

***Polymerase chain reaction in the
diagnosis and monitoring of
patients with PML-RAR α and
PLZF-RAR α gene rearrangement
in acute promyelocytic leukaemia***

March 2003

MSAC reference 9a (ii)

Assessment report

© Commonwealth of Australia 2003

ISBN (Print) 0 642 82358 8

ISBN (Online) 0 642 82359 6

ISSN (Print) 1443-7120

ISSN (Online) 1443-7139

First printed September 2003

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The Medical Services Advisory Committee is an independent committee which has been established to provide advice to the Australian Government Minister for Health and Ageing on the strength of evidence available on new and existing medical technologies and procedures in terms of their safety, effectiveness and cost-effectiveness. This advice will help to inform Government decisions about which medical services should attract funding under Medicare.

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Publication approval number: 3359

MSAC recommendations do not necessarily reflect the views of all individuals who participated in the MSAC evaluation.

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Executive summary

The procedure

The polymerase chain reaction (PCR) is a molecular technique that amplifies nucleic acid, producing millions of copies of an isolated portion of nucleic acid. Nucleic acid is extracted from body fluids (in this review bone marrow or peripheral blood samples) for detection of abnormality. A range of PCR techniques is available, including qualitative and quantitative methods. PCR is currently funded under the Medicare Benefits Schedule for a variety of non-haematological indications (for example, MBS items 69370, 69378, 69381, 69382, and 69442-69445).

Molecular laboratories are required to be NATA/RCPA accredited and must perform to a satisfactory level in appropriate external QA programs.

Medical Services Advisory Committee – role and approach

The Medical Services Advisory Committee (MSAC) is a key element of a measure taken by the Australian Government to strengthen the role of evidence in health financing decisions in Australia. MSAC advises the Australian Government Minister for Health and Ageing on the evidence relating to the safety, effectiveness and cost-effectiveness of new and existing medical technologies and procedures, and under what circumstances public funding should be supported.

A rigorous assessment of the available evidence is thus the basis of decision making when funding is sought under Medicare. A team from the New Zealand Health Technology Assessment (NZHTA) Research Unit, University of Otago, was engaged to conduct a systematic review of literature on nucleic acid amplification in haematological malignancy. A supporting committee with expertise in this area then evaluated the evidence and provided advice to MSAC.

MSAC's assessment of PCR in APL

The Australian Health Technology Advisory Committee (AHTAC) completed a review of nucleic acid amplification technology in 1997. The use of PCR was recommended for diagnosis and monitoring of acute promyelocytic leukaemia (APL) in that report but has not been implemented. MSAC was therefore approached by the Pathology Services Table Committee (PSTC) to review the use of nucleic acid amplification (NAA) in APL. The NAA supporting committee developed the following questions for APL:

- Does PML-RAR α and PLZF-RAR α detection increase the proportion of patients who are recognised to have a specific disease entity that defines a specific therapeutic strategy?
- Does repeated qualitative and/or quantitative PCR testing in APL predict haematological relapse and/or influence therapeutic decision making?

Detection of other cytogenetic abnormalities has important prognostic and therapeutic implications, so PCR testing is a supplementary rather than a replacement test.

Clinical need

APL is a morphologically distinct sub-type of AML (acute myeloid leukaemia) with unique clinical features, including potentially life-threatening coagulopathy. It is associated with chromosomal translocations that disrupt the gene encoding for the retinoic acid receptor alpha (RAR α) on chromosome 17. In approximately 99 per cent of cases, this disruption results in the fusion of RAR α with the PML gene of chromosome 15 (t(15;17)). The second most common variant of APL results from the fusion of PLZF (chromosome 11) with the RAR α gene (t(11;17)). This occurs in approximately 0.5 per cent of APL cases. Cells expressing PLZF- RAR α fail to differentiate in response to ATRA. In contrast, PML- RAR α is sensitive to ATRA. Therefore, correct disease characterisation is vital given the difference in responsiveness to ATRA in APL subgroups.

APL is a rare disease in Australia. Data from the Australian Institute of Health and Welfare indicate that in Australia the crude incidence of APL in 1999 was 0.2 per 100,000 population. There were 18 new cases of APL in Australian men (0.2 per 100,000) and 24 new cases in Australian women (0.3 per 100,000).

Reference standard

Clinical diagnosis of APL (usually by morphology) in combination with cytogenetic status and response to ATRA (all-*trans* retinoic acid) was used as the reference standard in the diagnostic part of the review. Specifically, a clinical diagnosis of APL and response to ATRA was used as the reference standard for the estimation of diagnostic accuracy of PCR for PML-RAR α . For PLZF-RAR α , the reference standard was a combination of clinical diagnosis and either the presence of t(11;17) or the absence of t(15;17) on cytogenetic testing.

Cytogenetic and haematological relapse was used as the reference standard for the monitoring part of the review.

Safety

The PCR assays discussed in this review are unlikely to directly increase risk to patients. The required quantity of blood or marrow is minimal and would usually be collected concurrently with other routine blood or marrow tests.

Effectiveness

Diagnostic accuracy in APL diagnosis

PCR was evaluated for its ability to correctly identify patients with PML-RAR α and PLZF-RAR α APL at presentation. The 13 studies identified and included were all case series (level IV evidence). Combined cytogenetic and PCR testing had an estimated

sensitivity for the detection of PML-RAR α and PLZF-RAR α of 99 per cent compared with 92 per cent for cytogenetic testing alone, in the studies where cytogenetic testing was successful. PCR was estimated to have a specificity of 100 per cent (95% CI 85, 100) in the five studies providing sufficient data to estimate this measure.

Diagnostic accuracy in APL monitoring

PCR was evaluated for its ability to predict subsequent cytogenetic and haematological relapse in the monitoring studies. Sixteen studies were eligible for the review. All were case series (level IV evidence) and all but one used qualitative RT-PCR techniques. The pooled diagnostic odds ratio (DOR) from the 13 studies where study DORs could be estimated was 103 (95% CI 57, 186). A DOR of 103 is consistent with, for example, a sensitivity of 92 per cent and specificity of 90 per cent.

Change in management

The use of PCR at presentation provides a sensitive method of confirming the diagnosis of APL. Moreover, it is able to differentiate ATRA-sensitive and ATRA-resistant translocations and establishes a target for minimal residual disease (MRD) detection.

The early use of salvage therapy is supported in the literature, hence detection of relapse through PCR monitoring potentially improves health outcomes.

Conclusions about the effect of additional PCR testing on patient outcome

There was evidence suggesting an incremental improvement in diagnostic accuracy from the addition of PCR to cytogenetic testing at diagnosis. Trial-based data has found a survival advantage for patients treated with ATRA and chemotherapy, compared with chemotherapy alone. Identification of PML-RAR α by PCR testing directs therapeutic options to ATRA-based therapy. Therefore, an incremental improvement in diagnostic accuracy at presentation, plus improved differentiation of PML-RAR α from PLZF-RAR α APL, could be expected to produce improved health outcomes in patients with this rare disease.

Monitoring with PCR was associated with early detection of relapse and pooled DOR significantly different from one, indicating improvement in diagnostic classification resulting from use of the test. One study was identified that compared the use of salvage therapy at molecular relapse, detected by PCR, with salvage therapy at haematological relapse, in a historical control series. This study supported improved outcomes with early therapy, therefore implying an expectation of improved patient outcomes with the use of PCR in the monitoring of APL.

Cost effectiveness

The economic analysis evaluating the use of PCR in the diagnosis of APL found the incremental cost per life year saved was \$329 for cytogenetic and PCR testing compared with cytogenetic testing alone. The incremental cost per life year saved was \$6,418 for monitoring with cytogenetic plus PCR testing combined, compared with cytogenetic testing alone.

Sensitivity analysis suggested the incremental cost effectiveness ratio in the diagnostic model was insensitive to inpatient management cost, management strategies for the cytogenetic test negative group and life expectancy estimates, including proportion of early deaths in patients treated with non-ATRA containing regimens as a result of false negative diagnostic tests. The monitoring model was sensitive to variation in PCR cost, life expectancy estimates and change in inpatient cost estimates. The maximal incremental cost per life year saved across a plausible range of sensitivity analyses in the monitoring model was \$16,269.

Overall, although the cost estimates were higher in the combined PCR and cytogenetic testing strategy compared with cytogenetic testing alone, in both the diagnosis and monitoring models the extra benefit in terms of life years saved suggest favourable indicative cost-effectiveness ratios for the use of combined cytogenetic and PCR testing.

Recommendation

MSAC recommended that on the strength of evidence pertaining to safety, effectiveness and cost effectiveness of polymerase chain reaction (PCR) testing in the diagnosis and monitoring of acute promyelocytic leukaemia (APL), public funding should be supported for this procedure.

- The Minister for Health and Ageing accepted this recommendation on 8 August 2003 -

Introduction

The Medical Services Advisory Committee (MSAC) has reviewed the use of nucleic acid amplification (NAA), which is a diagnostic test for acute promyelocytic leukaemia (APL). MSAC evaluates new and existing health technologies and procedures for which funding is sought under the Medicare Benefits Scheme in terms of their safety, effectiveness and cost-effectiveness, while taking into account other issues such as access and equity. MSAC adopts an evidence-based approach to its assessments, based on reviews of the scientific literature and other information sources, including clinical expertise.

MSAC's terms of reference and membership are at Appendix A. MSAC is a multidisciplinary expert body, comprising members drawn from such disciplines as diagnostic imaging, pathology, surgery, internal medicine and general practice, clinical epidemiology, health economics, consumer health and health administration.

This report summarises the assessment of current evidence for nucleic acid amplification in APL.

Background

Nucleic acid amplification

How it works

Nucleic acid amplification (NAA) includes a range of methods for detecting, identifying and amplifying minute amounts of nucleic acid. Two general types can be distinguished: amplifying the nucleic acid present in the sample and amplifying the signal produced by nucleic acid through binding to larger molecules. The polymerase chain reaction (PCR) is an example of amplifying nucleic acid. The branched DNA (bDNA) assay is an example of amplifying the signal. A breakdown of NAA techniques is shown in Figure 1.

While variants of the PCR have been developed for use in the management of haematological malignancy, this is not the case for other NAA methods. Therefore, this review focused on PCR testing.

The originally described PCR method produces millions of copies of an isolated portion of DNA through amplification of DNA with a single pair of primers. Reverse transcriptase is used to convert RNA into complementary DNA before using the methods described for PCR. This method has the ability to improve the limit of detection since a single gene can form many copies of mRNA.

A technique that can be applied to both PCR and reverse transcriptase PCR (RT-PCR) takes the product of the first round of amplification and adds a second amplification cycle using another pair of primers directed to sequences within that first round product. This method is known as nested PCR/RT-PCR.

A further variation is one where multiple primer pairs specific for different targets can be included in the same amplification reaction, making it possible to detect two or more nucleic acid sequences at the same time. This is known as multiplex PCR/RT-PCR.

The PCR techniques can be divided into qualitative and quantitative methods.

Qualitative techniques include PCR, RT-PCR (reverse transcriptase polymerase chain reaction) and multiplex PCR. RT-PCR is the most relevant qualitative technique in the context of this review.

Quantitative techniques include real time (cycle-cycle) quantitation, which estimates the level of PCR products as they accumulate, and competitive techniques which require addition of a known amount of competitor to the target gene or transcript. The bDNA assay is also an example of a quantitative technique but is not relevant to this review.

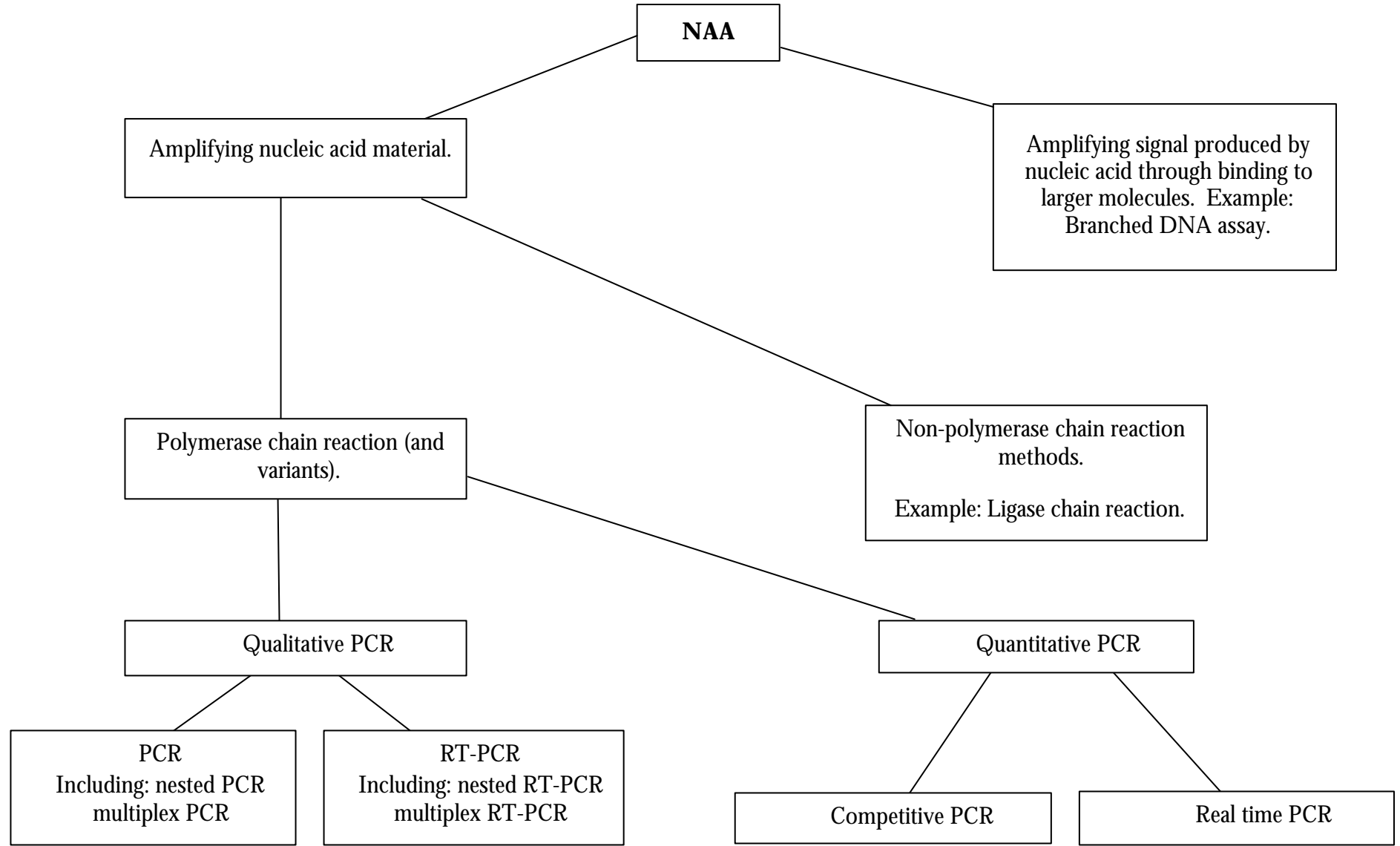


Figure 1 Nucleic acid amplification techniques

Requirements for PCR

Laboratory equipment required includes sterilisation facilities, centrifuges, biohazard cabinets and thermal cyclers. The use of separate rooms is required for different stages of the process. These precautions are required to minimise the risk of false positive results given the sensitivity of PCR techniques.

Specific measures to control contamination include:

- the competency of staff at performing laboratory tasks;
- the design of the laboratory;
- the choice of PCR method;
- the routine use of controls to detect contamination.

Three areas are required in each nucleic acid amplification laboratory: an area for extraction of nucleic acid and addition of sample DNA to tubes containing master mix before PCR amplification, a clean area for reagent preparation and a contained area for amplification and product detection. The normal airflow pattern for each of these regions should be known and the laboratory layout should be designed to minimise the potential for aerosol cross-contamination.

Key points about sample preparation include maintaining the integrity of the DNA or RNA, removal of potential inhibitors and avoiding contamination.

Selection of appropriate target regions and primers are critically important in the process.

The thermal cycling temperatures are of key importance. Three temperature settings are used in each PCR cycle: denaturing temperature, annealing temperature and the extension temperature.

The National Pathology Accreditation Advisory Council (NPAAC), which was established in 1979, has a major role in formulating standards, and initiating and promoting guidelines and educational programs relating to the performance of pathology tests.

NPAAC, as recently as 2000, published a set of standards and guidelines entitled Laboratory Accreditation Standards and Guidelines for Nucleic Acid Detection Techniques (Australia. National Pathology Accreditation Advisory Council, 2000). This publication is used by National Association of Testing Authorities (NATA)/Royal College of Pathologists of Australasia (RCPA) in assessing ISO17025 level competence in laboratories performing PCR tests in haematological malignancy, as in other areas of molecular pathology.

This guideline covers such topics as:

- ethics in diagnostic molecular pathology;
- laboratory methods and reporting;

- quality systems, including internal and external QC and QA;
- staffing skills and training;
- laboratory facilities and layout.

More recently, in 2002, NPAAC formed a new sub-committee to develop specific guidelines for the validation of in-house tests. This is particularly relevant for PCR testing, where a significant proportion of assays being used for both diagnostic and therapeutic monitoring may be assays developed in-house rather than commercial test kits. The Therapeutic Goods Administration (TGA) is also represented on this committee.

The TGA established an expert advisory group, the National Coordinating Committee for Therapeutic Goods Working Group on In Vitro Diagnostic Devices (IVDs), during 2002. The purpose of this group is to develop a framework regulating IVDs, and also one that addresses:

- the inequities between the level of scrutiny of in-house tests and commercial tests;
- the rapid development of increasingly complex genetic tests;
- the increasing availability of home-use IVDs.

As with any other type of human pathology laboratory, molecular laboratories must be NATA/RCPA accredited, and must perform to a satisfactory level in appropriate external QA programs. In addition, the BDO/Corrs report into Pathology Accreditation has recently been completed and presented to the Minister. The recommendations in this report aim to strengthen accreditation requirements and conformity to NPAAC standards (see Appendix C). Of the 37 recommendations, 24 have been implemented, seven are outstanding and no further action is to be taken with six of them.

Issues in evaluation of PCR

Intended purpose

In October 1997 the Australian Health Technology Advisory Committee (AHTAC) completed a review of nucleic acid amplification technology. This report included the use of PCR in haematological malignancy. Particular indications considered included: chronic myeloid leukaemia (CML), acute promyelocytic leukaemia (APL), immunoglobulin and T-cell receptor gene rearrangements in lymphoid malignancies, translocations in lymphoid leukaemia and lymphoma, and other non-random cytogenetic abnormalities of AML. AHTAC recommended use of the PCR in all indications apart from other non-random cytogenetic abnormalities of AML. However, the recommendations made by AHTAC have not been implemented and MSAC was formally approached by the Pathology Services Table Committee (PSTC) to review the use of NAA in haematological malignancy.

Specific indications to be considered included the diagnosis and monitoring of:

1. PML-RAR α and PLZF-RAR α in APL;
2. BCR-ABL in CML;
3. BCR-ABL in ALL (acute lymphocytic leukaemia);
4. AML1-ETO and CBF β -MYH11 in AML;
5. Monoclonal immunoglobulin gene rearrangements and monoclonal T-cell receptor gene rearrangements in lymphoid malignancies of B-cell and T-cell lineage respectively;
6. T-cell receptor gene rearrangements in pre-B ALL;
7. BCL-2 in B cell lymphoid neoplasms;
8. BCL-1 in mantle cell lymphoma.

Research questions

The research questions for APL were:

- Does PML-RAR α and PLZF-RAR α detection increase the proportion of patients who are recognised to have a specific disease entity that defines a specific therapeutic strategy?
- Does repeated qualitative and/or quantitative PCR testing in APL patients in remission predict haematological relapse and/or influence therapeutic decision making?

Principles of diagnostic test evaluation

There are three components in assessing the usefulness of diagnostic tests (Jaeschke et al., 1994b). These are:

1. diagnostic test performance;
2. therapeutic impact;
3. patient health outcomes.

Diagnostic test performance

The validity (sensitivity, specificity and positive and negative likelihood ratios) and reliability of the test indicate diagnostic test performance.

Therapeutic impact

A test has therapeutic impact if the treatment decision is changed, ie new therapy is added or the need for therapy is averted, as a result of the information provided by the test.

Patient health outcomes

The ultimate goal of diagnostic testing is to contribute to improvement in the health of patients. If a diagnostic test is to be beneficial, the diagnostic test performance needs to be satisfactory, the diagnostic test results should have an impact on therapy, and the therapy should be effective.

Clinical need/burden of disease

APL is a rare disease in Australia. Data from the Australian Institute of Health and Welfare indicates that in Australia the crude incidence of APL in 1999 was 0.2 per 100,000 population. There were 18 new cases of APL in Australian men (0.2 per 100,000) and 24 new cases in Australian women (0.3 per 100,000).

Existing procedures

Cytogenetic testing is currently listed on the Medicare Benefits Schedule (MBS) and is used in the diagnosis and monitoring of haematological malignancies.

Comparator

Cytogenetic testing was selected as the appropriate comparator for this review on the basis of it being the technique used most frequently in current practice.

Reference standard

Clinical diagnosis of APL (usually by morphology) in combination with cytogenetic status and response to ATRA was used as the reference standard in the diagnostic part of the review. Specifically, a clinical diagnosis of APL and response to ATRA was used as the reference standard for the estimation of diagnostic accuracy of PCR for PML-RAR α . For PLZF-RAR α , the reference standard was a combination of clinical diagnosis and either the presence of t(11;17) or the absence of t(15;17) on cytogenetic testing.

Cytogenetic and haematological relapse was used as the reference standard for the monitoring part of the review. Thus, the ability of PCR to predict cytogenetic and haematological relapse was assessed.

Marketing status of the technology

There are commercial PCR test kits available which are exempt from Parts 3 and 4 of the Therapeutic Goods Act, 1989. However, most Australian laboratories performing the tests are currently using tests that have been developed in-house.

Current reimbursement arrangement

PCR testing is available on the MBS schedule for a range of indications. The reimbursement price is variable for specific indications. Examples include:

- *Neisseria gonorrhoeae* (MBS item 69370) – reimbursement \$32.80;
- quantitation of HIV viral RNA (MBS items 69378, 69381 and 69382) – reimbursement \$176;
- hepatitis C virus testing (MBS items 69442-69445) – reimbursement price ranges from \$90 to \$200 for these indications.

However, the techniques used for PCR testing in haematological malignancy are more labour intensive than those listed above.

The comparator used in this review (cytogenetic testing) is listed on the MBS schedule (MBS item 73287) and its current reimbursement value is \$354 per test.

Approach to assessment

Review of literature

The medical literature was searched by an Information Specialist to identify studies and systematic reviews examining the accuracy of PCR for diagnosis and monitoring of PML-RAR α and PLZF-RAR α APL. Searches were updated in November 2002. Searches were conducted using the following sources:

- Medline
- Embase
- Current Contents
- Cancerlit
- Cochrane Library (Systematic Reviews & Controlled Trials Register)
- NHS Centre for Reviews and Dissemination databases (DARE, HTA, NHS EED)
- Website sources as detailed in Appendix D.

