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Clinical relevance of CD10 expression in childhood ALL

RITA CONSOLINI,* ANNALISA LEGITIMO,* ROBERTO RONDELLI,° CESARE GUGLIELMI,# ELENA BARISONE,@ ALMA LIPPI,^ ANGELO CANTÙ-RAJNOLDI,§ MAURIZIO ARICÒ,** VALENTINO CONTER,§ MARIA GRAZIA COCITO,°° MARIA CATERINA PUTTI,°° ANDREA PESSION,° GIUSEPPE MASERA,§ ANDREA BIONDI,§ GIUSEPPE BASSO##

**Istituto di Clinica Pediatrica, Università di Pisa; °III Clinica Pediatrica, Università di Bologna; #Cattedra di Ematologia, Università La Sapienza, Rome; @Clinica Pediatrica, Università di Torino; ^Ospedale Meyer, Firenze; §Clinica Pediatrica, Università di Milano, Ospedale San Gerardo, Monza; **Clinica Pediatrica, Università di Pavia; °°Dipartimento di Pediatria, Università di Padova; ##Dipartimento di Pediatria, Università di Torino, for the Italian Association for Pediatric Hematology and Oncology (AIEOP)*

Abstract

Background and Objective. **Previous studies have considered the prognostic significance of CD10 expression in childhood acute lymphoblastic leukemia (ALL) and showed its linkage to a more favorable prognosis. The aim of this study was to assess the independent significance of CD10 expression in a large population of ALL patients.**

Design and Methods. **We revised the independent clinical relevance of CD10 expression in 2038 children with acute lymphoblastic leukemia (ALL), who were consecutively entered in 4 sequential trials of the Italian Association for Pediatric Hematology and Oncology (i.e. AIEOP studies 82, 87, 88, 91); 1142 were males and 896 females, age ranged between 1 and 14 years (yrs) at diagnosis. Of the whole group, 1471 children (72.2%) were defined as having standard risk, 567 (27.8%) as having a high risk.**

Results. **CD10 was detected in blast cells from 1706 of 1784 (95.6%) patients with B-lineage ALL and 46 of 254 (18.1%) with T-cell ALL. In the B-lineage subgroup CD10 expression was associated with presenting features such as age < 9 yrs and inclusion in the standard risk category. No significant differences were found between $CD10^+$ and $CD10^-$ cases in T-lineage ALL, concerning presenting features, except for FAB L2 in the former group. We compared the event-free survival (EFS) rates for patients with T-ALL or B-lineage ALL, regarding CD10 positivity, overall and by individual study. Patients with T-ALL fared worse than those with B-lineage ALL (5 and 10 yrs EFS: 46.8% vs. 68.5% and 44.5% vs. 63.7% respectively, p=0.0001). In multivariate analysis of B-lineage subgroup poorer EFS was associated with male sex, higher WBC ($\geq 20\times10^9$/L), age > 9 yrs. Only WBC $\geq 20\times10^9$/L and age > 9 yrs were parameters linked to poorer EFS in the T-lineage subgroup. Finally, we compared EFS rates for four groups of patients categorized as having high or standard risk, and according to $CD10^+$ and $CD10^-$ expression. High-risk patients fared statistically worse than standard risk patients both in the $CD10^-$ and in the $CD10^+$ groups (42% vs. 50.7% and 63.6% vs. 66.8%, respectively).**

Interpretation and Conclusions. **CD10 expression does not have independent prognostic significance in either the larger subgroup of B-ALL patients or in T-cell ALL.**

Correspondence: Rita Consolini, M.D., Istituto di Clinica Pediatrica, Università di Pisa, via Roma 67, 56100 Pisa, Italy. Phone: international +39.050.992222 • Fax: international +39.050.550595.

