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BIPHENOTYPIC CHARACTERISTICS, CELL SIZE AND PROGNOSIS IN CHILDHOOD ACUTE MYELOBLASTIC LEUKEMIA*

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In order to determine the prognostic significance of cell size together with expression of biphenotypic markers in childhood acute myeloblastic leukemia (AML), we evaluated the cell size of children with AML, 12 with and 21 without biphenotypic markers. The patients were followed up for at least 12 months. The cells which were stained with FITC conjugated surface marker antibodies were divided into small, middle or large cell groups according to their mean channel number of forward scatter by flow cytometry. Nine of 12 biphenotypic and 15 of 21 non-biphenotypic children either died or relapsed within the first 12 months. The percentages of the small, middle and large cells were similar in children and in deceased patients, regardless of whether or not they expressed biphenotypic markers. We believe that biphenotypic marker expression is a poor prognostic factor regardless of cell size. *Key words: cell size, biphenotypic expression, childhood, acute myeloblastic leukemia.*

The known prognostic factors for acute myeloblastic leukemia (AML) are not as definite as they are for acute lymphoblastic leukemia (ALL)[1]. Although many factors have been examined for their prognostic role, the number of studies which have examined biologic and prognostic significance of myeloblast size is very few[2,3], (unpublished data), unlike with ALL[4].

Although we have shown that the percentage of large cells and their ratio to small cells were higher in nAML children with very short survival than in children who lived[3], the extensive form of this study, together with Kawada et al'.s[2] study, demonstrated no definite relationship between individual cell size and prognosis. Kawada et al.[2] established a relationship between myeloblast size and prognosis dependent on some surface markers. We established a relationship between blast cell size and prognosis dependent on high leukocyte count (unpublished data) in children with AML. Biphenotypic appearance of AML (presence of 2

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or more lymphoid markers) is known as a poor prognostic factor[1]. We carried out this study to evaluate the significance of cell size together with biphenotypic expression.

To our knowledge, this study is the first to investigate the prognostic significance of cell size together with biphenotypic expression in childhood AML.

Material and Methods

Patients: Children who were diagnosed as AML and underwent flow cytometric evaluation at admission between November 1993 and June 1996 were included in the study (median age: 11; mean age: 9.80; range: 1.5-16; 16 female, 17 male). Of these, 32 were de novo AML; one converted to AML from Fanconi's aplastic anemia [diepoxybutane (DEB) positive]. According to French-American-British (FAB) criteria, the patients had M1 (7 cases), M2 (14 cases), M3 (4 cases), M4 (5 cases), M6 (2 cases), M7 (1 cases) types.

Twenty-four of the patients died or relapsed from the 5^{th} day to the 12^{th} month of the treatment (median age: 10.5; mean age: 9.29; range: 2-15; 12 female, 12 male). Nine of the patients survived more than 12 months (median age: 13; mean age: 11.16, range: 15-16; female, 5 male): five for 13 months, two for two years and two for three years. Therapy of two patients was stopped two months before the completion of this study. Period of follow-up has been one to three years.

Of 33 patients, 12 did (median: 13; mean: 10.4; range: 2-16; 8 female, 4 male) and 21 did not (median: 10; mean: 9.59; range: 18 months-16 years; 8 female, 13 male) express biphenotypic markers. Of these, nine of the biphenotypic patients and 15 of the non-biphenotypic patients died or relapsed within the first 12 months. The others were alive and in remission when the study ended. According to French-American-British classification, the deceased biphenotypic patients had M4 (3 cases), M2 (5 cases, 1 being granular), and M1 (1 cases) types; the three living biphenotypic patients had M1 (one case), M2 (1 case), and M4 with eosinophilia (1 case).

Twenty-eight of these children were diagnosed and treated at Hacettepe Universiy, İhsan Doğramacı Children's Hospital, and five in Dr. Sami Ulus Children's Hospital. Thirty-two patients received the Hacettepe AML protocol[5] (11 received the 1993 version of this protocol and 21 the 1995 version) and one the Denver protocol[6].

Cytogenetic analysis was available in 10 of the 24 patients who died or relapsed within the first year, and eight of nine patients who lived for more than 12 months. In the former group abnormal cytogenetic results were as follows: 20 percent hypodiploidy, t(4;8) (q31,2;p23,1); trisomy 22; and trisomy 8 plus 22 in the same patient, dup (17)(q24), del (Y)(q11,23), t(8;21). For the latter group abnormal results were: del 13 (q12,1) and t(X;21)(q27,3;q21,1) in the same patient and trisomy 22 in another.

