CD54 and CD62L expression by lymphoid cells in acute lymphoblastic leukaemia in children

F. M. HAFEZ*, H. HASSAB*, Z. MORAD† and E. A. M. FARAG*
Departments of Pediatrics and Clinical Pathology†, Faculty of Medicine, Alexandria University, Egypt*

Accepted: 25 July 2003

ABSTRACT

Altered expression or function of adhesion molecules on leukaemic blasts may contribute to the evolution and biological behaviour of acute leukaemia. This work studies the expression of CD54 and CD62L by lymphoid cells and the serum level of the shed form of L-selectin (sL-selectin) in children with acute lymphoblastic leukaemia (ALL) at initial diagnosis and after first remission, and their relationship to disease activity and subtype. The study is conducted on 20 children (age range 2-10 years) newly diagnosed with ALL and admitted to Alexandria University Children's Hospital. Ten apparently healthy children of matched age and sex serve as a control group. Expression of CD54 and CD62L on mononuclear cells is detected by monoclonal antibodies using flow cytometry. Serum sL-selectin is measured by enzyme-linked immunosorbent assay (ELISA). B-cell ALL was the most common subtype (45%), followed by T-ALL (35%) and C-ALL (20%). CD54 and CD62L mean cellular expression, as well as serum sL-selectin level, were significantly higher at diagnosis than both after remission and in the control group. Univariate analysis showed that the presence of mediastinal mass, high leucocyte count, central nervous system involvement and low CD54 were significant predictors of mortality in children with ALL.

KEY WORDS: Intercellular adhesion molecule-1. Leukemia, lymphocytic, acute. L-Selectin.

Introduction

Acute lymphoblastic leukaemia is a biologically heterogeneous disorder that may develop as a consequence of malignant transformation of a single abnormal progenitor cell at any point during the multiple stages of normal lymphoid differentiation.[1] Normal haematopoiesis occurs within bone marrow (BM) under the influence of a marrow microenvironment that includes cellular, cytokine and extracellular matrix interactions, all of which contribute to an environment that allows the haematopoietic progenitor cells to proliferate and differentiate normally.[2]

Adhesive interaction between haematopoietic cells and the specialised BM microenvironment plays a critical role in regulating normal haemopoiesis and egress of mature blood cells into the circulation. Therefore, it is hypothesised that altered expression or function of adhesion molecules on leukaemic blasts could contribute to the evolution and biological behaviour of acute leukaemias,[3-5] and determine the egress of blasts into peripheral blood and the homing of others to extramedullary sites.[6,7]

Thus, in acute leukaemias, egress of blasts into peripheral blood might be due to alteration of the adhesive capacity of leukaemic cells. Three families of cell adhesion molecules (CAMs) that may mediate cell-cell or cell-matrix interactions have been identified: immunoglobulins, integrins and selectins. They are important for normal haematopoiesis as well as for initiation of immune response to inflammatory processes.[8]

The present work studies the expression of CD54 and CD62L by lymphoid cells and the serum level of shed L-selectin (sL-selectin) in children with acute lymphoblastic leukaemia (ALL) at initial diagnosis and after first remission, and the relationship to disease activity and subtype.

Materials and methods

The study was conducted on 20 children, newly diagnosed with ALL, admitted to Alexandria University Children's Hospital. Ten healthy children of matched age and sex served as a control group.

Correspondence to: Dr Hoda Hassab
16, Roushdy Pasha Street, Roushdy, Alexandria, Egypt
Email: drhoda@doctor.com

All cases included in the study were subjected to thorough investigation of the duration of the disease and the major presenting symptoms. Physical examination looked at manifestations of the disease such as fever, purpura, leg pain, pallor, hepatosplenomegaly and lymphadenopathy.

