Eur J Cancer Clin Oncol, Vol. 23, No. 8, pp. 1125–1129, 1987.
Printed in Great Britain

0277-5379/87$3.00+0.00

Relation Between Leukaemic Cell Count and Degree of Maturation in Acute Myeloid Leukaemia

M. VAN DER WEIDE, M.M.A.C. LANGENHUIJSEN, P.C. HUIJGENS, L.M.F.M. IMANDT, F.C. DE WAAL,* J.J. MOL, D.J. VAN RHENEN and D A.P.M. KESTER†

*Departments of Haematology, *Pediatrics and †Medical Statistics, Free University, Amsterdam, The Netherlands*

Abstract—*In acute myeloid leukaemia the peripheral leukocyte count is known to be a prognostic factor. The preserved capacity of leukaemic cells to mature has also been suggested to be one. In a series of* 179 *cases of adult acute myeloid leukaemia peripheral leukaemic cell count and degree of maturation were found to be inversely correlated. As the degree of maturation of leukaemic cells in peripheral blood was lower than that in bone marrow in the majority of cases, blast cells appear to be released more easily from the marrow than cells that have matured to some extent in the direction of the larger promyelocytic or promonocytic cell type. In a series of* 35 *cases we found peripheral blast cells to be smaller than those in bone marrow. Moreover, central blast cell diameter and peripheral leukaemic cell count were inversely correlated. Therefore, leukaemic cell size or some factor related to it may contribute to the preferential egress of small immature cells from the marrow. Differences in proliferative activity could not account for the inverse correlation between degree of maturation and leukaemic cell count.*

INTRODUCTION

THE PRESERVED capacity of leukaemic cells to mature varies a great deal between cases of acute myeloid leukaemia. It has been suggested to be inversely correlated with the peripheral leukocyte count [1, 2], which is considered a prognostic factor [3–6]. This would be in line with the favourable influence of residual maturation on prognosis, that has been suggested before [2, 7]. Elsewhere we present evidence from the literature and our own experience for such an influence [8]. In the study presented here, after having proved the leukaemic cell count to be significantly inversely related to maturation in a large series of patients, we went into the mechanisms of this relationship by estimating the influence of blast cell diameter and proliferative activity.

MATERIALS AND METHODS

Patients

To assess the suspected negative relationship between degree of maturation and peripheral leukaemic cell count, we reviewed data from 267 consecutive cases of untreated adult (> 15 years) acute myeloid leukaemia, whose material was sent to the Morphology Committee of the Dutch Leukaemia Working Group, a reference committee for the making of diagnostic decisions. After having demonstrated that relationship we went into the mechanisms underlying it in a different group of 43 consecutive cases of untreated adult acute myeloid leukaemia, referred to the Department of Haematology of the Free University Hospital or to the Department of Haematology of the Academic Medical Centre of the University of Amsterdam.

Morphology

The degree of maturation is expressed as the Maturation Index (MI), introduced before [9] as a quantitative aid to the FAB classification [10]. MI is defined as

$$\frac{\text{promyelocytes and/or promonocytes}}{\text{blasts + promyelocytes and/or promonocytes}} \times 100$$

and it is derived from the differential count of 200 cells in peripheral blood or 500 bone marrow cells in May–Grünwald–Giemsa stained smears. In FAB type M6 the MI was considered inappropriate because of the considerable contribution of the erythroid cell line to the leukaemic process. In the first part of the study, counts in each of 267 cases were done by one out of five haematologists. In the second part, including 43 cases, each case was studied by two observers. Whenever the indices

Accepted 2 March 1987.
Correspondence and requests for reprints: M. van der Weide, Department of Haematology, Free University Hospital, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands.

between them differed more than 5 (MI ≤ 30) or 10 (MI > 30) the smears were studied by a third observer. The final MI is the mean of the results from the two observers with the smallest difference. All counts were made without knowledge of laboratory data.

The leukaemic cell count in peripheral blood was calculated from the leukocyte count and the percentage of blasts, promyelocytes and promonocytes in the differential count. The bone marrow percentage of leukaemic cells was defined as the percentage of blasts, promyelocytes and promonocytes. In both leukaemic cell counts and MI more mature cells were neglected, as their relation to the leukaemic cell population is difficult to assess.