Flow Cytometric Analysis: Heparinized peripheral blood (PB) (n:10) or aspirated bone marrow (BM) (n: 23) samples were obtained before the treatment. The mean percentage of leukemic blasts which were determined morphologically was 66.47 percent for BM samples and 76 percent for PB samples. The size of blast cells were determined by flow cytometry (Becton Dickinson FAC Scan, Lysis I program) together with surface markers (CD2, CD3, CD5, CD7, CD22, CD10, CD20, CD19, HLA-DR) using fluorescein-isothiocyanate (FITC) conjugated antibodies[7]. Before analysis, the gated cells were examined on cytospin slides and the blast population was confirmed as more than 90 percent. The cells between 200-400, 400-600, 600-800 on forward scatter were considered as small, middle, or large cells, respectively, and cells were determined as a percentage. When more than 20 percent of blast cells expressed two or more lymphoid markers together with myeloid markers, these patients were considered biphenotypic. Two classifications were made to determine the significance of biphenotypic expression to cell size. In the first classification, the percentage of small, middle or large cells of the biphenotypic patients were compared with those of patients who were not biphenotypic. In the second classification, only the deceased-relapsed patients were evaluated. Among these, the percentages of small, middle or large cells of biphenotypic patients were compared with those of the patients who were not biphenotypic.

For statistical analysis, Mann-Whitney U test was used to compare the groups.

Results

In the first classification, the small, middle and large cells of the biphenotypic patients were compared with those of the patients who were not biphenotypic (Table I). It was striking that the percentages of small, middle and large cells of the patients were similar whether or not they expressed biphenotypic markers (34.19 ± 3.18 vs 39.39 ± 4.60; 41.67 ± 3.64 vs 38.78 ± 3.31; 24.12 ± 3.17 vs 22.03 ± 3.16, respectively) (p: 0.76; 0.82; 0.48).

Table I: Dispersion of Different-Sized Cells According to Biphenotypic Expression*

	Small		Middle		Large	
	B*Ψ	NB∂	B	NB	B	NB
N	12	21	12	21	12	21
Mean ± SE	34.19 ± 3.18	39.39 ± 4.60	41.67 ± 3.64	38.78 ± 3.31	24.12 ± 3.17	22.03 ± 3.16
Range	17.85-49.72	10.85-86.77	17.66-64.95	5.54-57.98	8.84-34.13	1.53-53.30
P		0.76		0.82		0.48

* Nine of the 12 (75%) biphenotypic patients died during the first year; three have been in remission for more than 12 months.

Ψ: Biphenotypic.
∂: NB: Non-biphenotypic.

In the second classification, only deceased patients were considered. The small, middle and large cells of the patients who were biphenotypic were compared with those of patients who were not biphenotypic (Table II). The percentages of the small, middle and large cells of patients who were biphenotypic were found similar to those of the patilents who were not biphenotypic (31.69 ± 3.19 vs 35.10 ± 4.19; 40.76 ± 4.81 vs 43.59 ± 2.60; 27.53 ± 3.24 vs 21.59 ± 3.53) (p: 0.70; 0.61; 0.20).

Table II: Dispersion of Different-Sized Cells in Deceased Patients According to Biphenotypic Expression

	Small		Middle		Large	
	B$^{\Psi}$	NB$^{\partial}$	B	NB	B	NB
N	9	15	9	15	9	15
Mean ± SE	31.69 ± 3.19	35.10 ± 3.19	40.76 ± 4.81	43.59 ± 2.60	27.53 ± 3.24	21.59 ± 3.53
Range	17.85-42.69	10.85-66.03	17.66-64.95	27.01-57.98	17.18-42.97	1.53-46.14
P		0.70		0.61		0.20

Ψ: Biphenotypic.
∂: Non-biphenotypic.

Discussion

In ALL, the size of the blasts are known to have prognostic significance. Presence of blasts with a large diameter (more than 2 times that of the erythrocyte diameter of the same patient established by peripheral blood smear examination) is a poor prognostic factor for survival independent of age and total leukocyte count[4]. In patients with lymphoma, cell size has been examined to determine any relationship with surface antigen expression[8,9]. In AML, the significance of blast cell size has been the subject of a few studies[2,3,10], (unpublished data). The first study was conducted by Kawada et al.[2], who planned to establish the role of cell size on heterogeneity of myeloblasts and to determine the relationship between cell size and surface markers in 23 adults with AML. It was demonstrated that the percentage and/or mean fluoresclence intensity (MFI) of surface markers (CD13, CD33, CD38, CD34, HLA-DR) were higher on the large cells than on the small cells. Furthermore, patients with a low ratio of MFI of antigen expression (CD13, CD38, CD33, HLA-DR) on the large cells to antigen expression on the small cells as a percentage exhibited longer remission durations or survival periods. The second study was ours[3]. In this preliminary study consisting of seven children with AML it was shown that the average percentage of large cells and the average ratio of large cell percentage to small cell percentage was higher in deceased patients than in patients who lived; surface markers were not taken into account[3]. And, in another study which was an extensive form of this study, we could not establish a definite relationship between cell

size at admission and prognosis (unpublished data). However, we determined that cell size of the blasts affected prognosis dependent on high leukocyte count (unpublished data). Kwong et al.[10] demonstrated near tetraploidy in large blasts of an adult AML patient.

