

Immunophenotypic, Cytogenetic and Clinical Features of 113 Acute Lymphoblastic Leukaemia Patients in China

Haixia Tong,¹MD, Jihong Zhang,¹MD, Chunwei Lu,²BMSc, Zhuogang Liu,³MMSc, Yingchun Zheng,¹MMSc

Abstract

Introduction: The analysis of immunophenotype of the leukaemic cells has been of great importance for the diagnosis, classification and prognosis of acute lymphoblastic leukaemia (ALL). **Materials and Methods:** One hundred and thirteen Chinese patients with ALL were immunophenotyped by flow cytometry and 74 cases were also subjected to karyotype analysis by G-banding technology. **Results:** Of the 113 Chinese ALL patients, 14.2% were identified as T-ALL and 85.8% as B-ALL. Myeloid antigen (MyAg) expression was documented in 34.9% of the cases analysed and CD13 was most commonly expressed MyAg in ALL patients (23.6%). MyAg positivity was higher in adult with ALL (47.6%) than in children with ALL (26.6%). Abnormal karyotypes were detected in 39 out of 74 (52.7%) cases. The clinical and biological characteristics of ALL patients between MyAg⁺ and MyAg⁻ groups showed that increased white blood count (WBC) ($>50 \times 10^9/L$), higher CD34 positivity and higher percentage of adult patients were found to be correlated with MyAg⁺ ALL. **Conclusion:** Our results indicate that the immunophenotype did have relevance to the abnormal cytogenetic changes and clinical features in ALL. Flow cytometry immunophenotype has become the most important method for diagnosis and typing of ALL.

Ann Acad Med Singapore 2010;39:49-53

Key words: Diagnosis and typing, Flow cytometry, Karyotype analysis

Introduction

Acute lymphoblastic leukaemia (ALL) is a heterogeneous disease with abnormal proliferation and accumulation of immature lymphoblasts within the bone marrow (BM), peripheral blood and lymphoid tissues,¹ and is composed of different genetic, biological, and clinically relevant subtypes. Morphological and cytochemical methods were the main tools for diagnosis and classification of acute leukaemia before identification of monoclonal antibodies. ALL patients are subdivided into 3 subsets – L1, L2 and L3 – according to the French American British (FAB) co-operative group criteria. There is no correlation between L1 and L2 morphology and immunophenotype, but the L3 subtype was found to be closely associated with the mature B-cell phenotype.^{2,3} Developments in flow cytometric techniques and the availability of lineage-associated monoclonal antibodies have permitted characterisation of normal and leukaemia cells and affirmed the immunophenotypic heterogeneity in ALL.⁴

Materials and Methods

One hundred and thirteen patients with newly diagnosed ALL were enrolled in the study and their BM were collected from January 2007 to July 2008 in Shengjing Hospital, China Medical University. There were 65 male and 48 female patients with a median age of 18.1 years (range, 1 to 72). All the patients entered were preliminary diagnosed and had not received any treatment. Eighty-seven patients had extramedullary involvement (including liver, spleen, lymphoid node, bone joint, central nervous system and testicle), including 60 children and 27 adults.

Immunophenotype Detection of Leukaemia Cells

Fluorescein isothiocyanate (FITC), phycoerythrin (PE) or peridinin chlorophyll-protein (Per-CP) labelled 19 mAbs (Table 1) were provided by Becton-Dickinson Bioscience in the United States. Cells were acquired and analysed with Cell Quest and Paint-A-Gate software with FACS Calibur flow cytometer (BD Bio). Abnormal populations were

¹ Laboratory of Hematology, Shengjing Hospital of China Medical University

² Section of Health Testing, College of Public Health, China Medical University

³ Department of Hematology, Shengjing Hospital of China Medical University

Address for Correspondence: Dr Haixia Tong, Shengjing Hospital of China Medical University, No. 39 Huaxiang Road, Tiexi District, Shenyang, China 110022.

