HTA Moleculaire Diagnostiek in België
Supplements

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Federaal Kenniscentrum voor de Gezondheidszorg
Centre Fédéral d'Expertise des Soins de Santé
2005

Het Federaal Kenniscentrum voor de Gezondheidszorg

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HTA Molecular Diagnostics
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CONTENT:

HTA Molecular Diagnostics Supplement I: Molecular testing for hepatitis C: a review of the existing evidence

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Inhoudstafel

1. INTRODUCTION

Hepatitis C is caused by the hepatitis C virus (HCV), a single-stranded RNA virus of the Flaviridae family[1]. Until its etiologic agent was described in 1989, hepatitis C was formerly defined as non-A, non-B hepatitis[2].

It has been estimated that currently 170 million people worldwide are infected with the hepatitis C virus, corresponding to a global prevalence of approximately 3%.[3]

In 2002, Van Damme et al[4] found a 1% prevalence for hepatitis C in Belgium. The prevalence of anti-HCV antibody in a French survey including 14 416 subjects 18 to 80 years old from April 2003 to March 2004 was 0.86% [5](table 1).

Table 1: Prevalence of anti-HCV antibodies

		95% CI
France Apr. 2003 – Mar. 2004	0,86 %	0,66 – 1,10
Europe	0,69 %	0,49 - 0,96
North Africa	1,26 %	0,39 - 3,95
Middle East	11,28 %	1,90 - 45,45
Sub-saharian Africa	1,92 %	0,91 - 3,97
Pacific-Asia	1,54 %	0,47 - 4,86
North & South America	1,69 %	0,17 - 14,27

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Transmission is mainly associated with infected blood products or intravenous drug abuse, although other less common routes such as nosocomial transmission through dialysis or colonoscopy have been reported.[6] Whereas genotype 1 is the most prevalent genotype in patients with chronic hepatitis C, new infections are now often associated with intravenous drug abuse and caused frequently by genotype 3 virus.[7]

Following initial HCV infection, it is estimated that up to 85% of patients will develop chronic hepatitis while only a small proportion of patients overcome the infection. The prognosis for those chronically infected is highly variable- with many never experiencing any adverse long-term effects at all.[3] However, it is likely that up to 20% will develop cirrhosis and a small number of these patients will develop hepatocellular carcinoma. Within the 70 new hepatocellular carcinomas reported in Belgium for the year 2003, the underlying liver disease was hepatitis C virus related in 41% (29/70) [8]

In many countries, HCV is now the most common cause for liver transplantation.[9, 10]

Patients with chronic hepatitis C can be treated with interferon. Whereas at first patients were treated with standard interferon monotherapy, and later with combination therapy of interferon and ribavirin, combination therapy of pegylated interferon with ribavirin is currently considered first choice for patients with moderate to severe chronic hepatitis C[11].

Patients with genotype 1 have much lower sustained response rates than patients with genotype 2 or 3. After treatment with peginterferon alfa-2a and ribavirin, sustained viral response for genotype 1 is 46%, compared to 76% for genotype 2 and 3[12] After treatment with peginterferon alfa-2b and ribavirin, sustained viral response is 42% for genotype 1 and 82% for genotype 2 and 3[13]. This illustrates the importance of genotyping the virus before treatment.

Molecular tests can be used for the detection or confirmation of HCV, for quantifying the level of viral load and for determining the genotype. The most important clinical

utility of these tests lies in the support of treatment decisions and assessing treatment success.

The aim of this report is to summarize the existing evidence on molecular testing in patients with hepatitis C. The report focuses on the accuracy of these tests, diagnostic strategies in patients with suspected or confirmed hepatitis C infection and possible diagnostic alternatives and the clinical utility of using molecular tests to support treatment decisions in hepatitis C patients. In addition, the cost-effectiveness of these tests is assessed on the basis of their impact on the cost-effectiveness of HCV treatment regimens. Finally, the cost of routine use of molecular testing for hepatitis C in Belgium is estimated from the perspective of the health care payer. Screening for hepatitis C in either blood products or the general population is not considered in this review.

