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RELATIONSHIP BETWEEN HIGH LEUKOCYTE COUNT AND CELL SIZE IN CHILDHOOD ACUTE MYELOBLASTIC LEUKEMIA*

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SUMMARY: Olcay L, Ertem U, Okur H, Etikan İ, Tuncer AM. (Hematology Unit, Department of Pediatrics and Department of Biostatistics, Hacettepe University Faculty of Medicine and Division of Oncology, Dr. Sami Ulus Children's Hospital, Ankara, Turkey). Relationship between high leukocyte count and cell size in childhood acute myeloblastic leukemia. Turk J Pediatr 1999; 41: 437-445.

In order to determine the significance of cell size together with high leukocyte count (>30x10^9/L) in acute myeloblastic leukemia (AML), we evaluated the percentages of small, medium and large cells in 33 children with AML. All of the 10 patients with a high leukocyte count and 14 of the 23 patients with a low leukocyte count (<30x10^9/L) died or experienced a relapse within the first year. The mean small cell percentage of patients with high leukocyte counts was significantly lower than that of patients with low leukocyte counts ($p<0.05$). The percentages of small, medium and large cells of patients with high leukocyte counts and of patients with low leukocyte counts who died or experienced a relapse within the first year were similar. The percentage of medium cells of patients with high leukocyte counts was significantly higher than that of surviving patients with low leukocyte counts ($p<0.05$). The mean percentages of small, medium and large cells were similar in patients who died or experienced a relapse and surviving patients with low leukocyte count. We conclude that cell size has prognostic significance when the leukocyte count at admission is over 30x10^9/L, although confirmation seems necessary with a larger population of patients. *Key words: cell size, acute myeloblastic leukemia, high leukocyte count.*

The known prognostic factors for acute myeloid leukemia (AML) are not as definite as for acute lymphoblastic leukemia (ALL)[1].

High leukocyte count (total leukocyte count >100x10^9/L) at admission is known to be a poor prognostic factor for patients with AML. High leukocyte counts are generally encountered in FAB M4 and M5 subtypes, and M4 patients with high leukocyte counts are especially at high risk for extramedullary infiltration and central nervous system involvement[2].

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However, the number of studies examining the biologic and prognostic significance of myeloblast size are few in contrast to studies with ALL[3-8].

The aim of this study was to determine whether myeloblast size has prognostic significance in relationship to a high leukocyte count at admission of children with AML.

To our knowledge, this is the first such study.

Material and Methods

Patients: Children who were diagnosed as AML between November 1993 and June 1996 were included in the study (mean age: 9.80; range 1.5-16; years 16 female, 17 male). Of these, 32 were de novo AML, and one was secondary to Fanconi's aplastic anemia (diepoxybutane [DEB] positive). According to French-American-British (FAB) criteria, seven cases had M1, 14 cases M2, four cases M3, five cases M4, two cases M6, and one case M7.

In 10 patients, the leukocyte count at admission was found over $30x10^9$/L (33.8-480.0x10^9/L, mean: 119.1x10^9/L). The age range was 2-14 (mean: 7.3 years).

In 23 patients, the leukocyte counts were below $30x10^9$/L.The leukocyte count ranged between 1.4-26.4x10^9/L (mean 9.6x10^9/L); the age of the patients ranged between 18/12-16 (mean: 10.8 years).

Five of the 33 patients were diagnosed and treated in Dr. Sami Ulus Children's Hospital and 28 in Hacettepe İhsan Doğramacı Children's Hospital. Thirty-two patients received Hacettepe AML protocol[9] (11 received the 1993 and 21 the 1995 version of the same protocol) and one received Denver protocol[10].

Leukocyte counts over $30x10^9$/L were accepted as a "high leukocyte count" and below $30x10^9$/L as a "low leukocyte count", although a leukocyte count over $100x10^9$/L is generally accepted as high and a poor prognostic factor[2]. All of the patients in our study with leukocyte counts over 3010^9/L died or experienced a relapse within the first 12 months.

