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Minimal Residual Disease in Acute Leukaemia

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THE PERSISTENCE of leukaemic cells throughout remission of the disease is increasingly recognised by modern methods. In particular polymerase chain reaction methodology allows to demonstrate the persistence of a few leukaemic cells (0.004–2.6%) in bone marrow of some patients with complete remission (CR) of acute lymphoblastic leukaemia (ALL) [1]. The disadvantage of this method is that it is too time consuming and cumbersome for the needs of clinical screening since individual diagnostic probes have to be sequenced and synthesised. We have used a technically less demanding procedure, namely cell cultures, which has already been successfully applied to detect residual disease in ALL [2].

We applied cell culture techniques in combination with immunological staining of the colonies to patients with adult acute myeloid leukaemia (AML) using a procedure which allows the reaction *in situ* in the agar without the necessity of picking colonies from the semi-solid medium [3]. Using an enzyme immunoassay we found that AML clones are characterised by the expression of early differentiation markers (e.g. CD10, CD20, CD34) which never occur on normal myeloid clones [4]. To our surprise such clones of leukaemic immunological phenotype were detectable repeatedly in 17 of 19 patients studied during complete remission of the disease [4]. 5 of these cases had clonal markers detectable cytogenetically or by Southern blot analysis which were identified only in the cultures of remission bone marrow. The percentage of such clones was higher than could be expected from the studies with molecular genetic markers, varying between 3 and 60%, and was significantly correlated to the clinical course since patients who relapsed within 6 months following the investigation had significantly higher proportions of colonies with immunologically leukaemic phenotype [4].

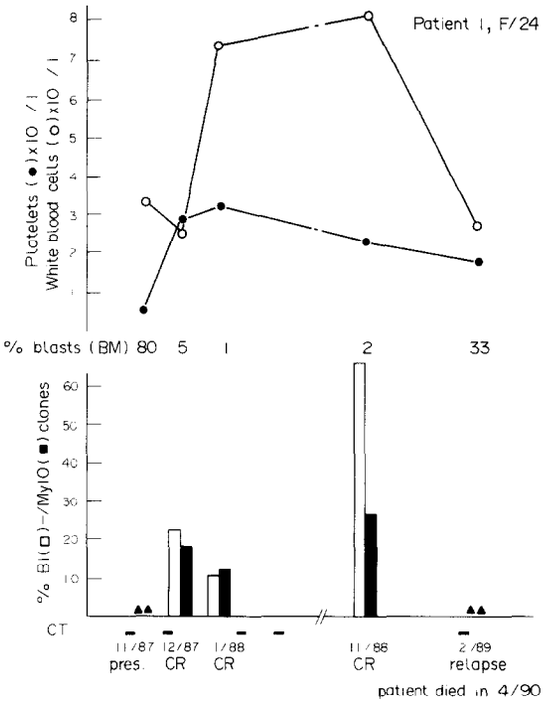

Fig. 1. Results and clinical course of AML patient 1, who died in April 1990. CT = chemotherapy, BM = bone marrow, pres. = presentation and ▲ = not done.

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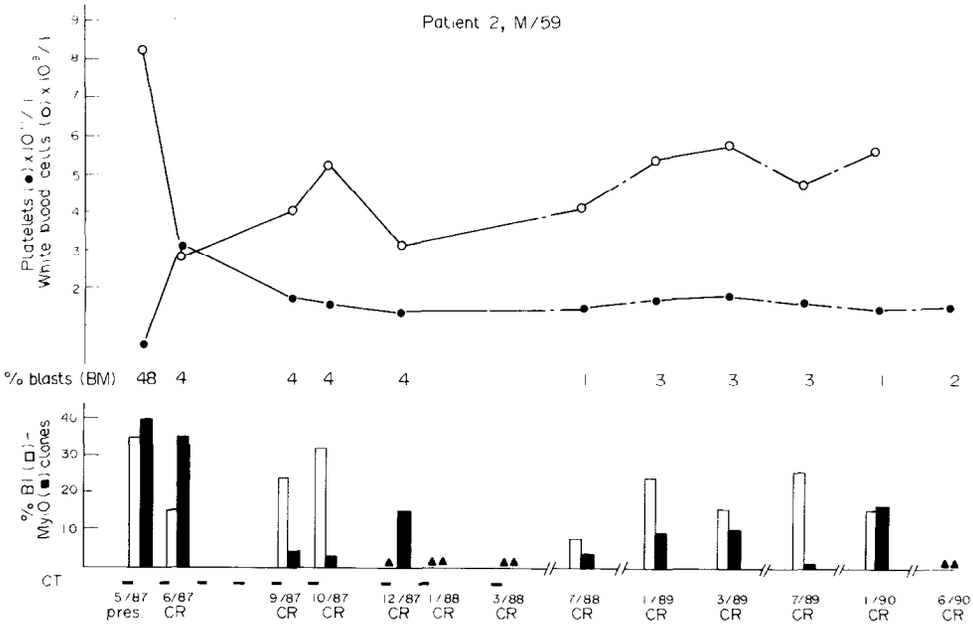

Fig. 2. Results and clinical course of AML patient 2.