2. METHODS

2.1. SEARCH STRATEGY

We performed an iterative literature search, more precisely we searched for existing health technology assessments (HTA) first, subsequently for systematic reviews and finally for original diagnostic research.

HTA reports were searched in the HTA database. We searched for systematic reviews in the CRD database, Medion, Medline and Embase databases. Original research was identified in Medline and Embase, the search limited to studies published after the literature search of any HTA report or systematic review. Studies that report the performance of a diagnostic strategy were included as well.

In addition, we checked the Food and Drugs Administration website to identify the tests that have received an FDA approval.

The economic value of molecular tests for hepatitis C was assessed on the basis of economic evaluations of hepatitis C treatments. Studies that make a comparison between a treatment guided by molecular testing and a treatment not guided by such tests are the basis for the assessment; they allow an estimate of the incremental cost associated with testing, the savings along the treatment path and the outcomes in terms of clinical benefit. The qualitative conclusions about the impact of testing on the cost-effectiveness of treatment are more important than the quantitative results of individual studies, as it is not the treatment regimens that are being studied here.

The search date was August 2004.

2.2. SEARCH TERMS

The search term used for HTA, CRD and Medion was "hepatitis C" or "hepacivirus".

The search-string we used in PubMed is listed below:

("Cytogenetic Analysis"[MeSH] OR "Molecular Probe Techniques"[MeSH] OR "Molecular Diagnostic Techniques"[MeSH]) AND ("Hepatitis C"[MeSH] OR "Hepacivirus"[MeSH] OR hepatitis C OR hepacivirus) AND _sensitive*[Title/Abstract] OR sensitivity and specificity[MeSH Terms] OR diagnos*[Title/Abstract] OR diagnosis[MeSH:noexp] OR diagnostic * [MeSH:noexp] OR diagnosis differential[MeSH:noexp] OR diagnosis[Subheading:noexp])

In Embase, we used an adapted version of the same search-string:

((((diagnos*) in AB)or((diagnos*) in TI)) or ("sensitivity-and-specificity" / all SUBHEADINGS in DEM DER DRM DRR) or (((_sensitive*) in AB)or((_sensitive*) in TI)) or ("differential-diagnosis" / all SUBHEADINGS in DEM DER DRM DRR) or (((diagnostic) in DEM)or((diagnostic) in DER)) or ("diagnosis-" / all SUBHEADINGS in DEM DER DRM DRR)) and ((hepacivirus) or (hepatitis c) or ("hepatitis-C" / all SUBHEADINGS in DEM DER DRM DRR) or ("Hepatitis-C-virus" / all SUBHEADINGS in DEM DER DRM DRR)) and ((explode "genetic-procedures" / all SUBHEADINGS in DEM DER DRM DRR) or (explode "molecular-probe" / all SUBHEADINGS in DEM DER DRM DRR) or ("chromosome-analysis" / all SUBHEADINGS in DEM DER DRM DRR) or (explode "gene-amplification" / all SUBHEADINGS in DEM DER DRM DRR))

2.3. QUALITY ASSESSMENT

To assess the quality of HTA reports, we used the HTA Checklist from the International Network of Agencies for Health Technology Assessment (INAHTA). The quality of systematic reviews and prognostic studies were assessed using the checklists of SIGN (www.sign.ac.uk).

The QUADAS tool was used for the quality assessment of original diagnostic research on patients.[14] For original, analytical studies, we assessed items on validity of the panel used, on test execution and analysis using a self-constructed checklist (see appendix).

Economic evaluations were assessed using the checklist of Drummond et al.[15] See appendix for all quality assessment checklists used in the review.

Quality assessment is summarized in the evidence tables as good quality, fair or poor quality.