Cytogenetic analysis was done in 18 patients. Abnormal cytogenetic findings were as follows: In patients with leukocyte counts over 3010^9/L, trisomy 22 and trisomy 8 plus 22 were established in one patient. In patüients with leukocyte counts below $30x10^9$/L, 20 percent hypodiploidy, t(4;8)(q31,2;p23,1) dup(17)(q24); del (Y)(q11,23); t(8;21) and del 13 (q12,1) and t(X;21)(q27,3:q21,1) were established in the same patient and trisomy 22 in another one.

Flow Cytometric Analysis: Heparinized peripheral blood or aspirated bone marrow samples were obtained before the treatment. The mean percentage of leukemic blasts determined morphologically was 66.5 percent for bone marrow samples and 76.0 percent for peripheric blood samples. Bone marrow and peripheral

blood were prepared for flow cytometric analysis using the density gradient separation technique. The size of the blasts were determined by flow cytometer by Lysis II (FAC Scan, Becton Dickinson Immunology Systems, BDIS, San Jose, CA, USA). All PMT and forward scatter standardization were performed using calibration standard beads (5, 10, 15 μm latex beads, immunopreb and lymphotreb). The blast concentrations of the samples were increased by a back-gating procedure using a mixture of CD14+CD45 and CD33+CD34 prior to gating. The MoAbs used were acute leukemia phenotyping kit (Becton Dickinson Immunology Systems, San Jose, CA, USA) and fluorescein-isothiocyanate conjugated antibodies (Ecx. CD15FITC, CD14PE and CD34PE). In addition, by separating the cells by ficoll gradient[11], the concentration of the blasts increased considerably. Hence, blast concentrations on the slides of the cytospin material made up completey of mononuclear cells exceeded 90 percent.

Cells between 200-400, 400-600, and 600-800 on forward scatter were considered as "small", "medium" and "large" cells, respectively (Fig. 1). Cells were determined as percentage. The percentages of small, medium and large cells were determined for each patient.

Statistical Analysis: Mann-Whitney U test was used for comparison. For determination of differences between the percentages of small, medium and large cells in individual groups, the Wilcoxon rank sum test was used.

Results

All 10 patients with leukocyte counts over $30x10^9$/L died or experienced a relapse within the first 12 months (7^{th} day-10^{th} month) of therapy. Fourteen of 23 patients with leukocyte counts below $30x10^9$/L (60.8%) died or experienced a relapse between the 5^{th} day-12^{th} month of treatment; nine have survived for more than twelve months. The characteristics of the patients are summarized in Tables I and II.

In patients with leukocyte counts over $30x10^9$/L, the percentage of medium cells was found higher than that of large cells (45.96±4.44 vs 24.81±3.63, $p<0.05$), but the percentages of large and small cells (24.81±3.63 vs 29.65±5.11) and of small and medium cells (29.65±5.11 vs 45.96±4.44) were similar ($p>0.05$) and >0.05). In the group of patients with leukocyte counts below $30x10^9$/L, the percentage of medium cells was greater than that of large cells (36.87±2.78 vs 20.91±2.91, $p<0.01$) and the percentage of small cells was greater than that of large cells (41.28±3.60 vs 20.91±2.91, $p<0.05$), but the percentages of small and medium cells were similar (41.28±3.60 vs 36.87±2.78, $p>0.05$).

In our first classification made to evaluate together with cell size the prognostic role of leukocyte counts over $30x10^9$/L at admission, small, medium and large

