EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 3 Number 1 (January-2015) pp. 115-123

www.ijcrar.com

A 10 Years Survey of Acute Lymphoblastic Leukemia in Northwest of Iran: Immunophenotyping Assessment and its Relation to Induction Therapy

Seyed Hadi Chavoshi[1*], Jamal Eivazi Ziaei[1], Ali Akbar Movasaghpour Akbari[1], Seyed Mohammad Reza Chavoshi[2], Hadi Hamedfar[2], Hosein Mazouchian[2] and Roya Dolatkhah[1]

[1]Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
Corresponding author

KEYWORDS

Acute Lymphoblastic Leukemia, Immunophenotyping, Induction Therapy

A B S T R A C T

Acute leukemia is a heterogeneous group of neoplastic diseases and is categorized into two main subgroups, myeloid and lymphoid. Therapy and prognosis differ based on the immunological type of Acute Lymphoblastic Leukemia (ALL). Therefore, the first step for ALL patients is the identification of the immunological subgroup by flow cytometry. The statistical population of the study constituted 134 ALL patients over 12 years old, who were morphologically diagnosed and for whom flow cytometry was performed. Laboratory data and patient records were studied and the values of hematological indices specified by flow cytometry were recorded. In terms of immunological subgroups, the following prevalence was observed from only 86 patients who had acceptable flow cytometry results: 19 (16%) patients were pro-B cell ALL, 63 (51%) were pre-B cell^{+} B-cell and 41 (33%) were pre-T cell^{+} T-cell. A total of 24 cases of patients in group B-ALL and 11 cases of patients in group T-ALL achieved complete remission, which was not statistically significant. Duration of remission in these patients was a secondary finding that ranged from 28 to 546 days in group B and from 45 to 220 days in group T, which was not statistically significant. Almost all the studies seem to agree that immunophenotyping is extremely important in the treatment and prognosis of ALL patients. Flow cytometry is a powerful and cost effective technique by which such antigens can be identified.

Introduction

Acute lymphoblastic leukemia (ALL) is a form of leukemia, or cancer of the white blood cells, characterized by excess lymphoblasts. Immature white blood cells continuously multiply and are overproduced in the bone marrow. Acute leukemia is a heterogeneous group of neoplastic diseases and is categorized into two main subgroups,

myeloid and lymphoid. The prognosis for ALL in adults is poor. Despite the significant progress in diagnosis and therapeutic methods in recent years, the rate of mortality in adults is high, and different studies have estimated the rate of successful treatment to be 25%–40%. Although standard prognostic variables for acute lymphoblastic leukemia include age, WBC count, immunophenotype, and cytogenetic, since the discovery of cell markers and antigens, they have been used in the definite diagnosis of blasts. Although the expression of these antigens in immature cells is not always specific, it is helpful in diagnosing different types of leukemia and ALL (Onciu, 2009; Appelbaum, 2004; Longo, 2008; Patkar *et al.,* 2012).

Cells surface antigens are useful in determining the type of leukemia. CD13, CD14, CD33, and CD34 are found in both normal immature myeloid cells and myeloblasts. Notably, in megakaryocyte and erythroid leukemia (AML), platelet and erythroid antigens are observed. In addition, in myeloid leukemia, HLA-DR is expressed by blasts (Belurkar *et al.,* 2013; Ching-Hon Pu, 2006). Intra-cytoplasmic Immunoglobulin (Ig) exists in 10% of B-ALL patients, and they are classified as pre-B cell ALL.

If the Ig is expressed on the surface of the cell, it is defined as a mature B-cell ALL and constitutes less than 5% of B-ALLs (Itaruka and Coutre, 2009; Tong *et al.,* 2012; Rosenthal *et al.,* 2012). In rare cases, leukemia patients' blasts don't express any antigen, which is called undifferentiated leukemia. If both lymphoid and myeloid markers are present on one blast, it is called biphenotypic, and if myeloid and lymphoid blasts exist simultaneously, they are called bilineage. Generally, the prognosis of patients in these three groups is much worse than with known leukemia.