Exclusion criteria for HTA reports or systematic reviews were insufficient search of the literature and no quality assessment of included studies. Diagnostic accuracy studies were excluded in case of an invalid or unreliable reference test or an insufficient sample size (n<10).

2.4. DATA EXTRACTION

Test characteristics are not absolute. Variables such as setting, spectrum or demographic features of the population studies, are known to influence test characteristics. In addition, when bias was introduced into the study methodology, this will distort the study results and give biased test characteristics. The most important forms of bias in diagnostic research are inappropriate case-control design leading to spectrum bias and unblinding when reading the test results[16].

Therefore, it is important to report these variables and study characteristics together with the test characteristics. Finally, any funding, whether partly or fully, by any commercial source was noted.

The following data were extracted from the clinical studies:

- Demographic characteristics of the population studied: setting, co-morbidities (mainly co-infection with HIV or hepatitis B), age and gender.
- Design: cross-sectional cohort study, prospective cohort study, case-control study.
- Results: sensitivity, specificity, odds ratio, negative en positive predictive value, correlation coefficients, mean difference and 95% limits of agreement, linearity.
- Remarks on the funding of the study.

We did not perform a formal meta-analysis, but present an overall review of the test characteristics and prognostic value.

3. THE DIFFERENT TESTS AND THEIR CLINICAL INDICATION

Several molecular test technologies have been developed which are being used for hepatitis C; the most important are the reverse transcriptase-PCR (rt-PCR), transcription-mediated amplification (TMA) and the branched DNA (b-DNA) technique.

In reverse transcriptase PCR, a conserved RNA region of the virus is transcribed into DNA and then amplified. As it is a highly sensitive test, rt-PCR can be prone to false positive results due to cross-contamination.

Transcription mediated amplification is a nucleic acid amplification method that relies on the enzymes reverse transcriptase and T7 RNA polymerase to generate detectable levels of RNA product from either an RNA or a DNA template.

The branched DNA assay is based on a sandwich nucleic acid hybridization technique that uses synthetic oligonucleotide capture and amplifier probes to ultimately detect and quantify viral RNA.

Molecular tests on hepatitis C fall into three categories: qualitative HCV RNA, quantitative HCV RNA and genotyping.

Qualitative HCV RNA tests detect directly the presence of viral RNA, resulting in a positive or negative test result. Quantitative HCV RNA tests measure the viral load, the results can be expressed in copies/ml or international units[17]. The genotype of the hepatitis C virus is highly predictive of treatment success. At present, six major genotypes have been identified (1-6), and more than 50 subtypes. Clinical decisions regarding interferon treatment are based on the major genotypes only. Therefore, studies on subtypes were not included in this review.

Currently, three molecular tests have an FDA approval for use in patients with hepatitis C infection: Amplicor HCV (rt-PCR), Versant HCV RNA qualitative assay (TMA) and Versant HCV RNA 3.0 (b-DNA).

The clinical indications for the tests are summarized on the basis of several, national and international guidelines.[18, 19, 11]

- Patients with a suspicion of hepatitis C are tested with a third generation ELISA test. These tests have an accuracy of more than 99%, thus rendering confirmation by RIBA unnecessary. Qualitative HCV RNA tests with a sensitivity of 50 IU/ml are used to confirm active HCV infection. A negative test indicates a past infection. In rare cases, such as known occupational exposure or immunosuppression, the qualitative HCV RNA test can be used to detect infection.
- Qualitative HCV RNA tests are additionally used to assess the sustained viral response after treatment. In some cases, qualitative tests are used during treatment for the evaluation of an early viral response and subsequent decisions on the continuation of the treatment.
- The success of treatment is highly dependent on the genotype of the hepatitis C virus. As a consequence, treatment regimens are different for genotype 2 and 3 compared to the other genotypes. Therefore, genotyping is an essential test prior to treatment. Patients entering treatment should receive genotyping once; patients not considering treatment do not need genotyping, unless monitoring for epidemiological reasons within the context of scientific research and not for patient management. This is not considered further in this review.
- A quantitative HCV RNA test is performed prior to treatment, to have a baseline value. Monitoring using a quantitative test during treatment is dependent on the genotype.