Search strategy

The search strategy used to identify relevant papers is outlined in Appendix E.

The evidence presented in the selected studies was assessed and classified using the dimensions of evidence defined by the National Health and Medical Research Council (NHMRC, 2000).

These dimensions (Table 1) consider important aspects of the evidence supporting a particular intervention and include three main domains: strength of the evidence, size of the effect and relevance of the evidence. The first domain is derived directly from the literature identified as informing a particular intervention. The last two require expert clinical input as part of its determination.

Table 1 Evidence dimensions

Type of evidence	Definition
Strength of the evidence	The study design used, as an indicator of the degree to which bias has been eliminated by design.* The methods used by investigators to minimise bias within a study design. The <i>p</i> -value or, alternatively, the precision of the estimate of the effect. It reflects the degree of certainty about the existence of a true effect.
Level	
Quality	
Statistical precision	The <i>p</i> -value or, alternatively, the precision of the estimate of the effect. It reflects the degree of certainty about the existence of a true effect.
Size of effect	The distance of the study estimate from the "null" value and the inclusion of only clinically important effects in the confidence interval.
Relevance of evidence	The usefulness of the evidence in clinical practice, particularly the appropriateness of the outcome measures used.

*See Table 2

The three sub-domains (level, quality and statistical precision) are collectively a measure of the strength of the evidence. The designations of the levels of evidence are shown in Table 2.

Table 2 Designations of levels of evidence*

Level of evidence	Study design
I	Evidence obtained from a systematic review of all relevant randomised controlled trials
II	Evidence obtained from at least one properly-designed randomised controlled trial
III-1	Evidence obtained from well-designed pseudorandomised controlled trials (alternate allocation or some other method)
III-2	Evidence obtained from comparative studies, including systematic reviews of such studies, with concurrent controls and allocation not randomised, cohort studies, case-control studies, or interrupted time series with a control group
III-3	Evidence obtained from comparative studies with historical control, two or more single arm studies, or interrupted time series without a parallel control group
IV	Evidence obtained from case series, either post-test or pre-test/post-test

*Modified from (NHMRC, 1999).

Eligibility criteria

Inclusion criteria

Studies were included for critical appraisal if:

- they were relevant to a review question;
- PCR testing was performed;
- the sample size was more than 20 human subjects;
- the study was published in English;
- a suitable reference test was used.

Exclusion criteria

Studies were excluded if:

- they were published in letter or abstract form only;
- the paper was a comment, editorial or non-systematic review;
- the paper focused on monitoring after the original diagnosis but PCR testing was not used to predict subsequent relapse. Specifically, monitoring studies that identified people who were PCR positive but cytogenetic negative, and did not follow such patients after initial PCR positivity for at least three months to identify subsequent cytogenetic or haematological relapse, were excluded from the review.

Assessment of validity

The diagnostic test performance includes consideration of validity and reliability of the test (PCR). Specifically, sensitivity, specificity, and positive and negative likelihood ratios were calculated when possible to assess the validity of PCR testing. These measures are calculated based on presentation of results as shown in Table 3.

Table 3 Assessment of validity of a diagnostic test

		Reference test	
		Positive	Negative
Diagnostic test	Positive	a	b
	Negative	c	d
Total sample size		n_1	n_2

Based on Table 3, measures of validity (and 95% confidence intervals) were calculated using the following formulae:

$$\text{Sensitivity} = a/(a+c)$$

$$= a/n_1$$

$$\text{Confidence interval for sensitivity: } p \pm 1.96(pq/n_1)^{1/2}$$

$$\text{Where } p = a/(a+c)$$

$$q = c/(a+c)$$

$$\text{Specificity} = d/(b+d)$$

$$= d/n_2$$

$$\text{Confidence interval for specificity: } p \pm 1.96(pq/n_2)^{1/2}$$

$$\text{Where } p = d/(b+d)$$

$$q = b/(b+d)$$

If either $n \cdot p$ or $n(1-p)$ were less than five for sensitivity or specificity, confidence intervals based on the normal approximation to the binomial distribution using the formulae above were considered unreliable, and exact methods based on the binomial distribution were used to calculate the confidence interval. Stata version 7.0 was used for these calculations.

'Sensitivity' can also be used as a reference to the limit of detection of the assay. For example, in PCR testing the number of nucleic acid sequences as a ratio of total sequences required to detect the target of interest. For clarity, the term 'limit of detection' will be used to describe this concept in this report.

A **positive likelihood ratio** (LR+) can be calculated by:

$$LR+ = \text{sensitivity} / (1 - \text{specificity})$$

The corresponding 95% confidence interval is calculated from (Simel et al., 1991):

$$\text{Exp}\{\ln[\text{sensitivity}/(1 - \text{specificity}) \pm 1.96[(\text{sensitivity}/c) + (\text{specificity}/b)]^{1/2}]\}$$

A **negative likelihood ratio** (LR-) can be calculated by:

$$LR- = (1 - \text{sensitivity})/\text{specificity}$$

The corresponding 95% confidence interval is calculated from (Simel et al., 1991):

$$\text{Exp}\{\ln[(1 - \text{sensitivity})/(\text{specificity})] \pm 1.96[(\text{sensitivity}/c) + (1 - \text{specificity})/d]^{1/2}\}$$

Normally these measures of validity of the test are assessed against the reference standard at approximately the same time as the diagnostic test. This was the approach used for the diagnostic phase of the review. However, for monitoring, the reference standard was subsequent relapse. Therefore, the ability of PCR to predict cytogenetic and haematological relapse was assessed in the monitoring phase of this review and these forms of relapse may have occurred considerable periods of time after PCR testing.

Inter-observer and **intra-observer reliability** were also presented when these measures were estimated in the original studies.

Diagnostic odds ratios (DOR) were estimated using Stata version 7 software. The DOR is estimated from:

$$DOR = ad/bc$$

Where a, b, c, and d are as listed in Table 3.

When pooling of diagnostic odds ratios (DOR) was conducted, the method of Peto was used (Yusuf et al., 1985) with Stata version 7 software.

Assessment of quality

Assessment of quality was based on internationally accepted criteria (Jaeschke et al., 1994a, Irwig et al., 1994, Irwig et al., 1996) and were recommended by MSAC in their

application and assessment guidelines (Medicare Services Advisory Committee, 2000). These were:

- there was an independent, blind comparison with a reference gold standard;
- the results of the test and reference standard were assessed independently of each other;
- the sample of patients in the study included an appropriate spectrum of patients, similar to those in whom the test is likely to be used in Australia;
- the results of the test being evaluated did not influence the decision to perform the standard reference test (verification bias); and
- there was sufficient detail in the study report to permit replication of the test.

Expert advice

A supporting committee with expertise in PCR testing in haematological malignancy was established to evaluate the evidence and provide advice to MSAC from a clinical perspective. In selecting members for supporting committees, MSAC's practice is to approach the appropriate medical colleges, specialist societies and associations and consumer bodies for nominees. Membership of the supporting committee is provided at Appendix B.

Results of assessment

Is it safe?

The PCR assays discussed in this review are unlikely to directly increase risk to patients. The required quantity of blood or marrow is minimal and would usually be collected concurrently with other routine blood or marrow tests.

Is it effective?

Clinical problem

APL is a morphologically distinct sub-type of AML with unique clinical features including potentially life-threatening coagulopathy and a sensitivity to retinoic acid and arsenic trioxide therapy. It is associated with chromosomal translocations that disrupt the gene encoding for the retinoic acid receptor alpha (RAR α) on chromosome 17. In approximately 99 per cent of cases, this disruption results in the fusion of RAR α with the PML gene of chromosome 15 (t(15;17)). The second most common variant of APL results from the fusion of PLZF (chromosome 11) with the RAR α gene (t(11;17)). This occurs in approximately 0.5 per cent of APL cases. Both rearrangements result in an oncogene that causes maturation arrest at the promyelocytic stage of myeloid development and a characteristic proliferation of undifferentiated promyelocytes and blasts in the bone marrow.

APL with the PML- RAR α fusion and PLZF- RAR α fusion are clinically similar but differ in their sensitivity to all-*trans* retinoic acid (ATRA). Cells expressing PLZF- RAR α fail to differentiate in response to ATRA. In contrast, PML- RAR α is sensitive to ATRA. Therefore, correct disease characterisation is vital given the difference in responsiveness to ATRA in APL subgroups.

Treatment

The treatment of APL is evolving. Treatment advances in this disease have coincided with an explosion of knowledge of the molecular pathology of APL.

During the late 1980s and early 1990s some studies reported high remission rates using ATRA alone for induction and first relapse therapy (Huang et al., 1988, Castaigne et al., 1990, Degos et al., 1990, Chen et al., 1991, Warrell et al., 1991). However, responses were short lived and newly diagnosed APL patients were subsequently treated with ATRA followed by intensive chemotherapy (Fenaux et al., 1992, Warrell Jr et al., 1994, Kanamaru et al., 1995, Fenaux et al., 1993, Fenaux et al., 2000, Tallman et al., 1997). Four year survival was 76 per cent after ATRA plus subsequent chemotherapy, compared with 49 per cent in the chemotherapy alone arm in the APL91 trial (Fenaux et al., 2000). In Tallman et al (1997), the median survival in the chemotherapy alone arm was three years.

More recently, trials have been conducted examining concurrent ATRA and chemotherapy (Fenaux et al., 1999). The APL93 trial estimated the two-year event-free survival in patients receiving concurrent ATRA and chemotherapy to be 84 per cent compared with 77 per cent in the ATRA followed by chemotherapy group (Fenaux et al., 1999). Concurrent therapy is currently considered optimal for patients with de novo APL.

Consolidation therapy is used in APL but the optimal treatment is not clear. The all-*trans* retinoic acid and idarubicin (AIDA) protocol involving the induction of ATRA and chemotherapy concurrently, followed by three consolidation courses, was associated with an actuarial event-free survival of 79 per cent at two years (Mandelli et al., 1997). Data presented at the International Conference on APL and Differentiation Therapy in Rome, 2001, estimated 77 per cent seven-year survival in this study (Iland, personal communication, 2002).

Maintenance therapy with chemotherapy and/or ATRA has been associated with improved outcome (Tallman et al., 1997, Fenaux et al., 1999). In the latest study, two-year survival following complete remission (CR) was 94 per cent after maintenance chemotherapy comprising post-remission chemotherapy and intermittent ATRA, of two years duration (Fenaux et al., 1999).

Patients in first complete remission (CR1) who subsequently relapse can be salvaged with ATRA and/or arsenic trioxide therapy. However, the long term outcome of patients initially treated with ATRA who relapse and achieve a second complete remission (CR2) after retreatment with ATRA, with or without chemotherapy, is considered to be poor (Douer, 2000). There are concerns relating to ATRA resistance in this group. Two possible interventions include liposomal ATRA for reversible ATRA resistance (Tobita et al., 1997, Takeuchi et al., 1998, Takeuchi et al., 1997) and arsenic trioxide for irreversible ATRA resistance (Soignet et al., 2001). Once in CR2, autologous or allogeneic transplantation should also be considered. Allogeneic transplantation is also considered at time of relapse from CR1.

The estimated disease-free survival in people treated with arsenic trioxide after clinical relapse has been estimated at 66 per cent after 18 months (Soignet et al., 2001). Overall survival has been estimated at 50 per cent after two years (Niu et al., 1999), 53 per cent after five years (Hu et al., 1999) and approximately 50 per cent after five years (Fenaux et al., 2000).

The one-year survival for relapsed patients who were considered potentially sensitive to ATRA, that is, had not received ATRA for more than one year or no previous ATRA therapy, was 62.5 per cent. In patients considered as likely to be resistant to ATRA, who had received ATRA within one year, the one-year survival rate was 23 per cent after treatment with liposomal ATRA (Douer et al., 2001).

Overall, survival after transplantation in CR2 has been estimated in a range of studies. Meloni et al (1997) estimated 40 per cent survival after median follow up of 28 months in a cohort of autologous transplants. Soignet et al (2001) estimated 66 per cent survival at 18 months in a cohort where eight patients received an allogeneic transplant and three received an autologous transplant. Data from the International Bone Marrow Transplant Register was presented in a review article where the six-year survival was estimated as 48 per cent after autologous transplantation and 58 per cent after allogeneic transplantation (Nabhan et al., 2001). Ferrant et al (1997) also reported 83 per cent three-year survival

after autologous transplantation and 53 per cent three-year survival after allogeneic transplantation in CR1.

In conclusion, APL is potentially the most curable form of AML, with complete remission rates of 90 per cent and two-year relapse rates under 10 per cent. Up to 75 per cent of APL patients can be cured with current therapy. Reducing the risk of relapse and attainment of molecular remission are now the major therapeutic goals in APL (Tallman, 1998, Lo Coco et al., 1999, Degos and Wang, 2001, Fenaux et al., 2001).

Potential value of PCR

PCR testing has the ability to detect abnormal cells in large numbers of normal cells, typically one leukemic cell among up to 10^4 to 10^5 normal cells. This is in contrast to cytogenetic testing with a limit of detection of one leukemic cell in approximately 25-30 normal cells. Cytogenetic detection requires dividing cells for analysis of metaphase chromosomes, and thus is best performed on bone marrow samples since they have more proliferating cells than peripheral blood. Typically, about 25 to 30 metaphase cells are examined in a cytogenetic analysis. Because of the low number of cells examined the limit of detection is high for cytogenetic testing compared with PCR. Therefore, PCR testing has the potential to identify genetic abnormalities associated with APL with greater accuracy than cytogenetic testing.

It is important to identify patients with APL because of their unique sensitivity to ATRA. Routinely, blood and bone marrow morphology, cytochemistry and flow cytometry are used to diagnose APL. Cytogenetic testing identifies the presence of the t(15;17), confirming the diagnosis and ATRA responsiveness. Cytogenetic testing is often sufficient to establish the diagnosis in cases of suspected APL but RT-PCR can identify cases not detected by standard cytogenetics. It is also important to reliably identify APL cases lacking PML-RAR α and accurately characterize their underlying molecular lesions. PLZF-RAR α APL, for example, is resistant to ATRA and arsenic trioxide therapy (Zelent et al., 2001).

Potentially, PCR can be used to monitor patients with APL but it is important to know that a PCR detectable translocation is present at diagnosis if monitoring with RT-PCR is to be performed. Accurate characterisation, through identification of the breakpoint in the PML gene at diagnosis, simplifies the process of subsequent MRD detection through the use of breakpoint specific primer systems (Lo Coco et al., 1998).

Ideally, there should be evidence that early therapy is more effective than late therapy after relapse if monitoring with PCR is to have an impact on survival. Improved outcome associated with early treatment is biologically plausible since treatment is provided when the disease burden is reduced. Monitoring patients with APL by RT-PCR was associated with improved survival compared with haematological monitoring in one study (Lo Coco et al., 1999). In this study, the two-year survival after salvage therapy for molecular relapse was 92 per cent (95% CI 61-98) compared with 44 per cent (95% CI 35-52) in a previous series following haematological relapse. In recognising molecular relapse may be detected on average three months earlier than cytogenetic relapse, lead-time bias should be considered as an explanation for the difference in survival between these groups. However, given the magnitude of the difference in outcome, lead-time bias is unlikely to fully explain the difference in these survival estimates.

One study has also documented the role of PCR testing in estimating prognosis before autologous transplantation after second complete haematological remission (Meloni et al., 1997). In this series of 15 consecutive cases, prognosis was determined in seven patients who were PCR positive and eight patients who were PCR negative before transplantation. The PCR positive group had a poorer prognosis than those who were PCR negative, with seven of seven relapsing within 14 months compared with one of eight in the PCR negative group, $P < 0.001$. Therefore, this study indicated the need to tailor the management of patients in second complete haematologic remission based on the results of PCR testing.

PCR testing is viewed as supplementary to cytogenetic testing. Cytogenetic testing identifies not only the t(15;17) abnormality that is fundamental to the pathogenesis of APL, but also identifies additional cytogenetic abnormalities in approximately 30 per cent of APL patients at initial diagnosis (Lo Coco et al., 1998). The significance of these additional cytogenetic abnormalities is currently under investigation, with data both for and against their role in determining prognosis. With regard to monitoring, cytogenetic analysis is still required to identify the emergence of additional cytogenetic abnormalities associated with clonal evolution.

Results

Papers selected

Twenty-four articles were identified that met the eligibility criteria for the review and these articles were critically appraised. Details of the selection process are shown in Figure 2.

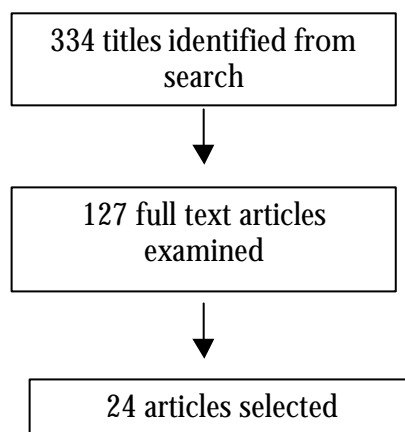


Figure 2 Study selection process for APL papers

Reasons for exclusion of studies that were examined in full text are stipulated in Table 4.

Table 4 Reasons for exclusion of APL papers examined in full text

Reason for exclusion	Number
Non-systematic review	39
Not relevant to research question	29
Sample size not stated or ≤ 20	15
Comparator inappropriate or absent	12
Published as a letter/abstract	6
Unable to retrieve	1
Interim study report of appraised paper	1

The selected studies consisted of:

- thirteen that assessed the validity of RT-PCR for the diagnosis of APL, including five with patients being monitored;
- sixteen that examined the validity of RT-PCR in monitoring, including five with patients examined at presentation.

Details about the selected studies are included in Appendix F.

Methodological issues

All studies selected were case series (level IV evidence). Only two of the 24 studies (8 per cent) documented blinding between PCR testing and the outcome of interest, usually relapse or diagnosis of APL. In general, relapse was poorly defined in most of the monitoring studies. The method of patient selection and clarification between prospective and retrospective study designs was inadequately described in some studies. Ten of the 13 studies (77 per cent) included in diagnosis and 11 of the 16 studies (69 per cent) included in monitoring had a sample size under 50. There was often limited demographic data on the patients included in the eligible studies, increasing the difficulty of interpreting these studies in the context of the Australian setting.

However, there was documentation that RT-PCR was conducted independently from the reference standard in 11 diagnostic studies (85 per cent) and seven monitoring studies (44 per cent). Documentation of avoidance of verification bias was provided in 12 of the diagnostic studies (92 per cent) and six of the monitoring studies (38 per cent).

In the monitoring review the PCR was assessed for its ability to predict cytogenetic and haematological relapse. PCR testing is characterised by an ability to detect one abnormal cell in numbers of normal cells that are orders of magnitude higher than for cytogenetic testing. Therefore, PCR is able to predict cytogenetic relapse before such relapse is detected through cytogenetic testing. Given the expected early prediction of relapse through PCR monitoring, the length of follow up after detection of PCR positivity is of considerable importance. In some cases, this length of follow up may not be sufficiently long to detect subsequent relapse, in which case the estimated specificity would be underestimated.

Diagnostic performance

Diagnosis

Studies included

There were 13 studies identified that included patients at diagnosis in the sample population. Five of the 13 studies also included patients who were being monitored.

In 12 studies, the study sample included patients who were t(15;17) positive by cytogenetic testing. These studies included 421 patients tested by PCR. Three studies included patients who were t(11;17) positive by cytogenetic testing. There were 13 patients tested by PCR in this group with t(11;17).

Eight studies were identified that included patients with evidence of being negative for t(15;17) and t(11;17). These patients were in two groups: either negative by cytogenetic testing or non-responsive to ATRA. The latter was a proxy for being t(15;17) negative in patients evaluated for PML-RAR α by PCR. There were 36 patients tested by PCR in this “negative cytogenetic testing group”.

There were six studies (29 patients) that included samples where cytogenetic testing failed but PCR testing was conducted.

Eleven studies could be evaluated for the sensitivity of cytogenetic testing alone and in combination with PCR testing. The former included 452 patients and the latter 370 patients.

The specificity of PCR was assessable in five studies: three based on non-response to ATRA in patients tested for PML-RAR α and two based on patients without APL. There were 23 patients in this category.

Reference standard

Clinical diagnosis of APL, usually by morphology, in combination with cytogenetic status and response to ATRA was used as the reference standard in the diagnostic part of the review. Specifically, a clinical diagnosis of APL and response to ATRA was used as the reference standard for the estimation of diagnostic accuracy of PCR for PML-RAR α . For PLZF-RAR α , the reference standard was a combination of clinical diagnosis and either the presence of t(11;17) or the absence of t(15;17) on cytogenetic testing.

Overall results

APL is associated with two genetic abnormalities that are routinely detected by PCR testing: PML-RAR α and PLZF-RAR α . Different primers are required for the detection of these abnormalities and the studies identified have tended to focus on PML-RAR α detection. Therefore, although PLZF-RAR α is uncommon, evaluation of PCR against the diagnosis of APL would be expected to miss some cases due to the non-evaluation of PLZF-RAR α . Cytogenetic testing could detect both translocations associated with these abnormalities so a study comparing PCR for PML-RAR α with cytogenetic testing slightly favours the latter, due to the specificity of PCR for PML-RAR α . In the clinical setting, when both PML-RAR α and PLZF-RAR α were evaluated, this limitation would be avoided. PLZF-RAR α transcript was identified subsequently to PML-RAR α so some of the earlier studies that evaluated response to ATRA may actually be evaluating two groups: ATRA responders may represent patients with PML-RAR α and non-responders

patients with PLZF-RAR α . Therefore, specificity could be assessed in these studies (Chen et al., 1992, Miller et al., 1992, Xiao et al., 1993).

Despite the above limitation, sensitivity of PCR was usually higher than cytogenetic testing in the studies identified. The median sensitivity for PCR was 100 per cent (range 89-100 per cent) and for cytogenetic testing was 91 per cent (range 75-100 per cent) based on APL diagnosis as the reference standard (usually by morphology). If response to ATRA was used as the surrogate for PML-RAR α (APL diagnosis by standard clinical criteria used otherwise) the range of sensitivity estimates was 92 to 100 per cent for PCR testing.

However, the quality of the diagnostic studies was variable. Two studies (15 per cent) documented blinding between PCR results and the reference standard (Grimwade et al., 2000, Gu et al., 2001). One of these was a workshop report where APL cases were restricted to those lacking the t(15;17) translocation (Grimwade et al., 2000). APL patients lacking the t(15;17) translocation represent less than 10 per cent of all APL cases. Avoidance of verification bias was documented in 12 studies (92 per cent). The spectrum of patients was adequate in eight studies (62 per cent) and independence between PCR testing and the reference standard was documented in seven studies (54 per cent).

The limitations outlined are likely to result in the overestimation of sensitivity for RT-PCR testing. However, the sensitivity of combined cytogenetic and PCR testing was estimated at 99 per cent (95% CI 97, 99) compared with 92 per cent (95% CI 89, 95) in the cytogenetic testing only group.