Diagnosis of leukaemia was based on routine morphological evaluation and cytochemical staining of smears, and used the French–American–British (FAB) classification and immunophenotyping with a panel of monoclonal antibodies against various lymphoid antigens.[9]

Chest X-ray for the presence of mediastinal lymph nodes and cerebrospinal fluid (CSF) examination for blast cells were performed at the time of initial diagnosis.[10] Expression of CD54 and CD62L on mononuclear cells was detected by immunophenotyping using flow cytometry,[9] and serum sL-selectin was measured by enzyme-linked immunosorbent assay (ELISA).[11]

Cell separation for immunophenotyping

Heparinised samples were used and mononuclear cells (MNC) from peripheral blood (or bone marrow when the

Table 1. Expression of CD54 and CD62L on mononuclear cells in childhood ALL and serum sL-selectin level (before and after remission) and their controls

	Leukaemic children		Control	F test
	At presentation (n=20)	At remission (n=16)	(n=10)	
CD54 (%)				
-Min – Max.	0.4 – 4.4	0.1 – 3.0	0.3 – 0.9	11.758*
-Mean (SE)	2.06 (0.310)#@	0.69 (0.187)	0.57 (0.072)	P<0.001
CD62L (%)				
-Min – Max	1.6 – 42.0	0.6 – 33.0	3.3 – 5.4	19.939*
-Mean (SE)	23.34 (2.493)#@	9.1 (2.122)	4.26 (0.278)	P<0.001
sL-selectin (mg/L)				
-Min – Max	13.1 – 85.5	0.70 – 3.10	0.68 – 2.20	36.838*
-Mean (SE)	35.82 (4.836)@#	1.85 (0.192)	1.45 (0.192)	P<0.001

*Significant, P<0.05

#Significant from the controls

@Significant from after remission

number of blasts was >50%) were separated by density gradient centrifugation using Lymphoprep (density 1.077 g/mL; Nycomed Pharma AS, Oslo, Norway). Heparinised samples were layered on equal volumes of Lymphoprep and the tubes were centrifuged at 2000 rpm for 30 min at room temperature. MNCs at the interface between the Lymphoprep and the plasma layer were removed carefully and transferred to another tube, to which was added Hank's balanced salt solution (HBSS) or RPMI 1640. The tubes were centrifuged at 2000 rpm for 10 min at room temperature. After centrifugation, the supernatant was removed and the cell pellet was washed (x3) in HBSS. Cell concentration was then determined.

Immunofluorescence and flow cytometry

Viable MNCs were incubated with unlabelled mouse primary antibody and a second layer of fluorescein-conjugated goat anti-mouse immunoglobulin. All incubations were performed at room temperature and 2% pooled human group AB serum was added at all steps to avoid non-specific binding of monoclonal antibody to Fc receptors.

A volume of MNC cell suspension containing 0.5–1 x 10^6 cells/mL was added to each tube with an automatic pipette. The cells were centrifuged (Immunofuge II) for 2 min at 2000 rpm. The cell pellet was resuspended in 50 µL 2% AB serum and then the recommended volume of the relevant, optimally titrated antibody (CD54 or CD62L) was added and incubated for 10 min at room temperature.

After incubation, the cells were washed in an automatic washing centrifuge (x3) for 2 min each. A second layer (5 µL) of fluorescein-conjugated rabbit anti-mouse immunoglobulin was added and incubated for 10 min room temperature. The cells were washed again (x3) in the automatic centrifuge. The cells were then resuspended in 500 µL Isoton II (Coulter) prior to flow cytometric analysis in a FACScan instrument.

Several negative controls were included in order to compensate for the autofluorescence of cells and the non-specific binding of antibodies. Data were analysed after gating of the MNC population in the light scatter dot plot, using the normal counterpart pattern.

Results

The age range of the children with ALL studied was from two to 10 years (mean 4.40±1.81) and 75% were males. The most common presenting symptom was pallor (85%), followed by fever (75%). Hepatomegaly was detected in all ALL children, while splenomegaly and lymphadenopathy were encountered in 85% of those studied. Mediastinal mass (diagnosed by X-ray) was detected in only 15% of the ALL children (T-cell subtype). Blast cells were present in CSF in just two (10%) cases of ALL (T-cell subtype). Of the three ALL subtypes, B-ALL was the most common (45%), followed by T-ALL (35%), and C-ALL (20%).