4. RESULTS

HTA reports

We found one HTA report on the clinical and economic value of polymerase chain reaction (PCR)-based tests.[20] It was published in 2000 by the Australian Medicare Services Advisory Committee (MSAC) and has not been updated since. Secondly, we used the HTA report of the National Coordinating Centre for Health Technology Assessment (NCCHTA) of the UK, which was published in October 2004, on the use of pegylated interferon.[11] This report includes the value of diagnostic testing in decisions on and monitoring of treatment, although the diagnostic value of molecular tests itself has not been addressed.

Systematic reviews

In preparation of the NIH Consensus Statement on the Management of Hepatitis C: 2002[21], the literature was systematically reviewed and published in peer-reviewed journals.[22] We excluded 16 reviews that reported no methods for literature search or synthesis and were primarily based on expert opinion[23-38].

Original research

The search for original clinical research was limited to studies published after the last, high-quality, systematic review or HTA report, which was 2002. Original economic studies were searched between 1998 and 2004.

Studies included in the review were categorized in analytical or clinical studies. Analytical studies comprise those studies that evaluate the test in laboratory conditions, to assess its reproducibility or linearity in serum panels. Clinical studies assess the value of the test in patients, in a clinical situation. We included 27 original studies, of which 12 analytical and 15 clinical. All included studies are referenced in the evidence tables.

In total, we excluded 19 studies. Main reason for exclusion was methodological flaws (9 studies)[39-47]. Two studies were excluded because they were set up to produce a reference panel[48, 17], two because they used liver samples[49, 50] and three because they assessed some specific technical aspects of the tests only[51-53]. Finally, we excluded two studies because they reported incidences of the different genotypes in a specific population[54, 55] and one because it assessed the subtypes of genotypes only[56].

4.1. ANALYTICAL ACCURACY

The HTA report excluded all analytical studies; the systematic review and the NIH Consensus Statement contain some remarks on the analytical performance of the tests.

Additionally, we identified 12 original studies published after the last systematic review, referenced in table 9. Overall quality of the studies included in this review was fair to poor. Most studies were at least partly funded by manufacturers of the molecular tests. In general, the reported analytical performance of the tests in patients with HIV or HBV co-infection is not sufficiently addressed.

4.1.1. Qualitative tests

In the NIH Consensus Statement, it is stated that FDA-approved qualitative tests have a limit of detection of around 50-100 IU/ml. Additionally, we identified two studies on a qualitative TMA test, in which the authors found a very low detection limit of 5,6 - 9,5 IU/ml. Specificity was 95,5-99,6%, indicating a relatively small percentage of false positive results.

4.1.2. Quantitative tests

According to the NIH Consensus Statement, testing for HCV RNA levels provides accurate information on viral levels. In order to permit normalization of the reported viral load levels, an HCV RNA standard has been introduced: IU/ml[17]. This IU does not represent the actual number of viral particles in a preparation.

However, despite the use of this international standard, there remains significant variability between available assays. The reportable range, accuracy and precision of each assay needs to be monitored, and appropriate dilutions of sample material should be performed. The clinical utility of serial viral levels depends on the continued use of the same assay.

In addition, we identified 7 studies on quantitative HCV RNA tests. Tests used are b-DNA technology or rt-PCR techniques. Specificity is reported to be 98% in all studies. Linearity approximates the line of identity. Within-run variability varies between 2,4 and 38,4%, between-run variability 2,7 and 43,8%. One study on real time PCR and subsequent genotyping, found only moderate to poor agreement, when comparing this test to the b-DNA Quantiplex. One study on a competitive rt-PCR test reported a very low detection limit of 47 IU/ml.