Table 5 Validity of RT-PCR and cytogenetic testing in the diagnosis of APL

Reference	Sample characteristics	QS ^a	Results ^b	Comments
Biondi et al., (1992)	Case series N=35 Diagnosis and monitoring Morphological evidence of APL (diagnosed on FAB ^c criteria)	BC:U I:A S:U V:A M:A	Sensitivity (PCR versus APL diagnosis): 100% (95% CI 90, 100) Sensitivity (cytogenetic testing versus APL diagnosis): 93% (95% CI 76, 99)	<ul style="list-style-type: none"> Sample selection methods unclear Nested RT-PCR on bone marrow and peripheral blood
Burnett et al., (1999)	Prospective patients N=239 Diagnosis Morphological evidence of APL	BC:U I:I S:A V:A M:I	Sensitivity (PCR versus APL diagnosis): 92% (95% CI 88, 96) Sensitivity (cytogenetic testing versus APL diagnosis): 94% (95% CI 90, 97)	<ul style="list-style-type: none"> Primarily a study evaluating treatment Cytogenetics performed in all patients but PCR only conducted in 85% No control group to allow full assessment of validity
Chen et al., (1992)	Case series N=32 Diagnosis APL by FAB ^c criteria	BC:U I:A S:A V:A M:A	Sensitivity (PCR versus APL diagnosis): 97% (95% CI 84, 100) Sensitivity (PCR versus response to ATRA): 100% (95% CI 86, 100) Sensitivity (cytogenetic testing versus APL diagnosis): 100% (95% CI 87, 100) – 5 not evaluable Specificity (PCR) based on response to ATRA and on non t(15;17): 100% (95% CI 2, 100)	<ul style="list-style-type: none"> Sample selection methods unclear Nested RT-PCR on bone marrow and peripheral blood
Grimwade et al., (2000)	Case series N=90 (11 PLZF-RAR α) Diagnosis Cases referred to workshop lacking t(15;17)	BC:A I:A S:U V:A M:A	Sensitivity (PCR versus APL diagnosis): 100% (95% CI 72, 100) Sensitivity (cytogenetic testing versus APL diagnosis): 82% (95% CI 48, 98) Specificity (PCR) based on non APL cases (by morphology): 100% (95% CI 40, 100)	<ul style="list-style-type: none"> Sensitivity data presented is restricted to patients with PLZF-RARα Highly selected patients – non-consecutive and criteria for choice of patients to present at the workshop not stipulated Groups investigated represent <10% of APL patients Cases provided from 42 institutions and 6 countries
Gu et al., (2001)	Case series N=31(APL) + 11 controls Diagnosis and monitoring APL by FAB ^c criteria	BC:A I:A S:A V:A M:A	Sensitivity (PCR versus APL diagnosis): 100% (95% CI 86, 100) Specificity (PCR) based on non APL cases (by morphology): 100% (95% CI 72, 100)	<ul style="list-style-type: none"> All APL patients t(15;17) positive Selection methods unclear Real time RT-PCR on bone marrow and peripheral blood

Table 5 Validity of RT-PCR and cytogenetic testing in the diagnosis of APL (continued)

Reference	Sample characteristics	QS ^a	Results	Comments
Huang et al., (1993)	Case series N=97 (53 with results at diagnosis) Diagnosis and monitoring APL by FAB ^c criteria	BC:U I:A S:A V:A M:A	Sensitivity (PCR versus APL diagnosis): 100% (95% CI 93, 100)	<ul style="list-style-type: none"> • Selection methods unclear • Unable to estimate specificity • Nested RT-PCR on bone marrow
Kane et al., (1996)	Retrospective, non-consecutive patients N=22 Diagnosis only APL only	BC:U I:A S:A V:A M:A	Sensitivity (PCR versus APL diagnosis): 100% (95% CI 85, 100) Sensitivity (cytogenetic testing versus APL diagnosis): 91% (95% CI 71, 99)	<ul style="list-style-type: none"> • No control group to allow full assessment of validity • Retrospective and non-consecutive patients produces potential selection bias • Study restricted to paediatric population
Lim et al., (2000)	Consecutive patients N=30 Diagnosis APL by FAB ^c criteria	BC:U I:A S:A V:A M:I	Sensitivity (PCR versus APL diagnosis): 100% (95% CI 88, 100) Sensitivity (cytogenetic testing versus APL diagnosis): 96% (95% CI 82, 100)	<ul style="list-style-type: none"> • Unclear whether study was prospective • No control group to allow full assessment of validity
Lo Coco et al., (1992)	Non consecutive, retrospective case series N=35 Diagnosis and monitoring Newly diagnosed or relapsed APL	BC:U I:A S:U V:U M:I	Sensitivity (PCR versus APL diagnosis): 100% (95% CI 88, 100)	<ul style="list-style-type: none"> • Inadequate selection methods • Nested RT-PCR on bone marrow
Mancini et al., (1995)	Case series N=28 Diagnosis APL diagnosed on morphological and cytochemical grounds	BC:U I:U S:A V:A M:A	Sensitivity (PCR versus APL diagnosis): 100% (95% CI 87, 100) Sensitivity (cytogenetic testing versus APL diagnosis): 90% (95% CI 68, 99) – 6 not evaluable, 4 positives had additional cytogenetic abnormalities	<ul style="list-style-type: none"> • Selection criteria unclear • RT-PCR on bone marrow
Miller et al., (1992)	Prospective case series N=36 Diagnosis and monitoring APL based on M3 morphology. Enrolled in retinoic acid trial	BC:U I:A S:U V:A M:I	Sensitivity (PCR versus APL diagnosis): 89% (95% CI 74, 97) Sensitivity (PCR versus response to ATRA): 100% (95% CI 88, 100) Sensitivity (cytogenetic testing versus APL diagnosis): 75% (95% CI 57, 89) – 4 not evaluable Specificity (PCR) based on response to ATRA: 100% (95% CI 40, 100)	<ul style="list-style-type: none"> • Sample selection methods unclear • Nested RT-PCR on bone marrow

Table 5 Validity of RT-PCR and cytogenetic testing in the diagnosis of APL (continued)

Reference	Sample characteristics	QS ^a	Results	Comments
Shivakumar et al., (2002)	Case series N=29 Diagnosis APL by morphology	BC:U I:A S:A V:A M:A	Sensitivity (PCR versus APL diagnosis): 100% (95% CI 85, 100) Sensitivity (cytogenetic testing versus APL diagnosis): 88% (95% CI 69, 97) – 4 not evaluable, 5 positives had additional cytogenetic abnormalities	<ul style="list-style-type: none"> • Selection methods unclear • Nested RT-PCR on bone marrow and peripheral blood
Xiao et al., (1993)	Case series N=26 samples (25 patients) Diagnosis APL by FAB ^c criteria	BC:U I:A S:U V:A M:I	Sensitivity (PCR versus APL diagnosis): 92% (95% CI 75, 99) Sensitivity (PCR versus response to ATRA): 100% (95% CI 86, 100) Sensitivity (cytogenetic testing versus APL diagnosis): 77% (95% CI 56, 91) Specificity (PCR) based on response to ATRA: 100% (95% CI 16, 100)	<ul style="list-style-type: none"> • Primarily investigating pulsed field gel electrophoresis • Sample selection methods unclear • RT-PCR on bone marrow and peripheral blood

^aQS-quality score, BC-blind comparison, I-independent assessment of diagnostic and reference test, S-spectrum of illness, V-verification bias, M-description of diagnostic testing methodology. A-adequate, U-unclear, I-inadequate.

^b Clinical diagnosis of APL (usually by morphology) in combination with cytogenetic status and response to ATRA was used as the reference standard

^c FAB-French-American-British

Sensitivity of PCR for PML-RAR α at diagnosis in patients with evidence of t(15;17) APL

Twelve studies provided sufficient data to estimate the proportion that was PCR positive in the presence of t(15;17) by cytogenetic testing at diagnosis (see Table 6).

Overall, 421 of 421 (100 per cent, 95% CI 99, 100) tested PCR positive in this group.

These data were limited by:

- cytogenetic testing and PCR testing were not necessarily performed simultaneously;
- potential biases in the individual studies (outlined in Table 5);
- potential publication bias with studies that had a low proportion testing PCR positive being less likely to be published.

Nevertheless, the proportion testing PCR positive was 100 per cent in this group of patients despite the studies being conducted in different regions, with different methods and at different times.

Table 6 Proportion testing PCR positive in patients who were t(15;17) positive

Reference	Results		
	Number tested	Proportion PCR positive (%)	95% confidence interval
Biondi et al., (1992)	26	100	87, 100
Burnett et al., (1999)	158	100	98, 100
Chen et al., (1992)	26	100	87, 100
Gu et al., (2001)	25	100	86, 100
Huang et al., (1993)	52	100	93, 100
Lo Coco et al., (1992)	28	100	88, 100
Mancini et al., (1995)	18	100	81, 100
Miller et al., (1992)	24	100	86, 100
Xiao et al., (1993)	18	100	81, 100
Kane et al., (1996)	20	100	83, 100
Lim et al., (2000)	26	100	87, 100
Shivakumar et al., (2002)	18	100	81, 100
Summary	421	100	99, 100

Sensitivity of PCR for PLZF-RAR α at diagnosis in patients negative for t(15;17) APL by cytogenetic testing

Three studies were identified that included PCR testing for PLZF-RAR α (Grimwade et al., 2000, Burnett et al., 1999, Huang et al., 1993). In the 11 patients tested in the Grimwade study the sensitivity of PCR was 100 per cent (95% CI 72, 100). One patient was identified with t(11;17) in Burnett et al and in Huang et al and those patients were positive by RT-PCR for PLZF-RAR α .

Overall, the sensitivity of PCR in this group was 100 per cent (95% CI 75, 100).

These data were limited by:

- small number of patients assessed in this category;
- cytogenetic testing and PCR testing were not necessarily performed simultaneously;
- potential biases in the individual studies (outlined in Table 5);
- potential publication bias with studies that had a low proportion testing PCR positive being less likely to be published.

Sensitivity of PCR for PML-RAR α at diagnosis in patients negative for t(15;17) and t(11;17) APL

Seven studies provided sufficient data to estimate the proportion PCR positive in the absence of t(15;17) and t(11;17) by cytogenetic testing at diagnosis (see Table 7). Patients who did not respond to ATRA were excluded from this analysis since they were unlikely to have PML-RAR α APL, and so were not a satisfactory group for assessing the sensitivity of the PCR for PML-RAR α .

Overall, 36 of 36 (100 per cent, 95% CI 90, 100) tested PCR positive in this group.

These data were limited by:

- cytogenetic testing and PCR testing were not necessarily performed simultaneously;
- potential biases in the individual studies (outlined in Table 5);
- potential publication bias with studies that had a low proportion testing PCR positive being less likely to be published.

Nevertheless, all patients in this group tested positive by PCR, indicating the potential benefit of PCR in cytogenetic test negative patients. The effect of the first two limitations is likely to be small but the latter two may result in overestimation of the sensitivity of PCR.

Table 7 Proportion testing PCR positive in patients who were t(15;17) negative (or ATRA non-responsive) and t(11, 17) negative

Reference	Results		
	Number tested	Proportion PCR positive (%)	95% confidence interval
Biondi et al., (1992)	2	100	16, 100
Mancini et al., (1995)	2	100	16, 100
Miller et al., (1992)	4	100	40, 100
Xiao et al., (1993)	6	100	54, 100
Burnett et al., (1999)	18	100	81, 100
Kane et al., (1996)	2	100	16, 100
Lim et al., (2000)	2	100	16, 100
Shivakumar et al., (2002)	3	100	29, 100
Summary	36	100	90, 100

Sensitivity of PCR for PML-RAR α at diagnosis in patients failed cytogenetic testing

Six studies provided sufficient data to estimate the proportion that was PCR positive in the group of patients who failed cytogenetic testing (see Table 8). Thus this group constituted patients in whom cytogenetic testing was uninformative since a positive or negative classification could not be assigned.

Overall, 28 of 29 (97 per cent, 95% CI 82, 100) tested PCR positive in this group.

These data were limited by:

- potential biases in the individual studies (outlined in Table 5);
- potential publication bias with studies that had a low proportion testing PCR positive being less likely to be published.

Nevertheless, all but one patient in this group tested positive by PCR, indicating the potential benefit of PCR in patients with inadequate cytogenetic samples.

Table 8 Proportion testing PCR positive in patients who failed cytogenetic testing

Reference	Results		
	Number tested	Proportion PCR positive (%)	95% confidence interval
Chen et al., (1992)	5	100	48, 100
Mancini et al., (1995)	6	100	54, 100
Miller et al., (1992)	4	100	40, 100
Burnett et al., (1999)	11	91	59, 100
Lim et al., (2000)	2	100	16, 100
Shivakumar et al., (2002)	1	100	2, 100
Summary	29	96	82, 100

Sensitivity of cytogenetic testing

Eleven studies provided sufficient data to estimate the proportion that was cytogenetic test positive in the group of patients diagnosed with APL (see Table 9). Two studies were excluded from this analysis, since the assumption was made in the study publication that all APL patients were t(15;17) positive.

Overall, 417 of 452 (92 per cent, 95% CI 89, 95) tested cytogenetic positive in this group. Three hundred and forty five patients were positive for t(15;17), 11 were positive for t(11;17) and the remainder had other abnormalities. The 11 positive results for t(11;17) should not be interpreted as representative of the APL population since the majority of these cases were based on a study examining patients without t(15;17).

These data were limited by:

- potential biases in the individual studies (outlined in Table 5);
- potential publication bias with studies that had a low proportion testing PCR positive being less likely to be published.

Table 9 Proportion testing cytogenetic positive in patients diagnosed with APL

Reference	Results		
	Number tested	Proportion cytogenetic positive (%)	95% confidence interval
Biondi et al., (1992)	28	93	76, 99
Burnett et al., (1999)	206	94	90, 97
Chen et al., (1992)	26	100	87, 100
Grimwade et al., (2000)	11	82	48, 98
Huang et al., (1993)	53	100	93, 100
Kane et al., (1996)	22	91	71, 99
Lim et al., (2000)	28	96	82, 100
Mancini et al., (1995)	20	90	68, 99
Miller et al., (1992)	32	75	57, 89
Shivakumar et al., (2002)	25	88	69, 97
Xiao et al., (1993)	26	77	56, 91
Summary	452	92	89, 95

Sensitivity of PCR and cytogenetic testing at diagnosis

Eleven studies allowed an estimation of the sensitivity of cytogenetic and PCR testing combined (see Table 10). The studies excluded from analysis of the sensitivity of cytogenetic testing were also excluded from this analysis to allow comparison of cytogenetic and PCR testing with cytogenetic testing alone.

Overall, 417 of 423 were cytogenetic or PCR positive in this group (sensitivity 99 per cent, 95% CI 97, 99). However, the six negative PCR tests were from patients who were non-responsive to ATRA. PCR evaluation was limited to detection of PML-RAR α in these six patients. Since it is likely these six patients had PLZF- RAR α APL then a more accurate estimate of the sensitivity may be 423 of 423 (100 per cent, 95% CI 99, 100).

These data were limited by:

- cytogenetic testing and PCR testing were not necessarily performed simultaneously;
- potential biases in the individual studies (outlined in Table 5);
- potential publication bias with studies that had a low proportion testing PCR or cytogenetic positive being less likely to be published.

Nevertheless, the proportion testing PCR and/or cytogenetic positive was high and consistent across studies that were conducted in different regions, with different methods and at different times. The one group not identified was those not responsive to ATRA (probably patients with PLZF-RAR α) who were only evaluated for PML-RAR α . Therefore, the probability of obtaining a false negative result using a strategy encompassing cytogenetic testing and PCR testing for PML-RAR α and PLZF- RAR α is low.

Table 10 Proportion testing cytogenetic and PCR positive in patients diagnosed with APL

Reference	Results		
	Number tested	Proportion PCR positive (%)	95% confidence interval
Biondi et al., (1992)	28	100	88, 100
Burnett et al., (1999)	177	100	98, 100
Chen et al., (1992)	26	100	87, 100
Grimwade et al., (2000)	11	100	72, 100
Huang et al., (1993)	53	100	93, 100
Kane et al., (1996)	22	100	85, 100
Lim et al., (2000)	28	100	88, 100
Mancini et al., (1995)	20	100	83, 100
Miller et al., (1992)	32	89	71, 96
Shivakumar et al., (2002)	22	100	85, 100
Xiao et al., (1993)	26	92	75, 99
Summary	370	98	97, 99

Specificity of PCR in APL diagnosis

Specificity of PCR for PML-RAR α was assessable in five studies: three based on non-response to ATRA (Chen et al., 1992, Miller et al., 1992, Xiao et al., 1993) and two based on diagnosis other than APL (Grimwade et al., 2000, Gu et al., 2001). Overall, the specificity was estimated as 100 per cent (95% CI 85, 100) in these studies.

These data were limited by:

- small number of patients in which the specificity was estimated;
- potential biases in the individual studies (outlined in Table 5);
- potential publication bias with studies that had a low proportion testing PCR or cytogenetic positive being less likely to be published.

However, the control groups used were appropriate. One non-APL group was based on a group of patients that had initially caused some confusion from morphological analysis. The other non-APL group included control samples selected from other patients with leukaemia. The ATRA non-responsive group is a key group to identify by PCR since this group is treated using different therapeutic agents to the ATRA responsive group.

Summary

Overall, the median sensitivity was estimated to be 100 per cent in the PCR group and 91 per cent in the cytogenetic group, based on the 13 studies included in this section.

Combining patient results from the relevant studies produced the following estimates:

- PCR was 100 per cent (95% CI 99, 100) sensitive in patients t(15;17) positive by cytogenetic testing;

- PCR was 100 per cent (95% CI 75, 100) sensitive in patients t(11;17) positive by cytogenetic testing;
- PCR was 100 per cent (95% CI 90, 100) sensitive in patients t(15;17) negative (or non-responsive to ATRA) and t(11;17) negative by cytogenetic testing;
- PCR was 97 per cent (95% CI 82, 100) sensitive in patients who failed cytogenetic testing;
- Cytogenetic testing was 92 per cent (95% CI 89, 95) sensitive in patients with a morphological diagnosis of APL;
- Combined PCR and cytogenetic testing was 99 per cent (95% CI 97, 99) to 100 per cent (95% CI 99, 100) sensitive at diagnosis (range based on incomplete PCR testing for PLZF-RAR α);
- PCR was 100 per cent (95% CI 85, 100) specific in APL diagnosis and in distinguishing ATRA responsive from ATRA non-responsive patients.

These results need to be considered in conjunction with the limitations relating to the individual studies and potential publication bias.

Nevertheless, the consistency of the results suggests PCR testing produces more valid results overall than cytogenetic testing.

Monitoring

Sixteen studies were identified that included disease relapse as an endpoint. Cytogenetic and haematological relapse was used as the reference standard for the monitoring part of the review. Thus, the ability of PCR to predict cytogenetic and haematological relapse was assessed.

The studies were characterised by lack of blinding, poor definition of relapse and mixed handling of verification bias. However, the same reference test was used irrespective of RT-PCR classification. The absence of blinding is of lesser significance in the prospective than the retrospective studies given relatively objective outcome measures (relapse) in the studies included.

The strongest study was characterised by a prospective design, sampling at defined times and repeat sampling when a positive RT-PCR result was obtained (Diverio et al., 1998). In this study, the 95 per cent confidence intervals for positive and negative likelihood ratios excluded one, indicating statistically significant results allowing improved prediction of subsequent cytogenetic or haematological relapse, in this study.

The specificity of PCR is likely to be underestimated overall. As discussed previously, specificity is reliant on accurate determination of the reference standard (cytogenetic or haematological relapse). It is not possible to be certain that sufficient time has elapsed in these studies to allow an accurate determination of relapse status. Thus, some cases that have been classified as false positive cases by PCR may have been classified as true positives if the follow up had been longer.

Sensitivity and specificity estimates for PCR monitoring have been converted into an odds ratio for each study to allow the estimation of an overall effect in the studies selected. These data are summarised in Figure 3. The overall estimate was based on the fixed effects model using the method of Peto (Yusuf et al., 1985). The chi squared test for heterogeneity was non-significant overall. An odds ratio of one indicates a diagnostic test with no utility. The estimated DOR from pooling of the selected studies was 103 (95% CI 57, 186). A DOR of 103 is consistent with, for example, a sensitivity of 92 per cent and specificity of 90 per cent.

Pooled sensitivity and specificity estimates were also calculated. Pooled sensitivity was estimated to be 76 per cent (95% CI 67, 84) and pooled specificity to be 93 per cent (95% CI 90, 96). Pooling sensitivity and specificity individually can lead to underestimation of diagnostic performance if there is variation in the diagnostic threshold (Egger et al., 2001). The pooled DOR does not have this limitation.

One study (Miller et al., 1993) allowed the estimation of PCR validity using three methods of classification. The overall estimate above is based on patients who were post-cytotoxic therapy. Pooled DOR based on two consecutive positive results in Miller et al was 71 (95% CI 30, 172) and pooled DOR based on PCR conversion from negative to positive in Miller et al was 97 (95% CI 37, 253).

A median of three months elapsed between PCR positivity and relapse overall.

One study examined the relationship between PML breakpoint location at diagnosis and subsequent risk of relapse (Gonzalez et al., 2001). This study allowed an assessment of

prognosis at diagnosis. Monitoring by RT-PCR post-therapy was not conducted in this study.

One further study did not allow assessment of validity of RT-PCR in predicting relapse but presented five-year relapse-free survival data based on RT-PCR result (Hu et al., 2000). There was a statistically significant difference in relapse-free survival between the groups classified by RT-PCR, with patients that had negative RT-PCR results having improved outcome.

The results are summarised in Figure 3 and Table 11.

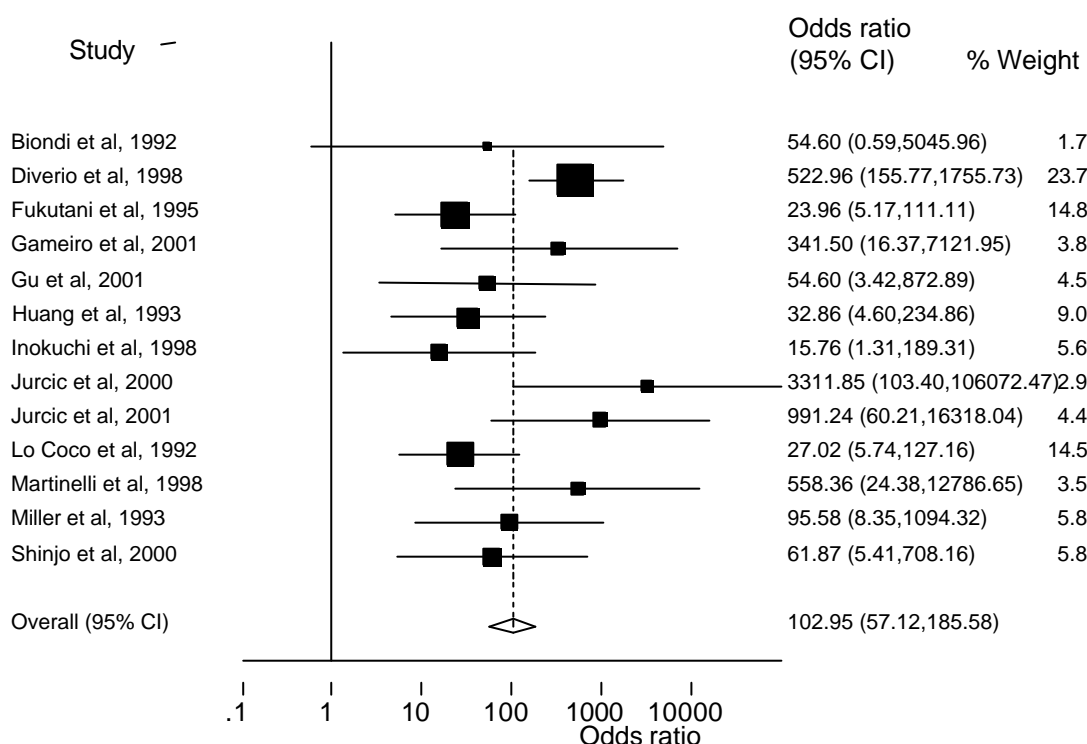


Figure 3 Validity of PCR results for predicting subsequent cytogenetic or haematological relapse, by study

Summary

Sixteen studies were identified that included patients with APL being monitored by PCR testing. A DOR could be estimated in 13 of these studies. The pooled DOR was 103 (95% CI 57, 186), indicating strong diagnostic utility. A diagnostic test that was not useful would have a DOR of one. A DOR of 103 is consistent with, for example, a sensitivity of 92 per cent and specificity of 90 per cent.