Mean cellular expression of CD54 was significantly higher at diagnosis than after remission and in the control group (P<0.001), but there was no significant difference between cases of ALL in remission and that of the control group (Table 1). Mean cellular expression of CD62L was significantly higher in ALL children, both before and after remission, compared with the control group. This difference was statistically significant (P<0.001)), but there was no significant difference between cases of ALL in remission and the controls (Table 1).

Serum sL-selectin level was significantly higher before remission than after remission and in the control group (P=0.001). There was no significant difference between cases of ALL in remission and the control group (Table 1).

Children with a mediastinal mass had significantly lower CD54 expression (t = 3.790, P=0.0013) and higher sL-selectin levels (t = 3.27, P=0.0043) than did those without mediastinal mass. Also, children with positive CSF findings had significantly lower CD54 (t = 3.38, P=0.0033) and CD62L (t = 7.06, P>0.001) expression than those with a negative CSF (Table 2).

No significant correlation was found between cellular expression of CD54 and CD62L and serum sL-selectin level with WBCs, blast cells, spleen size and liver size (Table 3). T-ALL cases showed lower cellular expression and B-ALL showed higher cellular expression; however, a significant difference was found only between T-ALL and C-ALL cases

Table 2. Cellular expression and sL-selectin level by clinical, radiological and CSF findings

	CD54 Mean (SE)	CD62L Mean (SE)	sL-selectin Mean (SE)
Lymphadenopathy			
– No (n=3)	2.09 (0.826)	22.11 (6.592)	37.16 (13.204)
– Yes (n=17)	1.70 (0.352)	28.25 (2.363)	30.48 (4.206)
Student t-test	0.6301	0.3353	0.5958
Mediastinal mass			
– No (n=17)	2.23 (0.344)	24.48 (2.512)	32.02 (4.966)
– Yes (n=3)	0.80 (0.152)	16.83 (9.084)	57.40 (5.865)
Student t-test	0.0013*	1.10	0.0043*
CSF			
– Negative (n=18)	2.16 (0.333)	25.07 (2.439)	33.24 (4.951)
– Positive (n=2)	0.75 (0.250)	7.75 (0.250)	59.05 (9.750)
Student t-test	0.0033*	>0.001*	0.0298

*Significant, P<0.05

Table 3. Correlation between CD54 and CD62L expression and sL-selectin level and the studied parameters before remission

	CD54	CD62L	sL-selectin
Age	0.0792 P=0.7399	-0.2761 P=0.2387	0.2427 P=0.3025
Spleen size	0.2196 P=0.3522	–0.2775 P=0.2362	0.0886 P=0.7103
Liver size	–0.2104 P=0.3733	–0.3627 P=0.1160	0.1554 P=0.5130
WBCs	–0.3088 P=0.1853	–0.1340 P=0.5733	0.1967 P=0.4058
Peripheral blasts	0.0190 P=0.9366	–0.1293 P=0.5532	–0.094 P=0.6934
Marrow blasts	0.0220 P=0.9266	0.2216 P=0.3478	0.2768 P=0.2374

with CD54 (P=0.0112). Serum sL-selectin level did not differ significantly by the ALL subtype (P=0.8418; Table 4).

Univariate analysis showed that mediastinal mass, positive CSF, high leucocyte count and low CD54 expression were significant predictors of mortality from ALL (Table 5); however, multivariate analysis showed that only mediastinal mass was a significant predictor of mortality from ALL (Table 6).

Discussion

This study showed that serum sL-selectin and cellular expression of CD54 and CD62L were high in children with newly diagnosed ALL, and that levels decreased significantly during the first remission stage to reach those found in the control group.