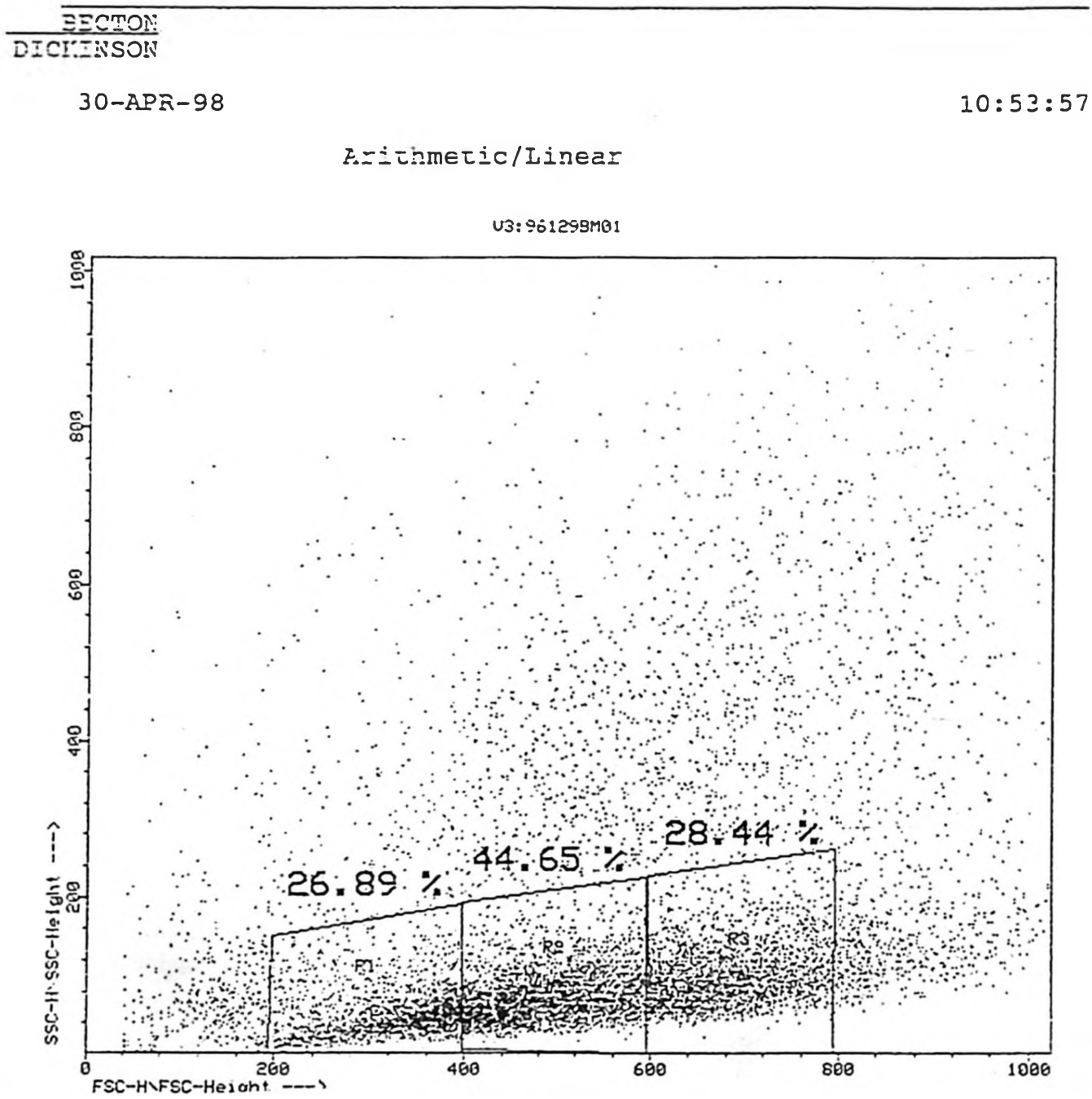

Fig. 1: The flow cytometric appearance of the cells. The cells between 200-400, 400-600 and 600-800 on forward scatter were considered as "small", "medium" and "large" cells, respectively.

cells of the patients with leukocyte counts over $30x10^9$/L were compared with those of the patients with leukocyte counts below $30x10^9$/L (Table IIIa). It was striking that the mean small cell percentage of patients with leukocyte counts over $30x10^9$/L (29.65±5.11) was significantly lower than that of patients with leukocyte counts below $30x10^9$/L (41.28±3.60) ($p<0.05$), while the mean percentages of medium and large cells of patients in both groups were similar ($p>0.05$) (Table IIIa).

