3790 FANC A mutation) was heterozygous for the G58A transition. No other mutation or deletion was found among these patients. It is important to note, however, that other large deletions or promoter region mutations could not be detected if beyond the limits of our primers. The G58A substitution was previously described in one patient with constitutional aplastic anemia and in two with acquired aplastic anemia by Vulliamy *et al.*[6] Yamaguchi *et al.*[8] also found two Black patients with myelodysplastic syndrome and one patient with acquired aplastic anemia who were heterozygous for this substitution. More recently, Wilson *et al.*[9] described a 2-year old Black myelodysplastic patient heterozygous for the G58A transition. Four of eight relatives of this patient carry this gene variant, but none presents any hematologic abnormality. The prevalence of the G58A substitution varies from 4 to 20% in African-Americans and is not a disease-causing mutation, but a common polymorphism, especially among normal Black subjects.[8,9] In the present study, the only patient carrying this substitution was an African-Brazilian. BM failure was diagnosed at the age of 3 years, and the patient had a short stature, low weight, narrow eyelid gap, tenar hypotrophy, and *café-au-lait* spots. No HLA-matched donor was available, but the BM showed relative response to treatment with oxymetholone and the boy became transfusion independent.

Nucleotide G58, located immediately 3' of the template, is not phylogenetically conserved in vertebrates, does not influence the telomerase RNA tertiary structure, and does not influence telomerase activity *in vitro*.[11] Taken together, these data strongly suggest that this polymorphism is unlikely to play a role in the pathogenesis of BM failure in FA or in other BM failure syndromes.

In conclusion, although excessive telomere length shortening is observed in some patients with FA and seems to predict evolution to aplastic anemia, mutations in the *hTERC* gene do not contribute to this phenomenon in the setting of FA. However, mutations in other genes encoding other components of the telomerase complex might play a role. Alternatively, increased hematopoietic proliferative demands and/or the oxidative stress in response to BM failure may ultimately lead to increased telomere shortening in FA.

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Funding: This work was supported in part by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), grant 98/14247-6.

Key words: Fanconi's anemia, bone marrow failure, telomerase, telomere.

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Acute Lymphoblastic Leukemia

Molecular diagnosis of leukemic cerebrospinal fluid cells in children with newly diagnosed acute lymphoblastic leukemia

Cytomorphology and IgH/T-cell receptor γ clonal gene rearrangements detected by polymerase chain reaction (PCR) homo/heteroduplex analysis and direct sequencing were evaluated in cerebrospinal fluid (CSF) free of red-blood cells at diagnosis of 37 children with acute lymphobalstic leukemia. Molecular CSF involvement was greater as detected by molecular analysis than observed by morphologic criteria (45.9% vs 5.4%). The 4-year event-free survival was lower in the group with molecularly detected CSF involvement (p = 0.01).

haematologica 2004; 89:1013-1015
(*http://www.haematologica.org/2004/8/1013)*

In the majority of treatment centers the diagnosis of central nervous system (CNS) involvement is defined by the presence of pleocytosis of more than 5 cells per cubic millimeters in the cerebrospinal fluid (CSF), with blast cells or the presence of cranial nerve palsies. The detection of CNS involvement has been associated with a high risk of relapse.[1,2] Some studies have also demonstrated that patients with any identifiable blast cells in CSF, as well CSF contamination by blastic cells during traumatic lumbar puncture, have an increased risk of CNS relapse.[3-5] Others, however, did not find this association.[6,7] Conventional cytological analysis has proven useful, but the analysis of cells in CSF, especially when the cell count is low, is more difficult than is widely admitted and nor is it always conclusive.

We analyzed 37 of 66 children with acute lymphoblastic leukemia (ALL) admitted to our institution (18 were classified as having standard risk and 19 as high risk). Twenty-nine patients were excluded, 13 because of the presence of red blood cells in the CSF, suggesting puncture accident, 10 because of the absence of stored CSF or bone marrow at

Table 1. Correlation between age, CSF molecular/morphologic involvement and outcome of 39 patients analyzed in this study.