Email: tonghaixia18@yahoo.com.cn

recognised by CD45/SSC gating, which was the base of calculating the positive rate of leukaemia-related antigens expressed on the abnormal cells. We regarded $\geq 20\%$ of cell expression in a tube as positive expression, unless cytoIgM, cytoCD3 or cytoCD79a ($\geq 10\%$).

Cytogenetic Analysis

BM cells were cultured for 24 hours to prepare for conventional chromosome detection, and karyotype was analysed with G-banding technique. Each karyotype was named according to International Human Chromosomes Nomenclature (ISCN 1995).

Statistical Method

Statistical analysis was done using SPSS 11.5 software. Statistical differences of various clinical and laboratory parameters between the groups were evaluated by chi-square or the Fisher's Exact tests. To compare the mean of 2 or more groups, the 2 independent sample *t*-test and ANOVA were used. $P < 0.05$ was considered significant.

Results

Immunophenotype

The immunophenotypic features in different subtypes of the 113 ALL patients are summarised in Table 1. Of 113 patients with ALL, 16 cases (14.2%) were identified as T-ALL and 97 cases (85.8%) were identified as B-ALL. Among B-ALL, 14.4% were identified as Pro-B ALL, 56.7% as Com-B ALL, 25.8% as Pre-B ALL and 3.1% as Mature B-ALL. B-lineage markers: CD19, CD10, CD22 and CD20 were expressed in the vast majority of B-ALL samples at the rate of 99.0%, 82.5%, 78.4% and 37.5%, respectively. Of 97 B-ALL, only 1 out of 25 cases of Pre-B ALL did not express CD19 but expressed cytoCD79a and cytoIgM. There was high expression of stem/progenitor cell markers CD34, CD38 and HLA-DR in B-ALL at the positive rate of 71.1%, 85.6% and 100%, respectively. Of the 16 T-ALL cases, T-lineage marker CD3 was expressed in 9/16, and the cytoCD3 was positive in the other 7/16 cases. CD7 was found to be expressed most commonly in T-ALL (100.0%), followed by CD5 (80.0%), CD2 (62.5%) and CD3 (56.3%). CD10 was positive in only 3 cases of T-ALL and there was no B-lineage marker in the other

Table 1. Immunophenotype of the 113 ALL Patients

Antigen	Pro-B (cases)	Com-B (cases)	Pre-B (cases)	Mature-B (cases)	Total-B (cases)	T-ALL (cases)	Positive rate (%)
CD34	11/14	46/55	12/25	0/3	69/97	5/16	65.5
CD10	0/14	55/55	24/25	1/3	80/97	2/16	72.6
CD19	14/14	55/55	24/25	3/3	96/97	0/16	84.9
CD2	0/14	0/55	0/25	0/3	0/97	10/16	8.8
CD14	0/14	0/55	0/23	0/3	0/97	0/16	0
CD13	3/12	17/55	3/23	0/3	23/93	2/13	23.6
HIA-DR	14/14	55/55	25/25	3/3	97/97	4/16	98.2
CD33	2/11	4/52	1/21	0/3	7/87	3/13	10.0
CD7	0/14	0/51	0/25	0/3	0/97	16/16	14.2
CD38	13/14	44/55	24/25	2/3	83/97	16/16	87.6
CD20	0/13	24/55	10/25	2/3	36/96	0/16	32.1
CD22	10/14	44/55	19/25	3/3	76/97	0/16	67.3
CD3	0/14	0/55	0/25	0/3	0/97	9/16	8.0
cytoCD3	0/14	0/55	0/25	0/3	0/97	7/16	6.2
CD15	7/9	4/28	0/16	0/3	11/56	2/6	20.9
cytoIgM	0/14	0/55	25/25	3/3(sIgM)	25/97	0/16	22.1
cytoCD79a	14/14	35/35	14/14	3/3	66/66	0/16	80.5
CD117	0/10	0/47	0/19	0/3	0/79	4/12	4.4
CD5	0/14	0/55	0/25	0/3	0/97	12/15	10.7

The 113 ALL patients included 14 cases of Pro-B, 55 cases of Com-B, 25 cases of Pre-B, 3 cases of Mature-B, 16 cases of T-ALL. All values of positive expression were presented as positive/studied cases. All values of positive rate were calculated on the basis of the 113 ALL patients.

