Minimal residual disease in leukaemia and lymphoma

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Introduction

The leukaemias and lymphomas may be contrasted sharply with most other malignancies by the fact that, despite wide spread dissemination at presentation many patients may be returned to normality, sometimes permanently following therapy. Survival correlates closely with the extent to which the disease has been eliminated, or the completeness of remission. It is of considerable practical importance to define the response to therapy as precisely as possible. Thus any technique permitting the detection of disease at a lower level than conventionally possible might be of enormous benefit, possibly allowing modification of therapy to convert a 'complete remission' destined to relapse (the majority) into cure, or alternatively the termination of therapy in a patient already cured.

Minimal Residual Disease (MRD) is a clumsy and contentious term, by definition describing the lowest level of disease detectable with the methods available. It is however in common use. The most sensitive techniques are often able to demonstrate molecular and cellular change in the neoplastic cells, in morphologically and cytologically normal tissue. The determination of tumour specific markers [1-3] is important in order to devise a strategy for detection of MRD. There is a need for rapidity, ease and certainty of results and techniques involving enzymatic amplification (PCR) [4] offer the best opportunity of attaining these aims. Southern analysis allows similar specificity however there is a much lengthier procedure with a marked loss of sensitivity [5-7].

Methods to detect MRD

Polymerase chain reaction (PCR). The ability to exponentially amplify a target sequence of DNA in vitro by enzymatic synthesis (PCR) was first described in 1985 [4]. Two synthetically prepared oligonucleotides primers hybridise to opposite strands flanking the region of interest in the target DNA. The intervening sequences are generated by extension from these primers by a DNA polymerase, thus duplicating the target DNA. There is theoretical doubling of DNA yield for every cycle leading to an amplification of the target DNA by a million-fold \(2^{20}\) over 20 cycles, a level detectable by gel electrophoresis or Southern analysis. The application of PCR to the detection of minimal residual disease requires the target DNA to be a marker from the tumour cells. This is illustrated well by the t(14; 18) translocation associated with 85% of follicular non-Hodgkins lymphomas (NHL) and 20% of high grade diffuse NHL [8-11]. The translocation involves the Bcl-2 gene at one of two sites (the major breakpoint region and minor cluster region) on chromosome 18 and the immunoglobulin heavy chain joining region on chromosome 14. The breakpoints cluster within a few hundred base pairs on both chromosomes enabling detection of disease by this technique and has the sensitivity to detecting at least one tumour cell in \(10^5\) or \(10^6\) cells [8]. The sensitivity may be increased by either enrichment of the sample for tumour cells by the use of flow cytometry followed by PCR or by a 'booster' PCR technique [12] or utilising two sets of primers flanking the target DNA.

In contrast many tumours show alterations over much longer segments of DNA, sometimes over hundreds of kilobases [13-15]. However the length of DNA sequence may consist of mainly intron sequence that is not expressed in the messenger RNA (mRNA) transcript. This mRNA may be short enough to facilitate the use of the PCR technique to look for alterations over a considerable length within the transcribed exon DNA. Abnormal expression of mRNA by tumour cells acts as a marker of disease [13]. This is demonstrated well in chronic myeloid leukaemia (CML), some forms of acute lymphocytic leukaemia (ALL) and occasionally in acute myeloid leukaemia (AML) where a chimaeric mRNA (BCR-ABL), from the Philadelphia chromosome, is found exclusively in the leukaemic cells [13, 15]. The BCR exons involved in the translocation may be separated by over a hundred kilobase of intron sequence [13-15]. Utilising a modified PCR technique it is possible to detect this abnormal mRNA marker of the disease. Complimentary DNA (cDNA) from the chimaeric mRNA is produced by an initial step involving a reverse transcriptase enzyme, followed by the PCR procedure for DNA. Resultant amplification of the cDNA occurs [13-15]. Amplification from the mRNA is advantageous in that interference from intron sequence does not occur and therefore analysis of changes within longer sections of DNA is possible [13-15].