Patient	*Age (months)*	*WBC count/dL*	*Immunophenotyping*	*Molecular CSF involvement*	*Morphologic CSF involvement*	*Outcome (months)*
1-L310	65	19600	COMMOM	–	–	CCR-102
2-L336	44	92700	COMMOM	–	–	UE-2
3-L339	51	2500	COMMOM	–	–	CCR-98
4-L341	117	100000	T	+	–	UE-4
5-L360	114	1300	COMMOM	–	–	UE-32
6-L389	22	442000	EPB	+	–	UE-6
7-L390	17	13200	NP	–	–	CCR-92
8-L478	44	5200	COMMOM	–	–	CCR-80
9-L481	21	75000	COMMOM	–	–	CCR-80
10-L523	72	8500	T	+	+	UE-22
11-L559	31	41200	T	+	–	UE-21
12-L604	61	24800	COMMOM	–	–	CCR-68
13-L628	60	2100	COMMOM	–	–	CCR-65
14-L707	93	4700	COMMOM	–	–	UE-51
15-L722	74	12200	COMMOM	–	–	CCR-63
16-L726	106	71800	T	+	–	CCR-63
17-L732	35	17300	COMMOM	+	–	UE-6
18-L751	129	45500	T	–	–	CCR-62
19-L753	29	36700	T	+	–	UE-15
20-L798	42	60000	COMMOM	+	–	CCR-59
21-L834	164	29700	COMMOM	+	–	CCR-56
22-L857	130	3100	COMMOM	+	–	CCR-54
23-L871	38	72700	COMMOM	+	–	UE-31
24-L874	7	404000	EPB	+	–	UE-13
25-L931	41	8100	COMMOM	+	–	CCR-50
26-L960	54	18300	COMMOM	+	–	CCR-48
27-L972	32	43600	COMMOM	–	–	CCR-46
28-L1015	146	254000	T	+	+	UE-0
29-L1022	52	3200	COMMOM	–	–	CCR-40
30-L1030	66	5800	COMMOM	–	–	CCR-39
31-L1055	14	28300	COMMOM	+	–	CCR-37
32-L1069	7	606000	EPB	+	–	UE-0
33-L1104	20	34600	COMMOM	–	–	CCR-35
34-L1111	67	17400	COMMOM	–	–	CCR-30
35-L1163	67	3100	COMMOM	–	–	CCR-30
36-L1191	43	21700	COMMOM	–	–	UE-8
37-L1206	71	6700	Biphenotype	–	–	CCR-27

CCR: complete continuous remission; UE: unfavorable event; EPB: early pre-B ALL; NP: no performed.

diagnosis, and 6 due to poor quality CSF DNA. The patients had been classified and treated in accordance to criteria from the Brazilian Group for the Treatment of Leukemia in Childhood (GBTLI 93). The characteristics of the eligible patients studied are shown in Table 1.

CSF samples with more than 5 cells/mm^3 were placed in a Cytospin sample chamber, cytocentrifuged, stained by Wright-Giemsa and analyzed by at least 2 examiners. The criterion used for the diagnosis of CNS leukemia was the presence of at least 5 mononuclear cells/mm^3 with leukemic blastic cells detected in the cytocentrifuged samples, or presence of a cranial-nerve palsy.[1] DNA from bone marrow and CSF free of red cells at diagnosis were analyzed retrospectively by polymerase chain reaction (PCR) with homo/heteroduplex analysis using consensus primers to IgH and T-cell receptor (TCR) γ and direct sequencing as described previously.[8-10] The bone marrow and CSF sequences obtained at the diagnosis compared against each other. The CSF samples were considered positive for molecular involvement when they had a clonal rearrangement identical to that found at diagnosis in the bone marrow.

