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Author(s)	Liang, RHS; Chan, DW; Kwong, YL; Chan, VNY
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Molecular detection of minimal residual disease for patients with leukaemia and lymphoma

R Liang, D Chan, YL Kwong, V Chan

Although a complete clinical remission can often be achieved with chemotherapy for patients with leukaemia and lymphoma, relapses still occur. Residual tumour cells probably have survived therapy and account for subsequent disease relapse. The sensitivity of conventioned ways of detecting residual tumour cells, such as morphological studies, immunophenotyping, and cytogenetics, is only about 1% to 5% and may be inadequate. Polymerase chain reaction technology has provided a simple and highly sensitive means for the detection of minimal residual disease. The technology has been successfully applied to study biopsy samples obtained from patients with leukaemia and lymphoma. Its clinical usefulness, however, requires further evaluation by prospective clinical studies.

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Key words: Neoplasm, residual; Leukaemia; Lymphoma; Polymerase chain reaction

Introduction

Although complete remission can often be achieved with chemotherapy for patients with leukaemia and lymphoma, relapses still occur. The chance of relapse depends on the kind of disease being treated, the therapy given, and on various prognostic factors.^{1,2} It has been shown that malignant cells found at relapse often exhibit cytological and phenotypic characteristics similar to those that were seen at the time of presentation.³ This suggests that residual tumour cells probably survived therapy and remained undetected. These may then account for subsequent disease relapse.^{1,2} Patients receiving high dose chemotherapy with autologous stem cell rescue may also relapse as a result of occult tumour contamination in the autologous stem cell or bone marrow given.^{2,4}

Conventional ways of detecting residual tumour cells such as morphological studies, immunophenotyping, and cytogenetics have a sensitivity of only about 1% to 5%.¹⁻⁶ This detection level may be inadequate for defining true remission following therapy.

The concept of minimal residual disease

Techniques with greater sensitivity in detecting minimal residual disease (MRD) are potentially useful to better define a patient's remission status and evaluate the effectiveness of therapy. There may also be important therapeutic implications. More aggressive treatment such as high dose therapy and stem cell transplantation may be offered to patients with persistent detectable MRD. On the other hand, those with undetectable disease, even with highly sensitive technology, are likely to be cured and further treatment associated with high mortality and morbidity is probably not advisable. By monitoring MRD, it may therefore be possible to tailor the treatment strategy to each patient.

The detection of MRD may also be useful for patients receiving high dose chemotherapy with autologous stem cell or marrow rescue. Highly sensitive

Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong

R Liang, MD, FRCP
D Chan, BSc (Hons)
YL Kwong, MD, MRCP
V Chan, PhD, FRCPath

Correspondence to: Dr R Liang

techniques can be used to identify occult tumour contamination in the autologous marrow or stem cell harvested for a transplant. It may also be used to assess the effectiveness of marrow purging if it is performed before a transplant.

For a technique to be clinically useful in the detection of MRD, it needs to satisfy some criteria. Firstly, it must have a high specificity and be able to discriminate malignant cells from normal cells with no false negative and positive results. The test must also be sensitive enough to detect the presence of a minute amount of tumour cells. Furthermore, it needs to be simple, quick, and easy to perform and the results must be reproducible.^{5,6}

The detection of minimal residual disease

The technique used for the detection of MRD must be able to recognise tumour cells from a mixture containing normal cells. A specific marker has to be identified on the malignant cells. It may be a protein, RNA or DNA located on the cell surface, in the cytoplasm, or in the nucleus.

The available techniques for the detection of MRD can be classified into four groups. Firstly, it may examine the overall characteristics of neoplastic cells by morphology or the "in vitro" colony growth pattern of tumour cells.⁷ Secondly, specific cell surface, cytoplasmic, or nuclear markers may be detected by immunological analysis.^{8,9} Thirdly, specific chromosomal aberrations can be found by standard cytogenetic analysis, flow karyotyping, or fluorescence in situ hybridization (FISH).^{10,11} Lastly, molecular techniques have been commonly used for the detection of MRD. They include the conventional DNA or RNA studies by Southern or Northern blotting technique and the highly sensitive polymerase chain reaction (PCR) gene amplification system.¹²