A significant association was found between sL-selectin level and clinical course, and supported the findings of Olejnic *et al.*, who observed that serum sL-selectin concentrations decreased significantly from diagnosis to the end of intensive chemotherapy, and increased during relapse. They suggested that monitoring of sL-selectin might be useful for evaluating leukaemia activity.[12]

Moreover, Spertini and colleagues showed that ALL patients in remission had plasma sL-selectin levels within the normal range, but at initial diagnosis and in those with therapy-resistant acute leukaemia or in relapse had significantly higher levels. They concluded that monitoring sL-selectin level was helpful for the diagnosis of relapse in patients with acute leukaemia.[13] Furthermore, an absence of normalisation of the sL-selectin level after induction chemotherapy was associated with failure to achieve complete remission.[14]

In the present study no correlation could be found between cellular expression of CD62L and serum level of L-selectin. Similar findings were reported by Simmons *et al.*[15] Expression of CD54 and CD62L proved to be significant predictors of remission in childhood ALL. However, the level during remission did not differ significantly from that in the control group. CD54 and CD62L expression was higher at initial diagnosis, as they reflect the greater number

Table 4. Relationship between CD54 and CD62L expression and serum sL-selectin level by ALL subtype

	T-ALL (n=7) Mean (SE)	B-ALL (n=9) Mean (SE)	C-ALL (n=4) Mean (SE)	F test (P)
CD54 (%)	0.89 (0.173)#	2.73 (0.453)	2.65 (0.67)	5.9174* (0.0112)
CD62L (%)	20.30 (5.488)	25.73 (3.676)	23.25 (0.625)	0.440 (0.6512)
sL-selectin (mg/L)	35.58 (8.054)	38.42 (9.08)	30.41 (2.105)	0.174 (0.8418)

#Significantly different from C-ALL

*Significant, $P<0.05$

of blast cells present at that time.[16] Similar results were reported by Tacyildiz and colleagues, who studied both serum levels and leukaemic cell-tumour tissue expression of ICAM-1/CD54 in children with acute leukaemia.[17]

Moreover, Hatzistilianou *et al*. found similar results in a study of the serum level of soluble ICAM-1, soluble vascular cell adhesion molecule-1 (s-VCAM-1) and soluble E-selectin, and they concluded that soluble circulating adhesion molecule levels could be used to monitor disease activity of ALL and its response to treatment, as well as the early detection of relapse. The strong linear correlations between the three soluble adhesion molecules tested suggested that each alone might be sufficient as an indicator.[18]

The level of cellular expression and serum sL-selectin differed among ALL subtypes. The present study revealed that children with T-ALL had the lowest CD54 level. Although patients with T-ALL showed lower CD62L cellular expression than those seen in patients with B-ALL and C-ALL, but the difference was below that of statistical significance. Also, children with T-ALL and B-ALL tended to have higher levels of sL-selectin, and similar findings were found by Liesveld *et al*.[2] and Mielcarek *et al*.[19]

Hara *et al*. analysed the expression pattern of adhesion molecules in children with B-cell-precursor acute lymphoblastic leukaemia (pre-B ALL) and in children with B-cell ALL/non-Hodgkin's lymphoma (B-ALL/NHL). They reported that the expression of CD54 was more frequent in B-ALL/NHL and that the presence of CD54 on the cell surface was an independent factor indicating a poor prognosis. They concluded that expression of adhesion molecules is dependent on the phenotype of B-lineage cells and that the expression of some of these molecules has clinical significance.[20] Furthermore, Herold *et al*. studied sCD62L plasma concentration in children with ALL at first relapse and found that high sCD62L was associated with circulating blasts and a T-cell phenotype.[21]

In the present study, the clinical characteristics of the cases studied concentrated on serum sL-selectin level at initial diagnosis. The results revealed a positive non-significant correlation with spleen and liver size, WBC count and peripheral blood blast cells. Non-significant negative correlation was found with BM blast cells. However, this non-significant correlation may have been due to the small numbers studied, as Liesveld *et al*. found a weak correlation between sL-selectin and blast cells in the blood.[2]

