Papers

Value of colony forming unit-granulocyte macrophage assay in predicting relapse in acute myeloid leukaemia

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Abstract

Aim—To evaluate the validity of the colony forming unit-granulocyte macrophage (CFU-GM) assay for predicting relapse in patients with acute myeloid leukaemia (AML).

Methods—The study population comprised 32 patients with AML in remission, followed for a median of 18 months. A mean of four studies was carried out per patient. Three patterns of in vitro growth based on the number of CFU-GM in normal bone marrow were defined: 1 = normal (normal number of CFU-GM and a cluster:colony ratio <2); 2 = hypoplastic (low number of CFU-GM and a cluster:colony ratio <2); 3 = anomalous (low or normal number of CFU-GM and a cluster:colony ratio >2).

Results—Eleven patients relapsed, all of whom had previously displayed an abnormal CFU-GM pattern: anomalous in nine and hypoplastic in two. The remaining 25 patients were in complete remission at the time of writing, 16 of whom had a normal growth pattern. The other nine had anomalous (eight patients) or hypoplastic (one patient) growth. The latter may be false positive results. The in vitro growth pattern was not constant during follow up analysis. All 15 patients in whom the growth pattern switched from abnormal to normal remain in complete remission. By contrast, of the five cases in whom the pattern changed from normal to abnormal, three have relapsed and the other two had other indicators of relapse. The growth pattern remained unchanged in the remaining 16 patients.

Conclusion—The present data show that the sequential investigation of the CFU-GM growth pattern may be of value in predicting relapse in patients with AML.

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Keywords: colony forming unit-granulocyte macrophage, acute myeloid leukaemia, relapse.

Despite significant improvements in the treatment of acute myeloblastic leukaemia (AML)

over the past 20 years, the majority of patients who have achieved complete remission will eventually relapse as a result of the persistence of residual leukaemic cells, below the detection limit of light microscopy.^{1,2} Several methods for detecting minimal residual disease have been developed and evaluated—for example, cytogenetics, immunological marker analysis and molecular biological techniques,³⁻⁶ and in vitro clonogenic assays.⁷⁻⁹ However, distinguishing between clonogenic leukaemic cells (colony forming unit-leukaemic (CFU-L)) and normal myeloid progenitor cells (colony forming unit-granulocyte macrophage (CFU-GM)) is problematic, because both types of cells respond to the same growth factors, despite displaying different growth characteristics in vitro.^{10,11} In order to identify precisely the existence of CFU-L, more sophisticated approaches have to be applied—for example, analysis of clonal gene rearrangements⁸ or in situ analysis of surface markers on the leukaemic colonies.⁹ In patients with preleukaemic syndromes, such as the myelodysplastic syndrome, alterations in the growth patterns of CFU-GM appear before the transformation into acute leukaemia.¹²

Methods

The study population comprised 37 patients with AML.^{13,14} Patients were uniformly treated with daunorubicin and Ara-C (3/7 days) plus intensive consolidation treatment with high dose Ara-C. Sequential bone marrow samples were obtained once the patients achieved complete remission. Five patients died and were excluded from the study. Four of the remaining 32 patients achieved remission for a second time; bone marrow samples from both remission episodes in these patients were included in the study, giving a total of 36. A mean of four studies was carried out per patient. Median follow up was 18 months.

Control bone marrow samples were obtained from 15 haematologically normal patients undergoing orthopaedic surgery who gave informed consent to bone marrow aspiration for scientific purposes.

COLONY ASSAY

Bone marrow samples were collected in sterile, preservative free heparin tubes and separated by Ficoll-Hypaque (d=1070) density gradient

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centrifugation.¹⁵ The CFU-GM assay was carried as described by Iscove *et al.*¹⁶ Briefly, 2×10^5 mononuclear cells/ml in Iscove's modified Dulbecco's medium (IMDM) were plated on 35 mm Petri dishes in 0.9% methylcellulose containing 10% phytohaemagglutinin stimulated leucocyte conditioned medium,¹⁷ 10% bovine serum albumin, and 10% human AB serum. Cultures were incubated at 37°C in a fully humidified atmosphere with 5% CO₂ and scored on day 14 under an inverted microscope. Aggregates containing more than 40 cells were scored as colonies; aggregates containing four to 40 cells were scored as clusters.