Blast cell diameter

Leukaemic cells from peripheral blood and bone marrow samples, anticoagulated with heparin, were separated by Ficoll Hypaque density gradient centrifugation (d = 1.077 g/cm^3). The interphase was collected and washed twice with Hank's balanced salt solution with 0.1% foetal calf serum. Cytocentrifuge (Shandon Cytospin 2) preparations were made and a differential count of 200 cells was done to calculate the MI of the enriched suspension. Only suspensions containing more than 80% leukaemic cells were taken into account. Blast cell diameters were measured in cell suspensions by means of an Elzone 80 XY Volume Counter (Particle Data, U.S.A.), which measures and plots cell diameters according to the coulter principle in 128 channels. The standard deviation of the measurements was one channel (0.09 μ). When the MI of the material was more than 10, diameters were only considered to be derived from blast cells if promyelocytes and promonocytes could be clearly separated from blasts in the histogram.

Pulse cytophotometry

Enriched samples from blood and bone marrow, prepared as described above, were fixed in 70% ethanol, treated with RNase and pepsin and stained with ethidium bromide (Hoechst 33342). DNA histograms were obtained using the ICP 11 impulse cytophotometer (Phywe, F.R.G.) and percentages of G-cells, S-phase cells and G + M-cells were calculated as previously described [10].

Statistical methods

For comparison of the peripheral and bone marrow MI the sign test was used. Peripheral and central blast cell diameters or S-phase cells were compared by the Wilcoxon signed rank test. All other correlations were tested by Spearman's correlation coefficient.

RESULTS

Maturation and leukaemic cell count

In 69 out of 267 cases cytologic details in bone marrow smears were considered inadequate, in four cases information on laboratory data at presentation was lacking, and 15 cases were considered unclassifiable. The distribution of the remaining 179 cases according to FAB type, was M1: 38, M2: 57, M3: 10, M4: 39, M5: 28, M6: 7 cases, respectively. For comparison of peripheral and bone marrow MI sufficient data were available in 137 cases, for the correlations between peripheral leukaemic cell count and bone marrow MI in 143 cases, between peripheral leukaemic cell count and bone marrow percentage of leukaemic cells in 151 cases, between bone marrow MI and bone marrow percentage of leukaemic cells in 170 cases.

The peripheral MI appeared to be lower than the MI in the marrow in the majority of cases, reaching significance in FAB type M2, and if all cases were taken together (Table 1). Even if all cases with an unknown peripheral MI, for reasons as listed in Table 1, were assigned to the group in which the peripheral MI exceeded the central MI, differences shown to be significant remained so. For further analysis the bone marrow MI only has been taken into account.

As can be seen from Table 2 a significant inverse relationship could be demonstrated between peripheral leukaemic cell count and bone marrow MI in FAB types M1–M3 (all cases assigned to the myeloid subtypes M1, M2 and M3), in M4 and in all cases categorized as M1–M5 taken together.

The peripheral leukaemic cell count appeared to be related also to the percentage of leukaemic cells in the marrow (Table 3). A significant positive correlation existed in FAB types M1–M3 and M4 and if all cases M1–M6 were taken together.

The relationship between bone marrow MI and percentage of bone marrow leukaemic cells proved to be significant, although to a lesser degree, in FAB types M1–M3 and M1–M5 (Table 4).

Cell diameter and proliferative activity

In eight out of 43 cases of newly diagnosed acute myeloid leukaemia the percentage of leukaemic cells after isolation on Ficoll Hypaque was less than 80. The distribution of the remaining 35 cases, according to FAB type was M1: 6, M2: 13, M3: 4, M4: 8, M5: 4 cases respectively. The blast cell diameter in peripheral blood was significantly lower than that in bone marrow ($P < 0.01$).