As sL-selectin may influence the homing of host cells by inhibiting their attachment to an activated endothelium,[2] the rates of shedding and synthesis of L-selectin might prove variable among leukaemic cells and could be upregulated by cytokines either produced by the blast cells or present in their microenvironment. However, the absence of a significant correlation with the individual clinical manifestations at initial diagnosis might be explained by the fact that plasma sL-selectin concentration does not only depend on the level of L-selectin expression.[2]

In the present study, CD62L pattern in acute leukaemic patients correlated neither with clinical presentation nor with extramedullary involvement. Similar findings have been reported elsewhere[7,22,23] but Kawasaki examined 24 lymphoma/leukaemia cell lines and found that L-selectin increased with the presence of lymph node metastasis.[24] Also, Tanaka *et al*. suggested that in adult T-cell leukaemia (ATL), L-selectin expression was significantly higher on peripheral ATL cells in patients with lymphadenopathy than in those without it. Thus, selectin expression may result in tissue-specific migration.[25]

In the present study, CD54 showed a positive correlation with spleen size and blast cells (in peripheral blood and BM), and a negative correlation with liver size and WBC count; however, these were not statistically significant. Other studies have revealed a negative correlation between CD54 expression and peripheral leucocyte count.[15] Mielcarek *et al*. observed a significant positive correlation between low CD54 expression and high peripheral leucocyte count and splenomegaly at the time of diagnosis. In addition, they observed that CD54 expression was a favourable but not independent prognostic factor.[19]

Retention of leukaemic blasts in the marrow and increased susceptibility to host tumour surveillance might have contributed to the lower peripheral blast counts seen in the CD54-positive patients. Also, CD62L did not show any significant correlation with the studied parameters.[15]

Lymphoblasts frequently infiltrate the central nervous system (CNS) and two cases (10%) in the present study showed blast cells in CSF at initial diagnosis. Both cases had a fatal outcome and the two patients also showed the lowest CD54 expression in the present study.

Simmons *et al*.[15] reported a 2.7% incidence of initial CNS involvement among ALL children, but this was significantly higher in CD54-negative patients. Mielcarek *et al*. found a

Table 5. Factors affecting outcome of ALL in children

	Outcome		Test
	Alive (n=16)	Dead (n=4)	
Age			Z = 0.095
– Mean (SE)	4.44 (0.497)	4.25 (0.435)	P=0.9203
Sex			Fisher = 1.0000
Male	11 (68.8%)	3 (75.0%)	
Female	5 (31.3%)	1 (25.0%)	
Liver size			Z = 0.3043
– Mean (SE)	4.06 (0.487)	4.00 (0.41)	P=0.7642
Spleen size			Z = 0.7269
– Mean (SE)	2.75 (0.545)	3.00 (0.705)	P=0.4708
Mediastinal mass			Fisher = 0.0035*
– No	16 (100.0%)	1 (25.0%)	
– Yes	0 (0.0%)	3 (75.0%)	
CSF			Fisher = 0.03158*
– Negative	16 (100.0%)	2 (50.0%)	
– Positive	0 (0.0%)	2 (50.0%)	
Platelets			Z = 0.7106
– Mean (SE)	58.75 (11.296)	62.50 (14.477)	P=0.4795
WBCs			Z = 2.3623*
– Mean (SE)	52.69 (19.857)	224.00 (64.475)	P=0.0183
Lymphocytes (BM)			Z = 1.8201
– Mean (SE)	4.78 (0.965)	1.75 (0.63)	P=0.0689
Blast cells (BM)			Z = 1.4211
– Mean (SE)	86.44 (3.43)	94.50 (1.04)	P=0.1552
CD54			Z = 2.2298*
– Mean (SE)	2.39 (0.342)	0.78 (0.11)	P=0.0258
CD62L			Z = 0.0474
– Mean (SE)	24.01 (2.627)	20.63 (7.46)	P=0.9748
sL-selectin			
– Mean (SE)	32.80 (5.352)	47.91 (10.36)	Z = 1.4179
			P=0.1552
Leukaemia subtype			Fisher = 0.0072*
– T-ALL	3 (18.8%)	4 (100%)	
– B-ALL	9 (56.2%)		
– C-ALL	4 (25.0%)		