Based on the CFU-GM pattern observed in normal bone marrow, we defined three patterns of in vitro growth: 1 = normal (normal number of CFU-GM and a cluster:colony ratio <2); 2 = hypoplastic (low number of CFU-GM and a cluster:colony ratio <2); 3 = anomalous (low or normal number of CFU-GM and a cluster:colony ratio >2). Normal values were (mean (SD)) 142 (60) CFU-GM and a cluster:colony ratio of 1.22 (0.18).

Results

Eleven patients relapsed, all of whom previously displayed an abnormal CFU-GM pattern: anomalous in nine and hypoplastic in two. No false negative results were observed. The median time between detection of an abnormal growth pattern and relapse was four $\pm$ two months.

The remaining 25 patients were in complete remission at the time of writing: 16 had a normal CFU-GM growth pattern, whereas the other nine showed anomalous (eight patients) or hypoplastic (one patient) growth. These may be false positive results. Of the nine patients with an abnormal CFU-GM growth pattern, four also had other indicators of imminent relapse: in two patients the number of blast cells increased from undetectable levels by morphological and immunophenotypical methods to 1.3% and 4%, respectively; thrombocytopenia was diagnosed in the other two patients.

During the first three months of the study all except one patient had an abnormal CFU-GM growth pattern. Stable, normal CFU-GM growth patterns were observed a median of six months after diagnosis. All 15 patients whose growth pattern normalised remain in complete remission. By contrast, of the five patients whose pattern changed from normal to abnormal, three have relapsed and the other two showed either an increase in the number of morphological blast cells present or a decrease in their platelet count. The growth pattern remained unchanged in the remaining 16 patients: it was normal throughout the study in one and was always abnormal in the other 15. Eight of the latter 15 patients subsequently relapsed.

Discussion

Investigations of minimal residual disease may be of great help in managing patients with AML. Most investigations have focused on

analysis of growth of CFU-L in bone marrow cultures.⁸⁻⁹ Moore *et al.*¹⁸ studied the predictive value of analysing the in vitro growth pattern of CFU-L at diagnosis. Analysis of the growth pattern of CFU-GM has generally been used to assess residual haemopoietic damage as a result of treatment¹⁹ but not for predicting relapse. Our results show that sequential investigation of the CFU-GM growth pattern may be helpful in predicting relapse in patients with AML. In our experience the proliferation pattern does not remain constant during the evolution of the disease and frequent changes may occur. All patients who achieved a normal growth pattern remain in complete remission, but a median of six months elapsed before a stable, normal growth pattern was observed. By contrast, three of the five patients whose initial normal growth pattern became abnormal relapsed. The other two patients exhibited other indicators of imminent relapse.⁵

Sequential studies of the CFU-GM growth pattern are mandatory if this technique is to be used to predict relapse. Thus, patients whose CFU-GM growth pattern changes from normal to abnormal must be monitored frequently as they are at a high risk of relapse. It is highly probable that those patients with a persistent, normal CFU-GM growth pattern of one year's duration will remain in remission and generally will not need further intensive treatment. A larger patient cohort and a longer follow up are needed to confirm these observations.

The situation is not so clear for those patients with a persistently abnormal growth pattern, as only half have relapsed to date. Fluctuations in CFU-GM numbers have been observed previously in patients with AML and these have been interpreted differently. Chang *et al.*¹⁹ regarded these fluctuations as a result of haemopoietic damage following chemotherapy with no particular pathological significance. Sallefords and Olofson²⁰, however, suggested that a fall in the number of these progenitor cells could precede a relapse episode.

Chemotherapy results in a severe reduction in the number of clonogenic progenitor cells, which may persist for a long period of time after the patient has achieved complete remission.¹⁹⁻²¹⁻²² By contrast, Sallefords and Olofson²⁰ showed that most patients with AML in remission have a normal number of CFU-GM cells, which is in keeping with the data presented here.

In summary, sequential investigation of the CFU-GM growth pattern may be of value in predicting relapse in patients with AML. Patients with a persistently abnormal growth pattern are at high risk of relapse.

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