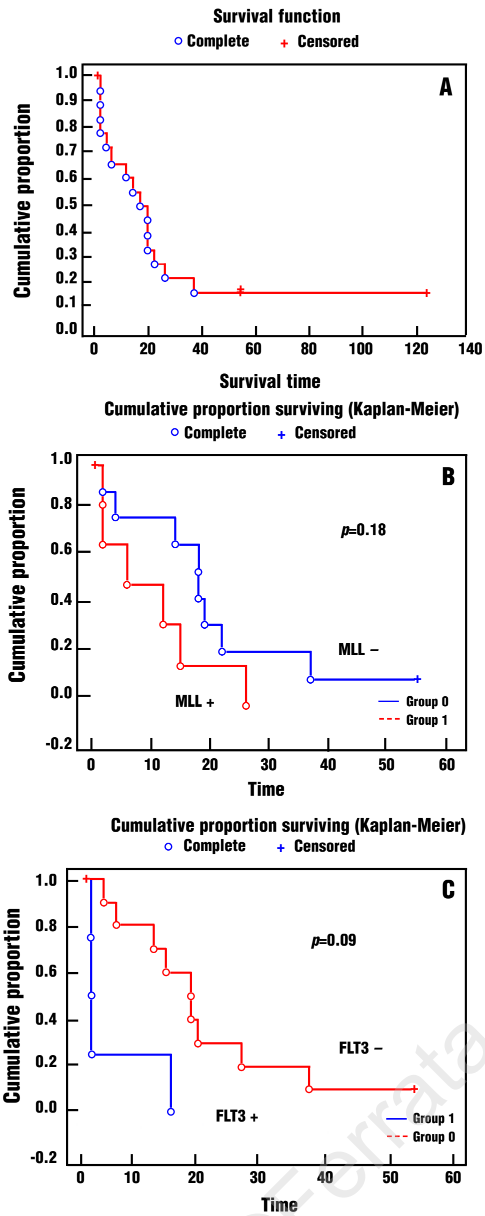

Figure 1. A. Kaplan-Meier survival curves of all patients with trisomy 11 (n=20). B. Group 0: patients negative for *MLL*-PTD (n=10); group 1: patients positive for *MLL*-PTD (n=7). C) group 0: patients negative for FLT3-ITD (n=11); group 1: patients positive for FLT3-ITD (n=5).

relevant in determining the poor outcome observed in patients with trisomy 11.

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References

1. Caligiuri MA, Strout MP, Schichman SA, Mrozek K, Arthur DC, Herzig GP, et al. Partial tandem duplication of ALL1 as a recurrent molecular defect in acute myeloid leukemia with trisomy 11. Cancer Res 1996;56:1418-25.
2. Nilson I, Lochner K, Siegler G, Greil J, Beck J, Fey G, et al. Exon/intron structure of the human ALL-1 (MLL) gene involved in translocations to chromosomal region 11q23 and acute leukemia. Br J Hematol 1996;93:966-97.
3. Jamal R, Taketani T, Taki T, Bessho F, Hongo T, Hamaguchi H, et al. Coduplication of the MLL and FLT3 genes in patients with acute myeloid leukemia. Genes Chromosomes Cancer 2001; 31:187-90.
4. Stirewalt DL, Radich JP. The role of FLT3 in haematopoietic malignancies. Nat Rev 2003;3:650-66.
5. Libura M, Asnafi V, Tu A, Delabesse E, Tigaud I, Cymbalista F, et al. FLT3 and MLL intragenic abnormalities in AML reflect a common category of genotoxic stress. Blood 2003;102:2198-204.
6. Steudel C, Wermke M, Schaich M, Schakel U, Illmer T, Ehninger G, et al. Comparative analysis of MLL partial tandem duplication and FLT3 internal tandem duplication mutations in 956 adult patients with acute myeloid leukemia.Genes Chromosomes Cancer 2003;37:237-51
7. Michaux L, Wlodarska I, Stul M, Dierlamm J, Mugneret F, Herens C, et al. MLL amplification in myeloid leukemias: a study of 14 cases with multiple copies of 11q23. Genes Chromosomes Cancer 2000;29:40-7.
8. Nakao M, Yokota S, Iwai T, Kaneko H, Horiike S, Kashima K, et al. Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. Leukemia 1996;10:1911-8.
9. Armstrong SA, Kung AL, Mabon ME, Silverman LB, Stam RW, Den Boer ML, et al. Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification. Cancer Cell 2003;3:173-83.
10. Poppe B, Vandesompele J, Schoch C, Lindvall C, Mrozek K, Bloomfield CD, et al. Expression analyses identify MLL as a prominent target of 11q23 amplification and support an etiologic role for MLL gain of function in myeloid malignancies. Blood 2004;103:229-35.