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The common acute lymphoblastic leukemia antigen (CALLA; CD10) originally described as a leukemia-associated antigen,[1] is now identified as a membrane-associated enzyme neutral endopeptidase[2] expressed on normal lymphoid progenitors[3] and neutrophils.[4] Previous studies have considered the prognostic significance of CD10 expression in childhood acute lymphoblastic leukemia (ALL)[5-16] and showed its linkage to a more favorable prognosis. The clinical significance of CD10 expression in a population of childhood ALL has been recently revised by Pui,[17] making allowance for poor prognosis subgroups, such as infants and patients with T-cell ALL or for the effects of improved therapy and biologic markers. That review showed that CD10 expression was associated with several good presenting features in childhood B-lineage ALL but is not an independent prognostic factor in B- or in T-cell lineage ALL.

Our study revises the independent significance of CD10 expression on a larger population of ALL patients, treated homogeneously according to AIEOP trials. Our results show that the lack of CD10 expression is not an independent adverse prognostic factor either in B- or in T-cell ALL.

Materials and Methods

The patient series comprised 2038 eligible children with newly diagnosed ALL enrolled from 1982 to 1991, referred to centers affiliated with the Italian Association for Pediatric Hematology and Oncology (AIEOP). We examined CD10 expression, clinical and biological features (i.e. age, sex, leukocyte count, morphologic and immunologic phenotype), risk and treatment outcome (i.e., EFS at 5 and 10 years) in the

entire patient population and in the $CD10^+/CD10^-$ subgroups. Karyotyping and DNA flow cytometric evaluation were not included in our study as they only became mandatory in 1995. Age ranged between 1 and 14 years. Infants were excluded from the study, but have already been analyzed taking into account intrinsic clinical and biological features peculiar to infantile leukemias.[18-20] The patients were consecutively enrolled in 4 sequential protocols of the AIEOP (i.e. AIEOP studies 82, 87, 88 and 91), which used increasingly intense therapy.[21] Risk factor (RF) was calculated on the basis of the number of initial leukemic cells and liver and spleen enlargement and delineated two groups: standard (SR) and high-risk (HR). The high-risk group included children with T-phenotype or WBC $\geq 50\times10^9$/L or leukemic-lymphoma syndrome in studies 82-87; RF $\geq$1.20 or t(9;22) or mediastinum > 1/3 of thoracic diameter or adenomegaly (single lymph node > 3 cm or multiple lymph nodes > 5 cm) or testicle involvement or bone marrow blasts $\geq$ 5% on the 42nd day of therapy identified high risk for protocol 8803 in the 88 study. Patients with CNS disease, in the same study, fell into the high-risk group for protocol 8833. In study 91, high risk included children with RF $\geq$ 1.70 or t(9;22) or t(4;11) or peripheral blasts $\geq$1000 on the 7th day of therapy or bone marrow blasts $\geq$ 5% on day 42 of therapy for protocol 9103. CNS disease indicated high risk in study 9133. Details of the treatment schedule used in the studies AIEOP-ALL 82,[22] AIEOP-ALL 87,[23] AIEOP-ALL 88[23] and the AIEOP-ALL 91 have been provided elsewhere.[24] In brief, treatment consisted of a classical Berlin-Frankfurt-Münster (BFM) backbone[25] with minor modifications. Patients in the HR group of study 91 received a block-type chemotherapy, derived from the BFM experience in relapsed ALL.[26]

Mean follow-up was 50 months (range 1-8 years); EFS was estimated at 5 and 10 years.