Table 11 Validity of monitoring for cytogenetic or haematological relapse in APL with RT-PCR methods

Reference	Sample characteristics	QS ^a	Results ^b	Comments												
Biondi et al., (1992)	Case series N=35 (5 in monitoring, 1 relapse) Diagnosis and monitoring APL by morphology	BC:U I:A S:I V:U M:A	Validity of RT-PCR compared with subsequent relapse: Sensitivity: 100% (95% CI 2, 100) Specificity: 100% (95% CI 29, 100) Time from PCR positivity to relapse 1 month	<ul style="list-style-type: none"> • Non consecutive sample • Median age 15 • One patient discarded because sample taken at relapse • Nested RT-PCR on bone marrow and peripheral blood 												
Diverio et al., (1998)	Prospective patients N=163 (28 relapses) Monitoring only PML/RAR α positive APL	BC:U I:A S:U V:A M:I	Validity of RT-PCR compared with subsequent haematological relapse: Sensitivity: 72% (95% CI 56, 89) Specificity: 99% (95% CI 98, 100) LR+: 98 (95% CI 14, 698) LR-: 0.28 (95% CI 0.15, 0.50) Median time from PCR positivity to relapse 3 months	<ul style="list-style-type: none"> • Bone marrow samples taken at set times • Second sample tested when PCR positive result obtained • Linkage between PCR result and change in management 												
Fukutani et al., (1995)	Case series N=27 (10 relapses) Monitoring only APL in CR more than 5 months post ATRA and chemotherapy. APL by FAB ^c criteria	BC:U I:U S:U V:U M:A	Validity of RT-PCR compared with subsequent relapse: Sensitivity: 100% (95% CI 69, 100) Specificity: 82% (95% CI 57, 96) LR+: 5.7 (95% CI 2.0, 15.8) LR-: 0	<ul style="list-style-type: none"> • Selection methods unclear • Variable follow up: PCR negative group median 9 months (range 2-18 months), PCR positive group median 5 months (range 3-16 months) • Relapse not defined • RT-PCR on bone marrow 												
Gameiro et al., (2001)	Case series N=47 Monitoring only APL by FAB ^c criteria	BC:U I:A S:U V:A M:I	Validity of RT-PCR compared with subsequent relapse: Sensitivity: 75% (95% CI 33, 100) Specificity: 100% (95% CI 80, 100) LR+: ∞ LR-: 0.25 (95% CI 0.05, 1.36)	<ul style="list-style-type: none"> • Relapse not defined • Selection methods unclear • Prediction of relapse best for monitoring after 10 months, therefore results presented restricted to this time frame. 												
Gonzalez et al., (2001)	Prospective, non-consecutive patients N=167 (12 relapses) Monitoring only Newly diagnosed PML/RAR α positive APL	BC:U I:A S:U V:A M:I	Risk of haematological relapse based on BCR isoform (BCR-1 baseline). <table border="1" style="margin-left: 20px;"> <thead> <tr> <th>Isoform</th> <th>Relapse risk</th> <th>Risk ratio</th> </tr> </thead> <tbody> <tr> <td>BCR-1</td> <td>0.05</td> <td>1</td> </tr> <tr> <td>BCR-2</td> <td>0.25</td> <td>5.19 (95% CI 1.12, 24.06)</td> </tr> <tr> <td>BCR-3</td> <td>0.08</td> <td>1.64 (95% CI 0.48, 5.58)</td> </tr> </tbody> </table>	Isoform	Relapse risk	Risk ratio	BCR-1	0.05	1	BCR-2	0.25	5.19 (95% CI 1.12, 24.06)	BCR-3	0.08	1.64 (95% CI 0.48, 5.58)	<ul style="list-style-type: none"> • Results represent risk of relapse based on isoform data at diagnosis • PCR testing in 12 laboratories using differing techniques • Potential selection bias due to patient selection methods
Isoform	Relapse risk	Risk ratio														
BCR-1	0.05	1														
BCR-2	0.25	5.19 (95% CI 1.12, 24.06)														
BCR-3	0.08	1.64 (95% CI 0.48, 5.58)														
Gu et al., (2001)	Case series N=25 (9 in monitoring with 3 relapses) Diagnosis and monitoring APL by FAB ^c criteria	BC:U I:U S:A V:U M:A	Validity of RT-PCR compared with subsequent relapse: Sensitivity: 100% (95% CI 29, 100) Specificity: 100% (95% CI 54, 100)	<ul style="list-style-type: none"> • Selection methods unclear • All t(15;17) positive patients • Real time RT-PCR on bone marrow and peripheral blood 												

Table 11 Validity of monitoring for cytogenetic or haematological relapse in APL with RT-PCR methods (continued)

Reference	Sample characteristics	QS ^a	Results	Comments
Hu et al., (2000)	Retrospective patients N=70 (23 relapses) Monitoring only APL by FAB ^c criteria	BC:U I:A S:U V:A M:I	Five-year relapse-free survival Negative PCR: 52.9% Positive PCR: 27.0% $P < 0.01$ Serial monitoring Continuously negative PCR: 76.4% Not continuously negative: 18.3%	<ul style="list-style-type: none"> Relapse not defined Patients classified as negative in non-serial results if one PCR result was negative after complete remission Timing of PCR testing was not clear in the serial monitoring group
Huang et al., (1993)	Case series N=97 (5 relapses) Diagnosis and monitoring APL by FAB ^b criteria	BC:U I:U S:A V:U M:A	Validity of RT-PCR compared with subsequent relapse: Sensitivity: 100% (95% CI 48, 100) Specificity: 75% (95% CI 64, 87) LR+: 4.1 (95% CI 2.6, 6.4) LR-: 0 Median time from PCR positivity to relapse 1 month	<ul style="list-style-type: none"> Only 62 of 97 assessed in PCR-relapse relationship (64%) Monitoring follow up 3-72 months At least 64% of the "false positive" PCR results were treated post PCR positivity, thus resulting in underestimation of specificity Selection methods and relapse definition were not clear Nested RT-PCR used on bone marrow
Inokuchi et al., (1998)	Case series N=23 (14 APL including 3 relapses) Monitoring only APL by FAB ^c criteria	BC:U I:U S:I V:U M:A	Validity of RT-PCR compared with subsequent relapse: Sensitivity: 100% (95% CI 29, 100) Specificity: 73% (95% CI 39, 94) LR+: 3.67 (95% CI 1.40, 9.62) LR-: 0 Median time from PCR positivity to relapse 6 months	<ul style="list-style-type: none"> Selection methods unclear Relapse not defined PCR classification based on PCR positive result post CR and not followed by a negative PCR result
Jurcic et al., (2001)	Consecutive, prospective patients N=82 (7 relapses) Monitoring only APL by FAB ^c criteria	BC:U I:A S:U V:A M:I	Validity of RT-PCR compared with subsequent relapse: Sensitivity: 57% (95% CI 20, 94) Specificity: 100% (95% CI 91, 100) LR+: ∞ LR-: 0.43 (95% CI 0.18, 1.01) Median time from PCR positivity to relapse 5 months	<ul style="list-style-type: none"> Relapse not defined
Jurcic et al., (2000)	Prospective, non-consecutive patients N=35 (3 relapses) Monitoring only APL	BC:U I:A S:U V:A M:I	Validity of RT-PCR compared with subsequent relapse (based on single positive PCR result post treatment): Sensitivity: 100% (95% CI 29, 100) Specificity: 100% (95% CI 82, 100) LR+: ∞ LR-: 0 Median time from PCR positivity to relapse 3 months	<ul style="list-style-type: none"> Data appears to be based on haematological relapse Eligibility criteria changed during the study (the inclusion criteria were expanded) Main purpose of the study was assessment of therapy

Table 11 Validity of monitoring for cytogenetic or haematological relapse in APL with RT-PCR methods (continued)

Reference	Sample characteristics	QS ^a	Results	Comments
Lo Coco et al., (1992)	Non-consecutive, retrospective case series N=35 (11 relapses) Monitoring alone Newly diagnosed or relapsed APL	BC:U I:U S:U V:U M:I	Validity of RT-PCR compared with subsequent relapse: Sensitivity: 100% (95% CI 72, 100) Specificity: 86% (95% CI 57, 98) LR+: 7.0 (95% CI 1.9, 25.3) LR-: 0 Median time from PCR positivity to relapse 3 months	<ul style="list-style-type: none"> Relapse not defined Selection methods inadequate Nested RT-PCR used
Martinelli et al., (1998)	Consecutive patients N=24 (4 relapses) Diagnosis and monitoring APL with evidence of t(15:17)	BC:U I:U S:U V:U M:A	Validity of RT-PCR compared with subsequent relapse (based on RT-PCR results post-third round consolidation therapy): Sensitivity: 75% (95% CI 19, 99) Specificity: 100% (95% CI 82, 100) LR+: ∞ LR-: 0.25 (95% CI 0.05, 1.36)	<ul style="list-style-type: none"> Basis of APL diagnosis not stated Relapse not defined Limited age range in study population
Miller et al., (1992)	Case series N=36 (6 relapses) Diagnosis and monitoring APL (M3 morphology), included in retinoic acid trial	BC:U I:U S:U V:U M:I	Validity of RT-PCR compared with subsequent relapse: Sensitivity: 100% (95% CI 54, 100) Specificity: NE LR+: NE LR-: NE	<ul style="list-style-type: none"> Only 6 studied in monitoring No description of sample selection Relapse not defined Nested RT-PCR on bone marrow

Table 11 Validity of monitoring for cytogenetic or haematological relapse in APL with RT-PCR methods (continued)

Reference	Sample characteristics	QS ^a	Results	Comments
Miller et al., (1993)	Case series N=33 Monitoring alone APL morphology and RT-PCR positive for PML-RAR α at diagnosis	BC:U I:U S:U V:U M:I	Validity of RT-PCR compared with subsequent relapse (based on two consecutive positive assays post CR): Sensitivity: 87% (95% CI 60, 98) Specificity: 80% (95% CI 52, 96) LR+: 4.3 (95% CI 1.5, 12.2) LR-: 0.2 (95% CI 0.0, 0.6) Validity of RT-PCR compared with subsequent relapse (based on PCR conversion from negative to positive): Sensitivity: 100% (95% CI 29, 100) Specificity: 100% (95% CI 79, 100) Validity of RT-PCR compared with subsequent relapse (based on single positive post cytotoxic therapy): Sensitivity: 100% (95% CI 40, 100) Specificity: 93% (95% CI 68, 100) LR+: 15.0 (95% CI 2.3, 99.6) LR-: 0 Time from PCR positivity to relapse 1-5 months (depending on classification system)	<ul style="list-style-type: none"> • Selection methods unclear • Relapse not defined • Number of relapses evaluable dependent on PCR classification used: 15 relapses with two consecutive positives, 3 relapses with conversion and 4 relapses post-cytotoxic therapy • Nested RT-PCR on bone marrow
Shinjo et al., (2000)	Prospective case series N=25 Monitoring alone APL – achieved CR post ATRA and subsequently relapsed	BC:U I:U S:U V:U M:A	Validity of RT-PCR compared with subsequent relapse: Sensitivity: 100% (95% CI 40, 100) Specificity: 100% (95% CI 63, 100)	<ul style="list-style-type: none"> • Primarily evaluating Am80 (a retinoid) • Four patients relapsed • RT-PCR used

^aQS-quality score, BC-blind comparison, I-independent assessment of diagnostic and reference test, S-spectrum of illness, V-verification bias, M-description of diagnostic testing methodology. A-adequate, U-unclear, I-inadequate.

^b cytogenetic and haematological relapse was used as the reference standard

^c FAB-French-American-British

Change in management

There is evidence of a change in management resulting from the PCR results in diagnosis and monitoring of APL.

The management of APL at diagnosis relies on identifying patients with ATRA sensitive APL. Thus, PCR has two roles:

1. providing a sensitive method of confirming the diagnosis of APL;

2. differentiating between ATRA-sensitive and ATRA-resistant translocations, for example PML-RAR α and PLZF-RAR α , emphasising the importance of determining the underlying abnormality in morphologically documented APL.

Furthermore, it is useful to establish a target for MRD detection by assessing the presence of a PCR-detectable abnormality at diagnosis. It is especially important to identify the location of the breakpoint in the PML gene at diagnosis in order to simplify the process of subsequent MRD detection through the use of breakpoint-specific primer systems (Lo Coco et al., 1998).

Change in management after PCR positive results during monitoring is documented or implied in a number of studies (Diverio et al., 1998, Lo Coco et al., 1999, Jurcic et al., 2001). Specifically, the GIMEMA and AIEOP groups have amended the AIDA protocol to test a repeat bone marrow sample after a positive PCR result following consolidation therapy. These groups now institute salvage therapy following confirmation of the positive PCR result (Diverio et al., 1998). The current ALLG (Australasian Leukaemia and Lymphoma Group) trial (APML3), which was opened in 1997, prospectively incorporated molecular monitoring with PCR-determined therapeutic intervention for molecular relapse.

Usefulness of early salvage therapy was suggested by Lo Coco et al (1999). In this study, the two-year survival after salvage therapy for molecular relapse was 92 per cent compared with 44 per cent after haematological relapse in a historical control group. The window of opportunity provided by molecular detection of relapse is only about three months on average; in other words, cytogenetic/haematological relapse occurs about three months after molecular relapse, and therefore lead-time bias does not explain the improvement in two-year survival in this study.

Conclusions on the role of PCR testing on patient outcome in patients with APL

Diagnostic testing improves patient outcome if the test is accurate: it results in change in management (when appropriate) and the instituted therapy is effective. The best study design for the evaluation of a diagnostic test is a randomised controlled trial (RCT) comparing patient outcome in those randomised to receive the diagnostic test with those randomised not to receive the test. However, no such RCT was identified in the evaluation of cytogenetic and PCR testing compared with cytogenetic testing alone, either at the time of diagnosis of APL or monitoring of APL. Therefore, the evaluation of PCR testing in APL relied on evaluating studies that focused on diagnostic performance, influence on patient management and the effect of therapeutic intervention on patient outcome individually.

Diagnosis

The 13 studies appraised suggested that combined PCR and cytogenetic testing had a sensitivity of 99 per cent (95% CI 97, 99) compared with a sensitivity of 92 per cent (95% CI 89, 95) for cytogenetic testing alone at presentation. The specificity of PCR testing was estimated as 100 per cent (95% CI 85, 100) in the five studies using a study design, where this could be calculated. All the studies were case series (level IV evidence) and most were characterised by small sample sizes. Two studies documented the presence of blinding and 11 documented independence between PCR testing and the reference standard. There was no evidence of verification bias in 12 studies. Therefore,

the results suggest improved diagnostic accuracy using combined cytogenetic and PCR testing compared with cytogenetic testing alone.

This improved accuracy allows more appropriate therapy to be used in the additional cases of APL detected by PCR testing. Treatment of APL evolved through the 1990s. The APL91 trial estimated four-year survival of 76 per cent in the ATRA plus chemotherapy arm, compared with 49 per cent with chemotherapy alone. These results established the effectiveness of ATRA-based therapies. ATRA and concurrent chemotherapy is now considered to be optimal therapy for de novo APL, with the exception of PLZF-RAR α APL.

An improvement in patient outcome could be expected due to:

- the incremental benefit from the addition of PCR testing as well as its ability to differentiate ATRA-sensitive and ATRA-resistant disease;
- trial data supporting the effectiveness of ATRA-based regimens that are employed as a result of the PCR and cytogenetic testing results.

Monitoring

Both PCR (molecular relapse) and cytogenetic testing (cytogenetic relapse) define forms of relapse. Haematological relapse is recognised as a further type of relapse. For the purposes of this review, PCR testing was assessed for its ability to predict cytogenetic or haematological relapse. However, specificity is likely to be underestimated using this approach. As discussed previously, the specificity is reliant on accurate determination of the reference standard (cytogenetic or haematological relapse). It is not possible to be certain that sufficient time has elapsed in these studies to allow an accurate determination of relapse status. Thus, some cases that are classified as false positive PCR results may have become true positives if the follow up had been longer.

There were 16 papers that examined the validity of monitoring, with only one using quantitative PCR methods. The pooled DOR from the 13 studies, where this could be estimated, was 103 (95% CI 57, 186). This indicated strong diagnostic utility using this test.

The quality of the studies identified for the evaluation of PCR performance in monitoring of patients with APL was variable but all were case series. There were biases present that would act in both directions on the overall effect measurement. For instance, lack of blinding would result in overestimation of PCR validity and lack of control over verification bias may have the same effect, but short follow up would result in underestimation of its validity.

Overall, monitoring with PCR has resulted in early detection of relapse (by three months) but at the cost of some false positive results that are likely to result from short follow up in some of the study participants.

Early detection of relapse allows early institution of salvage therapy and thus has the potential to improve patient outcome. One study was identified that compared the use of salvage therapy given at molecular relapse with therapy given at haematological relapse. The two-year survival following salvage therapy in molecular relapse was 92 per cent (95% CI 61, 98) compared with 44 per cent (95% CI 35, 52) in a previous series

following haematological relapse. Some of this difference will be due to lead-time bias, but it seems unlikely that this bias fully explains the variation in survival, given the relatively small time of three months between detection of PCR positivity and relapse in the studies appraised.

Therefore, there is support for the early detection of relapse through the use of PCR testing and there is low grade evidence supporting the benefit of early therapy. This implies an expected improvement in patient outcome with the use of PCR testing for the monitoring of APL.

What are the economic considerations?

Introduction

The purpose of this economic appraisal is to evaluate the value for money of PCR testing in combination with cytogenetic testing for APL. Decision analysis is used to model predicted health care costs of combined cytogenetic and PCR testing for APL in the Australian health care system.

We are not aware of any cost effectiveness studies evaluating the use of PCR for APL testing in Australia. However, there are some relevant studies from a methodological perspective investigating the cost of PCR for various sexually transmitted diseases such as chlamydia, gonorrhoea and herpes (Tebas et al., 1998, Shafer et al., 1999, Howell et al., 1998, Gift et al., 1999). Other studies using decision analysis modelling include Scott et al (1998), Rau and Libman (1999), and Nigrovic and Chiang (2000) covering illnesses such as lung cancer, tuberculosis, or detection using cerebrospinal fluid respectively.

The specific objectives for the cost effectiveness analysis of APL were to:

- identify and review recent published studies reporting economic evaluations of PCR testing strategies;
- identify what approach should be used in the economic evaluation, ie cost minimisation, cost-effectiveness, cost-utility, or cost-benefit;
- identify the detection parameters, treatment success probabilities, and cost estimate parameters, and the appropriate modeling device for outcome analysis;
- identify the quantifiable benefits of PCR and cytogenetic testing over cytogenetic testing alone;
- identify the incremental cost differences between PCR and cytogenetic testing over cytogenetic testing alone;
- evaluate the robustness of the results as judged by sensitivity analysis.

There appear to be two major benefits of using the PCR in testing for APL. Firstly, the PCR is more sensitive than cytogenetic testing for initial diagnosis and characterization of the underlying genetic abnormality. Secondly, the PCR is able to predict cytogenetic and haematological relapse earlier than the current cytogenetic-based testing strategy. The second benefit is therefore particularly useful in the monitoring for relapse.

To establish the incremental cost differentials between PCR and cytogenetic testing with cytogenetic testing alone, it is necessary to determine costs per test as well as the testing frequency for each strategy. For the full cost-effectiveness analysis it is also necessary to include flow-on costs such as variation in management costs associated with earlier or more accurate diagnosis.

Cost-effectiveness analysis (CEA) was selected as the most appropriate evaluation technique for this review. In the absence of relevant clinical trials, outcomes and costs have been estimated using literature-based estimates, existing data and expert opinion. The source of cost estimates is summarised in Appendix G. The total inpatient costs were based on the number of patients receiving “high cost” and “low cost” management. High cost and low cost profiles were derived by evaluating patterns of inpatient care in patients with APL. Existing New Zealand¹ data were used to develop a profile of inpatient separations that were limited to haematological DRG codes. AR-DRG 4.1 (2000-2001) cost estimates were applied to these profiles to derive an estimate for inpatient costs in the high cost and low cost categories. Estimates for patient outcomes were derived from the literature. If estimates were not identified in the literature, expert opinion was used.

The cost estimates were derived from the perspective of the Australian Government Department of Health and Ageing.

In the absence of a cost estimate for haematological PCR testing, a shadow price based on a survey of Australian laboratories conducted in the year 2000 was used. There was wide variation in the estimates across laboratories.

Diagnosis model

PCR and cytogenetic testing are viewed as complementary tests. PCR potentially provides additional information due to its increased sensitivity (see effectiveness section, pp24 and 25). Cytogenetic testing provides additional information relating to other cytogenetic abnormalities. These abnormalities may have therapeutic and prognostic implications.

The decision tree model for diagnosis is shown in Figure 4. Combined cytogenetic and PCR testing was compared with cytogenetic testing alone for a period of 2.5 years from the time of diagnosis. This time period was selected since patient management was at least partially determined by the results of diagnostic testing for the next 2.5 years using current treatment protocols (Burnett et al., 2002). The summary results for APL diagnosis are shown in Table 12.

¹ New Zealand data were used due to the availability of existing data for inpatient separations in patients with APL. It is assumed similar in-patient separations occur in Australian APL patients, although these were not available or presented in a suitable form to use for this review.