T-ALL cases. CD34 positivity (31.3%) and HLA-DR positivity (25.0%) were much lower in T-ALL than those in B-ALL (71.1% and 100%, respectively) ($P = 0.002$ and $P = 0.000$, respectively).

Analysis of Myeloid Antigen (MyAg) Expression in ALL Patients

MyAg expression was documented in 34.9% of the 106 ALL cases analysed. CD13 was the most commonly expressed myeloid antigen in ALL patients (23.6%), followed by CD15 (20.9%), CD33 (10.0%) and CD117 (4.4%). CD14 was negative in all ALL patients, and CD117 was negative in all B-ALL patients but positive in 33.3% T-ALL patients. CD13 expression was found most commonly in Com B-ALL (30.9%), and CD15 in ProB-ALL (77.8%). Though MyAg was more frequently associated with T-ALL (46.2%) than with B-ALL (33.3%), there was no significant difference ($P = 0.364$) between the 2 groups. MyAg expression was also compared in the subtypes of B-ALL. MyAg positivity in Pro B-ALL (66.7%) was higher than that in Com B-ALL (34.5%,

$P = 0.040$) and Pre B-ALL (17.4%, $P = 0.007$). We also found that MyAg positivity was much higher in adult patients with ALL (47.6%) than in children with ALL (26.6%) ($P = 0.020$).

Cytogenetics

The karyotype studies among 74 of the 113 ALL patients showed that a total of 39 (52.7%) had an abnormal karyotype (Table 2). Numerical abnormality of chromosome was found in 19 cases, including 16 cases of hyperdiploid and 3 cases of hypodiploid. Structural abnormality of chromosome was found in 20 cases, and 14 cases of t(9;22)(q34;q11) were found in Pro-B, Com-B and Pre-B, 2 cases of t(8;14)(q24;q32) were found in mature B-ALL, 3 cases of t(4;11)(q21;q23) were found in Pro-B, and 1 case of t(11;14)(p13;q11) was found in T-ALL.

Clinical and Biological Features in MyAg⁺ and MyAg⁻ ALL and Different Immunophenotype of ALL Patients (Table 3)

On the basis of the positivity for at least 1 myeloid marker, we stratified the patients into 2 groups: MyAg⁺ and MyAg⁻.

Table 2. Karyotype of 74 ALL Patients According to Different Immunophenotype

Karyotype	Pro-B (cases)	Com-B (cases)	Pre-B (cases)	Mature-B (cases)	Total-B (cases)	T-ALL (cases)
Normal	4	17	7	1	29	6
t(9;22)q34;q11	1	9	4	0	14	0
t(8;14)(q24;q32)	0	0	0	2	2	0
t(4;11)(q21;q23)	3	0	0	0	3	0
t(11;14)(p13;q11)	0	0	0	0	0	1
Hyperdiploid	0	8	4	0	12	4
Hypodiploid	0	1	2	0	3	0

The 74 ALL patients included 8 cases of Pro-B, 35 cases of Com-B, 17 cases of Pre-B, 3 cases of Mature-B, 11 cases of T-ALL.