Acute Lymphoblastic Leukemia

γδ and αβ T-cell acute lymphoblastic leukemia: comparision of their clinical and immunophenotypic features

γδ-T-cell acute lymphoblastic leukemia (ALL) is a rare variant of ALL. The comparison of some clinical and laboratory features in children and adults with γδ-T-ALL or αβ-ALL showed that in γδ-T-ALL the CD45RA$^-$/CD45RO$^+$ phenotype was predominant, the hemoglobin concentration was lower in children and the presence of splenomegaly and the white cell counts was higher in adults.

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T-cell acute lymphoblastic leukemia (T-ALL) expresses the αβ, or γδ T-cell receptor (TCR) in less than 35% of the cases.[1] Previous studies have shown that sub-classification of T-ALL according to immunophenotype and TCR complexes provides valuable biological, clinical and prognostic information.[2,3] Based on morphological, cytochemical and immunophenotypic criteria, we diagnosed 202 cases of T-ALL (from 961 ALL cases), and compared some clinical and laboratory features of 19 cases of γδ T-ALL (9 children, 10 adults) with those of 22 selected cases αβ T-ALL (11 children and 11 adults). Bone marrow malignant cells were studied by flow cytometry with the panel of monoclonal antibodies listed in Table 2. Results were considered positive if 20% or more of the cells expressed a particular antigen.

The overall incidence of T-ALL in Brazil is 12.5 cases/10^6 people-years: 25.5 cases/10^6 for children and 6.2 cases/10^6 for adults[4]. T-ALL represents about 20% of all ALL cases (21% in this series), and about 35% of the T-lineage ALL have the TCR complex on membrane[1] (20% in this series), whereas only 2% of all ALL cases express the γδ TCR (2% in this series). Among our 202 T-ALL cases, we found 19 γδ T-ALL (9%), a proportion similar to those in the series presented by Macintyre *et al.*[2] (9%) and Schott *et al.*[3] (12%). These proportions are high when compared to the low percentage (about 1%) of γδ T cells in the normal thymus.[1] This finding remains unexplained, raising the possibility that some γδ leukemic T-cells derive from extrathymic pathways of T-cell differentiation or, alternatively, that these cells have an increased potential for malignant transformation[1].

Comparison of the clinical and laboratory data from the αβ and γδ T-ALL revealed that only the hemoglobin concentration was lower in children with γδ T-ALL, as previously shown by Schott *et al.*[3]; whereas in adults, both the presence of splenomegaly and the white cell counts were higher in γδ T-ALL (Table 1). Together, these findings suggest an increased tumor burden in γδ T-ALL.

The immunophenotypic profile was similar in αβ, and γδ T-ALL for children and adults (Table 2), except for the expression of the CD45 isoforms. The CD45RA+/CD45RO- phenotype was the most common in γδ T-ALL

Table 1. Clinical and laboratory characteristics of the children and adult subgroups with αβ, and δγ T-ALL.

	Children† (n=20)		*p value*	*Adults (n=21)*		*p value*
	αβ T-ALL (n=11)	*γδ T-ALL (n=9)*		*αβ T-ALL (n=11)*	*γδ T-ALL (n=10)*	
Age, years	6 (1-14)	5 (2-14)0.789	20 (16-44)	17.5 (15-26)		0.149
Sex (M/F)	8/3	7/2	1.000	9/2	8/2	1.000
Mediastinal mass	8/11 (73%)	3/9 (33%)	0.175	2/11 (18%)	2/10 (20%)	1.000
CNS involvement	2/11 (18%)	0/8 (0%)	0.486	0/11 (0%)	1/9 (11%)	0.450
Splenomegaly	8/11 (73%)	7/9 (80%)	1.000	1/11 (9%)	7/10 (70%)	0.007
WBC, 10^9/L	114.0 (8.5-200.0)	150.0 (37.0-384.0)	0.503	18.0 (2.3-156.9)	161.2 (15.7-360.0)	0.005
Blasts in BM (%)*	78.5 (58-98)	93 (70-98)	0.316	84.0 (50-94)	91 (20-98)	0.136
Hemoglobin, g/L	11.0 (7.0-13.9)	7.9 (6.6-11.1)	0.003	12.5 (4.7-15.7)	9.8 (5.0-16.4)	0.197
Platelets ×10^9/L	94.0 (30.0-299.0)	71.5 (24.0-95.0)	0.206	115.0 (9.0-350.0)	50.5 (12.0-113.0)	0.114
Overall survival (5 years)	8/11 (73%)	5/9 (60%)	0.443	6/11 (55%)	5/10 (50%)	1.000

Values represent median (range). B.M.: bone marrow. n = 15 for αβ, and 18 for δγ T-ALL. †Individuals with 14 years-old or less were considered to be children. Comparisons were performed using the Mann-Whitney test for the analysis of continuous variables and Fisher's exact test for the analysis of discrete variables. A two sided p value less than 0.05 was considered significant.*

Table 2. Immunophenotypic features of TCR positive cases.