The best therapeutic outcome is seen in the early pre-B cell $CD10^{+}$ group. Approximately 25% of ALLs express T-cell antigens on the blasts, and less than half of this percentage are pre-T-cell ALLs that express CD3, with or without CD4 and CD8 (Ching-Hon Pu, 2006; Itaruka and Coutre, 2009). However, most T-cell ALLs express CD3 with one of the CD4 and CD8 antigens on their blasts. The prognosis of T-cell ALL is better than pre-T cell ALL (Onciu, 2009; Appelbaum, 2004; Longo, 2008; Patkar *et al.,* 2012). About 25% of ALL patients express myeloid antigens and have worse prognosis and need more severe chemotherapy. Therapy and prognosis differ based on the immunological type of ALL. Therefore, the first step for ALL patients is the identification of the immunological subgroup by flow cytometry, followed by accumulation of prognostic factors to design a therapeutic protocol.

ALL is rarely asymptomatic and the signs and symptoms of ALL are almost always based on bone marrow and extra-medullary involvement. Sometimes, peripheral blood assessment alone is sufficient for disease diagnosis, but clinical and laboratory experiments are necessary for generating a therapeutic protocol and obtaining diagnostic information (Longo, 2008). Although about 25–40% of adults with ALL are cured, one-third adults over age 60 are afflicted with ALL, and age is still considered an important limiting factor in treatment efficacy. Most adult ALL patients have experienced disease relapse, and the rate of successful treatment strategy for relapsed disease is low (Longo, 2008). Over 80% of ALL patients with CNS involvement, show dissimilar recurrent symptoms, which may include headache, nausea, cervical rigidity, changes in intellectual abilities, and focal neurologic abnormalities. Other extra-medullary sites

involved are testis, retina, and derma. Additionally, the most prevalent findings on patient examination are paleness, petechia, and ecchymosis in the derma and mucosae (Onciu, 2009; Appelbaum, 2004; Longo, 2008).

Fortunately, flow cytometry and immunophenotyping studies on leukemia patients had been performed ten years ago in the Shahid Ghazi Hematology and Oncology Center. Therefore, we decided to study the ALL patients at this center to determine the efficiency of immunophenotyping in the successful diagnosis of immunological subgroups. Additionally, we expected to study the changes and specialized markers that are needed for definite diagnosis of ALL. The treatment protocol of the mentioned cases and the response to treatment based on immunological subgroups were also assessed.

Materials and Methods

This research was a descriptive and analytical study. The statistical population of the study constituted ALL patients over 12 years old who were hospitalized at the Shahid Ghazi Hematology and Oncology Center in Tabriz for acute leukemia, who were morphologically diagnosed and for whom flow cytometry was performed. A total of 134 ALL patients were selected from among individuals who were hospitalized in this center over 10 years, by means of in-access consecutive sampling.

Laboratory data and patient records were studied and the values of hematological indices specified by flow cytometry were recorded. Demographic information, clinical symptoms of patients and immunophenotyping indices according to peripheral blood study, aspiration, and bone marrow biopsy (which was the basis for dividing ALL patient into pre-B cell ALL, B-cell ALL, and T-cell ALL) were all studied and organized in groups.

The correlation between factors such as age and sex with response rate to therapy and general response rate up to complete remission were studied. The induction therapeutic regimen that was to be assessed in this study was a 28-day protocol regimen, containing Vincristine, Danurubisin, Cyclophosphamide, L- asparaginase, and Prednisolone according to the approved guidelines of the ward.

To ensure the accuracy of the markers and also to ensure a positive response of the blasts in the cellular sample, the cellular population of the target blasts was first selected according to size and specifications of intracellular appendages (Forward Scatter, Side Scatter), and all marker-related analyses were conducted on the selected population. All the purchased markers were then assessed according to the standard protocol recommended by the manufacturer (batch-control). Further, IgG-PE was utilized for all negative control examinations, including IgG-FITC. The number of cells in the experiment was adjusted between 5 and 10 thousand per microliter and 100 microliters of the sample was transferred to the flow cytometry cylinder (Becton Dickinson). Ten microliters of each of the antibodies conjugated with FITC (Fluorescein Isothiocyanate) and PE (PhycoErythrin) were added to each sample cylinder, then incubated in the dark at room temperature for 45 minutes, and the results were assessed with the Cell Quest software.