Table 3. Clinical and Biological Features in Chinese ALL Patients

Factors	MyAg ⁺	MyAg ⁻	Pro-B	Com-B	Pre-B	Mature-B	Total-B	T-ALL
Sex (female/male)	17/20	30/39	8/6	21/34	12/13	2/1	43/54	5/11
Mean age (y)	20.3 ± 19.3	16.6 ± 19.3	24.2 ± 21.6	13.9 ± 15.2	17.4 ± 19.8	47.7 ± 39.2	17.4 ± 19.1	23.7 ± 19.7
Adult/Children	20/17	22/47*	8/6	19/36	7/18	2/1	36/61	8/8
Mean WBC count *10 ⁹ /L	66.6 ± 94.6	47.8 ± 89.5	85.7 ± 92.3†	50.7 ± 97.9	20.5 ± 22.7	21.7 ± 16.8	47.1 ± 84.3	86.6 ± 104.6‡
Mean haemoglobin (g/L)	79.7 ± 25.8	78.3 ± 27.4	83.4 ± 25.0	75.1 ± 27.9	75.0 ± 21.8	108.3 ± 37.5	77.3 ± 26.7‡	92.6 ± 25.7
Mean platelet count *10 ⁹ /L	82.6 ± 109.7	68.7 ± 87.2	144.6 ± 173.0	49.8 ± 62.2	78.5 ± 92.5	188.0 ± 163.3	75.2 ± 101.9	81.2 ± 82.2
WBC count *10 ⁹ /L (>50/<50)	18/19	14/55*	7/7	13/42	3/22	0/3	23/74	6/10
Mean bone marrow blasts (%)	79.8 ± 18.3	72.9 ± 21.3	78.3 ± 20.0§	78.1 ± 17.9§	64.2 ± 23.1	62.6 ± 25.9	74.1 ± 20.5	77.6 ± 20.1§
CD34+/CD34-	31/6	37/32*	11/3	46/9	12/13	0/3	69/28%	5/11
Cytogenetics (normal/abnormal)	15/14	20/25	5/3	18/17	9/8	2/1	34/29	5/6
B- ALL/T- ALL	30/7	64/5	-	-	-	-	-	-

* $P < 0.05$, compared with MyAg⁺ group; † $P < 0.05$, compared with Pre-B group(WBC count); ‡ $P < 0.05$, compared with T-ALL group; § $P < 0.05$, compared with Pre-B group (BM blasts); || $P < 0.05$, compared with Pro-B group(platelet count)

The percentage of patients with high WBC count ($>50 \times 10^9/L$) in MyAg⁺ (48.6%) was higher than that in MyAg⁻ ALL types (20.3%) ($P = 0.002$), and CD34 expression was found in 83.8% of MyAg⁺ and 53.6% of MyAg⁻ cases ($P = 0.002$). In addition, the percentage of adult ALL patients in MyAg⁺ group (54.1%) was higher than that in MyAg⁻ group (31.9%) ($P = 0.026$). No differences were found between the MyAg⁺ and MyAg⁻ groups with regard to other features. Analysis of the clinical and laboratory findings in different B-ALL subtypes and T-ALL failed to establish significant association for mean age, proportion of females/males, proportion of adult/children and cytogenetic abnormalities. However, mean WBC count was higher in Pro B-ALL ($P = 0.026$) or T-ALL ($P = 0.019$) than that in Pre B-ALL, and mean haemoglobin level in B-ALL was lower than that in T-ALL ($P = 0.034$). Higher mean platelet count was found in Pro B-ALL than that in Pre B-ALL ($P = 0.038$) and Com B-ALL ($P = 0.001$). For mean bone marrow blasts (%), the percentage in Pre B-ALL was lower than those in Pro B-ALL ($P = 0.034$), Com B-ALL ($P = 0.004$) and T-ALL ($P = 0.037$). Besides, CD34 positivity in B-ALL (71.1%) was higher than that in T-ALL (31.3%) ($P = 0.002$).