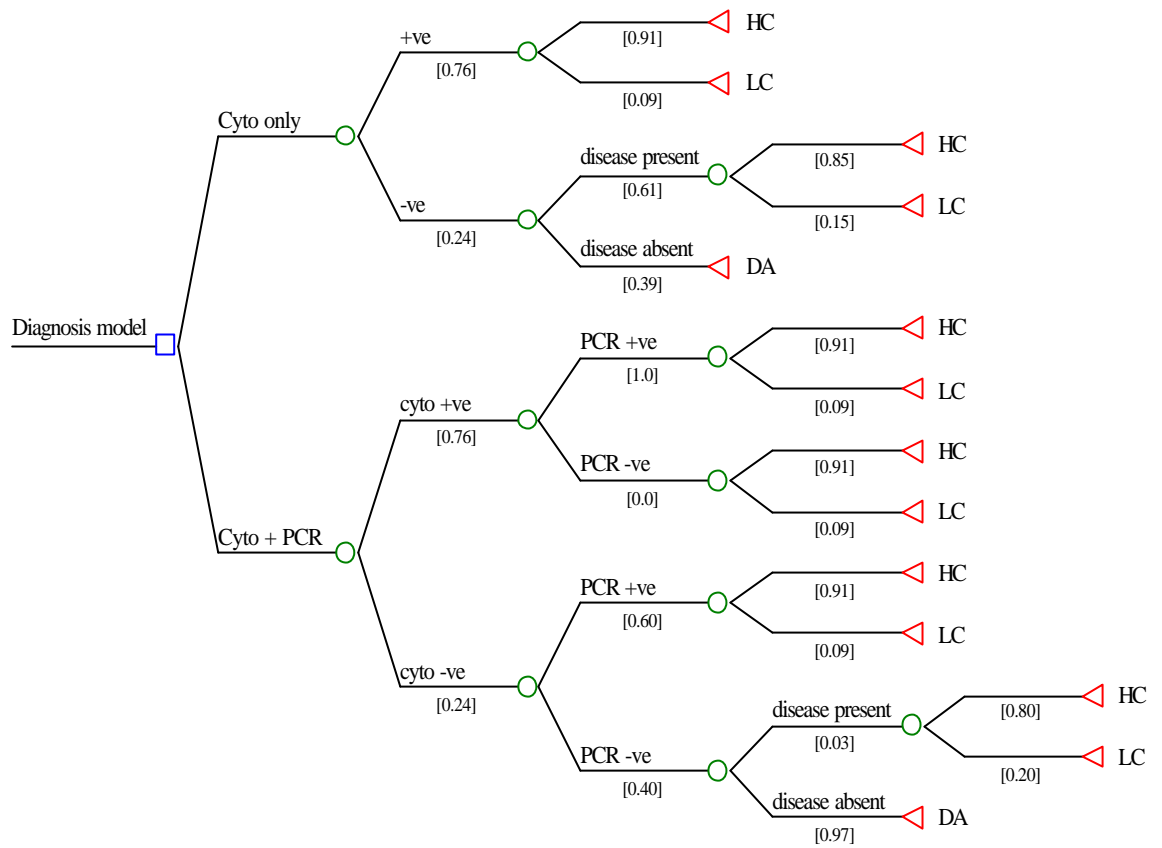


Figure 4 APL diagnosis economic model: patient proportions

Key to Figure 4

Cyto	Cytogenetic testing
PCR	Polymerase chain reaction testing
+ve	Positive result
-ve	Negative result
HC	High cost management category
LC	Low cost management category
Disease present	Patients who have APL that was not detected at initial testing
Disease absent (DA)	Patients who do not have APL
[]	Proportion of patients

Table 12 Diagnosis of APL: economic summary

Model	Number (proportion) of patients	Cost (\$)			Inc. ^a life year saved	Inc. ^a cost per life year saved (\$)
		Diagnostic testing	Management	Total (mean)		
<u>Cytogenetics only</u>	44.0 (1.000)	15,576	636,081	651,657		
Cyto positive	33.6 (0.764)		535,360	(14,810)		
HC	30.6 (0.695)		522,269			
LC	3.0 (0.069)		13,091			
Cyto negative	10.4 (0.236)		100,721			
Disease present	6.4 (0.145)		100,721			
HC	5.4 (0.124)		96,565			
LC	1.0 (0.022)		4156			
Disease absent	4.0 (0.091)					
<u>Cytogenetics and PCR</u>	44.0 (1.000)	25,916	637,380	663,296	0.80	329
Cyto positive	33.6 (0.764)		535,360	(15,075)		
PCR positive	33.6 (0.764)		535,360			
HC	30.6 (0.695)		522,269			
LC	3.0 (0.069)		13,091			
PCR negative	0.0 (0.000)		0			
HC	0.0 (0.000)		0			
LC	0.0 (0.000)		0			
Cyto negative	10.4 (0.236)		102,021			
PCR positive	6.3 (0.143)		99,934			
HC	5.7 (0.130)		97,490			
LC	0.6 (0.013)		2,444			
PCR negative	4.1 (0.094)		2,087			
Disease present	0.1 (0.003)		2,087			
HC	0.1 (0.002)		2,004			
LC	0.0 (0.000)		83			
Disease absent	4.0 (0.091)					

^aInc.=incremental

Patient numbers

The proportion of patients in each arm of the model is shown in Figure 4 and Table 12. The following assumptions were made in deriving patient numbers:

- total of 40 new cases of APL registered per year (AIHW data: 1990-1999);
- total cases tested for confirmation of suspected APL 44 per year (Iland, personal communication, 2002);
- eighty-four per cent of people with APL will be positive for t(11;17) or t(15;17) by cytogenetic testing at initial testing based on the sensitivity of cytogenetic testing and the proportion of patients with indeterminate cytogenetic test results (see effectiveness section, pp26 and 27).
- one hundred per cent of people cytogenetic positive for t(11;17) or t(15;17) will be PCR positive at initial testing (see effectiveness section, p23);
- ninety-eight per cent of people with APL who are negative by cytogenetic testing (or cytogenetic testing is unsuccessful) for t(11;17) or t(15;17) will be PCR positive at initial testing (see effectiveness section, p24);
- the proportion in the high and low cost groups varies between the early and late diagnosis groups:
 - at early diagnosis, 91 per cent of patients with APL are included in the high cost group. The high cost group represents those not classified as “early death” based on survival beyond 30 days after acute presentation (Lo Coco et al., 1998, Tallman et al., 1997, Sanz et al., 1999, Fenaux et al., 2000, Asou et al., 2001).
 - at late diagnosis, 85 per cent of patients with APL are included in the high cost group. A review identified that early deaths occurred in 15 to 30 per cent of patients treated with non-ATRA regimens (Degos, 1992). This was largely based on original articles published pre-1990. Since then, platelet support practices have changed in patients with APL and, to a lesser extent, patients with AML. This has resulted in a reduction in early deaths. It was noteworthy that randomised trials comparing ATRA plus chemotherapy versus chemotherapy alone in patients with APL did not find a significant difference in early deaths between the two study arms (Fenaux et al., 2000, Tallman et al., 1997). For these reasons it was assumed in the base model that the proportion of early deaths in the non-ATRA group was at the lower end of the 15 to 30 per cent spectrum, thus 20 per cent was assumed. Expert opinion suggests half of the cytogenetic test negative group will be treated with ATRA and chemotherapy while the other half will be treated with chemotherapy alone (see also cost estimates and life years saved sections), so in the base case we have assumed 15 per cent early deaths in the cytogenetic test negative group. In the PCR test negative group, it was assumed 20 per cent were early deaths.

Combined cytogenetic and PCR testing detected 6.3 (16 per cent) more cases of patients who had APL annually at initial testing than cytogenetic testing alone, based on the above assumptions. Patient numbers in each branch of the model are summarised in Table 12.

Cost estimates

The following assumptions were made in deriving cost estimates:

- the model duration is 2.5 years;
- cost estimates were adjusted to allow for a 10 per cent dropout rate per year due to the expected rate of relapse;
- the Medicare Benefits Schedule reimbursement fee for cytogenetic testing accurately reflects the cost of cytogenetic testing;
- the PCR cost estimate was derived from the median estimated cost in the RCPA Quality Assurance benchmarking exercise (\$235);
- the cost estimates for diagnostic testing were based on testing once only;
- baseline inpatient costs used private AR-DRG 4.1 (2000-2001) estimates;
- high cost estimates in those with disease not detected by cytogenetic testing were composed of 50 per cent treated as APL (assumed as chemotherapy with ATRA) and 50 per cent treated as non-APL (assumed chemotherapy without ATRA, plus 20 per cent receiving transplant)
- high cost estimates in those with disease not detected by cytogenetic and PCR testing were all treated as non-APL (assumed chemotherapy without ATRA plus 20 per cent receiving transplant);
- unit daily cost of ATRA therapy as an outpatient was estimated based on a dose of 80 mg/day given for two weeks every three months during maintenance therapy. This unit cost was converted to cost for the period of the model based on:
 - ten weeks therapy during 2.5 years;
 - ATRA taken seven days per week during those 10 weeks;
 - all patients in the high cost groups receive ATRA except as outlined for patients with APL not detected at initial diagnostic testing.
- other management costs, such as methotrexate, were not incorporated in the final assessment due to their low cost relative to other components.

The estimated inpatient costs are shown in Table 13.

Table 13 Estimated average inpatient costs for patients with APL

Diagnostic model group	Average inpatient cost per patient (\$)	
	Private sector	Public Sector
Diagnostic test positive		
High cost group	13,581	22,710
Low cost group	4,329	6,628
Cytogenetic test negative		
High cost group	16,001	27,157
Low cost group	4,329	6,628
Cytogenetic and PCR test negative		
High cost group	18,421	31,603
Low cost group	4,329	6,628

Note: costings have been calculated using final cost weights for AR-DRG 4.1, Round 4 (2000-2001)

The total cost was marginally higher in the PCR plus cytogenetic testing arm than cytogenetic testing alone (\$637,380 for combined testing compared with \$636,081 using cytogenetic testing alone). The increased cost was due to higher diagnostic testing costs.

The outpatient pharmaceutical component of the total cost was \$126,992, or 20 per cent of the combined testing total cost estimate. In contrast, the pharmaceutical component of the cytogenetic testing strategy alone was \$116,536 (18 per cent of the total). In the model, the pharmaceutical costs were based solely on ATRA therapy.

Life years saved

The following assumptions were made in deriving life years saved estimates:

- estimated median survival of 0.05 years in the low cost group after a positive diagnostic test result (based on early death within 30 days);
- estimated median survival of 15 years excluding early deaths post-concurrent ATRA and chemotherapy. This estimate is based on:
 - seventy-seven per cent seven-year survival in the AIDA study as presented at the International Conference on APL and Differentiation Therapy in Rome, 2001, as a follow up to earlier published data (Mandelli et al., 1997)(Iland, Personal communication, 2002);
 - seventy-seven per cent five-year survival in the modified AIDA study as presented at the International Conference on APL and Differentiation Therapy in Rome, 2001, as a follow up to earlier published data (Sanz et al., 1999)(Iland, Personal communication, 2002)
 - eighty-eight per cent five year-survival in the Australasian APML3 trial (Iland, Personal communication, 2002).
- estimated median survival of 3.5 years in the group treated with chemotherapy without ATRA (Fenaux et al., 2000, Tallman et al., 1997);

- estimated median survival of 9.3 years in the high cost group with APL initially missed on cytogenetic testing (Sanz et al., 1999, Mandelli et al., 1997, Fenaux et al., 2000, Tallman et al., 1997). This estimate is based on the assumption that half of this group will be treated with ATRA and chemotherapy while the other half will be treated according to non-APL protocols (as outlined in cost estimates);
- linear relationship between survival and time since diagnosis.

Incremental life years saved were estimated by calculating the difference in life expectancy between combined cytogenetic and PCR testing and cytogenetic testing alone. This baseline estimate was 0.80 incremental life years saved.

Cost per life year saved

The incremental cost per life year saved was calculated by estimating the difference in mean cost between combined cytogenetic plus PCR testing and cytogenetic testing alone and dividing this estimate by the incremental life years saved. Based on the previous assumptions, the incremental cost per life year saved was \$329 for combined cytogenetic and PCR testing when compared with cytogenetic testing alone.

Sensitivity testing

The baseline economic analysis used private AR-DRG 4.1 (2000-2001) cost estimates. There is wide variation between the private and public AR-DRG 4.1 (2000-2001) estimates for bone marrow transplantation and the public estimate is likely to be a more accurate reflection of the true cost, given characteristics of patients receiving transplantation privately. There is little absolute variation in cost per life years saved by public or private AR-DRG estimate (see Figure 5).

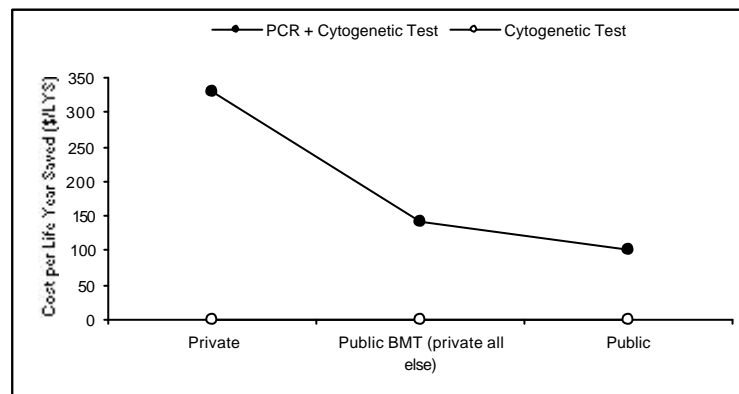


Figure 5 Estimated incremental cost per life years saved by private and public AR-DRG 4.1 (1999-2000) cost estimates: APL diagnosis

The baseline economic analysis assumed 50 per cent of patients who were cytogenetic test negative were treated as per APL protocols and the remaining 50 per cent were treated as AML patients, based on expert opinion (Iland, personal communication, 2002). Sensitivity analyses were performed using different proportions treated as AML. One estimate assumed all cytogenetic negative cases were treated as AML (reflecting the scenario where the decision on treatment was based on the diagnostic test results alone). Under this assumption, combined PCR and cytogenetic testing had an incremental cost per life year saved of \$92 (see Figure 6).

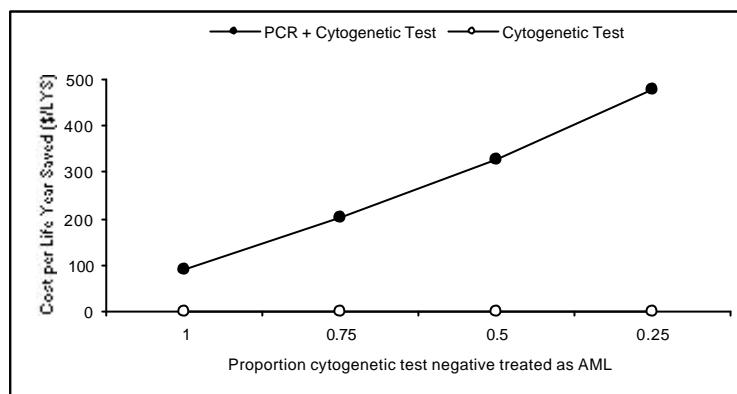


Figure 6 Estimated incremental cost per life years saved by proportion of cytogenetic test-negative cases treated as AML: APL diagnosis, private costs

The baseline economic analysis estimated incremental life years saved derived from life expectancy for specific therapeutic modalities, with the estimates derived from the literature. Given the lack of consistency in methodology and study populations between studies, caution needs to be applied in assessing the incremental cost-effectiveness ratio. The base model estimated incremental life years saved as 0.80. Sensitivity analysis indicated little absolute variation in cost per life year saved across a range of incremental life years saved estimates (see Figure 7), reflecting the low incremental cost derived from the model.

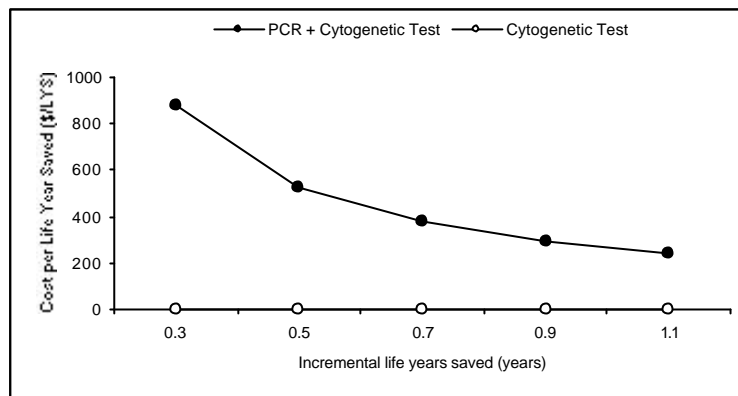


Figure 7 Estimated incremental cost per life years saved by incremental life years saved: APL diagnosis, private costs

There was some uncertainty about the proportion of early deaths in the groups negative by cytogenetic or PCR diagnostic testing. The base model assumed early deaths occurred in 20 per cent of patients treated with non-ATRA containing regimens. Variation of this estimate had little effect on the overall cost per life year saved (see Figure 8).

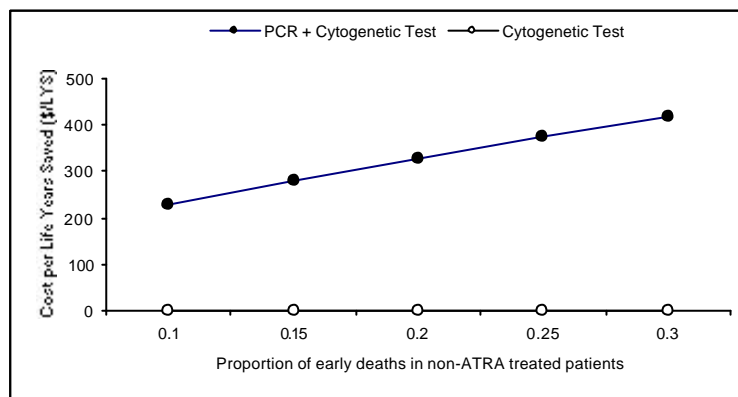


Figure 8 Estimated incremental cost per life years saved by proportion of early deaths in the cytogenetic-test negative group: APL diagnosis, private costs

Monitoring model

The decision tree model for monitoring of APL is shown in Figure 9. Combined cytogenetic and PCR monitoring was compared with cytogenetic monitoring alone. The model was run for three years from one month after diagnosis. The summary results for APL monitoring are shown in Table 14.

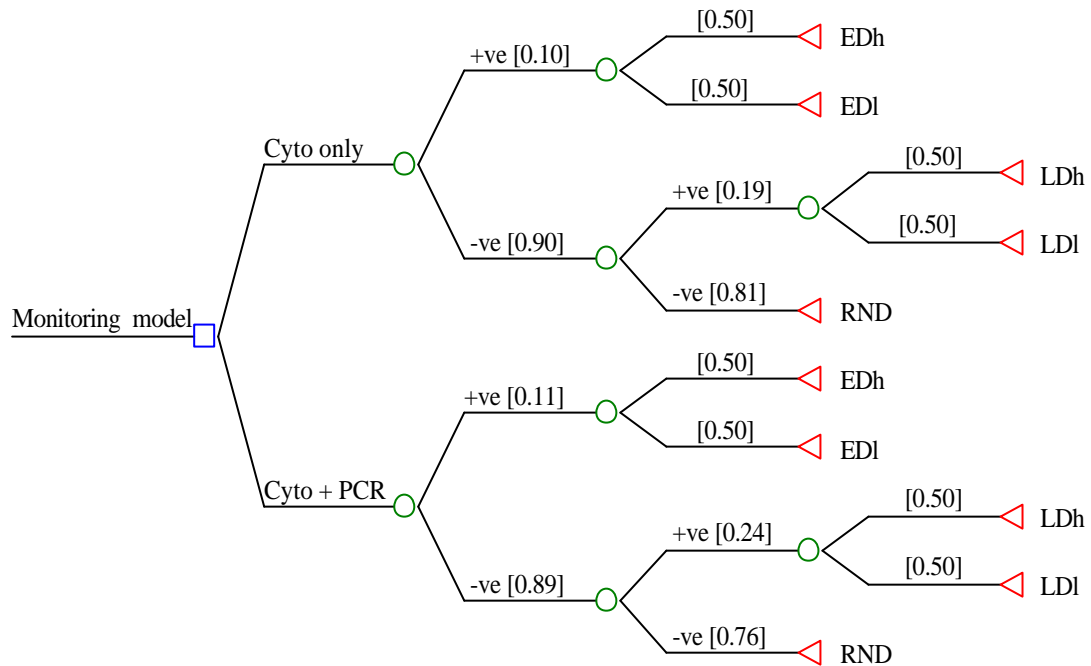


Figure 9 APL monitoring economic model: patient proportions

Key to Figure 9

Cyto	Cytogenetic testing
PCR	Polymerase chain reaction testing
+ve	Positive result
-ve	Negative result
EDh	Early detection high cost management category
EDl	Early detection low cost management category
LDh	Late detection high cost management strategy
LDl	Late detection low cost management strategy
RND	Relapse not detected at two years
[]	Proportion of patients

Table 14 Monitoring of APL: economic summary

Model	Number (proportion) of patients	Cost(\$)			Inc. ^{a,b} life years saved	Inc. ^a cost per life year saved (\$)
		Diagnostic testing (three years)	Management	Total ^b (mean)		
<u>Cytogenetics</u>	36.00 (1.000)	127,440	855,548	919,394		
Cyto positive	3.60 (0.100)		121,162	(25,539)		
Edh	1.80 (0.050)		88,373			
Edl	1.80 (0.050)		32,789			
Cyto negative	32.40 (0.900)		734,386			
Positive	6.16 (0.171)		271,206			
LDh	3.08 (0.086)		170,700			
LDI	3.08 (0.086)		100,506			
Negative	26.24 (0.729)		463,180			
<u>Cytogenetics and PCR</u>	36.00 (1.000)	212,040	899,374	1,036,528	0.51	6,418
Cyto or PCR positive	3.97 (0.110)		133,581	(28,792)		
Edh	1.98 (0.055)		97,431			
Edl	1.98 (0.055)		36,150			
Cyto and PCR negative	32.03 (0.890)		765,794			
Positive	7.59 (0.211)		334,470			
LDh	3.80 (0.105)		210,519			
LDI	3.80 (0.105)		123,951			
Negative	24.44 (0.679)		431,324			

^aInc.=incremental discounted value

^bdiscounted at 5 per cent

Patient numbers

The proportion of patients in each arm of the model is shown in Figure 9 and Table 14. The following assumptions were made in deriving patient numbers:

- a total of 36 cases starting monitoring one month after diagnosis, based on estimated number in the diagnosis model excluding early deaths;
- ten per cent of initially non-relapsed patients are detected in cytogenetic relapse by cytogenetic testing annually (Tallman et al., 1997, Fenaux et al., 2000);
- in concurrent PCR and cytogenetic testing, PCR predicts cytogenetic relapse three months before cytogenetic testing (see effectiveness section, p30);
- concurrent PCR and cytogenetic testing has a sensitivity of 84 per cent and specificity of 95 per cent for prediction of cytogenetic relapse three months before detection of that relapse by cytogenetic testing (see effectiveness section, p30);
- early detection of relapse is made by one year;
- late detection of relapse is made by three years;
- treatment after detection of relapse is composed of:
 - twenty-five per cent receiving chemotherapy and autologous BMT (high cost group);
 - twenty-five per cent receiving chemotherapy and allogeneic BMT (high cost group);
 - fifty per cent receiving chemotherapy alone, either ATRA and/or arsenic trioxide (low cost group).
- treatment in the non-relapse group is that applied in the diagnostic economic model, excluding treatment in the first month after diagnosis.

Combined cytogenetic and PCR testing detected 0.37 more early relapses than cytogenetic testing alone, although 0.19 of these were false positive cases. Patient numbers in each branch of the model are summarised in Table 14.