According to the criterion of the World Health Organization (WHO), if 20% of nucleate cells express an unusual antigen (marker) they are supposed to be positive.

Data obtained from this study was analyzed using the statistical software SPSS 13. Qualitative data were described as percentage and frequency, while central and dispersion statistics were used for description of quantitative data. The relation of qualitative variables was evaluated with Chi-squared test and Fisher exact test. The difference between the means of two groups was assessed with independent student t-Test, and data was considered significant at $p<0.05$.

Result and Discussion

Of 134 patients, 11 were omitted from the study because of uncertainty in ALL diagnosis, and of the remaining 123 cases, only 86 patients had acceptable flow cytometry results for determination of immunological subgroups (Table 2). Of the 123 patients assessed, 56 (45.5%) were women and 67 (54.5%) were men. Based on the immunological subgroups, although sex differences were insignificant, T-cell ALL was distinctively more common in men. The mean age of the patients was 28.5 ± 1.56 years, with a range of 12 to 80 years. There was no significant correlation between age and immunological and morphological subgroups.

In terms of geographical location, 61.3% of patients lived in urban areas and 38.7% lived in rural areas, and there was no significant difference between immunological and morphological subgroups and the place of residence. Disease incidence was almost the same in cold and hot seasons without any significant difference.

A mediastinal mass was observed in 3 (2.4%) cases and all the cases belonged to the T immunological subgroup. Splenomegaly was observed in 53 (43.5%) cases, while 60 (48.8%) patients had adenopathy for which the most common sites were cervix, submandibular, axillary, and inguinal regions. CNS involvement during the disease incidence was observed in 7 (5.7%) cases but was observed in a total of 25 (20.5%) of patients, regarding the immunologic subgroups, it was more frequently observed in B-cell rather than T-cell ALL patients. The existence of blast cells in the peripheral blood was seen in 75 (61%) patients without any significant difference among the various subgroups.

Tumor lysis syndrome was seen in 4 cases of all patients and was significantly more prevalent in the L3 morphological subgroup, although the immunological subgroups didn't present significant differences with this phenomenon. An elevated ESR was observed in 22 (17.8%) cases without a significant difference in subgroups. LDH increased in 96 (78.4%) patients without any significant difference (Table 2).

Morphologically, 3 (2.4%) patients were in group L1 and 115 (93.5%) were in L2 and 5 (4.1%) were in group L3. In terms of immunological subgroups, the following prevalence was observed: 19 (16%) patients were pro-B cell ALL, 63 (51%) were pre-B cell$^+$ B-cell and 41 (33%) were pre-T cell$^+$ T-cell.

None of the patients in subgroup L1 achieved complete remission after primary induction therapy, while in the L2 subgroup, 57.9% achieved complete remission and 100% in the L3 subgroup achieved complete remission. Duration of remission in B-cell ALL patients ranged from 28 to 546 days, with a mean of 177 days and it was 45 to 220 days in T-cell ALL patients, with a mean of 189 days.

A comparison of the survival rate among the subgroups showed that in group B, the survival rate ranged from 3 to 2000 days

with a mean of 588.42 days. In group T, of the 16 patients, the survival rate ranged between 9 to 950 days with a mean of 416.41 days. Of the 58 patients in the B-ALL group, 24 patients achieved complete remission, and of the 28 patients in the T-ALL group, 11 achieved complete remission. However, the difference between the groups was not statistically significant.