*Significant, P< 0.05
Z = Mann-Whitney U test
Fisher = Fisher exact test

positive correlation between low CD54 expression and CNS involvement at the time of diagnosis,[19] perhaps as a direct result of a higher number of peripheral tumour cells or a more specific sequence of adhesive events leading to migration across the blood-brain barrier.[26]

The tendency to splenomegaly in CD54-negative patients might be due to a shift in homing potential from marrow towards spleen, or it might be as a consequence of higher peripheral blast counts. There was also a trend toward lymphadenopathy and mediastinal enlargement in the CD54-negative group, even though the differences did not reach statistical significance.[15]

Univariate analysis showed that mediastinal mass, high leucocytic count, CNS involvement and low CD54 count were significant predictors of mortality in children with ALL. Adjusting for the confounding effects of the factors studied through a multiple logistic regression model showed that mediastinal mass was the most important prognostic risk predictor of mortality in children with ALL.

Similar results were reported by Simmons *et al.*, who found that event-free survival at 4.5 years was significantly better in CD54-positive than in CD54-negative patients. The authors also used multivariate analysis and showed that CD54 expression was not an independent risk factor, but was associated with an initial leucocyte count $<20 \times 10^9$/L, which is a well-established favourable prognostic sign.[27]

Table 6. Significant risk predictor of mortality from ALL in children

	Coefficient (B)	Wald test	Exp (B)
Mediastinal mass	12.9754	0.0187	431673.5
Constant	–2.7726	7.2351	
Predicting power of the model = 95%			

Nevertheless, this clearly demonstrates the close link between CD54 expression and both the course of the disease and overall prognosis.

In summary, an inverse correlation between CD54 expression and WBC, CNS involvement and splenomegaly was found in childhood ALL at the time of diagnosis. CD54-positive patients showed a better outcome but the marker was not of independent prognostic significance. □

References

1 Poplack DG. ALL. In: Pizzo PA, Poplack DG, eds. *Principles and practice of pediatric oncology*. Philadelphia: JB. Lippincott, 1989: 323–65.

2 Liesveld JL, Winslow JM, Frediani KE, Ryan DH, Abboud CN. Expression of integrins and examination of their adhesive function in normal and leukemic hematopoietic cells. *Blood* 1993; **81**: 112–21.

3 Archimband E, Thomas X, Campos L, Magaud JP, Dore JE, Fiere D. Expression of surface adhesion molecules CD54 (ICAM-1) and CD58 (LFA-3) in adult acute leukemia: relationship with initial characteristics and prognosis. *Leukemia* 1992; **6**: 265–71.

4 Bradstock KF, Gottlieb DJ. Interaction of acute leukemia cells with the bone marrow microenvironment: implication for control of minimal residual disease. *Leuk Lymphoma* 1995; **18**: 1–16.

5 Vila L, Thomas X, Campos L, Sabido O, Archimband E. Expression of VLA molecules on acute leukemia cells: relationship with disease characteristics. *Exp Hematol* 1995; **23**: 514–18.

6 Gordon MY, Dowdin CR, Riley GP, Goldman JM, Greaves MF. Altered adhesive interactions with marrow stoma of hematopoietic progenitor cells in chronic myeloid leukemia. *Nature* 1987; 328–42.

7 Liesveld L. Expression and function of adhesion receptors in acute myelogenous leukemia: parallels with normal erythroid and myeloid progenitors. *Acta Haematol* 1997; **95**: 53.

8 Takayuki I, Akihiro I, Kyoko T, Hirohumi S, Minoru O, Takashi U. E-selectin and vascular cell adhesion to endothelial cells. *Blood* 1993; **82**: 1590–8.

9 Rowan RM, Ban AIN, England JM, Hude K, Matutes E, Reilly JT. Immunophenotyping in diagnosis of acute leukaemias. *J Clin Pathol* 1994; **47**: 779–81.

10 Varley H, Growenlock AH, Bell H. General topics and common tests. In: *Practical clinical biochemistry* (Vol 1; 6th edn). William Heinemann, 1988: 80–126, 158–67, 349–93.