Cost estimates

The following assumptions were made in deriving cost estimates:

- the model is limited to three years of monitoring, starting one month after diagnosis;
- the Medicare Benefits Schedule reimbursement fee for cytogenetic testing accurately reflects the cost of cytogenetic testing;
- the PCR cost estimate was derived from the median estimated cost in the RCPA Quality Assurance benchmarking exercise of \$235;

- monitoring occurs at one month, three months and then every three months until 18 months, followed by testing every six months until 36 months (adapted from the AML 15-trial protocol), (Burnett et al., 2002);
- baseline inpatient costs used private AR-DRG 4.1 (2000-2001) estimates;
- other management costs (eg ATRA) were not incorporated in the final assessment due to their low cost relative to other cost components;
- costs were discounted at 5 per cent per annum.

The estimated inpatient costs are shown in Table 15.

Table 15 Estimated average inpatient costs for detection of relapse in APL

APL monitoring group	Average treatment (drug) cost per patient (\$) ^a							
	Private Sector				Public Sector			
	Year 1	Year 2	Year 3	Total ^b	Year 1	Year 2	Year 3	Total ^b
Early detection group								
High cost	26,522	11,287	11,287	49,096	50,289	18,504	18,504	87,297
Low cost	11,792	3,212	3,212	18,216	19,479	5,544	5,544	30,567
Late detection group								
High cost	17,649	26,522	11,287	55,458	29,513	50,289	18,504	98306
Low cost	17,649	11,792	3,212	32,653	29,513	19,479	5,544	54536
Non-relapse group	17,649	0	0	17,649	29,513	0	0	29,513

^acostings have been calculated using the final cost weights for AR-DRG 4.1, Round 4 (2000-2001).

^b undiscounted total costs

The total cost, discounted at 5 per cent, was higher in the combined PCR and cytogenetic testing arm than cytogenetic testing alone (\$1,036,528 using combined testing compared with \$919,394 using cytogenetic testing alone for the three year period). This was mainly due to the higher testing cost in the combined testing strategy.

Life years saved

Life years were estimated for both the cytogenetic alone testing strategy and the combined cytogenetic and PCR testing strategy by deriving overall estimates for each terminal arm of the model and imputing differences between the two testing strategies. The estimates were derived from the literature and the difference between the two diagnostic testing strategies was based on the study by Lo Coco et al (1999). The median estimated life expectancy was longer in the high cost arm (see assumptions below). A baseline difference in life expectancy was imputed as two years in the high cost group, based on an estimated two year difference in survival between patients detected at molecular relapse and patients detected at haematological relapse (Lo Coco et al., 1999).

A linear model was assumed in deriving this difference. In the low cost group, the median life expectancy was 2.5 years overall. The difference in life expectancy between

the two diagnostic strategies in the low cost group was based on a linearly proportional difference between the median estimate in the low cost group and the high cost group (Lo Coco et al., 1999). Specifically, since the difference was two years at a median survival of six years, it was assumed the difference at 2.5 years would be $(6 \times 2.5) / 2$ years, or 0.8 years. This was rounded to one year, reflecting the uncertainty in the estimate. Overall life expectancy estimates were then derived for each testing strategy and discounted at 5 per cent.

The median survival estimates were based on estimates from the studies referenced and weighted by sample size.

The following assumptions were made in deriving life years saved estimates:

- median survival of five years after transplantation post-relapse in the cytogenetic testing arm (Meloni et al., 1997, Soignet et al., 2001, Nabhan et al., 2001, Sanz et al., 2000, Ferrant et al., 1997, Lo Coco et al., 1999);
- median survival of seven years after transplantation post-relapse in the combined cytogenetic and PCR testing arm (Meloni et al., 1997, Soignet et al., 2001, Nabhan et al., 2001, Sanz et al., 2000, Ferrant et al., 1997, Lo Coco et al., 1999);
- median survival of two years after chemotherapy alone post-relapse in the cytogenetic testing arm (Niu et al., 1999, Shen et al., 2001, Hu et al., 1999, Fenaux et al., 2000, Lo Coco et al., 1999);
- median survival of three years after chemotherapy alone post-relapse in the combined cytogenetic and PCR testing arm (Niu et al., 1999, Shen et al., 2001, Hu et al., 1999, Fenaux et al., 2000, Lo Coco et al., 1999);
- median survival in the non-relapsed group based on estimates in the diagnostic model, excluding early deaths, and adjusting the estimate in the cytogenetic only arm to allow for the detection of relapse three months earlier in the combined PCR and cytogenetic testing arm.

Incremental life years saved were estimated by calculating the difference in life expectancy between combined cytogenetic and PCR testing compared with cytogenetic testing alone. This baseline estimate, discounted at 5 per cent, was 0.51 incremental life years saved.

Cost per life year saved

The incremental cost per life year saved was calculated by estimating the difference in discounted mean cost between combined cytogenetic plus PCR testing and cytogenetic testing alone, and dividing this estimate by the incremental life years saved. Based on the previous assumptions, the incremental cost per life year saved was \$6,418.

Sensitivity testing

The baseline economic analysis used private AR-DRG 4.1 (2000-2001) cost estimates. There is variation between these private estimates and the corresponding public estimates, with the public estimates usually being higher. However, particularly notable in the context of this review was the variation in estimates for BMT. The private estimate was \$15,235 per BMT DRG and the public estimate was \$31,785. The incremental cost-

effectiveness ratio was relatively sensitive to variation by private estimate, public estimate or public estimate for BMT, but private estimate for all other inpatient costs based on AR-DRG 4.1 (2000-2001) data (see Figure 10).

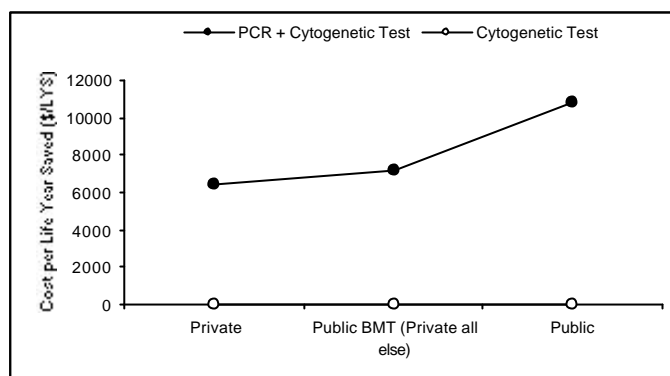


Figure 10 Estimated incremental cost per life year saved by private and public AR-DRG 4.1 (2000-2001) cost estimates: Monitoring in APL

The baseline economic analysis estimated the cost of PCR testing based on the median estimate from the RCPA quality assurance benchmarking program, with a baseline estimate of \$235. However, there was considerable variation in estimates by laboratory, ranging from \$67 to \$1,260. Incremental cost per life year saved was sensitive to changes in the PCR cost estimate with cost per life year saved increasing with rising PCR costs (see Figure 11).

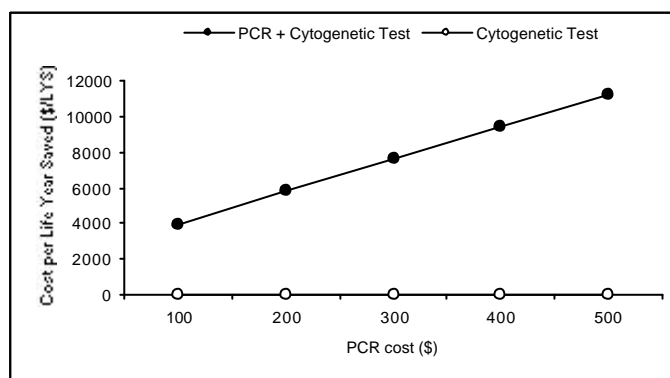


Figure 11 Estimated incremental cost per life year saved by PCR cost estimate: Monitoring in APL post-transplant, private costs

The baseline economic analysis estimated incremental life years saved using life expectancy for specific therapeutic modalities and clinical scenarios. The estimates were derived from the literature. One study examined the difference in survival in patients treated in molecular relapse versus cytogenetic or haematological relapse, allowing an estimation of the benefit resulting from early versus late treatment (see p15). Given the uncertainty resulting from reliance on this limited literature, caution needs to be applied in assessing the incremental cost-effectiveness ratio. The incremental cost-effectiveness ratio using costs and life expectancy, discounted at 5 per cent, was sensitive to variation in the incremental life years saved estimate with cost per life year saved decreasing with increasing incremental life years saved (see Figure 12).

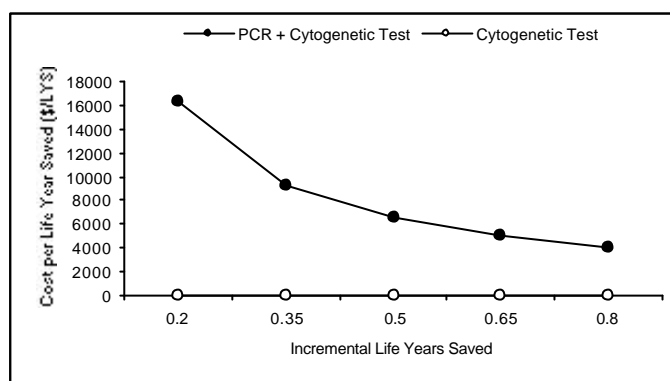


Figure 12 Estimated incremental cost per life year saved by incremental life years saved: APL monitoring, private costs

Summary of the economic evaluation

The objective of this economic evaluation was to determine the cost-effectiveness of cytogenetic and PCR testing for initial diagnosis and monitoring compared with cytogenetic testing alone.

The evaluation has been based on existing data rather than trial-based data. Economic evaluation is more robust when incorporated in or based on clinical trial data.

A further shortcoming of this analysis has been the use of laboratory “prices” as a proxy for costs. PCR prices have been quoted over a very large range, from \$67.30 to \$1259.79.

The cost-effectiveness analysis has been based on a decision tree model for each of the two major considerations: initial diagnosis of APL and monitoring for relapse after successful therapy. The incremental cost per life year saved for combined cytogenetic and PCR testing compared with cytogenetic testing alone was low in both models under baseline conditions. Costs were \$329 and \$6,418 per life year saved in the diagnosis and monitoring models respectively. There was little absolute change in cost per life year saved estimates across a plausible range of sensitivity analyses in the diagnostic model, including variation in inpatient cost, proportion of patients that were diagnostic negative

treated as non-APL, variation in incremental life years saved, and variation in the proportion of early deaths. The monitoring model was relatively sensitive to variation in PCR cost, variation in inpatient management cost and variation in incremental life years saved. However, all cost per life year saved estimates were less than \$17,000 across a plausible range of estimates.

The analysis focused on direct testing costs and flow-on therapy costs on the cost side, and life years saved on the effectiveness side. It was not practical to estimate societal costs and benefits, but use of PCR, in both diagnosis and monitoring of APL, provides both clinicians and patients with earlier and/or more accurate information. This enhanced detection will be of benefit to society as a whole, in that utility maximising behaviour will be improved.

In the diagnostic model, therapy cost differentials outweigh the cost of testing. Combined PCR and cytogenetic testing led to increased direct costs of diagnostic testing when compared with cytogenetic testing alone, but similar flow-on treatment costs. The direct benefit, however, is an estimated 0.80 life years gained. The estimated incremental cost per life year saved was \$329. Other outcomes, such as avoidance of relapse, were not assessed in the economic evaluation although it is an important outcome for the patient.

In monitoring, although the testing costs were lower than the inpatient management costs, they contributed a sizeable proportion of the total costs. The testing cost and the total inpatient management cost was higher in the combined cytogenetic and PCR testing program. The direct benefit was an improvement in survival. The estimated incremental cost per life year saved was \$6,418.

Overall, both the diagnosis and monitoring models suggest favourable indicative cost-effectiveness ratios for the use of combined cytogenetic and PCR testing.

Conclusions

Safety

PCR testing for haematological malignancy is conducted on peripheral blood or bone marrow specimens, though bone marrow is the better specimen and is the only specimen appropriate in monitoring for MRD. The required quantity of marrow is minimal and would usually be collected concurrently with other routine marrow tests. Therefore, there are few safety concerns associated with PCR testing.

Effectiveness

The specific research questions in relation to this review were:

- Does PML-RAR α and PLZF-RAR α detection increase the proportion of patients who are recognised to have a specific disease entity that defines a specific therapeutic strategy?
- Does repeated qualitative and/or quantitative PCR testing in APL predict haematological relapse and/or influence therapeutic decision making?

Diagnostic testing

It was concluded that:

- The sensitivity of PCR alone and cytogenetic testing alone was high at diagnosis but the sensitivity was higher for combined cytogenetic and PCR testing compared with cytogenetic testing alone, based on the 13 papers selected.
- Specificity of PCR testing was estimated to be 100 per cent (95% CI 85, 100) at diagnosis.
- The diagnostic studies were of variable quality. All 13 were case series (level IV evidence). Blinding was documented in two studies (15 per cent). Documentation of the independence of RT-PCR testing from reference testing was present in 11 studies (85 per cent) and avoidance of verification bias was present in 12 studies (92 per cent).
- The use of PCR at diagnosis provides a sensitive method of confirming the diagnosis of APL and is able to differentiate ATRA sensitive and ATRA resistant translocations. It also establishes a target for MRD detection.
- The increased sensitivity of PCR allows 7 per cent more cases to be identified and targeted for curative therapy in patients who had an interpretable cytogenetic test.

Monitoring

It was concluded that:

- There was a level of consistency throughout the studies selected that supported the use of PCR testing for monitoring of APL.
- Sixteen monitoring studies were appraised with 15 using qualitative RT-PCR and one using quantitative RT-PCR techniques.
- The pooled DOR was 103 (95% CI 57, 186) in the 13 studies where this could be estimated. A DOR of 103 is consistent with, for example, a sensitivity of 92 per cent and specificity of 90 per cent.

Change in management

Accurate identification of PML-RAR α and PLZF- RAR α APL at diagnosis is useful for confirming the diagnosis of APL and defining appropriate therapeutic strategies. PML-RAR α is responsive to ATRA-based therapy whereas PLZF- RAR α is not.

Detection of molecular relapse post-transplant, through PCR monitoring, allows the early institution of salvage therapy in this group. The studies available suggest early salvage therapy is associated with improved prognosis.

Effect of additional PCR testing on patient outcome

At diagnosis:

- Combined cytogenetic and PCR testing is more sensitive than cytogenetic testing alone.
- Detection of PML-RAR α and PLZF- RAR α APL is used to guide therapeutic interventions.
- Treatment options used preferentially in PML- RAR α include ATRA in the regimen. PML-RAR α is associated with approximately 99 per cent of APL. Trials comparing ATRA plus chemotherapy compared with chemotherapy alone support the use of ATRA-based regimens in patients with PML-RAR α .
- Therefore, the above conclusions support improved patient outcome in patients tested by PCR at diagnosis in patients with this rare disease.

During monitoring:

- PCR predicts cytogenetic and haematological relapse before cytogenetic testing, although with some false positives and false negatives.
- Early detection allows the institution of early salvage therapy.

- The literature on the effectiveness of early therapy compared with later therapy is limited but consistent with improved outcome beyond what could reasonably be attributed to lead-time bias.
- Therefore, the above conclusions support improved patient outcome in patients tested by PCR during monitoring of APL.

Cost-effectiveness

The economic analysis evaluating the use of PCR in the diagnosis of APL found the incremental cost per life year saved was \$329 for cytogenetic and PCR testing compared with cytogenetic testing alone. The incremental cost per life year saved was \$6,418 for monitoring with cytogenetic plus PCR testing combined compared with cytogenetic testing alone.

Limitations to the economic evaluation included:

- the use of existing data rather than trial based data;
- the use of laboratory price as a proxy for cost.

Sensitivity analysis suggested the incremental cost-effectiveness ratio in the diagnostic model was insensitive to inpatient management cost, management strategies for the cytogenetic test negative group and life expectancy estimates. These life expectancy estimates included the proportion of early deaths in patients treated with non-ATRA-containing regimens as a result of false negative diagnostic tests. The monitoring model was sensitive to variation in PCR cost, life expectancy estimates and change in inpatient cost estimates. The maximal incremental cost per life year saved across a plausible range of sensitivity analyses in the monitoring model was \$16,269.

Overall, although the cost estimates were higher in the combined PCR and cytogenetic testing strategy compared with cytogenetic testing alone in both the diagnosis and monitoring models, the extra benefit in terms of life years saved suggest favourable indicative cost-effectiveness ratios for the use of combined cytogenetic and PCR testing.

Recommendation

MSAC recommended that on the strength of evidence pertaining to safety, effectiveness and cost effectiveness of polymerase chain reaction (PCR) testing in the diagnosis and monitoring of acute promyelocytic leukaemia (APL), public funding should be supported for this procedure.

- The Minister for Health and Ageing accepted this recommendation on 8 August 2003 -

Appendix A MSAC terms of reference and membership

MSAC's terms of reference are to:

- advise the Minister for Health and Ageing on the strength of evidence pertaining to new and emerging medical technologies and procedures in relation to their safety, effectiveness and cost-effectiveness and under what circumstances public funding should be supported;
- advise the Minister for Health and Ageing on which new medical technologies and procedures should be funded on an interim basis to allow data to be assembled to determine their safety, effectiveness and cost-effectiveness;
- advise the Minister for Health and Ageing on references related either to new and/or existing medical technologies and procedures; and
- undertake health technology assessment work referred by the Australian Health Ministers' Advisory Council (AHMAC) and report its findings to AHMAC.

The membership of MSAC comprises a mix of clinical expertise covering pathology, nuclear medicine, surgery, specialist medicine and general practice, plus clinical epidemiology and clinical trials, health economics, consumers, and health administration and planning:

Member	Expertise or Affiliation
Dr Stephen Blamey (Chair)	general surgery
Professor Bruce Barraclough	general surgery
Professor Syd Bell	pathology
Dr Paul Craft	clinical epidemiology and oncology
Professor Ian Fraser	reproductive medicine
Professor Jane Hall	health economics
Dr Terri Jackson	health economics
Ms Rebecca James	consumer health issues
Professor Brendon Kearney	health administration and planning
Mr Chris Sheedy	Assistant Secretary, Diagnostics and Technology Branch, Australian Government Department of Health and Ageing
Associate Professor Richard King	internal medicine
Dr Ray Kirk	health research
Dr Michael Kitchener	nuclear medicine
Mr Lou McCallum	consumer health issues
Dr Ewa Piejko	general practice
Professor John Simes	clinical epidemiology and clinical trials

Professor Richard Smallwood	Chief Medical Officer, Australian Government Department of Health and Ageing
Dr Robert Stable	representing the Australian Health Ministers' Advisory Council
Professor Bryant Stokes	neurological surgery
Associate Professor Ken Thomson	radiology
Dr Douglas Travis	urology

Appendix B Supporting committee

Supporting committee for MSAC reference 9a

Professor Sydney Bell (Chair) MBBS, FRCPA, MD Area Director of Microbiology South East Sydney Area Health Service, NSW	member of MSAC
Associate Professor Mark Hertzberg MBBS, PhD, FRACP, FRCPA Senior Staff Specialist Haematologist Westmead Hospital, NSW	nominated by the Haematology Society of Australia and New Zealand
Associate Professor Harry Iland MBBS, FRACP, FRCPA Senior Staff Specialist Haematologist Kanematsu Laboratories & Institute of Haematology Royal Prince Alfred Hospital, NSW	co-opted member
Dr Paula Marlon MBBS (Hons I), FRACP, FRCPA Assistant Director of Haematology Senior Staff Haematologist, Princess Alexandra Hospital, Qld	nominated by the Royal College of Pathologists of Australasia
Dr Paul Craft MBBS, FRACP, MPH Director, Medical Oncology Unit The Canberra Hospital, ACT	MSAC member
Dr John Primrose (member until April 2002) MBBS (Hons), FRACP Senior Medical Adviser Health Access and Financing Division Department of Health and Ageing	medical adviser to MSAC until February 2002
Mr Michael Ralston FIBMS, FAIMS, FRCPA Managing Director Gippsland Pathology Service, Vic	nominated by the Pathology Services Table Committee

Appendix C BDO/Corrs report recommendations

Table C BDO/Corrs report recommendations

	Recommendation
2	The Australian Pathology Laboratory Accreditation Arrangements
2.1	That the current Australian pathology laboratory accreditation arrangements are fundamentally sound and should be maintained.
2.2	That the DHA, in partnership with State and Territory health authorities, undertakes a detailed evaluation of the need for, and potential costs and benefits of, additional legislation in all jurisdictions to complement the Australian pathology laboratory accreditation arrangements.
3	Standard Setting in Pathology
3.1	That in order to reinforce NPAAC's role as the standard setting body within the pathology industry, guidelines, standards or interpretive documents are only incorporated under the Principles after their explicit approval by NPAAC for that purpose.
3.2	That only documents that are incorporated under the Principles are used for the purposes of assessing a laboratory's accreditation status.
3.3	That cross representation is established between the governing body of Standards Australia and NPAAC.
3.4	That membership of NPAAC is reviewed to ensure appropriate representation of all stakeholder groups.
3.5	That NPAAC documents its processes for identifying and prioritising areas for standards development.
3.6	That the DHA contracts additional assistance for NPAAC as required, to enable NPAAC to focus on its policy and strategic responsibilities rather than on document drafting.
3.7	That NPAAC adopts and promulgates a standard process for standards development.
3.8	That NPAAC adopts consistent terminology in all of its documents.
3.9	That NPAAC ensures that the need for a new standard is rigorously assessed prior to its development.
3.10	That NPAAC documents a policy of adopting, where possible, international standards, and formally documenting its reasons when it modifies or does not adopt international standards.
3.11	That NPAAC standards be Clearly cross-referenced to relevant international standards.
3.12	That NPAAC pursues accreditation by Standards Australia as a Standards Development Organisation.
3.13	That NPAAC's Order in Council be amended to ensure its activities are focussed on standards development and policy, and that it does not engage in advising on the eligibility for accreditation of specific laboratories.
4	The NATA/RCPA Accreditation Process
4.1	That the NATA/RCPA accreditation assessment process is capable of reliably identifying laboratories that pose a significant threat to public health, and should continue to be the preferred mechanism by which such laboratories are identified for the purposes of the HIC initiating compliance measures.
4.2	That the composition of NATA/RCPA accreditation teams continues to include NATA staff officers and peer assessors.
4.3	That NATA provides incentives to all laboratories for participation in assessment processes to ensure that voluntary assessor participation more Closely balances utilisation of assessor resources.
4.4	That the current maximum three year interval between accreditation assessments is maintained, but that NATA/RCPA continues to develop more efficient means of assessment, including greater use of desktop audits and a flexible approach to the composition of assessment teams and the scope of assessments in laboratories with a history of excellent compliance.