With regards the response to therapy or complete remission followed by primary induction therapy in the morphological groups, both the patients in group L1 and L3 achieved complete remission. Since the number of patients in L1 and L3 was low, no specific conclusion could be reached; however, 60.4% in the ALL (L2) subgroup achieved complete remission which corroborates previous results. Considering that ALL remission varies based on prognostic factors, treatment of patients based on prognostic factor indices can be helpful in improving response to treatment.
In terms of responsiveness to induction therapy on classification into immunological subgroups, 36% of patients in the pro-B cell group, 73.9% in the pre-B cell group, and 28.6% in the T-cell group (both mature T-cell and pre-T cell) achieved complete remission, indicating a high complete-remission rate in pre-B cell patients. Further studies are warranted, as is a comparison with similar research, to determine whether the factors considered in our study improved the response to treatment, either alone or along with certain other factors.

Immunophenotyping now constitutes an important part of diagnosis and selection of the treatment protocol in leukemia patients and increasingly specialized markers are being introduced to facilitate definite diagnosis of different types of leukemia every day. Diagnosis of leukemia types according to their morphology has become a historic event in the progression of hematological science, and currently diagnosis and therapy are impossible in the absence of immunophenotyping, cytogenetic, identification of genes by FISH (fluorescence in situ hybridization), etc. But from a developing country point of view, the first step in achieving diagnosis and therapy would be developing a cost effective assay (Patkar *et al.*, 2012).

In a number of previous studies, ALL patients were organized in groups based on their immunophenotyping as follows: B-cell type ALL is divided into pre-pre-B cell, pre-B cell, and B-cell; T-cell type ALL is divided into pre-T cell and mature T-cell (Onciu, 2009; Appelbaum, 2004; Longo, 2008; Patkar *et al.*, 2012; Belurkar *et al.*, 2013; Ching-Hon Pu, 2006; Itaruka and Coutre, 2009). Although the determination of B and T subgroups was impossible because of limitations in procurement of the same antibodies (so that in some cases elimination of some patients from the study list was inevitable), the priority was to use the complete panel of markers to accurately determine the ALL subgroups. Unfortunately with immunological classification using flow cytometry, because of a scarcity of utilized markers and the absence of useful markers for the precise determination of new immunological subgroups, only the determination of B, T, and pre-B cell subgroups was possible, while distinguishing pre-T ALL from T-ALL and differentiating between mature B-cell ALL and pre-B ALL was impossible. Patient prognosis based on B-cell and T-cell groups is as follows: in the B-cell group, the more immature the blast cell is, the better is the prognosis and response to therapy, while in the T-cell group, prognosis of mature T-cell is better than pre-T cell. Regarding the importance of subgroups in choosing the treatment method of ALL patients and the

necessity for differentiation of subgroups in using novel therapeutic methods, identifying additional markers is considered more important. This is an important limitation of our current study.

In the remaining 86 patients, these results were obtained using flow cytometry criteria and cellular antigen expression: 14 patients (16.2 %) were in the pro-B cell group and 44 (51.3 %) were in the pre-B cell and mature B-cell group; however differentiation between them was impossible. Twenty-eight patients (32.5 %) were organized in the T-ALL group and of course differentiation between pre-T ALL and mature T-ALL was impossible. Different studies have identified 10%–50% expression of myeloid antigens in lymphoid blasts in adult ALL (Itaruka and Coutre, 2009; Tong *et al.*, 2012). Additionally, the expression of B markers on T-cell blasts and T markers on B-ALL was considered in this study.

With regards the expression of myeloid antigens, CD13 (the most prevalent myeloid marker) is expressed secondarily in lymphoid blasts. In our study 40% of B-ALL blasts and 50% of T-ALL blasts expressed the CD13 antigen. CD33 marker was aberrantly expressed at 29.3% in B-ALL and 31% in T-ALL, corroborating the results of previous studies (Longo, 2008; Ching-Hon Pu, 2006). In other words, the aberrant expression of myeloid markers CD13 and CD33 is more prevalent in T-ALL than B-ALL patients.

In another study at the Shahid Ghazi Center in Tabriz, prevalence of lymphoid markers on lymphoid blasts was reported as 45% for TdT, 28% for CD7, 21% for CD2, 14% for CD10, but unlike other studies CD19 (5%) and CD20 (9%) were less prevalent. The most prevalent myeloid markers expressed on lymphoid blasts were CD13 and CD33 with a prevalence of 16% and 10%, respectively. CD34 was expressed in 64% of lymphoid blasts, which translated as a good prognosis (Rosenthal *et al.*, 2012; Asvadi Kermani, 2002).