There seemed to be a positive correlation between blast cell diameter in peripheral blood or bone marrow and bone marrow MI but it did not reach statistical significance. A negative correlation was found between blast cell diameter in bone marrow

Table 1. Peripheral (P) as compared to central (C) MI in FAB types M1–M5

FAB type	P < C	P = C	MI P unknown*	P > C	Significance†
M1	15	10	0	6	NS
M2	41	1	4	4	< 0.0001
M3	4	0	4	0	NS
M4	18	1	1	12	NS
M5	13	4	0	8	NS
M1–M5	91	16	9	30	< 0.0001

*Only smears containing insufficient cells for a differential count or no leukaemic cells. In other patients PMI was unknown by absence or insufficient quality of smears (M1: 6, M2: 7, M3: 2, M4: 6, M5: 3). CMI was unknown by insufficient quality in two cases (M1, M4).
†Number of patients with P < C as compared to those with P > C.

Table 2. Peripheral leukaemic cell count correlated to bone marrow MI in FAB types M1–M5 (n = 143)

FAB groups	*n*	r_s^*	*P*
M1–M5	143	−0.33	< 0.0001
M1–M3	83	−0.44	< 0.0001
M4	34	−0.43	0.011
M5	26	0.18	NS

*Spearman's correlation coefficient.

Table 3. Peripheral leukaemic cell count correlated to bone marrow leukaemic cell percentage in FAB types M1–M6 (n = 151)

FAB groups	*n*	r_s^*	*P*
M1–M6	151	0.40	$< 10^{-6}$
M1–M3	84	0.43	< 0.0001
M4	35	0.40	0.016
M5	26	0.28	NS
M6	6	0.43	NS

*Spearman's correlation coefficient.

Table 4. Bone marrow MI correlated to bone marrow leukaemic cell percentage in FAB types M1–M5 (n = 170)

FAB groups	*n*	r_s^*	*P*
M1–M5	170	−0.22	< 0.005
M1–M3	104	−0.22	0.02
M4	38	−0.20	NS
M5	28	−0.15	NS

*Spearman's correlation coefficient.

and peripheral leukaemic cell count ($n32$, r_s −0.37, $P < 0.05$), peripheral blood blast cell diameter and peripheral leukaemic cell count were not significantly correlated.

Pulse cytophotometry was done on the same material. The percentage of cells in S-phase in peripheral blood was significantly lower than in bone marrow ($P < 0.01$). We found no correlation between bone marrow MI and percentage of S-phase cells in bone marrow or peripheral blood. A negative correlation was found between the percentage of S-phase cells in bone marrow and bone marrow percentage of leukaemic cells ($n30$, r_s −0.41, $P < 0.05$) and between the percentage of S-phase cells in bone marrow and peripheral leukaemic cell count ($n30$, r_s −0.37, $P < 0.05$). There seemed to be positive correlations between the percentage of S-phase cells in peripheral blood or bone marrow and blast cell diameter but they did not reach statistical significance.

DISCUSSION

In the present study we could demonstrate the peripheral leukaemic cell count to be significantly inversely related to maturation in acute myeloid leukaemia. This corroborates the results of a previous analysis of a smaller and completely different material [2], in which we found a non-significant trend of white blood cell counts and maturation to be negatively correlated. A statistically significant association between high total white blood cell counts and low degree of leukaemic cell differentiation, and vice versa, has been reported before in a small series of patients [1]. The relationship described could point to a preferential egress from the bone marrow of immature leukaemic cells as compared to cells that have matured to some degree. Cells are known to be delivered from the bone marrow to the circulation across the vascular sinus walls [12, 13]. Cell size, motility, deformability and chemotaxis are factors that may be essential for marrow egress [12]. In the normal state these factors work in favour of mature cells. Mature granulocytes are highly deformable, probably influenced by cytoplasmic maturation during granulopoiesis [14, 15]. Little is known on the release of cells from the bone marrow in pathological states like acute leukaemia. Possible mechanisms include a breakdown of the marrow blood barrier, as found in rat leukaemia by

some investigators [16], but not by others [17], or the acquisition by leukaemic cells of cell membrane or cytoplasmic properties that make them more motile than their normal counterparts. In our study, the MI in peripheral blood was shown to be lower than that in bone marrow. This supports the idea that blast cells are released more easily from the bone marrow than cells that have matured to the promyelocytic or promonocytic stage. An alternative, although less probable, explanation would be that promyelocytes or promonocytes have a shorter circulation time as opposed to blasts after release from the bone marrow in the proportions in which both cell types are present in that marrow. The possibility of a larger proliferative activity of extramedullary immature cells as opposed to their counterparts in the marrow seems remote as we found less S-phase cells in peripheral blood than in bone marrow.