Table C BDO/Corrs report recommendations (continued)

	Recommendation
5	External Quality Assurance Programs
5.1	That the DHA and the HIC seek the cooperation of the RCPA QAP to establish explicit external quality assurance performance criteria, initially in chemical pathology and gynaecological cytology, and a mechanism for the RCPA QAP to identify relatively poorly performing laboratories.
5.2	That RCPA QAP and other external quality assurance providers regularly submit to NATA reports identifying laboratories that are poorly performing according to these agreed performance criteria.
5.3	That the DHA and the HIC further consider whether the RCPA QAP should receive the benefit of statutory immunity during any pilot program and this is evaluated as a part of that program.
5.4	That NPAAC's Criteria for Assessment of External Quality Assurance Programs incorporates a requirement of inclusion of effective mechanisms to identify and deter gaming/cheating in quality assurance programs.
5.5	That NPAAC standards require: an effective adverse events monitoring, reporting, analysis and remediation program in all laboratories; that all pathologists and scientists undertake adequate continuing education (CE) and are subject to regular, effective performance appraisal; and that records of these processes are available for NATA/RCPA review at the time of laboratory assessment.
5.6	That the DHA more closely aligns compliance requirements for APAs, APPs and APLs in order to ensure that professionals who have been repeatedly associated with poorly performing APLs are identified and a more thorough assessment is undertaken of their suitability to continue as an APP or APA.
6	The Legal Basis of Compliance
6.1	That the objection process for NATA/RCPA assessment is streamlined, such that laboratories are provided with a single opportunity to object within a time limit of 14 days to up to three proposed assessors and up to two proposed dates for assessment.
6.2	That a new Deed between the HIC and NATA is drafted to incorporate: detailed key performance indicators; timeframes for assessment and reporting back; a specific reporting format and required content, attached as a schedule to the Deed; a confidentiality undertaking; and sanctions if NATA defaults under the Deed.
6.3	That the range of sanctions available to the HIC for dealing with non-compliant laboratories is increased, such that escalating sanctions are available in response to escalating levels of non-compliance. These sanctions should include: a requirement that a laboratory undergoes reinspection, as directed by the HIC, with the inspection being undertaken by an inspection team acting at the direction of the HIC; a requirement that a laboratory develops a quality management plan, the implementation of which would be directly overseen by NATA/RCPA with reports forwarded to the HIC; public notification by the HIC on its website of all laboratories whose rating is Non-Compliant with Moderate or Serious Risk; a discretion that the HIC may require a laboratory to directly notify referring medical practitioners and/or consumers of their assessed non-compliance with NPAAC standards when assessed as Non-Compliant with Moderate or Serious Risk; and revocation of eligibility for Medicare benefits.
6.4	That the HIC: establishes a position of HIC contract manager to manage the relationship between NATA and the HIC; considers options to more effectively utilise the expert panel available under section 12 of the Principles; and attends NATA/RCPA field inspections on a supernumerary basis.

Table C BDO/Corrs report recommendations (continued)

	Recommendation
6.5	That pending any initiatives by the Australian Council for Safety and Quality in Health Care to develop stewardship and overarching quality monitoring structures, the DHA allocates responsibility to a senior officer in the Diagnostics and Technology Branch to receive regular, structured reports from the HIC contract manager on the overall quality of pathology services, and to initiate any necessary policy responses to those reports.
6.6	That the DHA coordinates any necessary consumer/referring practitioner notification regarding a breakdown in quality of a screening or diagnostic test and that it develops a comprehensive policy to manage this process.
6.7	<p>That the Principles are amended to include:</p> <p>a specific provision for the Minister to approve the independent body qualified to assess the suitability of the premises to operate as an APL;</p> <p>a new Rating System incorporating the following ratings;</p> <p>Compliant – Approval with Merit;</p> <p>Compliant;</p> <p>Non-Compliant with Minor Risk;</p> <p>Non-Compliant with Moderate Risk; and</p> <p>Non-Compliant with Serious Risk.</p> <p>The sanctions available to the HIC as incorporated in Recommendation 6.3; and</p> <p>The HIC's right to require the approved independent body to undertake an inspection of a laboratory with or without notice where the laboratory is applying for Approval as an APL, is currently operating under an Approval as an APL or in any other circumstance as determined by the HIC.</p>
6.8	<p>That the HIC Application for Approval as an APL is amended to include:</p> <p>A statement that the HIC has the right to determine the nature and conditions of the inspections to be undertaken;</p> <p>A requirement that the laboratory consents to the independent assessment body providing reports to the HIC;</p> <p>A requirements that the laboratory consents to its external quality assurance providers sending exception reporting data to NATA/RCPA as required by the HIC and in the form nominated by the HIC;</p> <p>A requirement that the laboratory uses its best endeavours to cooperate with the NATA/RCPA assessment team and where relevant, the HIC appointed expert panel;</p> <p>A provision Clarifying that the HIC may order the inspection of the laboratory with or without notice;</p> <p>Provisions Clarifying and limiting the grounds upon which the laboratory may use arguments regarding conflict of interest to reject assessors;</p> <p>A requirements that the laboratory complies with specified assessment timeframes; and</p> <p>An information package setting out the rating system and detailing the range of options available to the HIC where NATA/RCPA or other approved assessment agency assesses the laboratory as non-compliance with the NPAAC accreditation materials.</p>

Table C BDO/Corrs report recommendations (continued)

	Recommendation
6.9	That NATA's application forms are amended to explicitly identify that information relating to accreditation assessments may be provided to both the RCPA (if that practice continues) and the HIC for the purposes of accreditation under the Health Insurance Act.
6.10	That NATA amends the document <i>Accreditation Enquiry Medical Testing</i> to clarify that NATA's role in the HIC Approval process is limited to providing reports to the HIC.
6.11	That NATA ensures that its by-laws do not prohibit it undertaking, at the request of the HIC, inspections of non-member laboratories without notice.
6.12	That the joint naming of the NATA/RCPA accreditation arrangements ceases, but that the RCPA continues to be represented on committees and forums within NATA where policy is determined and professional input is required.

Appendix D Website sources of information

Table D Website sources of information

HTA Organisations	Website URL
Agence d'Evaluation des Technologies et des Modes d'Intervention (AETMIS)	http://www.aetmis.gouv.qc.ca/
Agencia de Evaluacion de Tecnologias Sanitarias (AETS)	http://www.isciii.es/unidad/aet/caet.html
Agencia de Evaluacion de Tecnologias Sanitarias de Andalucia (AETSA)	http://www.csalud.junta-andalucia.es/orgdep/AETSA/
Alberta Heritage Foundation for Medical Research (AHFMR)	http://www.ahfmr.ab.ca/
Agency for Health Research Quality (AHRQ)	http://www.ahrq.gov
L'Agence nationale d'Accréditation et d'Évaluation en Santé	http://www.anaes.fr
L'Agence Nationale pour le Développement de l'Évaluation Médicale (ANDEM)	http://www.upml.fr/andem/andem.htm
British Columbia Office of Health Technology Assessment (BCOHTA)	http://www.chspr.ubc.edu.ca/bcohta
Catalan Agency for Health Technology Assessment (CAHTA)	http://www.aatm.es/
Canadian Coordinating Office for Health Technology Assessment (CCOHTA)	http://www.ccohta.ca
Centre for Clinical Effectiveness, Monash University	http://www.med.monash.edu.au/healthservices/cce/
Center for Medical Technology Assessment (CMT)	http://ghan.imt.liu.se/cmt/
College voor Zorgverzekeringen (CVZ)	
German Agency for Health Technology Assessment at the German Institute for Medical Documentation and Information (DIMDI)	http://www.dahta.dimdi.de/
Danish Centre for Evaluation and Health Technology Assessment (DACEHTA)	http://www.dihta.dk/
Danish Institute for Health Services Research (DSI)	http://www.dsi.dk/
ECRI (USA)	http://www.ecri.org
Unidad de Tecnologias de Salud (ETESA)	http://www.minisal.cl
EUROSCAN	http://www.ad.bham.ac.uk/euroscan/index.asp
Finnish Office for Health Care Technology Assessment (FinOHTA)	http://www.stakes.fi/finohta/
Health Council of the Netherlands (GR)	http://www.gr.nl/
Health Technology Board for Scotland	http://www.htbs.org.uk/
Minnesota Health Technology Advisory Committee (HTAC)	http://www.health.state.mn.us/htac/
Institute for Clinical Systems Improvement (ICSI)	http://www.icsi.org
Institute of Technology Assessment of the Austrian Academy of Science (ITA)	http://www.oeaw.ac.at/ita/hta/
International Network of Agencies for Health Technology Assessment (INAHTA)	http://www.inahta.org
International Society of Technology Assessment in Health Care	http://www.istahc.org
Medical Technology Assessment Group (M-TAG)	http://www.m-tag.net/
Medical Technology and Practice Patterns Institute	http://www.mtpipi.org/

HTA Organisations	Website URL
National Coordinating Centre for Health Technology Assessment (NCCHTA)	http://www.soton.ac.uk/~hta
National Horizon Scanning Centre (NHSC)	http://www.bham.ac.uk/PublicHealth/horizon
National Institute for Clinical Excellence (NICE)	http://www.nice.org.uk/
New Zealand Health Technology Assessment (NZHTA)	http://nzhta.chmeds.ac.nz
Medical and Health Research Council (MW-NWO)	http://www.nwo.nl
Basque Office for Health Technology Assessment (OSTEBA)	http://www.euskadi.net/sanidad/
Swedish Council on Technology Assessment in Health Care (SBU)	http://www.sbu.se
Norwegian Centre for Health Technology Assessment (SMM)	http://www.oslo.sintef.no/smm/
Swiss Science Council/Technology Assessment (SWISS/TA)	http://www.ta-swiss.ch/
TNO Prevention and Health (TNO)	http://www.tno.nl/homepage.html
University Health Consortium Technology Assessment Monitor	http://www.uhc.edu
Veterans' Affairs Technology Assessment Program (VATAP)	http://www.va.gov/vatap/
WHO Health Technology Assessment Programme (Collaborating Centres)	http://www.who.int/pht/technology_assessment/index.html
Other organisations	
Australian National Health & Medical Research Council	http://www.health.gov.au/nhmrc/index.htm
Centre for Health Program Evaluation (Monash University)	http://chpe.buseco.monash.edu.au/
Centres for Medicare and Medicaid Services (US Health Care Financing Administration)	http://www.hcfa.gov
Health Economics Research Group (Brunel University)	www.brunel.ac.uk/depts/herg/
Health Information Research Unit (McMaster University)	http://www.hiru.mcmaster.ca
Health Canada	http://www.hc-sc.gc.ca/
UK Department of Health publications	http://www.doh.gov.uk/publications/index.html
US Centers for Disease Control	http://www.cdc.gov
US National Cancer Institute	http://www.nci.nih.gov
Professional Associations/Societies	
Canadian Medical Association Infobase	http://mdm.ca/cpgsnew/cpgs/index.asp
American Society of Hematology	http://www.hematology.org/
British Society for Haematology	http://www.blackwell-science.com/uk/society/bsh/
European Hematology Association	http://www.ehaweb.org/
North West Haematology Group (UK)	http://www.nwhaems.co.uk/
Royal College of Pathologists	http://www.rcpath.org/
Haematology Society of Australia and New Zealand	http://www.hsanz.org.au
University Pathology Consortium (US)	http://www.upcmd.com/
Lymphoma Forum (UK)	http://www.lymphoma.org.uk/
Medmark Links (Haematology Section)	http://www.medmark.org/hem/
Medical Research Council Leukaemia Clinical Trials	http://leuktrials.uwcm.ac.uk/
Professional Associations/Societies	
Controlled Clinical Trials	http://www.controlled-trials.com/
Clinicaltrials.gov	http://www.clinicaltrials.gov

Appendix E Search Strategy

The following core strategy developed and implemented by an Information Specialist was used in Medline and CancerLit to identify relevant information on nucleic acid amplification techniques. The Medline strategy was adapted for Embase using the relevant subject headings, and simplified for use in the databases without indexing.

Table E.1 Core search strategy

	Search
1	exp polymerase chain reaction/
2	exp nucleic acid amplification techniques/
3	1 or 2
4	"sensitivity and specificity"/
5	false negative reactions/
6	false positive reactions/
7	disease free survival/
8	survival analysis/
9	follow-up studies/
10	neoplasm recurrence, local/
11	neoplasm, residual/
12	predictive value of tests/
13	prognosis
14	prospective studies/
15	recurrence/
16	reproducibility of results/
17	survival rate/
18	evaluation studies/
19	monitor\$
20	(ppv or npv or positive predictive value or negative predictive value).mp.
21	di.fs.
22	or/4-21
23	"costs and cost analysis"/
24	cost benefit analysis/
25	health care costs/
26	ec.fs.
27	cost of illness/
28	health expenditures/
29	cost control/
30	cost savings/
31	(cost or costs or costing\$).mp.
32	or/23-31
33	3 and 22
34	3 and 32
35	33 or 34
36	(news or letter).pt. or case reports/
37	35 not 36
38	limit 38 to English

The core strategy was then linked with each of the four conditions covered in this report (see below).

Table E.2 APL search terms

	Search
1	leukemia, myelocytic, acute/ or leukemia, promyelocytic, acute/
2	(acute myelo\$ leukemia or acute myelo\$ leukaemia).mp.
3	(acute promyelo\$ leukemia or acute promyelo\$ leukaemia).mp.
4	or/1-3
5	(plzf or zinc finger or raralpha or rar alpha or pml rar\$).mp.
6	(5;15 or 15;17 or 11;17).mp.
7	or/5-6
7	4 and 7

Appendix F Description of studies included in the review

Table F Characteristics of studies selected for appraisal in APL

Study Country	Study design	PCR type	Eligibility criteria	n	Patient characteristics
Biondi et al., (1992) Italy	Case series Diagnosis and monitoring	RT-PCR (Qualitative)	APL diagnosed by FAB criteria – AML (M3 and M3v)	35	No demographic details
Burnett et al., (1999) UK	Prospective case series Diagnosis	RT-PCR (Qualitative)	Morphological evidence of APL. Entered into MRC leukaemia working party trial.	239	Median age not stated. Male 120 Female 119
Chen et al., (1992) China and USA	Case series Diagnosis	RT-PCR (Qualitative)	APL diagnosed by FAB criteria – AML (M3)	32	Median age 31 years (Range 5-67) Male 19 Female 13
Diverio et al., (1998) Europe (multicentre)	Prospective case series Monitoring	RT-PCR (Qualitative)	PML-RAR α positive at AIDA entry. PCR negative post consolidation and follow up for \geq 6 months from this point.	163	No demographic details
Fukutani et al., (1995) Japan	Case series Monitoring	RT-PCR (Qualitative)	APL by FAB criteria. In CR more than 5 months post ATRA and chemotherapy	27	No demographic details
Gameiro et al., (2001) Portugal UK	Case series Monitoring	RT-PCR (Qualitative)	APL FAB criteria	47	Median age 27 years (Range 1-69) Male 25 Female 22
Gonzalez et al., (2001) Spain	Prospective, non-consecutive case series Monitoring	RT-PCR (Qualitative)	Newly diagnosed PML/RAR α positive APL enrolled in PETHEMA/LPA96 trial	167	Median age not stated (Range 0-74) Male 93 Female 74
Grimwade et al., (2000) Europe	Case series Diagnosis	RT-PCR (Qualitative)	Cases referred to a workshop. APL lacking t(15;17) using FAB criteria. Cytogenetic analysis successful and molecular analysis performed.	90	Demographic details not stated

Table F Characteristics of studies selected for appraisal in APL (continued)

Study Country	Study design	PCR type	Eligibility criteria	n	Patient characteristics
Gu et al., (2001) China	Case series Diagnosis and monitoring	Real time RT-PCR (Quantitative)	APL-M3 (FAB criteria)	31 (+11 non-APL controls)	Age range 8-74 years Male 19 Female 12
Hu et al., (2000) China	Retrospective case series Monitoring	RT-PCR (Qualitative)	Newly diagnosed APL (FAB criteria), evaluable PCR data, obtained complete remission.	70	Median age 36 years (Range 14-72) Male 39 Female 31
Huang et al., (1993) China	Case series Diagnosis and monitoring	RT-PCR (Qualitative)	APL by FAB criteria (AML M3)	97	No demographic details
Inokuchi et al., (1998) Japan	Case series Monitoring	RT-PCR (Qualitative)	AML by FAB criteria	23 (14 APL)	No demographic details
Jurcic et al., (2001) USA	Consecutive, prospective case series Monitoring	RT-PCR (Qualitative)	APL FAB criteria. Confirmed by RT-PCR ≥ 2 PCR assays 1-3 months apart post treatment	82	Median age 45 years (Range 7-80) Gender breakdown not stated
Jurcic et al., (2000) USA	Prospective, non-consecutive case series Monitoring	RT-PCR (Qualitative)	APL. Complete remission post induction treatment.	35	Median age 45 years (Range 17-71) Male 16 Female 19
Kane et al., (1996) USA	Retrospective, non-consecutive case series Diagnosis	RT-PCR (Qualitative)	APL. Cryopreserved diagnostic bone marrow sample available	22	Median age 13.5 years (Range 3-18) Male 7 Female 15
Lim et al., (2000) Singapore	Consecutive case series Diagnosis	RT-PCR (Qualitative)	APL by FAB criteria	30	Median age 39 years (Range 17-71) Male 18 Female 12
Lo Coco et al., (1992) Italy	Non consecutive, retrospective case series Diagnosis and monitoring	RT-PCR (Qualitative)	APL newly diagnosed or in relapse	35	No demographic details

Table F Characteristics of studies selected for appraisal in APL (continued)

Study Country	Study design	PCR type	Eligibility criteria	n	Patient characteristics
Mancini et al., (1995) Italy	Case series Diagnosis	RT-PCR (Qualitative)	APL on morphological and cytochemical grounds	28	Median age 34 years (Range 2-77) Male 12 Female 16
Martinelli et al., (1998)	Consecutive case series Diagnosis and monitoring	RT-PCR (Qualitative)	APL on AIDA trial. Age < 70 years. Cytogenetic or molecular evidence of t(15;17).	24	Median age 41 years (Range 15-59) Male 15 Female 9
Miller et al., (1992) USA	Case series Diagnosis and monitoring	RT-PCR (Qualitative)	APL by FAB (AML M3). On retinoic acid trial	36	No demographic details
Miller et al., (1993) USA	Case series Monitoring	RT-PCR (Qualitative)	APL by morphology and RT-PCR positive for PML/RAR α at diagnosis	33	No demographic details
Shinjo et al., (2000) Japan	Prospective case series Monitoring	RT-PCR (Qualitative)	APL – achieved CR post ATRA and subsequently relapsed	25	No demographic details
Shivakumar et al., (2002) India	Case series Diagnosis	RT-PCR (Qualitative)	APL by morphology	29	Median age 33 years (Range 2-57) Male 15 Female 14
Xiao et al., (1993) USA	Case series Diagnosis	RT-PCR (Qualitative)	APL by FAB criteria	26 samples, 25 patients	No demographic details

Appendix G Economic assessment

Source of cost estimates

Table G Cost sources and diagnosis related group (DRG) definitions for the major cost elements of APL patient management

Item for Costing	Cost Source	Cost: Private (Public)	Details/Comments
PCR Test	RCPA	\$ 235 per test	Quality Assurance benchmarking data
Cytogenetic Test	MBS	\$ 354 per test	Reimbursement price
Out-Patient Treatment			
ATRA	Hospital pharmacy estimation (January 2003)	\$ 50 per 80 mg dose	
Inpatient treatment			
Bone marrow transplant [‡]	2000-01 public & private sector, AR-DRG 4.1 estimated cost weights	A04Z - \$ 15,235 (31,785)	Very high cost procedures are assigned pre major diagnostic category DRGs, eg bone marrow transplant (A04Z)
Leukaemia & lymphoma DRGs [†]	2000-01 public & private sector, AR-DRG 4.1 estimated cost weights	R01A - \$ 13,315 (24,214) R01B - \$ 1,910 (7,499) R03A - \$ 9,465 (20,994) R03B - \$ 2,094 (3,391) R60A - \$ 12,012 (19,508) R60B - \$ 1,841 (4,999) R60C - \$ 1,668 (2,622) R61A - \$ 7,611 (10,656) R61B - \$ 1,884 (3,857) R61C - \$ 429 (634)	Any separation with a diagnosis of CML and coded to one of the following DRGs: Lymphoma & leukaemia with <i>major</i> operating room procedures (R01) Lymphoma & leukaemia with <i>other</i> operating room procedures (R03) Acute leukaemia (R60) Lymphoma & non-acute leukaemia (R61)

Table G Cost Sources and Diagnosis Related Group (DRG) definitions for the major cost elements of CML patient management (continued)

Item for Costing	Cost Source	Cost: Private (Public)	Details/Comments
Other related & haematological DRGs ^I	2000-01 public & private sector, AR-DRG 4.1 estimated cost weights	Q01Z - \$ 5,552 (9,338) Q02A - \$ 7,387 (13,812) Q02B - \$ 1,669 (2,257) Q60A - \$ 4,553 (6,239) Q60B - \$ 878 (1,231) Q61A - \$ 4,776 (5,113) Q61B - \$ 2,255 (2,491) Q61C - \$ 920 (1,088) Q62A - \$ 2,065 (2,633) Q62B - \$ 1,570 (1,601) R63Z - \$ 556 (613) T60A - \$ 5,304 (7,002) T60B - \$ 3,132 (3,497)	Any separation with a diagnosis of CML and coded to one of the following DRGs: Splenectomy (Q01Z) Other operating room procedures of blood and blood forming organs (Q02) Reticuloendothelial & immunity disorders (Q60) Red blood cell disorders, including leukocyte transfusion (Q61) Coagulation disorders (Q62) Chemotherapy (R63Z) Septicaemia (T60)

H Very high cost procedures such as bone marrow transplant are coded separately and do not have clinical complexity (CCL) grading.

I The adjacent DRGs are subdivided according to level of clinical complexity and co-morbidity based on a number of factors including complicating diagnoses or procedures, age and/or patient clinical complexity level, eg DRG R61 is coded as R61A (high CCL), R61B (medium CCL), or R61C (low CCL).