Immunologic phenotyping

The diagnosis of ALL was based on morphologic and cytochemical criteria set out by the French American British (FAB) Working Group.[20,27] Thus by definition, all patients had less than 3% blast cells positive for myeloperoxidase and Sudan black B, and less than 20 per cent positive for α-naphthyl-acetate-esterase (myeloid pattern); none of the cells contained Auer rods nor had FAB L3 morphology (i.e. B-ALL). Immunophenotype was extensively analyzed, including evaluation of lymphoid and myeloid-associated antigen expression by flow cytometry (Profile II, Coulter or Facscan, Becton Dickinson). The immunophenotype included the obligatory panel of mAbs established by BFM Family Cooperative Group.[28] This panel included mAbs against B-cell antigens (CD10, CD19; Coulter Clone), T-cell antigens (surface and cytoplasmic CD3, Ortho; CD7, Becton Dickinson: BDIS; CD1a, BDIS), myeloid antigens (CD13, CD33, CD65; Coulter Clone), and not-lineage specific antigens (HLA-DR; BDIS). Isotypical immunoglobulins were used as negative controls, at the same concentrations as the test antibodies. Blast cells were also tested for surface (sIg) and cytoplasmic (cyIg) immunoglobulins with polyclonal goat anti-human IgG and goat anti-human μ (Southern Biotechnology, Valter Occhiena, Torino, Italy) respectively. Terminal deoxynucleotidyl transferase (TdT, Valter Occhiena) was also assayed. Surface markers were considered positive when present on more than 20% of the blast cells; for cyIg a level of 10% was considered positive. TdT was evaluated on fixed marrow smears using polyclonal antibodies (10% being the limit for positivity).[28] Different immunologic groups were identified by positivity to different markers; thus the following categories were recognized: pre-pre-B ALL (TdT^+CD19^+ $HLA\text{-}DR^+CD10^-$ $cy\mu^-$), common ALL (TdT^+ $CD19^+$ $HLA\text{-}DR^+$ $CD10^+$ $cy\mu^-$), pre-B-ALL (TdT^+ $CD19^+$ $HLA\text{-}DR^+$ $CD10^{+/-}$ $cy\mu^+$), T-ALL. A case was considered T-ALL if the lymphoblasts expressed cyCD3, surface CD7 and TdT. T-ALL maturational stage was defined as follows: early thymocyte (TdT^+ $CyCD3^+$ $CD7^+$ $CD1a^-$ $sCD3^-$), intermediate stage (TdT^+ $CyCD3^+$ $CD7^+$ $CD1a^+$ $sCD3^-$), late stage (TdT+ CyCD3+ $CD7^+$ $CD1a^-$ $sCD3^+$). Patients with mature B-ALL were not eligible for these studies.

Statistical analysis

Data were collected on patient-oriented forms compiled by a physician at each center. All information was stored, controlled and analyzed by Venus, an integrated system of software facilities running on an IBM mainframe at the North-East Italian Interuniversity Computing Center (CINECA).

EFS was estimated using the Kaplan-Meier method.[29] Time to the study or time to terminal event was calculated from the day of diagnosis. Induction failure (resistant disease or death during induction), death in complete continuous remission and relapse were counted as failures. The log-rank test was used to assess differences in univariate analysis.[30]

Multivariate analysis was conducted using the Cox regression model to investigate the prognostic role of different variables in terms of duration of EFS.[31]

The significance of observed differences in proportions was tested using Fisher's exact test or the χ^2 test.[32] All reported *p* values are two-sided.

Results

Clinical and biological features according to CD10 expression in children with ALL

CD10 antigen was detected in blast cells from 1752 (86%) patients with ALL and was significantly associated with the following presenting features: female sex (p=0.0001), age range 1-9 years (p=0.0001), lower leukocyte count (p=0.0001), standard risk inclusion (p=0.0001) and immunophenotype B (p=0.0001). No significant differences were shown for FAB and proto-

Table 1. Presenting clinical and biological features according to CD10 expression in children with B-lineage ALL.

Feature	No. of patients	%	No. of patients CD10+ (%)	No. of patients CD10– (%)	p value
	1784	100	1706 (95.6)	78 (4.4)	
Sex					
F	831	46.6	795 (95.7)	36 (4.3)	0.94
M	953	53.4	911 (95.6)	42 (4.4)	
WBC (x10⁹/L)					
<20	1188	66.7	1143 (96.2)	45 (3.8)	0.114
*20	592	33.3	560 (94.6)	32 (5.4)	
FAB					
L1	1308	74.6	1259 (96.25)	49 (3.75)	0.09
L2	445	25.4	420 (94.4)	25 (5.6)	
Age (years)					
1-9	1503	84.25	1444 (96.0)	59 (4.0)	0.033
>9	281	15.75	262 (93.2)	19 (6.8)	
Risk					
Standard	1436	80.5	1389 (96.7)	47 (3.3)	0.0001
High	348	19.5	317 (91.1)	31 (8.9)	
Studies					
82-87	997	55.9	949 (95.2)	48 (4.8)	0.304
88-91	787	44.1	757 (96.2)	30 (3.8)	

Table 2. Presenting clinical and biological features according to CD10 expression in children with T-lineage ALL.