It has been suggested before that smaller leukaemic cells are more predominant in the blood than in the marrow [18]. In this study we present evidence that peripheral leukaemic cells are indeed significantly smaller. We also found a negative correlation between central blast cell diameter and peripheral leukaemic cell count. From these facts it may be derived that the egress of leukaemic cells from the marrow is influenced by the leukaemic cell size or some factor related to it in favour of small cells.

The number of peripheral leukaemic cells appeared to be significantly positively related to the percentage of leukaemic cells in the bone marrow, as has been reported before [19]. It is not surprising, therefore, that the percentage of leukaemic cells in the marrow and the bone marrow MI were found to be inversely related. The mechanism of this relationship is not clear. A possible explanation could be that there is a critical tumour volume beyond which the disease becomes clinically manifest. In a rather immature leukaemia with small blasts, this volume would be reached at a higher percentage of tumour cells than in a leukaemia that has larger tumour cells paralleling their capacity to mature. A larger proliferative activity leading to a larger number of leukaemic cells in cases with lower MI could be another explanation, but in this study we did not find a relation between MI and proliferative activity as assessed by pulse cytophotometry. On the contrary, there seemed to be a negative correlation between leukaemic cell numbers and S-phase cell percentages. Our finding of a significantly lower percentage of S-phase cells in peripheral blood as compared to that in bone marrow is in line with data from others [20, 21]. It is not certain whether the marrow leukaemic cell percentage contributes to the peripheral leukaemic cell count in itself or just by its relation to maturation degree.

Other factors than cell volume and cell number might play a role in the egress of tumour cells from the marrow. One of them is the ability of the cell to move. This may hold especially for the FAB type M5, that did not show the negative correlation between peripheral leukaemic cell count and MI nor the positive correlation with bone marrow percentage of leukaemic cells. As the release of leukaemic cells from the marrow can be considered to contribute to tumour mass, the capacity of leukaemic cells to leave the marrow may have an impact on therapeutic results. It may be related to the above-mentioned favourable influence on prognosis of the preserved capacity to mature. Since the patients were treated in different hospitals, according to different treatment regimens, the influence of MI on prognosis could not be evaluated in this material. It would be interesting to assess this in a group of patients treated according to a single protocol.

Acknowledgements—We wish to thank the following clinicians who allowed us to use data from their patients: Prof. Dr. J. Abels, Rotterdam; Dr. J.A. ten Bokkel Huinink, Zwolle; Dr. L.J. Bosch, Eindhoven; Dr. J.Th.M. Burghouts, 's Hertogenbosch; Dr. O. Cohen, Meppel; Prof. Dr. R. Goudsmit, Amsterdam; Prof. Dr. C.A.M. Haanen, Nijmegen; Dr. H.F.P. Hillen, Eindhoven; Dr. E. van Kammen, Dordrecht; Dr. H. Kerkhofs, 's Gravenhage; Dr. R.M.A. Kurstjens, 's Hertogenbosch; Prof. Dr. H.O. Nieweg, Groningen; Dr. G.J. den Ottolander, Leiden; Dr. D.M. Prenger, Oosterhout; Dr. K. Punt, Utrecht; Dr. J. de Regt, Delft; Dr. K.J. Roozendaal, Amsterdam; Dr. C.J. Russchen, Zwolle; Dr. R. Somers, Amsterdam; Dr. J. Steenbergen, Apeldoorn; Dr. W.F. Stenfert Kroese, Rotterdam; Dr. W. Sizoo, Rotterdam; Prof. Dr. R.L. Verwilghen, Leuven; Dr. J.W.J. van Wersch, Heerlen.

We further thank Prof. Dr. R. Goudsmit and Dr. A.E.G.Kr.v.d. Borne for putting cell material of their patients at our disposal W. Jalink and P. de Graaf for their valuable technical assistance, and A. Loonen and G. Broekema for pulse cytophotometry measurements.