4.1.3. Genotyping

Lau et al. have shown good concordance (94%) between the different genotyping techniques, although they demonstrated that whatever method is used there is a proportion of patients who can not be genotyped (3-17%).[57]

In addition, we identified one analytical study on two genotyping methods, INNO-Lipa and TRUGENE, which found a very high overall agreement. Discrepancies between the two assays were resolved with a sequence analysis of the NS5B region. This study found that the accuracy of the INNO-Lipa assay was 98,6% and that of the TRUGENE assay 98%.

Key messages :

- **In general, the analytical accuracy of nucleic acid based tests is good.**
- **The limit of detection of qualitative assays is 50-100 IU/ml, although some assays have a limit of 10 IU/ml.**
- **Quantitative tests have sufficient linearity across different viral load levels and reasonable within and between-run variability. The agreement between different assays is not sufficient.**

- **Genotyping is accurate, although some patients are not typable with any assay.**

4.2. CLINICAL ACCURACY

All 15 studies included as original studies on clinical accuracy are referenced in table 10.

4.2.1. Qualitative tests

The authors of the HTA report state that the absence of viral RNA after 4 or 12 weeks of interferon monotherapy is highly predictive of a long-term response to treatment. Very few patients with detectable RNA levels after 4 or 12 weeks of treatment will have a sustained viral response. It should be noted that this statement is applicable to interferon monotherapy only.

Additionally, we identified 3 original studies on the clinical accuracy of qualitative tests. One study compared an rt-PCR with a TMA test. Although the detection limit of the TMA test is lower than that of the rt-PCR test, the test characteristics of the TMA test in predicting a sustained viral response were lower than that of the rt-PCR test. In fact, the TMA test was reactive at 12 weeks of treatment in two patients who eventually proved to have a sustained viral response. This is probably due to the lower limit of detection of the TMA assay.

4.2.2. Quantitative tests used for patient selection

Viral load is predictive of a response to interferon therapy, but is not sufficient as an indicator to exclude patients from a trial or therapy. The HTA report of MSAC cites a pooled odds ratio of 11,8 (95% CI 4,7-16,8) of sustained response to interferon monotherapy in patients with low versus high viral load titres. In the NIH Consensus Statement, it is agreed that although there is little correlation between disease severity and disease progression with the absolute level of HCV RNA, quantitative determination provides important information on the likelihood of response. This was confirmed in the two pivotal trials on peginterferon, in which patients with lower viral load levels were more likely to achieve a sustained viral response[11]. However, in one study that compared a 1.0 and 2.0 version of the b-DNA assay, higher RNA levels with the 2.0 version were not associated with a lower rate of sustained viral response after correction for genotype.

4.2.3. Quantitative tests used to guide treatment decisions

Besides estimation of the probability of success prior to treatment, quantitative tests are also used to monitor treatment in patients with genotype 1. More precisely, patients with genotype 1 who fail to achieve an early viral response, defined as a minimum 2 log decrease in viral load after the first 12 weeks of treatment, have only a small chance in achieving a sustained viral response. Treatment of these patients is subsequently discontinued. It follows that the negative predictive value of a test used to discontinue treatment from patients should be as high as possible, to avoid withholding treatment from patients that would have achieved a sustained viral response had they continued treatment.

In an analysis of two trials on the effectiveness of peginterferon and ribavirin, the author found a negative predictive value (NPV) of 0,98 (95% confidence interval 0,96-0,99) for a sustained viral response, when the RNA levels showed a decrease of 2 log or undetectable RNA at 12 weeks after the start of the treatment. Patients with a 2 log decrease at 12 weeks, but still detectable RNA, can achieve a sustained viral response if RNA was undetectable after 24 weeks of treatment. Other studies found negative predictive values of 96-100%. Even in a study on patients who had relapsed after an initial treatment with interferon, the negative predictive value was high, despite a low overall sustained response rate.

Interestingly, the detection of HCV core Ag also has a negative predictive value of 97%.