Antigens	*Children*		*p value*	*Adults*		*p value*
	αβ T-ALL (n=11)	*γδ T-ALL (n=9)*		*αβ T-ALL (n=11)*	*γδ T-ALL (n=10)*	
CD2	7/11 (64)	8/9 (90)	0.319	9/11 (82)	7/10 (70)	0.635
CD3*	11/11 (100)	9/9 (100)	NA	11/11 (100)	10/10 (100)	NA
CD5	11/11 (100)	9/9 (100)	NA	10/11 (91)	10/10 (100)	1.000
CD7	11/11 (100)	9/9 (100)	NA	10/11 (91)	10/10 (100)	1.000
CD1	4/11 (36)	6/9 (67)	0.369	5/11 (45)	6/10 (60)	0.670
CD4+ CD8-	1/11 (9)	2/9 (22)	0.566	2/11 (18)	1/10 (10)	1.000
CD4- CD8+	1/11 (9)	1/9 (11)	1.000	1/11 (9)	1/10 (10)	1.000
CD4+ CD8+	5/11 (45)	4/9 (44)	1.000	3/11 (27)	5/10 (50)	0.387
CD4- CD8-	4/11 (36)	2/9 (22)	0.642	3/11 (27)	3/10 (30)	1.000
TdT	7/10 (70)	8/9 (90)	0.582	9/11 (82)	9/9 (100)	0.479
CD34	4/10 (40)	2/9 (22)	0.628	5/10 (50)	7/10 (70)	0.650
CD45	9/9 (100)	9/9 (100)	NA	10/10 (100)	9/9 (100)	NA
CD45RA+/CD45RO-	5/11 (45)	0/5 (0)	0.118	5/10 (50)	1/9 (11)	0.141
CD45RA-/CD45RO+	2/11 (18)	4/5 (80)	0.035	2/10 (20)	7/9 (78)	0.023
CD45RA+/CD45RO+	3/11 (27)	1/5 (20)	1.000	3/10 (30)	0/9 (0)	0.211
CD45RA-/CD45RO-	1/11 (9)	0/5 (0)	1.000	0/10 (9)	1/9 (11)	0.474
CD10	3/11 (27)	5/9 (56)	0.361	5/11 (45)	6/10 (60)	0.670

*Antigens (clone): CD1a (SK9), CD2 (S5.2), CD3 (SK7), CD4 (SK3), CD5 (L17F12), CD7 (4H9), CD8 (SK1),CD10 (W8E7), CD34 (8G12), CD45 (2D1), CD45RA (L48), CD45RO (UCHL1), TCR αβ, (WT31), TCR γδ (11F2) and anti-TdT (HT-6). NA: not applicable. *cCD3 and/or mCD3.*

in both children and adults, whereas the $CD45RA^+$/$CD45RO^-$ phenotype predominated in αβ T-ALL. There is a scarcity of data about the CD45 isoforms in αβ and γδ T-ALL, and the only two studies describing the predominance of the CD45RO isoform in T-ALL did not evaluate the αβ, or γδ-TCR status of leukemic cells[5,6]. Since most normal thymocytes express the $CD45RA^-$/$CD45RO^+$ phenotype,[7] the finding that $CD45RA^+$/$CD45RO^-$ was the most common phenotype in αβ, T-ALL was unexpected. Although CD45RA and CD45RO expression discriminates as naïve and memory subsets of T-cells, respectively this differentiation holds best for peripheral blood T cells. In the thymus, where the normal counterparts of T-ALL are found, the expression of these CD45 isoforms is associated with functions related to thymic maturation[7]. Clinically, the CD45 isoform status had no prognostic value when overall survival of patients whose blasts expressed the CD45RA or CD45RO antigens were compared (*data not shown*).

The expression of membrane CD3 (mCD3), expected to be always detected in TCR^+ ALL, was negative in two cases of αβ T-ALL, whereas cytoplasmic $CD3^+$ was present in all cases. Asnafi *et al.*[8] recently described some T-ALL cases with negativity for mCD3, but with cytoplasmic expression of the TCRβ, antigen, which they called pre-TCR T-ALL. We have no explanation for these $TCR\alpha\beta^+$/$cCD3^+$/$mCD3^-$ cases observed in our series.