Feature	No. of patients	%	No. of patients CD10+ (%)	No. of patients CD10– (%)	p value
	254	100	46 (18.1)	208 (81.9)	
Sex					
F	65	25.6	10 (15.4)	55 (84.6)	0.51
M	189	74.4	36 (19.05)	153 (80.95)	
WBC (x10⁹/L)					
<20	55	21.9	14 (25.45)	41 (74.55)	0.122
*20	196	78.1	32 (16.3)	164 (83.7)	
FAB					
L1	165	66.5	23 (13.9)	142 (86.1)	0.015
L2	83	33.5	22 (26.5)	61 (73.5)	
Age (years)					
1-9	184	72.4	29 (15.8)	155 (84.2)	0.115
>9	70	27.6	17 (24.3)	53 (75.7)	
Risk					
Standard	35	13.8	10 (28.6)	25 (71.4)	0.083
High	219	86.2	36 (16.44)	183 (83.56)	
Studies					
82-87	131	51.6	22 (16.8)	109 (83.2)	0.574
88-91	123	48.4	24 (19.5)	99 (80.5)	

col inclusion. In the B-lineage CD10 was expressed in 1706 out of 1784 patients (95.6%). The lack of CD10 expression in cases of B-lineage ALL was significantly associated with these clinical and biological features at presentation; CNS involvement, age > 9 yrs, adenomegaly, platelets ≥ 50×10^3/mmc and high-risk inclusion (p=0.001, 0.033, 0.016, 0.023, 0.0001 respectively) (Table 1). In contrast none of these characteristics significantly differed between CD10+ and CD10– cases in T-ALL except for L2 inclusion (p=0.015) (Table 2).

Clinical outcome

The EFS (±SE) at 5 years for CD10+ children was 68.8±1.4% (63.9±1.2% at 10 yrs) and 50.5±8% (46.6±8.3% at 7 yrs) among B- and T-lineage ALL respectively, while for the CD10– children it was 61.5±5.6% (57.6±5.9% at 10 yrs) and 45.9±3.6% (43.9±3.7% at 10 yrs) among B- and T-lineage ALL, respectively.

The EFS rates at 5-and 10-yrs of overall ALL population were significantly worse for patients with higher WBC (p=0.0001), male gender (p=0.0001), age > 9

Table 3. EFS for B-lineage ALL.

Feature	No. of patients	No. of events	% EFS (SE) 5 yrs	% EFS (SE) 10 yrs	Univariate p value	Multivariate p value
	1784	545	68.5 (1.2)	63.7 (1.4)		
Sex						
F	831	204	75.5 (1.6)	70.6 (1.9)		
					0.0001	0.0001
M	953	341	62.4 (1.7)	57.5 (2.0)		
WBC (x10⁹/L)						
<20	1188	325	72.4 (1.4)	66.4 (1.7)		
					0.0001	0.0001
*20	592	219	60.6 (2.1)	58.3 (2.2)		
FAB						
L1	1308	374	70.1 (1.35)	65.8 (1.6)		
					0.0067	0.055
L2	445	162	63.8 (2.4)	57.8 (2.7)		
Age (years)						
1-9	1503	430	70.8 (1.2)	65.8 (1.5)		
					0.0001	0.0001
>9	281	115	55.8 (3.2)	52.0 (3.6)		
Risk						
Standard	1436	389	63.3 (2.6)	61.2 (2.7)		
					0.0001	
High	348	156	53.6 (2.8)	51.2 (2.9)		
CD10						
Pos.	1706	514	68.8 (1.2)	63.9 (1.4)		
					0.095	
Neg.	78	31	61.5 (5.6)	57.6 (5.9)		
Studies						
82-87	997	365	65.1 (1.5)	60.6 (1.7)		
					0.0004	
88-91	787	180	73.3 (1.8)	67.6 (3.3)*		

**EFS at 7 yrs; SE: Standard Error*

Table 4. EFS for T-lineage ALL.

