Detection of minimal residual disease in leukaemia

The advent of modern intensive treatment has ensured that the majority of children with acute lymphoblastic leukaemia (ALL) will attain complete haematological remission. The length of this remission is, however, dependent on effective elimination of malignant cells as relapses are almost certainly due to residual leukaemic cells unresponsive to initial treatment. Such cells are referred to as minimal residual leukaemia or minimal residual disease (MRD) which is effectively the lowest number of abnormal cells identifiable with the available methods.

Detection of minimal residual disease
Remission in leukaemia is conventionally defined by a level of 5% leukaemic blasts or less in the bone marrow. However, identification of low numbers of leukaemic cells in a background of normal regenerating bone marrow precursors presents difficulties for even experienced haematologists. More sensitive and specific methods have been developed to complement morphology, and these will be discussed.

Immunological analysis
Immunological analysis has shown that aberrant or asynchronous surface markers are often present on leukaemic cells but absent from their normal counterparts. Two colour immunofluorescent techniques were initially developed to detect residual blasts in the bone marrow of patients with T cell acute lymphoblastic leukaemia as the phenotype CD7+/cytoplasmic CD3+/terminal transferase (TdT)+ is normally found only on thymic precursors. The coexpression of the CD10 antigen on blasts from patients with ‘common’ ALL together with the myeloid associated antigens CD13 or CD33, or of CD10 with the T cell markers CD2 or CD7, is a useful indicator of MRD which can be exploited using two colour immunofluorescence and fluorescence activated cell sorter analysis. The technique can detect one leukaemic cell in 10^4 normal cells. This technique is limited by the small numbers of leukaemias expressing such aberrant surface markers. In addition, the frequency of normal regenerating precursor cells expressing unusual combinations of surface markers has not been well studied and may be more common than realised, giving rise to false positive results.

Cytogenetic abnormalities
Consistent cytogenetic abnormalities in the malignant cells are useful markers to monitor MRD, but chromosome analysis is dependent on dividing cells, and in ‘remission’ the 1–5% abnormal cells present may not enter mitosis, thus limiting its use. The method is labour intensive, requires fresh samples, and at least 100 cells need to be screened for each sample.

Molecular analysis
Molecular analysis provides a sensitive and specific technique to monitor MRD, particularly in cases of ALL. The DNA rearrangements in the genes coding for the immunoglobulin heavy chain (IgH) in B cells, or the T cell receptor complex in T cells, can be detected in the majority of cases of ALL of T or B cell origin. Detection of clonally derived cells has been shown to be useful to evaluate the remission status of patients undergoing treatment and to predict early relapse.

B LINEAGE LEUKAEMIAS
In B lineage ALL, the IgH gene rearrangements are unique...
to each leukaemia and provide a tumour specific marker for individual patients. Using conventional Southern blotting, the level of sensitivity of detection of malignant cells is only 1-5% and it has not proved possible to identify all patients at risk of late relapse. Methods to improve the sensitivity have relied on the polymerase chain reaction (PCR) to amplify very small amounts of tumour marker DNA. This can then rapidly be visualised by gel electrophoresis. However, a number of problems may arise in applying PCR based techniques to the analysis of IgH gene rearrangements. IgH gene rearrangement involves the selection of one variable (V) gene out of more than a 1000 which is then joined to one of six joining (J) genes via an additional D (diversity) gene. Antibody specificity is encoded by the entire DJV segment and the number of gene rearrangements possible is very large. As these rearrangements are thus unique to individual leukaemias, clonospecific or patient specific probes are needed to detect MRD. Attempts to overcome these problems have relied on either specifically amplifying regions of the IgH gene which are commonly rearranged in B cell lymphoproliferative disorders or by using ‘consensus’ or framework primers to amplify and sequence the complementary determining region of IgH. The sensitivity of detection varies from one cell in 10^1 to one cell in 10^6 and can identify up to 95% of rearrangements. False positive and false negative reactions are found with all these techniques and also the inherent heterogeneity of leukaemic cells frequently results in a oligoclonal population of blasts showing multiple rearranged bands, not amenable to PCR analysis.

T LINEAGE LEUKAEMIAS

Rearrangements of the gamma chain of the T cell receptor complex occur in most T lineage and a proportion of B lineage ALLs. These rearrangements are much more restricted in their number of V regions and thus more amenable to PCR based analysis. The delta chain of the T cell receptor complex rearranges before the gamma chain and a higher percentage of delta chain rearrangements have been documented in B lineage leukemias. Restricted numbers of gene rearrangements have meant that these can also be monitored using PCR based methods of ‘remission’ or ‘marrow’ probes, and one cell in 10^8 can be detected many months after diagnosis and frequently precedes clinical relapse.

Currently this would appear to offer the best PCR based method for assessing MRD, the only problem being the 20% of B lineage leukemias with no detectable rearrangements of the TCR delta locus.

MOLECULAR CYTOGENETICS

Cloned translocation breakpoints will obviously provide the most sensitive and tumour specific MRD markers used in association with either DNA or RNA PCR methods. One example is the t(9;22) or Philadelphia translocation found in 98% of chronic myeloid leukemias, and 20% of adult and up to 5% of childhood ALLs. Cytoogenetically, the translocation appears identical in both diseases, but at the molecular level all chronic myeloid leukemic breakpoints are within the breakpoint cluster region (bcr) gene on chromosome 22. The ALL breakpoints are more widely dispersed. After the t(9;22) translocation, the abl oncogene on chromosome 9 is translocated onto chromosome 22 adjacent to the bcr gene and fuses with it, resulting in a chimeric gene. This produces a novel mRNA. The entire coding region for the bcr gene has been cloned; and oligonucleotide primers, which detect both the normal and the abnormal bcr genes or the abnormal transcripts have been used with PCR technology in cells from patients with chronic myeloid leukaemia after allogeneic bone marrow transplantation or cells from patients with Ph’ positive ALL in remission. The value of screening cyogenetically negative blast cells for concealed aberrant bcr transcripts was recently demonstrated in a large series of both adult and childhood ALLs, of whom 55% and 6% respectively, were positive.