Table I: Patients with Leukocyte Counts Over 30x10^9/L

Patient	Age (yrs) sex	Extramedullary involvement	Hb (g/L)	WBC (x10^9/L)	Platelet (x10^9/L)	FAB	Time of remission	Time of death or relapse
MT	3,M	right hilar LAP	80	67.2	25.0	M4	no remission	21st day, death
SÖ	5,F	pleura	122	40.2	22.0	M6	30th day	60th day, death
MA	13,F	No	95	73.2	10.0	M1	no remission	7th day, death
SK	2,F	lung	37	90.5	32.0	M1	8th day	9th month, relapse
MA	10,F	No	77	100.0	70.0	M2	4th month	10th month, relapse
Rİ	6,F	No	97	88.3	20.0	M2	36th day	4th month, relapse
AG	8,M	No	111	480.0	190.0	M2	no remission	2nd month, death
İZ	5,M	No	57	133.9	26.0	M2	no remission	2.5 month, death
RÇ	7,M	pericardium	73	84.0	32.0	M2	46th day	7th month, death
MÇ	14,F	No	67	33.8	27.0	M2	15th day	35th day, death

Hb: hemoglobin; WBC: white blood cells; FAB: French-American-British criteria; LAP: lymphadenopathy.

Table II: Patients with Leukocyte Counts Below 30x10^9/L

Patient	Age (yrs) sex	Extramedullary involvement	Hb (g/L)	WBC (x10^9/L)	Platelet (x10^9/L)	FAB	Time of remission	Time of death or relapse
MG	12,F	No	91	20.4	108.0	M4	2nd month	15th month, death
DÜ	13,F	No	79	14.2	44.0	M2	no remission	18th day, death
EG	13,M	No	55	1.4	12.0	M3	no remission	25th day, death
SÇ	13,F	No	80	10.0	32.0	M3	no remission	16th day, death
HH	4,F	No	60	11.0	25.0	M2	no remission	58th day, death
SU	15,M	No	53	9.2	20.0	M2	24th day	83rd day, death
SÖ	14,F	No	109	5.0	160.0	M2	1st month	6th month, death
İG	11,M	tonsilla, gingiva	81	28.6	23.0	M4	26th day	7th month, relapse 9th month, death
EU	10,M	No	76	8.0	22.0	M2	no remission	38th day, death
BT	11,M	No	87	1.8	15.0	M7	2nd month	5th month, death
LÇ	12,M	No	122	1.4	20.0	M3	no remission	5th day, death
MAK	2,M	gingiva, palatinum	78	6.0	30.0	M4	53rd day	12th month, death
İA	14,F	gingiva	34	12.8	25.0	M1	37th day	10th month, relapse
MEE	6,M	No	40	15.2	33.0	M2	15th day	8th month, death
NK	16,F	palatinum	74	26.4	10.0	M4	36th day	Alive and in remission for 4 years
BŞ	15,M	scapula, gingiva	89	5.5	11.0	M1		Alive and in remission for 2 years 11 months
SA	4,M	No	94	6.0	200.0	M3	30th day	Relapse at 21st month, (not remitted yet)
SMG	15,F	No	94	3.0	70.0	M1	9th day	Alive and in remission for 2 years
KP	15,M	No	70	6.5	80.0	M1	16th day	Relapse at 22nd month, (not remitted yet)
GD	16,F	No	94	2.6	27.0	M1	20th day	Relapse at 13th month, (not remitted yet)
MAT	10,M	bone	83	5.4	170.0	M6	5th day	Alive and in remission for 2 years
BY	13,F	No	41	19.1	25.0	M2	14th day	Alive and in remission for 4 years
AA	10,M	No	89	6.2	30.0	M2	8th day	2nd year 1st month, relapse 2nd year 4th month, death