Feature	No. of patients	No. of events	% EFS (SE) 5 yrs	% EFS (SE) 10 yrs	Univariate p value	Multivariate p value
	254	127	46.8 (3.3)	44.5 (3.4)		
Sex						
F	65	30	50.1 (6.5)	50.1 (6.5)		
					0.23	
M	189	97	45.7 (3.8)	42.7 (4.0)		
WBC (x10⁹/L)						
<20	55	19	64.0 (6.8)	60.8 (7.2)		
					0.0019	0.013
*20	196	106	41.9 (3.7)	39.8 (3.8)		
FAB						
L1	165	79	47.7 (4.2)	44.9 (4.4)		
					0.84	
L2	83	46	44.2 (5.6)	42.7 (5.6)		
Age (years)						
1-9	184	85	50.5 (3.9)	48.2 (4.1)		
					0.036	0.032
>9	70	42	36.9 (6.1)	34.8 (6.1)		
Risk						
Standard	35	10	68.9 (8.2)	68.9 (8.2)		
					0.0047	
High	219	117	43.3 (3.5)	41.0 (3.6)		
CD10						
Pos.	46	21	50.5 (8.0)	46.6 (8.3)*		
					0.53	
Neg.	208	106	45.9 (3.6)	43.9 (3.7)		
Studies						
82-87	131	70	44.9 (4.5)	41.9 (4.6)		
					0.64	
88-91	123	57	48.7 (4.8)	48.7 (4.8)*		

**EFS at 7 yrs; SE: standard error.*

yrs (p=0.0001) and high-risk inclusion (p=0.0001). Also T-immunophenotype (p=0.0001), lack of CD10 expression (p=0.0001), FAB L2 (p=0.0016) and treatment in 82-87 protocols (p=0.001) emerged as significantly linked to poorer outcome.

When we considered the same variables in a multivariate analysis only age (p=0.0001), sex (p=0.0001), and WBC (p=0.0001) were independently associated with poorer EFS. Similarly, for B- and T-lineage ALL, CALLA expression did not have an independent prognostic significance (Tables 3 and 4); while the classical prognostic factors, i. e. WBC, sex, age, were independently associated with EFS (p=0.0001, 0.0001, 0.0001, respectively) only in B-lineage patients (Table 3). In T-cell ALL the multivariate analysis attributed independendent significance only to age, (p=0.032) and WBC (p=0.013) (Table 4).

We also compared the EFS rates for four groups of patients $CD10^+$ high and standard risk and $CD10^-$ high and standard risk. $CD10^-$ high-risk patients fared significantly worse than standard risk patients either in the $CD10^-$ or in the $CD10^+$ group (p=0.004, p=0.0006 respectively) (Figure 1).

When we examined the EFS in the standard risk group, no significant difference was observed between $CD10^+$ versus $CD10^-$ ALL cases (p=0.66); in the high risk group, however, $CD10^+$ patients fared significantly worse than $CD10^-$ (p=0.019).

Discussion

This study evaluates CD10 expression in the largest series of consecutive newly diagnosed childhood ALL cases studied with flow cytometry, in order to assess the independent significance of CD10 expression and to clarify wide discrepancies in results given in previous reports.[5-9]

CD10 expression was found in 95.63% of the B-lineage and in 18.1% of the T-cell cases. Among our

