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.

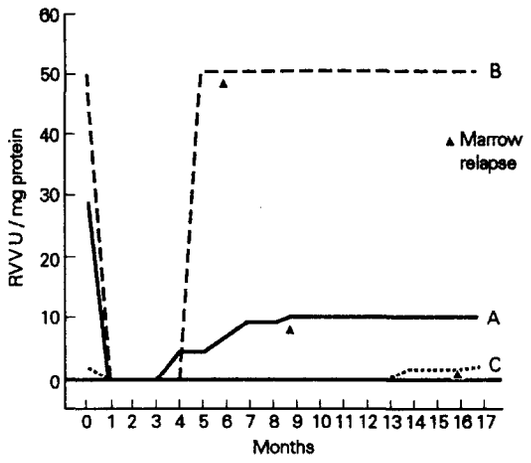


**Fig. 1. CP activity in the bone marrow cell extracts from 3 ANLL patients (A, B and C).**

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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Incubation with Con A failed to inhibit the procoagulant activity of the sample. Induction therapy (daunorubicin 80 mg/m$^2$ for 4 days) achieved a complete remission and complete disappearance of CP from the bone marrow cell extract (Fig. 1)

After the second consolidation cycle (etoposide, cytarabine, daunorubicin) in the presence of multiple cutaneous nodules, the lesions were biopsied and proved to be leukaemic relapses compatible with the M3 subtype. One nodule was utilised to obtain an extract which was tested for the presence of CP, of which a significant amount of CP was detected. 5 months later the patient relapsed in the bone marrow.

Subsequently, another two patients (B and C), have been studied (Fig. 1): in these two cases also the appearance of CP in bone marrow anticipated the relapse by 1–5 months.

Several methods for detection of minimal residual disease have been proposed [7, 8], mainly for acute lymphocytic leukaemia (ALL). Falanga *et al.* [4] recently described the production by ANLL cells of a particular procoagulant substance. This activity disappears upon obtaining complete remission. The presence of this factor in remission BMs could therefore be utilised as a marker of early relapse.

The data presented here show that in 1 ANLL patient the leukaemic relapse was evidenced by the bone marrow CP assay several months before bone marrow relapse and 1 month prior to a peculiar cutaneous recurrence of the disease. No other case of cutaneous localisation of M3 ANLL is available, to our knowledge. Similar data are now available from 2 other patients, with positive CP in the bone marrow anticipating the clinical relapse by 1 and 5 months, respectively (Fig. 1).

If these results are confirmed, this procedure could constitute the first assay for the detection of minimal residual disease in ANLL, a tumour in which molecular biology techniques, such as the detection of monoclonal rearrangement of immunoglobulin or T cell receptor genes, cannot be used.


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# Common Errors in Conducting and Reporting Clinical Trials in Non-Hodgkin Lymphomas and Patients' Age

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OLDER ADULTS are at higher risk of developing and dying from malignant tumours than their younger counterparts. However, it is only in recent years that a number of researchers and clinicians have focused their interest on the appropriate clinical management of cancer in the elderly, including non-Hodgkin lymphomas (NHL). It is important to recognise that there are several potential causes of bias in the performance on reporting of trials in NHL, with respect to age. The following are the most common.

Firstly the median age of the patient populations of series reported in the literature is usually between 50–55 years, sometimes between 45–50 years or 55–60 years, but rarely with a median age of more than 60 years. However, one third of NHL patients are more than 70 years and two thirds are over 65. Secondly, recently reported clinical trials where age over 70 is not an exclusion criterion includes the statement that there is no upper age limit for entry into the study. In practice, the number of patients over 70 is small and the median age usually ranges between 50 and 55 years. Therefore, this statement does not mean that the conclusions reached in the studies are applicable to elderly NHL patients.

Thirdly, patients may be grouped, for example, into those younger or older than 60 years, and complete response and survival rates compared. However, the median age of the older patients is usually not reported and hence the conclusion that older age does not influence complete response or survival rate is not acceptable. Fourthly, "age is not a prognostic factor" is another common assertion; however, patients tend to be selected for entry into the study mainly because of their age. Fifthly, conclusions almost never state that the results presented are valid for a patient population of that median age. As a result, the use of the conclusions of the trials in older patients may be associated with an increased percentage of treatment-related toxic deaths.

The quality of reporting of clinical trials in NHL, particularly with regard to age, should be improved.

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