One study assessed the correlation of bDNA 3.0 and rt-PCR in patients with HCV-HIV co-infection and in patients with HCV mono-infection. The authors found lower correlation of the two assays in patients with HCV-HIV co-infection.

4.2.4. Genotyping

Genotyping is predictive of a response to therapy. Sustained viral response rates with genotype 1 are much lower than those for genotype 2/3, whereas sustained viral response for genotypes 4, 5 and 6 appear to be between those of the genotypes 1 and 2/3 respectively. Treating patients with peginterferon alfa-2a and ribavirin, sustained viral response rate was 46% for genotype 1 and 76% for genotype 2/3. Treatment regimens are also dependent on the genotype, as patients with genotype 2 or 3 are treated for 24 weeks, other patients for 48 weeks.

Concordance found in recent studies between different assays is 100%. We found one study in which a PCR assay was compared to typing with serology. In this study, concordance was only 68,3%, with 10% of the samples not typable wit the serotyping and better concordance for genotype 1 (75%).

A small proportion of the patients can not be typed using molecular tests, ranging from 2-7%. However, in one study a TMA-Lipa procedure was able to type 83-93% of specimens that failed to be typed previously with another procedure.

Key messages:

- **Qualitative tests are used to assess the end of treatment response and sustained viral response after the completion of therapy.**
- **Patients with higher viral load levels have a smaller chance of achieving a sustained viral response.**
- **No decrease of 2 log or still detectable RNA at 12 weeks of treatment is highly predictive of not achieving a sustained viral response in patients with genotype 1.**
- **Genotype 2 and 3 is associated with higher sustained viral response rates. Concordance between genotyping assays is very high.**

5. ECONOMIC VALUE

5.1. QUALITATIVE TESTS FOR DIAGNOSIS OF ACUTE HCV

The evidence about the economic impact of qualitative HCV RNA testing is limited.

As a diagnostic tool to identify acute HCV hepatitis, the qualitative HCV-RNA test is in theory the most sensitive test. Moller & Krarup[58] investigated 2023 patients clinically suspected of having acute viral hepatitis. Of the 64 patients who were found positive for HCV-RNA, 51 proved to have a chronic hepatitis and 13 had an acute HCV infection. Twelve out of these 13 patients also had circulating anti-HCV antibodies at time of sampling. The authors therefore conclude that the presence of anti-HCV antibodies is a reliable marker of acute hepatitis C and is obtained at a considerably lower cost than qualitative HCV-RNA testing.

However, it should be noted that this study included patients with signs and symptoms suggestive of acute hepatitis. The results can therefore not be generalised to patients with needle stick accidents or other recent exposures, where RNA testing could be appropriate.

5.2. QUANTITATIVE TESTS USED FOR PATIENT SELECTION PRIOR TO TREATMENT

Viral load levels before the start of treatment are one of the factors that influence the chances of achieving a sustained viral response, even when patients are treated with peginterferon combination therapy. The result of a quantitative test could therefore be an element in the decision process of the patient and his treating physician whether or not to start treatment.

The Australian HTA report examined the cost-benefit of quantitative viral load testing and genotyping relative to no testing prior to treatment. Qualitative assessments of treatment response to interferon monotherapy were performed at 12 and at 48 weeks in both groups. Patients who had no early viral response (EVR) at the end of 12 weeks according to this test discontinued their treatment; others continued for another 12 weeks.

Combination therapy was only considered for patients with EVR at 12 weeks but in whom a relapse was detected at 48 weeks. The combination therapy would then be administrated for a further 24-week period. It was assumed that only 10% of the patients with relapse at 48 weeks would start a combination therapy (range in sensitivity analysis 5-20%). The patients who were tested before the start of the treatment could decline the treatment if the test results predicted a low probability of treatment success. It was assumed that about 6% would eventually decide against treatment (range 0-30%).

The costs (price year 1996) were estimated in a population of 1 000 patients. This figure is similar to the number of patients actually treated in Belgium.