Although normal γδ T-cells represent a very small proportion of normal thymocytes, this subset is enriched among CD4 and CD8 double-negative T cells[9]. Malignant γδ T-cells, however, expressed CD4, CD8 or both of them in 74% of the cases. Similar results have been previously described, contradicting the intuitive acceptance that the γδ T-ALL blasts should not express CD4 and CD8, as their normal counterparts. Van Dongen *et al.*[1] described a small subset of normal γδ T-cells in the peripheral blood which was either CD4 or CD8 positive, in addition to the vast majority of double negative cells. Therefore, perhaps this small subset of normal CD4 or CD8 γδ T-cells is particularly prone to malignant transformation. Alternatively, the expression of CD4 or CD8 antigens in γδ T-cells may represent a phenomenon related to the neoplastic transformation. Taken together, these data give support to the concept that γδ T-ALL represents a distinctive subtype of leukemia, with peculiar clinical, laboratory and immunophenotypic characteristics.

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Key words: δγ-T cells, flow cytometry, T-cell receptor, acute lymphoblastic leukemia.

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References

1. Van Dongen JJ, Comans-Bitter WM, Wolvers-Tettero IL, Borst J. Development of human T lymphocytes and their thymus-dependency. Thymus 1990;16:207-34.
2. Macintyre EA, Salloum E, Sigaux F. Comparison of αβ and γδ expressing $CD3^+$ acute lymphoblastic leukemias. Nouv Rev Fr Hematol 1990;32:95-9.
3. Schott G, Sperling C, Schrappe M, Ratei R, Martin M, Meyer U, et al. Immunophenotypic and clinical features of T-cell receptor $\gamma\delta^+$ T-lineage acute lymphoblastic leukaemia. Br J Haematol 1998;101:753-5.
4. Rego EM, Garcia AB, Viana SR, Falcão RP. Characterization of acute lymphoblastic leukemia subtypes in Brazilian patients. Leukemia Res 1996;20:349-55.
5. Falcao RP, Garcia AB. Expression of CD45RA (naive) and CD45RO (memory) antigens in T-acute lymphoblastic leukaemia. Br J Haematol 1993;85:483-6.
6. Schiavone EM, Lo Pardo C, Di Noto R, Manzo C, Ferrara F, Vacca C, et al. Expression of the leucocyte common antigen (LCA, CD45) isoforms RA and RO in acute haematological malignancies: possible relevance in the definition of new overlap points between normal and leukaemic haemopoiesis. Br J Haematol 1995; 91:899-906.
7. Pilarski LM, Deans JP. Selective expression of CD45 isoforms and of maturation antigens during human thymocyte differentiation: observations and hypothesis. Immunol Lett 1989; 21: 187-98.
8. Asnafi V, Beldjord K, Boulanger E, Comba B, Tutour PL, Estienne MH, et al. Analysis of TCR, pTα, and RAG-1 in T-acute lymphoblastic leukemias improves understanding of early human T-lymphoid lineage commitment. Blood 2003; 101:2693-703.
9. Moretta L, Ciccone E, Mingari, MC, Zeromski J, Bottino C, Ferrini, S, et al. Phenotypic and functional characterization of human T lymphocytes expressing a γ/δ T cell antigen receptor. Clin Exp Rheumatol 1989; Suppl 3:S9.

Chronic Lymphoproliferative Disorders

Absence of surface CD27 distinguishes hairy cell leukemia from other leukemic B-cell malignancies

Surface expression of CD27 was evaluated in 75 mature leukemic B-cell neoplasms. All cases other than hairy cell leukemia (HCL) expressed CD27. Intensity was significantly higher in chronic lymphocytic leukemia. Lack of CD27 in 17/17 HCL contrasted with expression of this marker in 5/5 splenic lymphomas with villous lymphocytes. Lack of CD27 is a new distinctive feature of HCL among B-cell malignancies.

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CD27 is a member of the tumor necrosis factor (TNF)-receptor family induced on B lymphocytes after antigenic challenge and interacts with CD70 to differentiate mature B cells into plasma cells.[1] CD27 was originally defined as a memory B-cell marker, mainly because of its expression on B-cells with mutated *VH*-genes.[1] However, CD27 is induced in centroblasts and centrocytes of the germinal center (GC) and retained by post-GC memory B cells.[2] CD27 is generally conserved after neoplastic transformation on mature B-cell neoplasms,[3] but, differently from the normal situation, is independent of *VH*-gene status.[4] In chronic lymphocytic leukemia (B-CLL), CD27 is also present as a soluble molecule correlating with tumor load.[3] Immunohistochemistry has confirmed expression of CD27 in mantle cell lymphoma (MCL), Burkitt's lymphoma, marginal zone lymphoma (MZL) and plasmacytomas/myelomas.[5]