In a study conducted in the Pathology Department of the Aga Khan University in Karachi Pakistan, 209 ALL patients were studied according to immunophenotyping classification and the amount of T-ALL were reported at 17.22%. The mean age of patients afflicted with ALL was 17.2 years and T-ALL was more prevalent in adults than in children (21.95% against 14.17%, respectively). T-ALL was more prevalent among men than women (69.40% against 25.36%, respectively). CD7 was the most sensitive marker observed in pediatric and adult T-ALL with a prevalence of 94.4%. The markers utilized in these patients were CD22, CD20, CD17, HLA-DR, CD33, CD13, CD7, CD5, CD1, and CD10 (11).

In another study conducted by Xu *et al.* (2003) at the hematology department of Nanfang hospital at the Medical University of China's army, the expression rate of myeloid antigens on blasts of 29 ALL patients was 34.5%. Additionally, the expression rate of lymphoid antigens on blasts of 71 AML patients was reported at 23.9 %. A study conducted by Zhu *et al.* (2002) in the West hospital of China's Sichuan University demonstrated that the expression rate of myeloid antigens in 28 patients was 30%; 10.3% of cases expressed CD13 and 20.7% expressed CD33. This study demonstrated that there is no difference in the expression rate of myeloid antigens between T-ALL and B-ALL types, while the CD33 expression was significantly higher in the T-cell type ($P<0.001$). However, in the study by Xu *et al.*, myeloid antigen expression in ALL patients who expressed CD34 was more than in the CD34-negative patients ($P = 0.036$, 13.3%

against 77.8%, respectively) (Zhu *et al.*, 2002). In our current study, since the flow cytometry file was incomplete, we couldn't evaluate the expression rate of myeloid antigens on lymphoid blasts based on the presence of CD34.

In a study conducted by Al Gwaiz *et al.*, in the hematology department of the medicine faculty of King Khalid University Hospital and King Saud University Medical College, using flow cytometry and Immuno-histochemistry (IHC), it was shown that of the 50 patients, 37 (74%) had pre-B ALL markers, 10 (20%) were T-ALL, and 3 (6%) were mature B-ALL (Al Gwaiz and Bassioni, 2008).

A few other studies have documented the prevalence rate of T-ALL at 20–25%, while in our study it is 32.5%, which is relatively high (Lenormand *et al.*, 1998; Chen *et al.*, 2007; Goldberg *et al.*, 2003). Due to the possibility of bone marrow and peripheral blood involvement in high degree lymphomas being misdiagnosed as ALL, the necessity of utilizing supplementary methods has gained importance. A few other studies have documented the percentage of pre-B cell, T-ALL, and B-ALL as 75%, 20%, and 5%, respectively (Onciu, 2009; Appelbaum, 2004; Itaruka and Coutre, 2009).

It is obvious that for identifying the role of immunological subgroups in adult ALL, it is essential to determine the expression of CD19, CD20, CD21, SIg, cIg, TdT, CD2, CD3, CD4, CD7, CD1a and to consider the common marker CD45 and several myeloid markers (CD13, CD33) (Belurkar *et al.*, 2013; Vitale *et al.*, 2006; Patte *et al.*, 2001; Hamouda *et al.*, 2007). Further, when the co-expression of special antigens is considered, performing the task can be helpful in determining the expression of B markers on T-cell and vice versa (Pui *et al.*, 2003; Qiu *et al.*, 2009). Two other studies, conducted in the hematology department of China's People University, analyzed the expression of T antigens (CD2, CD7) and demonstrated that T antigens are rarely expressed in B-ALL blasts (Qiu *et al.*, 2009; Wang *et al.*, 2003).