Morphological examination and cell culture

Morphological study of tissue biopsy specimen is a standard way of detecting the presence of neoplastic cells. It relies on examining the morphology of malignant cells under a light microscope and the detection limit is at best one to five neoplastic cells among 100 normal cells.⁷

The other technique involves "in vitro" cell culture and observation of neoplastic colony growth. For acute leukaemia, blood or marrow specimen samples can be obtained and cultured in plates containing haem-

atopoietic growth factors. Leukaemic progenitor cell colonies can be observed after a period of time. The presence of a residual leukaemic progenitor cell colony in a remission marrow has been shown to be a useful predictor of leukaemia relapse after autologous bone marrow transplantation.¹³ Although the sensitivity of this technique appears to be better than conventional morphological examination, it is a very time-consuming procedure. Also, contamination during culture is sometimes seen and may give a false negative result.

Immunophenotyping

Immunophenotyping using a panel of monoclonal antibodies has been used extensively in parallel with morphological analysis for diagnosing blood cancers. These monoclonal antibodies recognise tumour cells by binding to the specific antigens that are expressed on their cell surface. There may be a unique expression of some of these antigens by neoplastic cells and it may thus provide a specific marker for disease detection. Using a flow cytometer, the sensitivity of detection has been reported to be as high as one leukaemic cell per 10 000 normal cells.¹⁴ Double immunofluorescent staining technique has also been used and an even higher sensitivity of one in 100 000 has been achieved.^{8,9,15-17}

Cytogenetic analysis

Cytogenetic analysis has been used routinely to study chromosomal aberrations of various haematological malignancies. The conventional technique studies the abnormal chromosomal pattern of the tumour cells at their metaphase.¹⁸ The sensitivity of the technique is believed to be similar to morphological examination. Flow karyotyping is an alternative method that uses fluorescent staining and flow cytometry.⁵ Compared with conventional cytogenetic analysis, the flow karyotyping technique gives a ten-fold increase in sensitivity.⁵ Both techniques are limited, however, having low detection sensitivity and the need to study the tumour cells at metaphase. Hence, they are not widely used for the detection of MRD. Fluorescent in situ hybridization is a newer technique for studying chromosomal aberrations of tumour cells at interphase.^{11,19,20} The FISH technique has the advantage that mitotic cells are not required and the sensitivity of detection is higher,^{21,22} being reported to be at the level of one in 1000.⁵ If FISH is combined with flow cytometry, the sensitivity can be further enhanced to one in 10 000 to 1 million.⁵

Molecular technology

The progress of recombinant DNA technology has allowed us to study the unique molecular features of

monoclonal cellular populations; either the Southern blot or PCR techniques are used. Southern blotting has been used extensively to detect a specific DNA aberration associated with a malignant clone of cells by employing appropriate DNA probes.^{23,24} The technique may detect specific recombination of the oncogene associated with the tumour or clonal rearrangement of the normal gene such as the immunoglobulin (Ig) or T-cell receptor (TCR) genes.¹² Rearrangement of the Ig heavy chain gene in a wide range of B-cell malignancies can be detected with a positive detection rate ranging from 70% to more than 90%.^{25,26} A similar technique also detects rearrangement of TCR- β or TCR- γ genes in 80% to more than 90% of cases of T-cell malignancy.^{27,28} For TCR- δ gene rearrangement, up to 68% of the T-acute lymphoblastic leukemia are positive by the Southern technique.²⁹ Southern analysis, however, has several disadvantages. Firstly, the sensitivity of the technique is only approximately 1%.^{30,31} Secondly, a relatively large amount of DNA is required for the analysis. Thirdly, it is quite labour-intensive and time-consuming. Lastly, it involves radioactivity if a radiolabelled probe is used.

Polymerase chain reaction technology is a powerful technique by which repeated cycles of oligonucleotide priming and DNA polymerisation allow rapid amplification of short segments of DNA taken from a very small number of cells. This system can consistently detect cells at a concentration of one in 10 000 to 100 000 cells and is potentially useful for MRD detection. The PCR technique requires the design of sequence-specific oligonucleotide primers for amplification of the desired DNA segment. It is usually a tumour-specific nucleic acid sequence at the breakpoint of a genetic recombination, for example, the *bcl-2* gene rearrangement found in follicular lymphoma. Also, most cases of B-cell or T-cell malignancy have rearrangement of the Ig gene and/or TCR genes that are detectable by PCR technology.^{12,31,32} However, this technique has its limitations, which include easy cross-contamination by exogenous amplifiable template and hence, the production of false-positive results; this can often be avoided if special efforts are made to prevent contamination. False-negative results may also be seen as a result of clonal evolution, sampling effect (too small), or the degradation of nucleic acids.

