (Table 2). Spontaneous breakage has not been previously studied in CML, however fragile site induction has been reported occasionally. This is the first report of an increased frequency of spontaneous chromosme aberrations with a non-random distribution of breakpoints, suggesting that CML patients have chromosome instability. The *Human Gene Database* lists 2 rare and 2 common fragile sites located close to the Ph[1] breakpoints. However, there is only one report that identified a fragile site at 22q11 in one Ph[1+] patient and her father.[4] A coincidence between variant Ph[1] breakpoints and fragile site location has been reported;[5-7] but others observed different results.[8] Spontaneous expression of a BrdU-sensitive fragile site at 10q25 was enhanced in CML malignant cells, suggesting

Table 1. Spontaneous and induced chromosome instability in CML patients and controls.

Cultures	*Individuals*	*Abnormal cells* %	*Gaps* %	*Breaks* %	*Total CA* %
Untreated	Controls	3.6±1.5	2.0±1.2	2.5±1.1	4.5±2.2
	CML	13.2±3.9*	10.3±2.9°	9.8±3.6*	20±5.9°
FUdR	Controls	32.6±6.4	25.6±5.2	27.0±7.3	52.4±12.0
	CML	36.3±6.3	33.5±7.6	25.6±6.2	59.1±11.3
BrdU					
	Controls	21.4±3.2	18.9±2.8	12.2±3.8	31±6.3
	CML	33.6±5.7#	35.3±8.2#	24.6±7.4	59.9±13.1#

*CA=chromosome aberrations; FUdR=fluorodeoxyuridine; BrdU=bromodeoxyuridine; CML=chronic myeloid leukemia *Significant differences in spontaneous CA with respect to controls p <0.05; °Significant differences in spontaneous CA with respect to controls p <0.025; #Significant differences in BrdU-induced CA with respect to controls p <0.05.*

Table 2. Common fragile sites induced in controls and CML patients.

Controls				*CML cases*			
FudR c-fra[a]	*N.of CA/ Individuals*	*BrdU c-fra*[b]	*N.of CA/ Individuals*	*FudR c-fra*[c]	*N.of CA/ Individuals*	*BrdU c-fra*[d]	*N.of CA/ Individuals*
1p22	4/2	1p22	3/2	1p32	5/2	2q21	3/3
1q25	4/3	2q33	3/1	1p22	5/3	2q31	4/2
1q42	5/3	3p14	4/3	1q21	8/4	2q33	4/3
2p13	6/3	4q31	9/5	2q11	4/2	3p14	5/3
2q23	4/2	5q31	4/4	2q13	7/4	4p16	4/2
3p24	4/3	6p23	4/2	2q21	5/2	4q12	7/3
3p14	17/6	6p22	6/4	2q31	4/3	5p13	6/3
3p27	4/2	6q13	10/3	2q33	8/5	5q15	5/4
4q12	5/5	10q21	3/2	3p24	5/2	5q31	5/4
4q31	9/5	13q21	11/5	3p14	25/7	6q13	3/2
5p13	6/2	14q24	3/3	4q12	6/2	7q22	4/3
5q15	10/4	16q22	4/3	4q31	6/5	8q22	3/2
5q31	7/3	17q21	5/2	5p14	5/3	9q22	3/2
6p23	4/2			5q15	12/6	10q21	3/2
6p22	4/2			5q31	17/7	13q13	5/2
7q22	4/3			6q13	7/3	13q21	5/4
7q32	5/3			7q32	4/4	22q12	4/2
8q22	8/2			8q22	5/4		
13q21	5/3			10q24	4/3		
14q24	7/3			12q24	4/3		
15q22	5/3			13q21	4/2		
17q23	5/2			14q24	6/4		
19q13	7/4			16q22	4/3		
				17q21	5/4		

[a]23 fragile sites defined by χ^2 test (p <0.001); [b]13 fragile sites defined by χ^2 test (p <0.001); [c]24 fragile sites defined by χ^2 test (p <0.001); [d]17 fragile sites defined by χ^2 test (p <0.001); c-fra=common fragile site

that malignancy increased its expression.[9] Despite the extensive research performed, there is little evidence that either supports or refutes the role of fragile sites in cancer. Our results suggest that the origin of Ph[1] chromosome is independent of spontaneous instability or fragile site expression, because bands 9q34 or 22q11 were not observed in our series either spontaneously or by induced breakage. The proximity between BCR and ABL genes in specific cell cycle phases may explain the genesis of the translocation.[10] The increased chromosome instability affecting specific bands could be a systemic manifestation or a consequence of the leukemic process, possibly due to certain unknown clastogenic factors of the neoplastic cells.

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Vascular endothelial growth factor isoforms 121 and 165 are expressed on B-chronic lymphocytic leukemia cells

We used flow cytometry to analyze the expression of vascular endothelial growth factor (VEGF) on leukemic cells of 11 B-CLL patients using a monoclonal antibody directed against the 121 and 165 isoforms. All patients tested displayed a positive reaction for VEGF. Interestingly, mean fluorescence intensity (MFI) of cases with a progressive pattern of disease was higher than MFI of patients with stable disease. Cellular VEGF-expression may be involved in disease progression.

Sir,

An increasing body of evidence has been accumulated which suggests a central role for angiogenesis in the pathophysiology of hematopoietic malignancies.[1-6] Although most information comes from patients with multiple myeloma (MM) it has been recently shown that acute myeloid leukemia (AML) cells express vascular endothelial growth factor (VEGF), a potent inductor of angiogenesis.[7] Furthermore, elevated levels of basic fibroblastic growth factor (b-FGF) were detected in the urine of patients with acute lymphoblastic leukemia (ALL) and associated with increased bone marrow microvessel density.[6] In B-cell chronic lymphocytic leukemia (CLL), evidence for increased angiogenesis has been demonstrated by Kini *et al*.[8] and clinico-prognostic implications of of such a feature have been investigated by our group in a series of CLL patients with early disease.[9]

With respect to the source and mechanisms of production of serum VEGF in CLL much remains unproven. Chen *et al*.,[10] on the basis of results obtained in RT-PCR and Slot-blot analysis, showed that B-CLL cells express VEGF m-RNA and identified in VEGF 121 and VEGF 165 the two isoforms produced. We studied 11 B-cell CLL patients using flow cytometry and a monoclonal antibody anti-VEGF whose specificty covered