Other recently cloned breakpoints such as the t(1;19) seen in pre-B ALL and t (14;18) seen in 85% of centrosomal/centromycytic lymphomas and the retinoic acid receptor α gene rearrangements seen in the t(15;17) of promyelocytic acute myeloid leukaemia have also proved amenable to PCR based MRD detection. In the case of the latter, it is likely that the beneficial pharmacological effect of retinoic acid is mediated via the α gene fusion protein. Hence the method can be used to suggest treatment options as well as monitor response to all-trans retinoic acid treatment with promyelocytic acute myeloid leukaemia.

Fluorescence in situ hybridisation and chromosome painting

Probably one of the most interesting recent techniques to assess MRD in leukaemias with chromosome abnormalities is fluorescence in situ hybridisation, using either DNA probes located at or near translocation breakpoints or chromosome painting using whole chromosome DNA. The use of interphase cytogenetics involving non-bleomycin cells lends itself to the analysis of remission bone marrow samples which contain few dividing cells. Centromeric probes which recognise specific repetitive sequences have been used to detect and quantitate their respective chromosomes in interphase nuclei. In addition, probes which detect both numerical and structural cytogenetic abnormalities combined with morphological analysis of the relevant cells provide a rapid and accurate assessment of MRD.

Conclusion

Unfortunately, in some cases the leukaemic clone may persist for many months or even years without overt relapse or any associated adverse clinical features. Thus, the significance of MRD and its ultimate relevance to treatment and long term survival will require both a large cohort of patients and detailed long term study using a variety of methods. The clinical relevance of MRD will only become important when both haematological and molecular criteria indicative of remission are achieved and sustained. This will then enable earlier cessation or alteration of treatment for a number of patients.

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Stressful life events and childhood illness

An undesirable life event is defined as an adverse social experience with an identifiable outset, circumscribed course, and discernible end point, such as a death or permanent separation, the effects of which are likely to have an impact on an individual for a period of at least a few hours. In other words, exposure to the adversity is relatively brief (hours or days) but the impact is substantive. Undesirable life events constitute a subclass of all types of life events (desirable, undesirable, and neutral) and one form of environmental experience that children are exposed to in everyday life. Ongoing difficulties are another form of social adversity, and refer to those social experiences where beginnings and endings are difficult to discern but indicate that a child is continuously being exposed to an undesirable experience, such as chronic marital disharmony, whose intensity and impact may wax and wane over time. For nearly 50 years life events and ongoing difficulties have been the subject of extensive research as potential causes of mental and physical disorders in individuals of all ages. It is only relatively recently, however, that different forms of life experiences to which children may be exposed have been the subject of classification and detailed research.1

Life events measurement

Questionnaires have been developed for parents and children to indicate the number of events that have occurred over a defined time period, often the previous 12 months. Events have invariably been weighted for likely stressful effects. 2 3 A grading of the ‘stressfulness’ of events such as parental death, divorce, personal illness, house or school move is then obtained. Unfortunately, there are considerable methodological problems with the measurement of life events by this procedure. Firstly, questionnaires invariably fail to cover all forms of recent life experiences; secondly, the wording of events is often ambiguous; thirdly, they cannot take into account the personal nature and qualities of events for children in different social and physical circumstances; and fourthly, they fail to determine when events occurred in relation to the onset or change (for example relapse or recovery) in the nature or course of disorder under investigation. To interpret the meaning of life events considerable effort should be expended in establishing the exact nature of the event and its temporal relationship with the episode (or change) of illness.

Many of these methodological difficulties are overcome by using face to face interviews with parents and children.4 Interview procedures provide a more sensitive method for determining the prevalence of events as well as collecting detailed personal information about the event (who was involved, its nature, salience to the individual, duration, and outcome). Such information allows a judgment to be made on the likely impact of an event on the child knowing its full nature and circumstances. This also takes into account the appreciable individual differences that occur in children’s social circumstances even for so called identical events such as death of a parent. Ratings of the degree of undesirability of any event can be obtained from a number of sources including parents, child, interviewer, or another professional not involved in the collection of the life event data. In addition, the onset of events can be carefully noted and compared with the onset of disorder being investigated. These methodological and procedural advances have resulted in a much greater understanding of the causal role of recent undesirable life events in the onset and recovery of anxious and depressive disorders in school age children.5-7

Recent undesirable events and child psychiatric disorder

There is now considerable evidence to show that most children between the ages of eight and 16 experience between three and five life events per year. Desirable events appear to occur with equal frequency in psychiatric cases and controls.8 There is, however, a significantly greater exposure to undesirable life events in the 12 months before the onset of psychiatric disorders in school age children compared with controls.9 It is apparent, however, that undesirable events are neither necessary nor sufficient as causal factors in all cases of psychiatric morbidity. Indeed, when they occur in isolation there are significant but modest increases in the risk, approximately fivefold, for the occurrence of anxious and depressive disorders. For the majority