Table.1 The immunophenotyping of the various subgroups of patients was conducted based on the following criteria, according to previous reports:

Pro-B cell	$CD19^+$, $HLADR^+$, $CD34^+$
Early Pre-B cell	$CD19^+$, $CD10^+$, TdT^+, $CD22^+$,$CD79^+$
Pre-B cell	$CD19^+$, $CD20^+$, $CD21^+$, $CD10^-$
Mature B-cell	$CD19^+$, $CD20^+$, $CD21^+$, $CD22^+$,sIg M, IgD
Pre-T cell	$CD2^+$, $CD7^+$, $CD3^+$, $CD8^+$, $CD4^+$ or $CD2^+$, $CD7^+$, $CD3^+$, $CD8^-$, $CD4^-$
Mature T-cell	$CD2^+$, $CD7^+$, $CD3^+$, $CD4^-$, $CD8^+$, TCR^+ or $CD2^+$, $CD7^+$, $CD3^+$, $CD4^+$, $CD8^-$

Table.2 Immunophenotyping classification of ALL patients studied

ALL subtype	Frequency (%)	Mean ± Std
Pro-B cell	14(10.4%)	25.86±14.79
Pre-B cell	4(3%)	37.25±25.78
B-cell	32(23.9%)	29.19±18.67
Pro-T cell	1(0.7%)	12±0
T-cell	35(26.1%)	25.63±15.42
Total	86(64.2%)	27.35±16.99

Conclusion

The results of this study on the response to induced therapy are different from the studies that consider a good prognosis for T-cell ALL. The inability to differentiate pre-T cell ALL from T-cell ALL can primarily explain this difference, because the pre-T cell subgroup has a worse prognosis than T-cell ALL based on hematological studies. The expression of myeloid T-cell ALL markers is more prevalent than B-cell ALL. Almost all the studies seem to agree that immunophenotyping is extremely important in the treatment and prognosis of ALL patients. Unfortunately, in the Tabriz Shahid Ghazi hematology center, immunophenotyping faced certain failures and defects that need to be improved upon.

References

Al Gwaiz, L.A., Bassioni, W. 2008. Immunophenotyping of acute lymphoblastic leukemia using immunohistochemistry in bone marrow biopsyspecimens. *Histol. Histopathol.*, 23(10): 1223–8.

Appelbaum, F.A. 2004. The acute leukemia. In: Goldman L, Ausiello D (eds). Cecil text book of medicine, 22nd edn. WB Saunders, Philadelphia. Pp. 1161–1166.

Asvadi Kermani, I. 2002. Immunophenotyping of acute leukemia in northwestern Iran. *IJMS*, 27(3): 136–8.

Belurkar, S., Mantravadi, H., Manohar, C., Kurien, A. 2013. Correlation of morphologic and cytochemical diagnosis with flowcytometric analysis in acute leukemia. *J. Can. Res. Ther.*, 9: 71–9.

Chen, W., Karandikar, N.J., McKenna, R.W., Kroft, S.H. 2007. Stability of leukemia-associated immune phenotypes in precursor B-lymphoblastic leukemia/lymphoma: a single institution experience. *Am. J. Clin. Pathol.*, 127(1): 39–46.

Ching-Hon, Pu. 2006. Acute lymphoblastic leukemia. In: Lichtman, M.A., Beutler, E., Kipps, T.J., Seligsohn, U., Kaushansky, K., Prchal, J.T. (Eds). Williams hematology, 7th edn. Mc Graw-Hill, New York. Pp. 1321–42.

Goldberg, J.M., Silverman, L.B., Levy, D.E., *et al.* 2003. Childhood T-cell acute lymphoblastic leukemia: the Dana-Farber Cancer Institute acute lymphoblastic leukemia consortium experience. *J. Clin. Oncol.*, 21(19): 3616–22.

Hamouda, F., EL-Sissy, A.H., Radwan, A.K., *et al.* 2007. Correlation of karyotype and immunophenotype in childhood acute lymphoblastic leukemia; experience at the NationalCancer Institute, Egypt. *J. Egypt Natl. Canc. Inst.*, 19(2): 87–95.