11 Ishikawa E, Yoshitaks S. Use of antienzyme for enzyme immunoassay of antibodies. In: Ishikawa E, Kawai T, Miciai K, eds. *Enzyme immunoassay*. New York: Igaki-Shoin, 1981: 263.

12 Olejnik I. Serum soluble L-selectin in childhood acute lymphoblastic leukemia. *Pediatr Int* 1999; **41**(3): 246–8.

13 Spertini O, Patrizia C, Anne-Sophie C, Jacques H, Jean J, von Fliedner V. High levels of the shed form of L-selectin inhibit blast cell adhesion to activated endothelium. *Blood* 1994; **84**: 1249–56.

14 Spertini O, Schleiffenbaum B, White-Owen C, Ruiz PJ , Tendder TF. ELISA for quantitation of L-selectin shed from leukocytes *in vivo*. *J Immunol Methods* 1992; **156**: 115.

15 Simmons PJ, Zannettino A, Gronthos S, Leavesley D. Potential adhesion mechanisms for localization of hemopoietic progenitors to bone marrow stroma. *Leuk Lymphoma* 1994; **12**: 35–63.

16 Marco M, Sperling CH, Martin S, Hansjorg R. Expression of intercellular adhesion molecule-1 (ICAM-1) in childhood acute lymphoblastic leukemia: correlation with clinical features and outcome. *Br J Haematol* 1997; **96**: 301–7.

17 Tacyildiz N, Yavuz G, Gozdasoglu S *et al*. Serum levels and differential expression of intercellular adhesion molecule-1 in childhood leukemia and malignant lymphoma: prognostic importance and relationship with survival. *Pediatr Hematol Oncol* 1999; **16**:149–58.

18 Hatzistilianou M, Athanassiadou F, Agguridaki C, Catriu D. Circulating soluble adhesion molecule levels in children with acute lymphoblastic leukaemia. *Eur J Pediatr* 1997; **156**(7): 537–40.

19 Mielcarek M, Sperling C, Schrappe M, Meyer U, Riehm H, Ludwig WD. Expression of intercellular adhesion molecule 1 (ICAM-1) in childhood acute lymphoblastic leukaemia: correlation with clinical features and outcome. *Br J Haematol* 1997; **96**(2): 301–7.

20 Hara J, Matsuda Y, Fujisaki H *et al*. Expression of adhesion molecules in childhood B-lineage-cell neoplasms. *Int J Hematol* 2000; **72**: 69–73.

21 Herold R, Stibenz D, Hartmann R, Henze G, Buhrer C. Soluble L-selectin (sCD62L) in relapsed childhood acute lymphoblastic leukaemia. *Br J Haematol* 2002; **119**(3): 677–84.

22 Reuss-Bost MA, Burgring HJ, Klein G, Muller CA. Adhesion molecules on CD34+ hematopoietic cells in normal bone marrow and leukemia. *Ann Hematol* 1992; **65**: 169.

23 Mengarelli A, Zarcone D, Caruso R *et al*. Adhesion molecule expression, clinical features and therapy outcome in childhood acute lymphoblastic leukemia. *Leuk Lymphoma* 2001; **40** (5-6): 625–30.

24 Kawasaki N, Matsuo Y, Yoshio T *et al*. Metastatic potential of lymphoma/leukemia cell lines in SCID mice is closely related to expression of CD44. *J Cancer Res* 1996; **87**(10): 1070.

25 Tanaka Y, Wake A, Morgan KJ *et al*. Distinct phenotype of leukemic T cells with various tissue tropisms. *J Immunol* 1997; **158**(8): 3822.

26 Bleyer AW. Biology and pathogenesis of CNS leukemia. *Am J Pediatr Hematol Oncol* 1989; **11**: 57–63.

27 Riehm H, Feickest H.J, Lampert F. *Acute lymphoblastic leukemia. Cancer in children*. Berlin: Springer, 1986: 101–18.