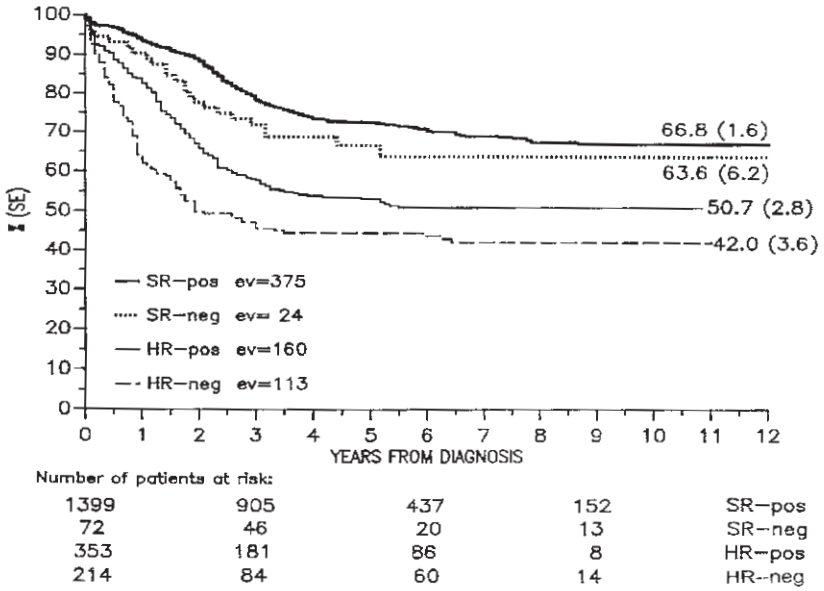

Figure 1. EFS for standard risk (SR) ALL vs. high risk (HR) ALL according to CD10 expression.

patients with B-lineage ALL, CD10$^+$ cases were characterized by a high proportion of children between 1 and 9 years of age, a larger leukemic burden and higher incidence of CNS leukemia at presentation than CD10$^+$ patients according to previous reports, in which a higher frequency of adverse genetic features, such as blast cells with a DNA index < 1.16 and pseudo-diploid karyotype were also reported.[16,17] In contrast, none of these presenting clinical and biological features significantly differed between CD10$^+$ and CD10$^-$ cases in T-ALL except for FAB classification. The analysis of the prognostic importance of CD10 expression in B-lineage in ALL has given contrasting results when infants are included.[4,6,7,8,17] In our study, from which infants were excluded, CD10 expression did not achieve independent prognostic significance in a multivariate analysis.

Studies of the prognostic significance of CD10 expression in T-cell ALL have yielded conflicting results.[8,11,16] Although its expression on leukemic blast cells was generally associated with a better outcome,[8,11] it did not achieve independent prognostic significance in multivariate analysis.[11,17] Cytogenetic and phenotypic differences between the two groups (CD10$^+$, CD10$^-$) were evocated by Pui's review,[17] in order to explain contrasting results. In our study, CD10 expression did not have independent prognostic significance, in accordance with previous studies by the AIEOP group,[24,33] in which higher WBC count was associated with unfavorable prognosis while steroid response with a remarkably good outcome.

We did not, however, analyze the correlation between myeloid antigen positivity and CD10 expression; in fact, the clinical relevance of myeloid antigen positivity has been excluded by two consecutive large studies.[34,35]

We conclude that CD10 expression is associated with several favorable presenting features in childhood B-lineage ALL and does not have prognostic value when related to risk inclusion. Finally our work stresses the lack of its independent significance either in B-lineage or T-cell lineage ALL.

Recently, CD10$^+$CD19$^+$CD34$^+$ immature B-progenitor immunophenotype has been associated with favorable characteristics for children with ALL and has identified a subset of infants who achieve favorable EFS outcomes.[36] That work suggested future investigations about the prognostic value of CD10 expression in ALL when associated with other unexplored markers.

Neutral endopeptidase (NEP) CD10 is expressed by human lymphoid malignancies with an immature phenotype and by normal human lymphoid progenitors that are either uncommitted or committed to only the earliest stages of B- or T- cell differentiation.[3,37-38] Studies performed on multiple organs and cell types indicate that the enzyme downregulates induced responses to peptide hormones.[3,39-42] Along B-cell ontogeny this enzyme participates in the regulation of stromal cell-dependent B-cell lymphopoiesis.[40] It is supposed that the enzyme may hydrolyze a peptide that promotes the initial proliferation of early lymphoid progenitors or cleaves a peptide precursor and generates a break-down product that inhibits early lymphoid development.[38] In this context, it is of interest that transin, a zinc metalloprotease belonging to the same superfamily as NEP, can be induced by oncogenes and is expressed more abundantly in malignant than in benign tumors.[43,44] Furthermore concomitant identification of natural NEP substrates, their receptors and functions, may contribute to our understanding of the variables that

influence self-renewal and differentiation of normal and leukemic lymphoid progenitors.[37,45]

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