The DNA synthesis rate and, therefore, cell proliferation rate, determined by tritiated thymidine uptake test in patients with infectious mononucleosis were demonstrated to be higher in the monocytes than in the large lymphocytes and higher in large lymphocytes than in small lymphocytes. In addition it was shown that a large mononuclear cell in infectious mononucleosis was equivalent to a myeloblast in the bone marrow[11]. DNA content in the band cells of normal people have been more than that found in the neutrophils[12]. Gavasto et al.[13], who examined the proliferation rate of blasts in connection with blast size in eight patients with different kinds of leukemia, demonstrated that thymidine uptake increased as blast size increased. Terasima et al.[14] demonstrated in synchronously dividing populations of HeLa cells that cell volume gradually increased during mitosis, reached maximum at the 18th hour and then gradually decreased. Although Minden et al.[15] showed in 1978 that the young progenitor cells were small in volume, contrary to general expectations, this has not been confirmed. In view of this, we consider the large cells determined by flow cytometry in our study as young cells with a high proliferation capacity.

In this study, death of nine of the 12 biphenotypic patients supports the theory that presence of biphenotypic expression is a poor prognostic factor. However, no difference could be established in the percentages of cells with different size, whether the patients were biphenotypic or not. Therefore, biphenotypy is a poor prognostic factor independent of cell size.

It has been reported that CD3 and CD7 were expressed most often on small (mature) cells, in patients both with good and poor prognosis (unpublished data). On the other hand, the CD3 percentage in small cells was found significantly higher than that of deceased-relapsed patients.

CD10 was found more often on the large (immature) rather than middle cells of the patients with a poor prognosis, while the percentages of CD10 on small, middle and large cells of patients with a good prognosis were similar. Moreover, the ratio of the percentage of CD20 expression on large and small cells was significantly higher in the deceased or relapsed patients compared to patients who lived. Therefore, we believe that CD10 and CD20 may be valuable parameters for poor prognosis.

On the other hand, the expression of CD10 more often on the large rather than middle cells in patients with a good prognosis and the similarity of CD19 expression on small, middle and large cells of patients with a poor prognosis (unpublished data) suggest that further studies in larger series are necessary to evaluate the prognostic role of expression of biphenotypy with each individual lymphoid marker.

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REFERENCES

1. Rowe JM, Liesveld JL. Treatment and prognostic factors in acute myeloid leukemia. In: Löwenberg B (ed). Bailliere's Clinical Haematology International Practice and Reseach. Acute Myelogenous Leukaemia and Myelodysplasia Vol: 9. London: W.B. Saunders Company Ltd; 1996: 87-105.
2. Kawada H, Ichikawa Y, Watanabe S, Nagao T, Arimori S. Flow cytometric analysis of cell-surface antigen expressions on acute myeloid leukemia cell populations according to their cell-size. Leukemia Res 1994; 18: 29-35.
3. Olcay L, Tuncer AM. Cell size and prognosis in childhood AML. Leukemia Res 1995; 19: 581-582.
4. Lee SL, Kopel S, Glidewell O. Cytomorphological determinants of prognosis in acute lymphoblastic leukemia of children. Semin Oncol 1976; 3: 209-217.
5. Hiçsönmez G, Tuncer AM, Çetin M, Özbek N, Gümrük F, Eight-year experiences with HDMP as a differentiation inducer in childhood AML. Differentiation therapy. In: Waxman S (ed). Challenges of Modern Medicine. New York: Ares-Serono Symposia Publications; 1995: 471-475.
6. Woods WG. Ruymann FB. Lampkin BC, et al. The role of timing of high-dose cytosine arabinoside intensification and of maintenance therapy in the treatment of children with acute nonlymphocytic leukemia. Cancer 1990; 66: 1106-1113.
7. Ormerod MG. Preparing suspensions of single cells. In: Ormerod MG (ed). Flow Cytometry (2nd ed). Oxford: Oxford University Pross; 1994: 45-65.
8. Ratech H. HLA-DR expression in B-cell non-Hodgkin's malignant lymphomas: a multiparameter flow cytometry study. Hum Pathol 1990; 21: 1275-1282.
9. Lanier LL, Warner NL. Cell cycle related heterogeneity of Ia antigen expression on a murine B lymphoma cell line: analysis by flow cytometry. J Immunol 1981; 126: 626-631.
10. Kwong YL, Lam CK, Chan AY, Lie AK, Chan LC. Cytogenetic triclonality in acute myeloid leukemia: a morphologic, immunologic and in situ hybridization study. Cancer Genet Cytogenet 1994; 72: 86-91.
11. Hale AJ, Cooper EH. DNa synthesis in infectious mononucleosis and acute leukemia. Acta Haematol 1963; 29: 257-266.
12. Hale AJ. The leukocyte as a possible exception to the theory of deoxyribonucleic acid constancy. J Path Bact 1963; 85: 311-326.
13. Gavasto F, Pileri A, Bachi C, Pegoraro L. Proliferation and maturation defect in acute leukaemia cells. Nature 1964; 203: 92-94.
14. Terasima T, Tolmach LJ. Growth and nucleic acid synthesis in synchronously dividing populations of HeLa cells. Exp Cell Res 1963; 30: 344-362.
15. Minden MD, Till JE, McCulloch EA. Proliferative state of blast cell progenitors in acute myeloblastic leukemia (AML). Blood 1978; 52: 592-600.