Discussion

Immunophenotypic characterisation of leukaemic cells has several goals including lineage assignment, evaluation of cell maturation and assessment of phenotypic aberrations.¹ Based on the expression of CD10, CD19, cytoIgM and sIgM, we classified our B-ALL cases into 4 subtypes.^{5,6} Comparison of different subtypes of B-ALL in this study has demonstrated a lower frequency of mature B subtype followed by Pro B and Pre-B subtype, and common B-ALL is the dominant subtype in B-ALL patients, which was similar to previous reports.⁷ In this study, we found that CD34 positivity (71.1%) was much higher in B-ALL than in T-ALL (31.3%). CD34 expression is related to good prognosis in B-ALL patients but has opposite prognostic significance in T-ALL patients, and the CR rates of CD34⁺ T-ALL patients were lower than those of CD34⁻ T-ALL patients.⁸

It has been thought that ALL cells with expression of myeloid antigens originate from initial stem cells earlier than lymph-directional stem cells and progenitor cells. MyAg positivity in Pro B-ALL (66.7%) was higher than that in Com B-ALL (34.5%) and Pre B-ALL (17.4%), and this may be one of the reasons that leukaemia cells in Pro-B ALL with poor prognosis originate from more initial stem cells. This was different from earlier reports that MyAg was not significantly associated with B-lineage ALL than with T-ALL.⁹ It may be due to the relatively small numbers of T-ALL patients in our study. MyAg expression was documented in 34.9% of the 106 ALL cases analysed, which is consistent with previous reports.⁹ CD15 positivity

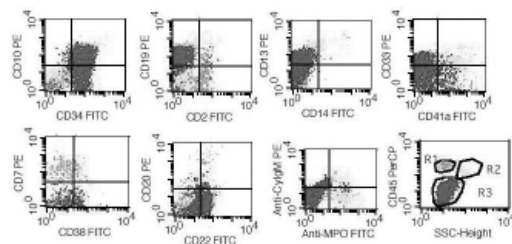


Fig. 1. Com-B ALL: The abnormal blasts (R3) are positive for CD34, CD10, CD19, CD13 and CD22.

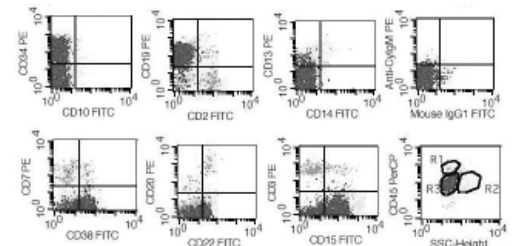


Fig. 2. Pro-B ALL: The abnormal blasts (R3) are positive for CD34, CD19, CD38, CD15 and CD22.

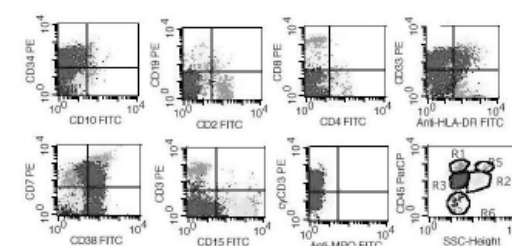


Fig. 3. T-ALL: The abnormal blasts (R3) are positive for CD34, CD33, CD7, CD38 and cytoCD3.

was highest in Pro B-ALL (77.8%) and the 3 cases with t(4;11)q21;q23 were all children with CD10-CD15⁺ which is consistent with a previous report that 11q23/MLL translocation occurred in CD10/CD15⁺ Pro-B ALL.¹⁰ CD13 was the most commonly expressed antigen in Com-B ALL with t(9;22) (q34;q11) or with CD34⁺ and HLA-DR⁺.

The clinical and biological characteristics of the 106 patients between MyAg⁺ and MyAg⁻ groups showed that patients with high WBC count ($>50 \times 10^9/L$), higher CD34 positivity and higher proportion of adult ALL were found to be correlated with MyAg⁺ ALL. But no differences were found with regard to other clinical features, which is different from an earlier report.⁹ It may be due to the fact that they analysed the expression of the MyAg only in 377 adult patients with ALL, while we analysed the expression of MyAg in both adults (39.6%) and children (60.4%). Some scholars reported that ALL patients with MyAg expression had poor prognosis.^{8,11} However, most reports indicate that MyAg expression in ALL patients is not associated with adverse presenting clinical and biological features and prognosis, especially in some cases based on high-dose chemotherapy.^{9,12,13} We also found that MyAg positivity

was much higher in adult patients (47.6%) than in children (26.6%) and this may be the reason that adults with ALL have poorer prognosis than children. The prognostic value of MyAg expression in ALL is still a controversial issue. The relatively small numbers of patients in ALL series and differences in treatment protocols have led to divergent results. Nonetheless, the evaluation of the expression of these antigens remains valuable for a more precise characterisation of the leukaemic population in each individual patient.