The clinical usefulness of our assay can be demonstrated by two typical cases: Fig. 1 gives the course of a patient who was investigated three times during CR. While the proportion of leukaemic clones was below 20% during maintenance chemotherapy it had increased to 60% 10 months later during CR. 3 months later, the patient relapsed clinically. In contrast, another patient maintained a proportion of 5–30% of phenotypically leukaemic clones at repeated investigations over a period of nearly 3 years without relapsing clinically (Fig. 2).

Our data clearly demonstrate that "complete remission" of adult AML represents a balance of leukaemic and normal hematopoiesis rather than eradication of leukaemia. They argue for the action of mechanisms which suppress the outgrowth of leukaemic progenitor cells *in vivo*. Our *in vitro* culture system is applicable to all cases of AML and not restricted to certain immunological constellations as the investigation of uncultured bm cells in ALL [5] and much more sensitive than the Southern blot methodology in those cases which have a gene rearrangement as a clonal marker [4, 6]. Since it is relatively easy to perform it can be a valuable tool in clinical trials of postremission therapy including alternative approaches (e.g. cytokines like interleukin-2 or autologous bone marrow transplantation) as well as for testing the effectivity of *in vitro* purging methods.

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Early Detection of Relapse in Acute Non-lymphoblastic Leukaemia Patients by Cancer Procoagulant Assay

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ACUTE NON-LYMPHOCYTIC LEUKAEMIA (ANLL) represents a highly drug sensitive tumour and complete response rates of 65–75% are commonly achieved [1]. Despite this high chemosensitivity, disease relapse is common and long-term survival is poor. Therefore, it is conceivable that early detection of relapse could permit prompt treatment and better therapeutic response.

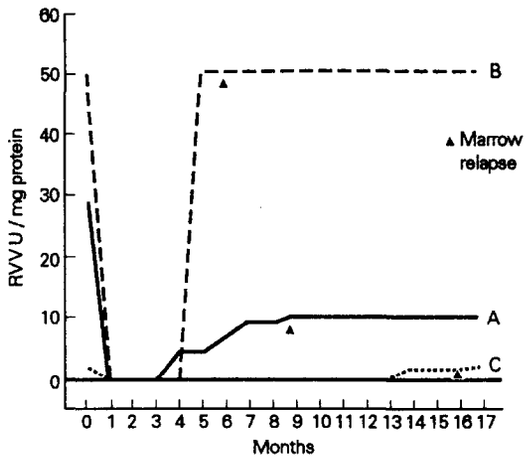

Fig. 1. CP activity in the bone marrow cell extracts from 3 ANLL patients (A, B and C).

The capacity of acute non-lymphocytic leukaemia (ANLL) cells, particularly the M3 subtype, to induce blood coagulation is well established [2, 3]. This procoagulant activity of leukaemic cells has been attributed to the production of thromboplastin or tissue factor (TF).

More recently a new procoagulant substance, distinct from TF, has been shown in ANLL cells [4], and similarly, in other neoplastic human cells [5]. This factor, named cancer procoagulant (CP) [6], a 67 000 kD cysteine proteinase, is not present in normal cells and is maximally produced by promyelocytic leukaemia cells [4]. No data are presently available regarding the use of CP as a disease marker in acute leukaemia.

Here we describe a case of ANLL (M3 subtype) in which the appearance of CP activity in the morphologically normal bone marrow of the patient anticipated the development of cutaneous relapse of the disease by 1 month, and the bone marrow relapse by 6 months.

A 25-year-old woman (Patient A) was admitted to our division with haemorrhagic diathesis of 10 days duration. Coagulation profile was consistent with disseminated intravascular coagulation. A bone marrow aspirate showed ANLL with a morphology compatible with the M3 subtype. The CP activity was determined on a leukaemic cell extract, by Falanga's method [4]. The factor VII independent procoagulant activity (CP) was expressed as Russell's viper venom (RVV) units/mg protein. The CP identification criteria were independence from factor VII and sensitivity to $HgCl_2$.

At the onset, the total procoagulant was 26.24 RVV U/mg protein; this activity was completely independent of factor VII and inhibited by $HgCl_2$, showing the characteristics of CP.

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