Abbreviations

95% CI	95 per cent confidence interval
AHTAC	Australian Health Technology Advisory Committee
AIDA	all <i>trans</i> retinoic acid and idarubicin
ALL	acute lymphocytic leukaemia
ALLG	Australasian Leukaemia and Lymphoma Group
AML	acute myeloid leukaemia
APL	acute promyelocytic leukaemia
AR-DRG	Australian Refined Diagnostic Related Groups
ATRA	all <i>trans</i> retinoic acid
BDNA	branched DNA
BMT	bone marrow transplant
CML	chronic myeloid leukaemia
CR	complete remission
DARE	Database of Abstracts of Reviews of Effectiveness
DLI	donor lymphocyte infusion
DNA	deoxyribonucleic acid
DOR	diagnostic odds ratio
EED	Economic Evaluation Database
Exp	power to base e
FISH	fluorescence in situ hybridisation
GVHD	graft versus host disease
HIV	human immunodeficiency virus
HTA	Health Technology Assessment
ln	natural logarithm
LR-	negative likelihood ratio
LR+	positive likelihood ratio

MRD	minimal residual disease
MSAC	Medicare Services Advisory Committee
NAA	nucleic acid amplification
NATA	National Association of Testing Authorities
NHMRC	National Health and Medical Research Council
NHS	National Health Service
NPAAC	National Pathology Accreditation Advisory Council
PCR	polymerase chain reaction
Ph	Philadelphia
PSTC	Pathology Services Table Committee
QA	Quality Assurance
QC	Quality Control
RCPA	Royal College of Pathologists of Australasia
RCT	randomised controlled trial
RNA	ribonucleic acid
RT-PCR	reverse transcriptase polymerase chain reaction
SCT	stem cell transplant

References

- Asou, N., Adachi, K., Tamura, J., Kanamaru, A., Kageyama, S., Hiraoka, A., Omoto, E., et al. (2001) 'Analysis of prognostic factors in newly diagnosed patients with acute promyelocytic leukemia: the APL92 study of the Japan Adult Leukemia Study Group (JALSG)', *Cancer Chemotherapy & Pharmacology*, 48(Suppl 1), S65-S71.
- Australia. National Pathology Accreditation Advisory Council (2000) *Laboratory accreditation standards and guidelines for nucleic acid detection techniques*, Commonwealth Department of Health & Ageing, Canberra.
- Biondi, A., Rambaldi, A., Pandolfi, P. P., Rossi, V., Giudici, G., Alcalay, M., Lo Coco, F., et al. (1992) 'Molecular monitoring of the myl/retinoic acid receptor-alpha fusion gene in acute promyelocytic leukemia by polymerase chain reaction', *Blood*, 80(2), 492-497.
- Burnett, A., Goldstone, A. H. and Wheatley, K. (2002) *AML15 Medical Research Council Working Parties on Leukaemia in Adults and Children. Acute myeloid leukaemia trial 15. Protocol for patients aged under 60 years. Trial reference 15RCTN 17161961*, University of Birmingham Clinical Trials Unit, Birmingham.
- Burnett, A. K., Grimwade, D., Solomon, E., Wheatley, K. and Goldstone, A. H. (1999) 'Presenting white blood cell count and kinetics of molecular remission predict prognosis in acute promyelocytic leukemia treated with all-trans retinoic acid: result of the Randomized MRC Trial', *Blood*, 93(12), 4131-4143.
- Castaigne, S., Chomienne, C., Daniel, M. T., Ballerini, P., Berger, R., Fenaux, P. and Degos, L. (1990) 'All-trans retinoic acid as a differentiation therapy for acute promyelocytic leukemia. I. Clinical results', *Blood*, 76(9), 1704-1709.
- Chen, S. J., Chen, Z., Chen, A., Tong, J. H., Dong, S., Wang, Z. Y., Waxman, S., et al. (1992) 'Occurrence of distinct PML-RAR-alpha fusion gene isoforms in patients with acute promyelocytic leukemia detected by reverse transcriptase/polymerase chain reaction', *Oncogene*, 7(6), 1223-1232.
- Chen, Z. X., Xue, Y. Q., Zhang, R., Tao, R. F., Xia, X. M., Li, C., Wang, W., et al. (1991) 'A Clinical and experimental study on all-trans retinoic acid-treated acute promyelocytic leukemia patients', *Blood*, 78(6), 1413-1419.
- Degos, L. (1992) 'All-trans retinoic acid (ATRA) therapeutical effect in acute promyelocytic leukemia', *Biomedicine & Pharmacotherapy*, 46(5-7), 201-209.
- Degos, L., Chomienne, C., Daniel, M. T., Berger, R., Dombret, H., Fenaux, P. and Castaigne, S. (1990) 'Treatment of first relapse in acute promyelocytic leukaemia with all-trans retinoic acid', *Lancet*, 336(8728), 1440-1441.
- Degos, L. and Wang, Z. Y. (2001) 'All trans retinoic acid in acute promyelocytic leukemia', *Oncogene*, 20(49), 7140-7145.
- Diverio, D., Rossi, V., Avvisati, G., De Santis, S., Pistilli, A., Pane, F., Saglio, G., et al. (1998) 'Early detection of relapse by prospective reverse transcriptase-polymerase chain reaction analysis of the PML/RARalpha fusion gene in patients with acute promyelocytic leukemia

enrolled in the GIMEMA-AIEOP multicenter "AIDA" trial. GIMEMA-AIEOP Multicenter "AIDA" Trial', *Blood*, 92(3), 784-789.

Douer, D. (2000) 'Transcription therapy for acute promyelocytic leukaemia', *Expert Opinion on Investigational Drugs*, 9(2), 329-346.

Douer, D., Estey, E., Santillana, S., Bennett, J. M., Lopez-Bernstein, G., Boehm, K. and Williams, T. (2001) 'Treatment of newly diagnosed and relapsed acute promyelocytic leukemia with intravenous liposomal all-trans retinoic acid', *Blood*, 97(1), 73-80.

Egger, M., Smith, G. D. and Altman, D. G. (Eds.) (2001) *Systematic reviews in health care: meta-analysis in context*, BMJ Books, London.

Fenaux, P., Castaigne, S., Dombret, H., Archimbaud, E., Duarte, M., Morel, P., Lamy, T., et al. (1992) 'All-transretinoic acid followed by intensive chemotherapy gives a high complete remission rate and may prolong remissions in newly diagnosed acute promyelocytic leukemia: a pilot study on 26 cases', *Blood*, 80(9), 2176-2181.

Fenaux, P., Chastang, C., Chevret, S., Sanz, M., Dombret, H., Archimbaud, E., Fey, M., et al. (1999) 'A randomized comparison of all transretinoic acid (ATRA) followed by chemotherapy and ATRA plus chemotherapy and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia. The European APL Group', *Blood*, 94(4), 1192-1200.

Fenaux, P., Chevret, S., Guerci, A., Fegueux, N., Dombret, H., Thomas, X., Sanz, M., et al. (2000) 'Long-term follow-up confirms the benefit of all-trans retinoic acid in acute promyelocytic leukemia', *Leukemia*, 14(8), 1371-1377.

Fenaux, P., Chomienne, C. and Degos, L. (2001) 'Treatment of acute promyelocytic leukaemia', *Bailliere's Best Practice in Clinical Haematology*, 14(1), 153-174.

Fenaux, P., Ledele, M. C., Castaigne, S., Archimbaud, E., Chomienne, C., Link, H., Guerci, A., et al. (1993) 'Effect of all transretinoic acid in newly diagnosed acute promyelocytic leukemia - results of a multicenter randomized trial', *Blood*, 82(11), 3241-3249.

Ferrant, A., Labopin, M., Frassoni, F., Prentice, H. G., Cahn, J. Y., Blaise, D., Reiffers, J., et al. (1997) 'Karyotype in acute myeloblastic leukemia: prognostic significance for bone marrow transplantation in first remission: a European Group for Blood and Marrow Transplantation study. Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT)', *Blood*, 90(8), 2931-2938.

Fukutani, H., Naoe, T., Ohno, R., Yoshida, H., Kiyoi, H., Miyawaki, S., Morishita, H., et al. (1995) 'Prognostic significance of the RT-PCR assay of PML-RARA transcripts in acute promyelocytic leukemia. The Leukemia Study Group of the Ministry of Health and Welfare (Kouseisho)', *Leukemia*, 9(4), 588-593.

Gameiro, P., Vieira, S., Carrara, P., Silva, A. L., Diamond, J., de Sousa, A. B., Mehta, A. B., et al. (2001) 'The PML-RAR alpha transcript in long-term follow-up of acute promyelocytic leukemia patients', *Haematologica*, 86(6), 577-585.

Gift, T. L., Pate, M. S., Hook, E. W., 3rd and Kassler, W. J. (1999) 'The rapid test paradox: when fewer cases detected lead to more cases treated: a decision analysis of tests for chlamydia trachomatis', *Sexually Transmitted Diseases*, 26(4), 232-240.

- Gonzalez, M., Barragan, E., Bolufer, P., Chillon, C., Colomer, D., Borstein, R., Calasanz, M. J., et al. (2001) 'Pretreatment characteristics and Clinical outcome of acute promyelocytic leukaemia patients according to the PML-RAR alpha isoforms: a study of the PETHEMA group', *British Journal of Haematology*, 114(1), 99-103.
- Grimwade, D., Biondi, A., Mozziconacci, M. J., Hagemeijer, A., Berger, R., Neat, M., Howe, K., et al. (2000) 'Characterization of acute promyelocytic leukemia cases lacking the CIassic t(15;17): results of the European Working Party', *Blood*, 96(4), 1297-1308.
- Gu, B. W., Hu, J., Xu, L., Yan, H., Jin, W. R., Zhu, Y. M., Zhao, W. L., et al. (2001) 'Feasibility and Clinical significance of real-time quantitative RT-PCR assay of PML-RARalpha fusion transcript in patients with acute promyelocytic leukemia', *Hematology Journal*, 2(5), 330-340.
- Howell, M. R., Quinn, T. C., Brathwaite, W. and Gaydos, C. A. (1998) 'Screening women for chlamydia trachomatis in family planning clinics: the cost-effectiveness of DNA amplification assays', *Sexually Transmitted Diseases*, 25(2), 108-117.
- Hu, J., Shen, Z. X., Sun, G. L., Chen, S. J., Wang, Z. Y. and Chen, Z. (1999) 'Long-term survival and prognostic study in acute promyelocytic leukemia treated with all-trans-retinoic acid, chemotherapy, and As2O3: an experience of 120 patients at a single institution', *International Journal of Hematology*, 70(4), 248-260.
- Hu, J., Yu, T., Zhao, W. L., Gu, B. W., Shen, Z. X., Li, X. S., Sun, G. L., et al. (2000) 'Impact of RT-PCR monitoring on the long-term survival in acute promyelocytic leukemia', *Chinese Medical Journal*, 113(10), 899-902.
- Huang, M. E., Ye, Y. C., Chen, S. R., Chai, J. R., Lu, J. X., Zhoa, L., Gu, L. J., et al. (1988) 'Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia', *Blood*, 72(2), 567-572.
- Huang, W., Sun, G. L., Li, X. S., Cao, Q., Lu, Y., Jang, G. S., Zhang, F. Q., et al. (1993) 'Acute promyelocytic leukemia: Clinical relevance of two major PML-RAR alpha isoforms and detection of minimal residual disease by retrotranscriptase/polymerase chain reaction to predict relapse', *Blood*, 82(4), 1264-1269.
- Inokuchi, K., Iwakiri, R., Futaki, M., Hanawa, H., Tanosaki, S., Nomura, T. and Dan, K. (1998) 'Minimal residual disease in acute myelogenous leukemia with PML/RAR alpha or AML1/ETO mRNA and phenotypic analysis of possible T and natural killer cells in bone marrow', *Leukemia & Lymphoma*, 29(5-6), 553-561.
- Irwig, L., Glasziou, P. and et al. (1996) *Screening and diagnostic tests: the Cochrane Methods Working Group on Systematic Review of Screening and Diagnostic Tests: recommended methods*, Cochrane Collaboration, Available from: <http://som.flinders.edu.au/fusa/cochrane>.
- Irwig, L., Tosteson, A. N., Gatsonis, C., Lau, J., Colditz, G., Chalmers, T. C. and Mosteller, F. (1994) 'Guidelines for meta-analyses evaluating diagnostic tests', *Annals of Internal Medicine*, 120(8), 667-676.
- Jaeschke, R., Guyatt, G. and Sackett, D. L. (1994a) 'Users' guides to the medical literature III. How to use an article about a diagnostic test. A. Are the results of the study valid', *JAMA*, 271(5), 389-391.

- Jaeschke, R., Guyatt, G. and Sackett, D. L. (1994b) 'Users' guides to the medical literature III. How to use an article about a diagnostic test. B. What are the results and will they help me in caring for my patients?', *JAMA*, 271(9), 703-707.
- Jurcic, J. G., DeBlasio, T., Dumont, L., Yao, T. J. and Scheinberg, D. A. (2000) 'Molecular remission induction with retinoic acid and anti-CD33 monoclonal antibody HuM195 in acute promyelocytic leukemia', *Clinical Cancer Research*, 6(2), 372-380.
- Jurcic, J. G., Nimer, S. D., Scheinberg, D. A., DeBlasio, T., Warrell, R. P. and Miller, W. H. (2001) 'Prognostic significance of minimal residual disease detection and PML/RAR-alpha isoform type: long-term follow-up in acute promyelocytic leukemia', *Blood*, 98(9), 2651-2656.
- Kanamaru, A., Takemoto, Y., Tanimoto, M., Murakami, H., Asou, N., Kobayashi, T., Kuriyama, K., et al. (1995) 'All-trans retinoic acid for the treatment of newly diagnosed acute promyelocytic leukemia. Japan Adult Leukemia Study Group', *Blood*, 85(5), 1202-1206.
- Kane, J. R., Head, D. R., Balazs, L., Hulshof, M. G., Motroni, T. A., Raimondi, S. C., Carroll, A. J., et al. (1996) 'Molecular analysis of the PML/RAR alpha chimeric gene in pediatric acute promyelocytic leukemia', *Leukemia*, 10(8), 1296-1302.
- Lim, L. C., Vellupillai, M. and Ghafar, A. A. (2000) 'Clinico-biological features of 30 patients with acute promyelocytic leukemia and response to combination induction chemotherapy with all-trans retinoic acid and anthracycline', *Medical Oncology*, 17(4), 301-306.
- Lo Coco, F., Diverio, D., Avvisati, G., Petti, M. C., Meloni, G., Pogliani, E. M., Biondi, A., et al. (1999) 'Therapy of molecular relapse in acute promyelocytic leukemia', *Blood*, 94(7), 2225-2259.
- Lo Coco, F., Diverio, D., Pandolfi, P. P., Biondi, A., Rossi, V., Avvisati, G., Rambaldi, A., et al. (1992) 'Molecular evaluation of residual disease as a predictor of relapse in acute promyelocytic leukaemia', *Lancet*, 340(8833), 1437-1438.
- Lo Coco, F., Nervi, C., Avvisati, G. and Mandelli, F. (1998) 'Acute promyelocytic leukemia: a curable disease', *Leukemia*, 12(12), 1866-1880.
- Mancini, M., Nanni, M., Cedrone, M., Diverio, D., Avvisati, G., Riccioni, R., De Cuia, M. R., et al. (1995) 'Combined cytogenetic, FISH and molecular analysis in acute promyelocytic leukaemia at diagnosis and in complete remission', *British Journal of Haematology*, 91(4), 878-884.
- Mandelli, F., Diverio, D., Avvisati, G., Luciano, A., Barbui, T., Bernasconi, C., Broccia, G., et al. (1997) 'Molecular remission in PML/RAR alpha-positive acute promyelocytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy. Gruppo Italiano-Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups', *Blood*, 90(3), 1014-1021.
- Martinelli, G., Ottaviani, E., Testoni, N., Visani, G., Diverio, D., D'Elia, G., Mandelli, F., et al. (1998) 'Disappearance of PML/RAR alpha acute promyelocytic leukemia-associated transcript during consolidation chemotherapy', *Haematologica*, 83(11), 985-988.
- Medicare Services Advisory Committee (2000) *Funding for new medical technologies and procedures: application and assessment guidelines*, Medicare Services Advisory Committee, Canberra.
- Meloni, G., Diverio, D., Vignetti, M., Avvisati, G., Capria, S., Petti, M. C., Mandelli, F., et al. (1997) 'Autologous bone marrow transplantation for acute promyelocytic leukemia in second

remission: prognostic relevance of pretransplant minimal residual disease assessment by reverse-transcription polymerase chain reaction of the PML/RAR alpha fusion gene', *Blood*, 90(3), 1321-1325.

Miller, W. H., Jr., Kakizuka, A., Frankel, S. R., Warrell, R. P., Jr., DeBlasio, A., Levine, K., Evans, R. M., et al. (1992) 'Reverse transcription polymerase chain reaction for the rearranged retinoic acid receptor alpha clarifies diagnosis and detects minimal residual disease in acute promyelocytic leukemia', *Proceedings of the National Academy of Sciences of the United States of America*, 89(7), 2694-2698.

Miller, W. H., Levine, K., Deblasio, A., Frankel, S. R., Dmitrovsky, E. and Warrell, R. P. (1993) 'Detection of minimal residual disease in acute Promyelocytic Leukemia by a Reverse Transcription-Polymerase Chain-Reaction Assay for the Pml Rar-Alpha Fusion Messenger-Rna', *Blood*, 82(6), 1689-1694.

Nabhan, C., Mehta, J. and Tallman, M. S. (2001) 'The role of bone marrow transplantation in acute promyelocytic leukemia', *Bone Marrow Transplantation*, 28(3), 219-226.

NHMRC (1999) *A guide to the development, implementation and evaluation of Clinical practice guidelines*, National Health and Medical Research Council, Canberra.

NHMRC (2000) *How to use the evidence: assessment and application of scientific evidence*, National Health and Medical Research Council, Canberra.

Nigrovic, L. E. and Chiang, V. W. (2000) 'Cost analysis of enteroviral polymerase chain reaction in infants with fever and cerebrospinal fluid pleocytosis', *Archives of Pediatrics & Adolescent Medicine*, 154(8), 817-821.

Niu, C., Yan, H., Yu, T., Sun, H. P., Liu, J. X., Li, X. S., Wu, W., et al. (1999) 'Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: Remission induction, follow-up, and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients', *Blood*, 94(10), 3315-3324.

Rau, N. V. and Libman, M. D. (1999) 'Laboratory implementation of the polymerase chain reaction for confirmation of pulmonary tuberculosis', *European Journal of Clinical Microbiology & Infectious Diseases*, 18(1), 35-41.

Sanz, M., Arcese, W. and de la Rubia, J. (2000) 'Stem cell transplantation (SCT) for acute promyelocytic leukemia in the ATRA era: a survey of the European Blood and Marrow Transplantation Group (EBMT)', *Blood*, 96 Supp 1522a Abstract 2247.

Sanz, M. A., Martin, G., Rayon, C., Esteve, J., Gonzalez, M., Diaz-Mediavilla, J., Bolufer, P., et al. (1999) 'A modified AIDA protocol with anthracycline-based consolidation results in high antileukemic efficacy and reduced toxicity in newly diagnosed PML/RAR alpha-positive acute promyelocytic leukemia', *Blood*, 94(9), 3015-3021.

Scott, W. J., Shepherd, J. and Gambhir, S. S. (1998) 'Cost-effectiveness of FDG-PET for staging non-small cell lung cancer: a decision analysis', *Annals of Thoracic Surgery*, 66(6), 1876-1883; discussion 1883-1885.

Shafer, M. A., Pantell, R. H. and Schachter, J. (1999) 'Is the routine pelvic examination needed with the advent of urine-based screening for sexually transmitted diseases?', *Archives of Pediatrics & Adolescent Medicine*, 153(2), 119-125.

- Shen, Y., Shen, Z. X., Yan, H., Chen, J., Zeng, X. Y., Li, J. M., Li, X. S., et al. (2001) 'Studies on the Clinical efficacy and pharmacokinetics of low-dose arsenic trioxide in the treatment of relapsed acute promyelocytic leukemia: a comparison with conventional dosage', *Leukemia*, 15(5), 735-741.
- Shinjo, K., Takeshita, A., Ohnishi, K., Sakura, T., Miyawaki, S., Hiraoka, A., Takeuchi, M., et al. (2000) 'Good prognosis of patients with acute promyelocytic leukemia who achieved second complete remission (CR) with a new retinoid, AM80, after relapse from CR induced by all-trans-retinoic acid', *International Journal of Hematology*, 72(4), 470-473.
- Shivakumar, S., Poonkhuzhali, B., Gunasekaran, S., Srivastava, A. and Chandy, M. (2002) 'Cytogenetic, fluorescent in situ hybridization & reverse transcriptase-polymerase chain reaction analysis in acute promyelocytic leukaemia patients', *Indian Journal of Medical Research*, 115(no issue number given), 59-67.
- Simel, D. L., Samsa, G. P. and Matchar, D. B. (1991) 'Likelihood ratios with confidence: sample size estimation for diagnostic test studies', *Journal of Clinical Epidemiology*, 44(8), 763-770.
- Soignet, S. L., Frankel, S. R., Douer, D., Tallman, M. S., Kantarjian, H., Calleja, E., Stone, R. M., et al. (2001) 'United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia', *Journal of Clinical Oncology*, 19(18), 3852-3860.
- Takeuchi, M., Yano, T., Omoto, E., Takahashi, K., Kibata, M., Shudo, K., Harada, M., et al. (1998) 'Relapsed acute promyelocytic leukemia previously treated with all-trans retinoic acid: Clinical experience with a new synthetic retinoid, Am-80', *Leukemia & Lymphoma*, 31(5-6), 441-451.
- Takeuchi, M., Yano, T., Omoto, E., Takahashi, K., Kibata, M., Shudo, K., Ueda, R., et al. (1997) 'Re-induction of complete remission with a new synthetic retinoid, Am-80, for relapse of acute promyelocytic leukaemia previously treated with all-trans retinoic acid', *British Journal of Haematology*, 97(1), 137-140.
- Tallman, M. S. (1998) 'Therapy of acute promyelocytic leukemia - all-trans retinoic acid and beyond', *Leukemia*, 12(Suppl 1), S37-S40.
- Tallman, M. S., Andersen, J. W., Schiffer, C. A., Appelbaum, F. R., Feusner, J. H., Ogden, A., Shepherd, L., et al. (1997) 'All-trans-retinoic acid in acute promyelocytic leukemia', *New England Journal of Medicine*, 337(15), 1021-1028.
- Tebas, P., Nease, R. F. and Storch, G. A. (1998) 'Use of the polymerase chain reaction in the diagnosis of herpes simplex encephalitis: a decision analysis model', *American Journal of Medicine*, 105(4), 287-295.
- Tobita, T., Takeshita, A., Kitamura, K., Ohnishi, K., Yanagi, M., Hiraoka, A., Karasuno, T., et al. (1997) 'Treatment with a new synthetic retinoid, Am80, of acute promyelocytic leukemia relapsed from complete remission induced by all-trans retinoic acid', *Blood*, 90(3), 967-973.
- Warrell Jr, R. P., Maslak, P., Eardley, A., Heller, G., Miller Jr, W. H. and Frankel, S. R. (1994) 'Treatment of acute promyelocytic leukemia with all-trans retinoic acid: An update of the New York experience', *Leukemia*, 8(SUPPL. 2), S33-S37.

Warrell, R. P., Frankel, S. R., Miller, W. H., Scheinberg, D. A., Itri, L. M., Hittelman, W. N., Vyas, R., et al. (1991) 'Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-trans-retinoic acid)', *New England Journal of Medicine*, 324(20), 1385-1393.

Xiao, Y. H., Miller Jr, W. H., Warrell Jr, R. P., Dmitrovsky, E. and Zelenetz, A. D. (1993) 'Pulsed-field gel electrophoresis analysis of retinoic acid receptor-alpha and promyelocytic leukemia rearrangements: Detection of the t(15;17) translocation in the diagnosis of acute promyelocytic leukemia', *American Journal of Pathology*, 143(5), 1301-1311.

Yusuf, S., Peto, R., Lewis, J., Collins, R. and Sleight, P. (1985) 'Beta blockade during and after myocardial infarction: an overview of the randomized trials', *Progress in Cardiovascular Diseases*, 27(5), 335-371.

Zelent, A., Guidez, F., Melnick, A., Waxman, S. and Licht, J. D. (2001) 'Translocations of the RAR alpha gene in acute promyelocytic leukemia', *Oncogene*, 20(49), 7186-7203.