Itaruka, H., Coutre, S.E. 2009. Acute lymphoblastic leukemiain adults. In: Greer, J.P., Foerster, J., Lukens, J.N., Rodgers, G.M., Paraskevas, F., Glader,

B. (Eds). Wintrobe's clinical hematology, 12th edn. Lippincott Williams &Wilkins, Philadelphia. Pp. 1820–1842.

Khawaja, M.R., Allana, S.S., Akbarali, A.N., *et al.* 2005. Flow cytometric and demographic analysis of t cell acute lymphoblasticleukemia in Pakistani population. *J. Ayub. Med. Coll. Abbottabad*, 17(4): 3–8.

Lenormand, B., Béné, M.C., Lesesve, J.F., *et al.* 1998. PreB1 (CD10-) acute lymphoblastic leukemia: immunophenotypic andgenomic characteristics, clinical features and outcome in 38 adults and 26 children. The Grouped EtudeImmunologique des Leucémies. *Leuk Lymphoma*, 28(3–4): 329–42.

Longo, D.L. 2008. Malignancies of lymphoid cells. In: Fauci, A.S., Braunwald, E., Kasper, D.L., Hauser, S.L., Longo, D.L., Jameson, J.L. (Eds). Harrison's principle of internal medicine, 17th edn. McGrow-Hill, New York. Pp. 687–99.

Onciu, M. 2009. Acute lymphoblastic leukemia. *Hematol. Oncol. Clin. North Am.*, 23(4): 655–74.

Patkar, N., Alex, A.A., Bargavi, B., *et al.* 2012. Standardizing minimal residual disease by flow cytometry for precursor B lineage acute lymphoblastic leukemia in a developing country. *Cytometry Part B*, 82B: 252–258.

Patte, C., Auperin, A., Michon, J., *et al.* 2001. The Société Française d'Oncologie Pédiatrique LMB89 protocol: highly effective multiagent chemotherapy tailored to the tumor burdenand initial response in 561 unselected children with Bcell lymphomas and L3 leukemia. *Blood*, 97(11): 3370–9.

Pui, C.H., Sandlund, J.T., Pei, D., *et al.* 2003. Results of therapy for acute lymphoblastic leukemia in black and white children. *JAMA*, 290(15): 2001–7.

Qiu, Y., Wang, G., Liu, Y.J., Shan, L.J. 2009. Analysis of immunophenotypes of acute lymphoblastic leukemia by three color flow cytometry. *Zhongguo Shi Yan Xue Ye XueZaZhi*, 17(2): 442–4.

Rosenthal, D.S., Schrier, S.L., Landaw, S.A. 2012. Evaluation of bone marrow aspirate smears [Up to Date]. Release: 20.5-c20.7: Up to Date Marketing Professional, Wolkers Kluwer Health; 2012.

Tong, H., Lu, C., Wang, Q., Wang, H., Wang, H. 2012. Immunophenotype of Chinese patients with T-lineage acute lymphoblastic leukemia and its association to biological and clinical features. *Acta Haematol.*, 127(4): 199–204.

Vitale, A., Guarini, A., Ariola, C., *et al.* 2006.Adult T-cell acute lymphoblastic leukemia: biologic profile at presentation and correlation with response to induction treatment in patients enrolled in the GIMENIA LAL 0496 protocol. *Blood*, 107(2): 473–9.

Wang, H., Liu, Y.R., Chen, S.S., *et al.* 2003. Analysis of immunophenotype of B-lineage acutelymphoblastic leukemia cells by 4-color flowcytometry. *Zhongguo Shi Yan Xue Ye XueZaZhi*, 11(2): 137–41.

Xu, B., Hu, C., Miao, X.D., *et al.* 2003. Immunophenotyping of 106 adult patients with acute leukemia by flow cytometry. *Di Yi Jun Yi Da XueXueBao*, 23(10): 1043–6.

Zhu, H., Niu, T., Meng, W., Xu, C., Lei, S. 2002. Immunophenotype of acute leukemia and its clinical significance. *Hua XiYi Ke Da XueXueBao*, 33(1): 118–20.