PCR is most suited for detection of target DNA of less than a 1.000 nucleotide base pairs [4]. The quantity of
DNA required is extremely small [4], 100 times less than for Southern analysis, while giving a least a $10^3$ times greater sensitivity [6–10, 16]. In addition PCR has been applied to small lengths of target DNA allowing the use of this method on degraded DNA, unsuitable for Southern analysis, such as paraffin embedded sections [17]. Retrospective examination of archival material for tumour markers and clonality is thus facilitated for prospective follow-up of patients with MRD. Sequencing reactions readily carried out directly on the PCR products allows the tumour specific alterations to be analysed down to the single nucleotide base level. In this way, sequence analysis from tumour material gives a very clear and precise clonal marker for the patients disease [10] and may be helpful in determining if recurrent disease shows the same clonality as the original tumour. In some cases it may be possible to use the PCR product as a unique marker for the patients tumour as shown with amplification of the T cell receptor in T cell lymphoid malignancies [18, 19]. The use of an immunoglobulin heavy chain variable region consensus sequence in combination with a heavy chain joining sequence has broadened the application of PCR to the majority of B cell lymphoid malignancies [20]. The detection of minimal residual disease by PCR has the advantage of rapidity (approximately 3 hours per reaction) greatly increased sensitivity over other techniques and therefore the ability to detect sub-clinical disease at a much earlier stage [8, 13]. The main disadvantage lies in the necessity for the neoplastic disease to have identified flanking sequences of DNA or mRNA that will allow amplification of a tumour specific marker. PCR is an extremely sensitive method for amplification of a target DNA [4, 8, 13], however is not directly quantitative. Adaptation of PCR to allow quantitation for detection of proviral DNA has been demonstrated [21]. Application of quantitation techniques to MRD could be of benefit in sequential follow-up, charting progression of disease from an early sub-clinical stage. Further clarification of tumour specific translocations, deletions and alterations will permit greater application of this technique to ascertain the presence or absence of MRD.

**Discussion**

The overall clinical relevance to patients with leukaemias and lymphomas of laboratory techniques able to detect minute quantities of tumour associated molecular abnormalities will depend on several factors, excluding the unanswered questions relating to false negative or positive results (the sensitivity of the techniques may allow contamination to contribute to rogue results).

Although non random chromosomal changes have been reported for an increasing number of haematological malignancies [1–3, 6], the use of molecular probes for Southern analysis [5] and subsequent development of PCR methods to detect these alterations [4, 8, 13] has only been applicable for the *bcr* gene in chronic myeloid leukaemia [13–15], the *bcl-2* gene in follicular lymphoma [8–11] and the immunoglobulin heavy chain [20] and T cell receptor genes in lymphoid malignancies [18, 19]. This clearly limits the applicability of the techniques to a relatively modest number of groups of patients at present, if however in no way limits the investigation of the hypothesis that 'molecular evidence of disease' after treatment equates with an inevitability of recurrence occurring sooner or later.

Monitoring of remission, with other, less sensitive and probably less 'disease specific' methods has only met with limited success [6, 7, 16, 22–24]. Follow up of patient in clinical and haematological remission of acute lymphoblastic leukemia using anti CALLA antibody to examine the marrow known to have been positive at presentation failed to be of use due to phenotype shift at relapse [27]. However a study, in which two immunological parameters, tailored to the original phenotype of the blast cells were utilised, produced results highly predictive of relapse [28]. Investigation of patients presenting with different stages of lymphoma or leukaemia using flow cytometric analysis for *κ* or *λ* light chain expression [29], or gene rearrangement analysis suggests that clonal evidence of disease may be found in the absence of clinical or morphological findings [6, 22]. Studies of the peripheral blood of patients in long term follow up of malignant lymphoma using RFLP (restrictive fragment length polymorphisms) or PCR for the *t(14; 18)* translocation show persistent abnormalities in a proportion despite continuing clinical remission [8, 30, 31]. Clearly if the abnormalities are present for many years without the evidence of recurrence, their clinical relevance must be queried [32].

Satisfactory methods for quantifying the amount of abnormal circulating DNA [21, 33] will obviously offer the opportunity to look for progression or regression over time.
Such techniques may have an application for determining the efficacy of in vitro treatment of bone marrow for autologous transplantation [34]. Much controversy surrounds the necessity or reliability of 'clean up' and is hampered by the difficulty of proving that the procedure either does anything, or contributes to outcome. Preliminary data using the PCR with probes for the bcl-2, suggest that in vitro treatment of bone marrow from patients with follicular lymphoma with anti-B-1 and complement depletes it by 10-50 fold, but rarely cleans it completely [35]. Similar results have been shown with cell lines or multiple antibodies [36].

Ultimately these techniques will only be of major clinical relevance when treatment is available which can convert a clinical but not molecular remission into a clinical and 'molecular' complete remission and thereby cure. In addition, it must be borne in mind that most cytotoxic therapies are dangerous, and the confirmation of remission at the molecular level might allow some patients to stop therapy sooner.

Many questions remain to be answered about 'minimal residual disease'. Indeed, this is a relatively small problem in haematological malignancy. A far greater problem concerns the inability of treatment to achieve even clinical remission for most patients. Under no circumstances must the investigation of molecular generation of leukaemia and lymphoma be inhibited by this. On the contrary it must be pursued along the current lines with vigour and enthusiasm. Greater understanding of the diseases continues to evolve from the laboratory and the fact that therapeutic advances are lagging behind should only serve as a stimulus for forward thinking.

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