Hb: hemoglobin; WBC: white blood cells; FAB: French-American-British criteria

Table IIIa: Percentage of Myeloblasts with Different Size According to Leukocyte Count

	Small		Medium		Large	
	WBC O*	WBC B**	WBC O	WBC B	WBC O	WBC B
N	10	23	10	23	10	23
Mean + SE	29.65 ± 5.11	41.28 ± 3.6	45.96 ± 4.44	36.87 ± 2.78	24.81 ± 3.63	20.91 ± 2.91
Range	10.85-64.72	17.97-86.77	17.66-64.95	5.54-53.9	7.24-41.8	1.53-53.3
P		0.04		0.06		0.31

* WBC O: Leukocyte count over $30x10^9$/L (10/10, 100% died or relapsed within the first 12 months).
** WBC B: Leukocyte count below $30x10^9$/L (14/23, 60.8%, died or relapsed within the first 12 months).

Table IIIb: Percentage of Myeloblasts with Different Size in Patients Who Died or Experienced Relapse Within the First 12 Months According to Leukocyte Counts Over and Below $30x10^9$/L

	Small		Medium		Large	
	WBC O*	WBC B**	WBC O	WBC B	WBC O	WBC B
N	10	14	10	14	10	14
Mean + SE	29.65 ± 5.11	36.79 ± 3.16	45.96 ± 4.44	40.07 ± 2.52	24.81 ± 3.63	23.11 ± 3.60
Range	10.85-64.72	17.97-66.03	17.66-64.95	27.01-53.9	7.24-41.8	1.53-46.14
R		0.14		0.21		0.76

* WBC O: Leukocyte counts over $30x10^9$/L (10/10, 100% died or relapsed within the first 12 months).
** WBC B: Leukocyte counts below $30x10^9$/L (14/14, 100%, died or relapsed within the first 12 months).

The mean percentages of small, medium and large cells of patients with leukocyte counts over $30x10^9$/L who died or experienced a relapse within the first 12 months were compared with those of the patients who died or experienced a relapse within the first 12 months and had leukocyte counts below $30x10^9$/L. The mean percentages of small, medium and large cells of these patients were similar ($p>0.05$) (Table IIIb). The mean percentage of medium cells of patients who died or experienced a relapse within the first 12 months of treatment and had leukocyte counts over $30x10^9$/L was significantly higher than that of surviving patients with leukocyte counts below $30x10^9$/L (45.96±4.44 vs 32.63±6.04) ($p<0.05$). Percentages of small and large cells of patients who died or experienced a relapse and had leukocyte counts over $30x10^9$/L were similar to those of surviving patients with leukocyte counts below $30x10^9$/L (Table IIIc). Percentages of small, medium and large cells in the group of patients who died or experienced relapse and had leukocyte counts below $30x10^9$/L were similar to those of surviving patients with leukocyte counts below $30x10^9$/L ($p>0.05$, >0.05, >0.05) (Table IIId).

Table IIIc: Percentage of Myeloblasts with Different Size in Patients Who Died or Experienced Relapse and Had Leukocyte Counts Over $30x10^9/L$ and in Surviving Patients with Leukocyte Counts Below $30x10^9/L$

	Small		Medium		Large	
	WBC O*	WBC B**	WBC O	WBC B	WBC O	WBC B
N	10	9	10	9	10	9
Mean + SE	29.65 ± 5.11	47.32 ± 8.12	45.96 ± 4.44	32.63 ± 6.04	24.81 ± 3.63	20.04 ± 5.13
Range	10.85-64.72	15.22-86.77	17.66-64.95	5.54-52.80	7.24-41.80	5.75-53.30
R		0.06		0.04		0.22

* WBC O: Leukocyte counts over $30x10^9/L$ (10/10, 100% died or relapsed within the first 12 months).
** WBC B: Leukocyte counts below $30x10^9/L$ (all 9 were alive for more than 12 months).