Studying the relationship among immunophenotype, cytogenetics, clinical features and prognosis in ALL patients may promote the recognition of the essence of ALL and its subtypes and provide better guidance for clinical diagnosis and therapeutic decision making.

Acknowledgements

Authors would like to thank the staff at the Laboratory and Department of Hematology, Shengjing Hospital, China Medical University for ALL samples.

REFERENCES

1. Foa R, Vitale A. Towards an integrated classification of adult acute Lymphoblastic leukemia. *Rev Clin Exp Hematol* 2002;6:181-99.
2. Bene MC. Immunophenotyping of acute leukaemias. *Immunol Lett* 2005;98:9-21.
3. Chan NP, Ma ES, Wan TS, Chan LC. The spectrum of acute lymphoblastic leukemia with mature B-cell phenotype. *Leuk Res* 2003;27:231-4.
4. Plasschaert SL, Kamps WA, Vellenga E, de Vries EGE, de Bont ES. Prognosis in childhood and adult acute lymphoblastic leukaemia: a question of maturation? *Cancer Treat Rev* 2004;30:37-51.
5. Campana D, Behm FG. Immunophenotyping of leukemia. *J Immunol Methods* 2000;243:59-75.
6. Paredes-Aguilera R, Romero-Guzman L, Lopez-Santiago N, Burbano-Ceron L, Camacho-Del Monte O, Nieto-Martinez S. Flow cytometric analysis of cell-surface and intracellular antigens in the diagnosis of acute leukemia. *Am J Hematol* 2001;68:69-74.
7. Asgarian Omran H, Shabani M, Shahrestani T, Sarafnejad A, Khoshnoodi J, Vossough P, et al. Immunophenotypic subtyping of leukemic cells from Iranian patients with acute lymphoblastic leukaemia: association to disease outcome. *Iran J Immunol* 2007;4:15-25.
8. Vitale A, Guarini A, Ariola C, Mancini M, Mecucci C, Cuneo A, et al. Adult T-cell acute lymphoblastic leukemia: biologic profile at presentation and correlation with response to induction treatment in patients enrolled in the GIMEMA LAL 0496. protocol. *Blood* 2006;107:473-9.
9. Vitale A, Guarini A, Ariola C, Meloni G, Perbellini O, Pizzuti M, et al. Absence of prognostic impact of CD13 and/or CD33 antigen expression in adult acute lymphoblastic leukemia. Results of the GIMEMA ALL 0496 trial. *Haematologica* 2007;92:342-8.
10. Attarbaschi A, Mann G, Konig M, Steiner M, Strehl S, Schreiberhuber A, et al. Mixed lineage leukemia-rearranged childhood pro-B and CD10-negative pre-B acute lymphoblastic leukemia constitute a distinct clinical entity. *Clin Cancer Res* 2006;12:2988-94.
11. Uckun FM, Sather HN, Gaynon PS, Arthur DC, Trigg ME, Tubergen DG, et al. Clinical features and treatment outcome of children with myeloid antigen positive acute lymphoblastic leukemia: a report from the Children's Cancer Group. *Blood* 1997;90:28-35.
12. Larson RA, Dodge RK, Burns CP, Lee EJ, Stone RM, Schulman P, et al. A five drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: Cancer and Leukemia Group B Study 8811. *Blood* 1995;85:2025-37.
13. Czuczman MS, Dodge RK, Stewart CC, Frankel SR, Powell BL, Sztatrowski TP, et al. Value of immunophenotype in intensively treated adult acute lymphoblastic leukemia: Cancer and Leukemia Group B. *Blood* 1999;93:3931-9.