Of the 37 patients analyzed, 2 (5.4%) had a morphologic diagnosis of CNS disease, both with a T immunophenotype, 19 had clonal TCR and/or IgH rearrangements in CSF cells when analyzed by PCR homo/heteroduplex, with 17 (45.9%) of these being identical sequences to those in the diagnostic bone marrow. In 2 patients the sequences were different from those of the bone marrow and considered as negative. Fisher's exact test showed that molecular involvement was more frequent in patients with a white cell count greater than 50,000/mm^3 (p=0.02) and CALLA—immunophenotype (p=0.01). The estimated global 4-year event-free survival using Kaplan Meier curves and a log rank test in the analyzed patients was 0.64. The event-free survivavl of the group with molecular involvement was 0.41 and whereas it was 0.84 for the group without molecular involvement (p= 0.01) (Figure 1). The duration of continuous complete remission ranged from 27 to 102 months (median: 56 months). The 4-year event-free survival in the whole group of 66 ALL children treated in the period was

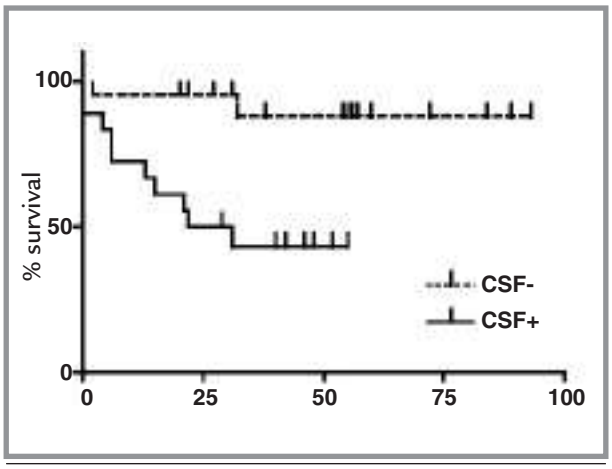

Figure 1. Kaplan-Meier analysis of event-free survival (EFS) according to the presence (CSF^+) or absence (CSF^-) of molecularly detected CSF involvement.

0.74 (0.82 and 0.61 in standard and high risk groups respectively). When molecular CNS detection at diagnosis was analyzed as a prognostic factor in association with age, white cell count and immunophenotyping by multivariate analysis using a Cox proportional model, no statistical significance was observed ($p = 0.38$).

Controversies exist about the prognosis of the children with low cell counts in CSF.[3,4,6,7] Our data suggest that molecular detection of blast cells in CSF at diagnosis could be associated with a poor prognosis in children with ALL in univariate analysis, in accordance with results described by some authors in patients with low CSF blast cell counts at diagnosis.[3,4] Although these data were not confirmed in multivariate analysis and need to be viewed with caution due to the relative small number of cases, the short follow-up of the study and the selected group of patients analyzed. From a clinical point of view, the present study supports the identification of a new level of CNS involvement and could suggest a revision of the standard definition of CNS leukemia. To confirm these initial data and analyze the real prognostic impact of molecularly detected CNS leukemia involvement a prospective study with a greater number of patients and a longer follow-up will be necessary.

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Funding: supported by grants from Fundaçáo de Apoio ao Ensino, Pesquisa e Assistància do HC-FMRP-USP (FAEPA) and the Federal Brazilian financial support agencies CAPES and CNPq.

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Malignant Lymphomas

Effect of interleukin-1β and glutathione S-transferase genotypes on the development of gastric mucosa-associated lymphoid tissue lymphoma

We tested whether polymorphic variations in glutathione S-transferase genes (*GSTM1*, *GSTT1*, *GSTP1*) and inyterleukin-1 (IL-1β and IL-1RN) genes confer susceptibility to mucosa-associated lymphoid tissue lymphomas (MALT) in a Chinese population. The rates of *GSTM1*, *GSTP1*, *IL-1β* and *IL-1RN* genotypes did not differ between patients and controls. However, *GSTT1* null genotypes were significantly more common in patients with MALT lymphomas (43/75 vs. 138/321, p=0.029; OR=1.8, 95% CI: 1.1~3.0) than in controls. Our results suggest that a glutathione S-transferase defect plays a role in MALT lymphoma.

haematologica 2004; 89:1015-1017
(*http://www.haematologica.org/2004/8/1015*)

Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is the most commonly encountered extranodal marginal-zone B-cell lymphoma. Accumulating evidence has indicated that *H. pylori* infection induces the formation of MALT and the subsequent occurrence of lymphoma.[1-3] *H. pylori* infection is also involved in the pathogenesis of gastritis, peptic ulcer, and gastric adenocarcinoma.[4] The factors determining why *H. pylori* can lead to different gastroduo-