Table IIIc: Percentage of Myeloblasts Size of Patients with Leukocyte Counts Below $30x10^9/L$ According to Prognosis

	Small		Medium		Large	
	Deceased	Alive	Deceased	Alive	Deceased	Alive
N	14	9	14	9	14	9
Mean + SE	36.79 ± 3.16	47.32 ± 8.12	40.07 ± 2.52	32.63 ± 6.04	23.11 ± 3.6	20.04 ± 5.13
Range	17.97-66.03	15.22-86.77	27.01-53.9	5.54-52.80	1.53-46.14	5.75-53.30
R		0.28		0.61		0.41

Discussion

In ALL, blast size is known to have prognostic significance. Presence of blasts with large diameters is an independent poor prognostic factor for survival in ALL[5]. In AML, which is heterogeneous morphologically and biologically, the significance of cell size has been the subject of few studies[3,4,6-8].

Kawada et al.[3] demonstrated that myeloblast size affected the prognosis being dependent on surface markers. In a preliminary study[4], we reported that the mean percentage of large cells and the mean ratio of large cell percentage to small cell percentage was higher in deceased than in surviving patients, but in another study[7], we could not establish any relationship between cell size at admission and prognosis. Moreover, we also determined that cell size did not have prognostic value according to biphenotypy[8].

Cell proliferation and DNA synthesis rate were higher in the larger hematopoietic cells than in the smaller cells in infectious mononucleosis[12] and healthy individuals[13]. It was demonstrated that DNA synthesis increased as blast size

increased[14] and that the cell volume gradually increased during mitosis, reaching a maximum at the 18th hour and then gradually decreasing[15]. Thus, we consider the large cells of AML determined by flow cytometry as young cells with a high proliferation capacity.

The mean percentage of small cells of patients with leukocyte counts below $30x10^9$/L was significantly higher than that of patients with leukocyte counts over $30x10^9$/L, all of whom died or experienced a relapse within the first 12 months of treatment.

These data suggest that the number of cells of different size varies according to high leukocyte counts. Establishment of no difference in cell size of surviving patients with leukocyte counts below $30x10^9$/L and deceased patients with leukocyte counts below $30x10^9$/L suggests that the distribution of the blasts with different size does not differ according to prognosis when the leukocyte count declines below $30x10^9$/L.

The mean percentage of medium cells of patients with leukocyte counts over $30x10^9$/L, all of whom died or experienced a relapse within the first 12 months, was significantly higher than that of surviving patients with leukocyte counts below $30x10^9$/L.

These data suggest that the distribution of the cells according to blast size is dependent on the leukocyte count at admission being higher or lower than $30x10^9$/L and thus on prognosis. One reason for the poor prognosis in our patients with leukocyte counts over $30x10^9$/L seems to be the reduction of the number of small cells, generally more mature than the medium and/or large cells, which have less proliferating capacity than the smaller cells, and enhancement of the number of the middle cells. Therefore, cell size has prognostic significance when the leukocyte count at admission is over $30x10^9$/L.

Another significant finding, in the group of patients with leukocyte, counts below $30x10^9$/L was that large cells were the smallest in number. This supports our consideration of large blasts as young with a high proliferation capacity. Absence of significant differences between small-large or small-medium cells of the group of patients with leukocyte counts over $30x10^9$/L may be due to the limited number of patients. It is interesting that there was no significant difference between the percentages of small, medium and large cells of patients with leukocyte counts below $30x10^9$/L, whether they survived or died or experienced a relapse within the first 12 months of treatment. Thus, cell size of the blasts does not seem to have prognostic significance when the leukocyte count at admission is less than $30x10^9$/L. On the other hand, establishment of no difference in the mean percentage of small, medium and large cells of the deceased or relapsed patients with leukocyte counts higher and lower than $30x10^9$/L suggests that there are

more poor prognostic factors influencing cell size other than leukocyte counts over $30x10^9$/L.

It appears necessary to confirm these findings in patients with higher leukocyte counts (higher than $100x10^9$/L) and with a larger patient population.

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