This technique can also be used for the detection of RNA expression. The reverse transcription PCR (RT-PCR) technique has made studying abnormal genetic recombination at RNA level possible, for example, the *abl-bcr* recombination in chronic myeloid leukaemia

(CML) or acute lymphoblastic leukaemia (ALL).¹² There have also been modifications to the PCR technique to further enhance its sensitivity. The technique of nested or semi-nested PCR has been used to improve its sensitivity in detecting Ig gene rearrangement.³⁰ The DNA sequencing of the PCR product enables the design and synthesis of a specific oligonucleotide for use as a primer for clonal specific PCR or as a clonal specific probe for Southern analysis and hence, further improves sensitivity.¹⁷

Clinical applications of molecular detection methods

Chronic myeloid leukaemia

The RT-PCR technique has been successfully used to detect MRD in patients with CML following either bone marrow transplantation or alpha-interferon therapy. Residual *bcr-abl* transcripts are often detectable in patients who are in apparent haematological and cytogenetic remission. Positive PCR results are especially common in the first six months immediately following marrow transplant. However, these positive results may not always be predictive of imminent haematological or cytogenetic relapse.³³⁻⁴² Studies have suggested that the detection of increasing amounts of the *bcr-abl* transcripts by quantitative PCR technique may be a better predictor of CML relapse.³⁸

Many explanations have been proposed for this finding of persistent *bcr-abl* transcripts despite apparently durable remission. Early positive PCR results following alpha-interferon therapy or marrow transplant may simply mean that these abnormal cells are not capable of re-populating the marrow and the survival advantage of normal cells over leukaemic cells may have been re-established. Alternatively, these abnormal transcripts may belong to clones of lymphoid cells that persist but do not contribute to marrow repopulation. With bone marrow transplantation, it is possible that disease relapse as a result of these PCR-positive cells may be prevented by the graft versus leukaemia effect. Therefore, the predictive value of positive PCR for *bcr-abl* transcripts as a means of follow up for CML remains to be established by long term studies.

Acute promyelocytic leukaemia

Studies have shown that PCR detection of MRD is strongly predictive of relapse for patients with acute promyelocytic leukaemia (AML-M3). The highly specific genetic marker, the t(15;17) translocation, has been used for RT-PCR detection. For patients with AML-M3, successful induction to complete remission

with all-trans retinoic acid (ATRA) alone often does not eradicate all PCR-positive cells.^{43,44} Durable remission is only possible when PCR negativity is achieved by further consolidation chemotherapy.

Acute myeloid leukaemia

Polymerase chain reaction detection is also used to detect the t(8;21) transcripts in patients with acute myeloid leukaemia (AML). In contrast to AML-M3, long term remission has been reported in patients with positive PCR findings and the biological explanation for this curious finding remains obscure.⁴⁵

Acute lymphoblastic leukaemia

For patients with ALL, PCR detection of MRD relies on amplification of the clonal rearrangement of Ig or TCR genes. For ALL with a t(9;22) translocation, the *bcr-abl* transcript may also be used as the target for PCR. It appears that PCR detection of MRD is highly predictive of leukaemic relapse for ALL and PCR positivity often precedes relapse by several months.⁴⁶⁻⁵²

Follicular lymphoma

For follicular lymphoma with a t(14;18) translocation, the *bcl-2* gene rearrangement may be used as a target for PCR detection. The technique has been used to evaluate patients receiving chemotherapy or autologous bone marrow transplantation and is found to be highly predictive of disease relapse. The PCR technique has also been used to assess the efficacy of a purging technique for autologous marrow.⁵³⁻⁵⁶ Persistent detection of residual lymphoma cells within the autologous marrow samples following purging is associated with a greater risk of post-transplant relapse.⁵⁵

Conclusion

The technique of PCR detection of MRD has been successfully established for different kinds of leukaemia and lymphoma. It is a simple and very sensitive technique. Its clinical usefulness, however, requires further evaluation by clinical trials involving larger numbers of patients.

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