REFERENCES

1. Brincker H. Clinical classification and evaluation of treatment response in acute myeloid leukaemia on the basis of differences of leukaemic cell differentiation. *Scand J Haematol* 1973, **1**, 383–390.
2. Van Rhenen DJ, Langenhuijsen MMAC. Clinical and laboratory data related to maturation in acute leukaemia. *Cancer* 1984, **53**, 1923–1926.
3. Weil M, Jacquillat CI, Gemon-Auclerc MF *et al.* Acute granulocytic leukemia. Treatment of the disease. *Arch Int Med* 1976, **136**, 1389–1395.
4. Keating MJ, Smith TL, Gehan EA *et al.* Factors related to length of complete remission in adult acute leukemia. *Cancer* 1980, **45**, 2017–2029.

5. Schwartz RS, Mackintosh FR, Halpern J, Schrier SL, Greenberg PL. Multivariate analysis of factors associated with outcome of treatment for adults with acute myelogenous leukemia. *Cancer* 1984, **54**, 1672–1681.
6. Zittoun R, Cadiou M, Bayle C, Suciu S, Solbu G, Hayat M. Prognostic value of cytologic parameters in acute myelogenous leukemia. *Cancer* 1984, **53**, 1526–1532.
7. Mertelsmann H, Thaler HT, To L *et al.* Morphological classification, response to therapy, and survival in 263 adult patients with acute non-lymphoblastic leukemia. *Blood* 1980, **56**, 773–781.
8. Langenhuijsen MMAC. Maturation and prognosis in acute myeloid leukemia. *Eur J Cancer Clin Oncol* (submitted).
9. Van Rhenen DJ, Verhulst JC, Huijgens PC, Langenhuijsen MMAC. Maturation index: a contribution to quantification in the FAB-classification of acute leukaemia. *Br J Haematol* 1980, **46**, 581–586.
10. Bennett JM, Catovsky D, Daniel MT *et al.* Proposals for the classification of the acute leukaemias. *Br J Haematol* 1976, **33**, 451–458.
11. Smets LA, Mulder E, de Waal FC, Cleton FJ, Blok J. Early responses to chemotherapy detected by pulse cytophotometry. *Br J Cancer* 1976, **34**, 153–161.
12. Lichtman MA, Chamberlain JK, Santillo PA. Factors thought to contribute to the regulation of egress of cells from the marrow. In: Gordon AS, Silber R, Lobus J, eds. *The Year in Haematology*. Plenum, 1978, 243–279.
13. Tavassoli M. The marrow-blood-barrier. *Br J Haematol* 1979, **41**, 297–302.
14. Lichtman MA, Weed RI. Alteration of the cell periphery during granulocyte maturation: relation to cell function. *Blood* 1972, **39**, 301–316.
15. Lichtman MA, Kearney EA. The filterability of normal and leukemic human leucocytes. *Blood Cells* 1976, **2**, 491–506.
16. Chen LT, Handler EE, Handler ES, Weiss L. An electron microscopic study of the bone marrow of the rat in an experimental myelogenous leukemia. *Blood* 1972, **39**, 99–112.
17. De Bruyn PPH, Becker RP, Michelson S. The transmural migration and release of blood cells in acute myelogenous leukaemia. *Am J Anat* 1977, **149**, 247–268.
18. Killmann S-A. Kinetics of leukaemic blast cells in man. In: Roath S, ed. *Clinics in Haematology*. London, Saunders, 1972, Vol. 1, 95–113.
19. Hart JS, George SL, Frei III E, Bodey GP, Mickerson RC, Freireich EJ. Prognostic significance of pretreatment proliferative activity in adult acute leukemia. *Cancer* 1977, **39**, 1603–1617.
20. Hillen H, Wessels J, Haanen C. Bone marrow proliferation patterns in acute myeloblastic leukaemia determined by pulse cytophotometry. *Lancet* 1975, **1**, 609–611.
21. Barlogie B, Spitzer G, Hart JS *et al.* DNA histogram analysis of human hemopoietic cells. *Blood* 1976, **48**, 245–258.