The authors found that viral load testing and genotyping prior to an interferon therapy would cost on average AUD 8 697 (€ 5 589) versus AUD 8 466 (€ 5 441) for an empirical interferon treatment. The incremental cost of pre-treatment viral load testing and genotyping is hence AUD 231 (€ 148). The authors concluded that pre-treatment viral load testing would be cost-saving if at least 15% of the patients decided to forego treatment with interferon on the basis of the quantitative HCV RNA test results.

The major weakness of this study is that it only looked at direct costs and savings of the tests, without any consideration of the effects in terms of quality of life improvement or deterioration due to (avoided) treatment. This precludes conclusions about the incremental cost-effectiveness of pre-treatment molecular testing relative to no testing. An intervention must not be cost-saving to be worthwhile. If for example QALYs (quality adjusted life years) can be gained by introducing pre-treatment quantitative PCR at an acceptable cost, it may be worth the investment.

The cost-per-QALY gained of quantitative HCV RNA testing was examined in one study.[59] The relevance of this study, which was done in 1995, is limited, however, given the current state-of-the art evidence on testing and treatment strategies. Pre-treatment testing was compared with empirical treatment and conservative treatment. The lowest incremental cost per QALY relative to conservative treatment was obtained with quantitative HCV RNA testing and treating only patients with a viral load < 3.5×10^5 genomes per millilitre. Empirical treatment with interferon (i.e. treating all HCV positive patients) was the least cost-effective alternative.[60]The authors conclude that the assessment of a 12-week viral response in genotype 1 hepatitis C infected patients can improve the efficiency of an empirical treatment by limiting the treatment duration in non responders, which is the strategy currently recommended in the clinical guidelines.

An alternative protocol was suggested by another study Kukuczka et al.[61] More specifically, they introduced the idea of a reflexive testing protocol for patients with positive anti-HCV tests. The routine combination of quantitative and qualitative tests results in redundant testing. A reflexive protocol would mean that a quantitative test is performed on all samples first, and that only those samples with a result below the limit of the quantitative test receive a qualitative test. The authors found that the number of qualitative tests was reduced by 59,4%, with a turn-around time of 8,1 days in case of both quantitative and qualitative testing.

5.3. QUANTITATIVE TESTS USED TO GUIDE TREATMENT DECISIONS

Quantitative HCV RNA testing is especially useful to guide the duration of an HCV treatment. In absence of quantitative data for HCV RNA, the duration of treatment must be standardised to 24 weeks for patients infected with genotype 2 or 3 and to 48 weeks with genotype 1. But non-responders at 12 weeks are unlikely to reach a SVR at 48 weeks[62]. Consequently, these patients will be treated too long. That will negatively impact their quality of life and increase costs, without improving the clinical outcome. Thus a quantitative HCV RNA assay after 12 weeks will be cost-saving if the cost of the test is lower than the cost of drug for 36 useless weeks. Testing is here a dominant strategy: less costly and equally (in clinical terms) or more (in QALY terms) effective than "not testing". However, this is only the tip of the iceberg. Even if quantitative HCV RNA testing should not be cost-saving, it will have an impact on the cost-effectiveness of the HCV treatment. It is worthwhile to consider whether testing increases or decreases the cost-effectiveness of HCV treatment.

From the literature, it can be concluded that limiting treatment duration to 24 weeks for genotype 2 or 3 patients and testing EVR at 12 weeks in genotype 1 patients in order to decide whether or not to continue treatment, positively impacts upon the cost-effectiveness ratio of HCV treatments.[22, 63] This applies to different types of treatment, including dual therapy with non-pegylated interferon (IFN) & ribavirin (RBV), dual therapy with pegylated interferon (pegIFN) & 800 mg RBV and dual therapy with pegIFN & RBV $\geq$ 10.6mg.kg^{-1} RBV, and irrespective of the comparator.[60] To illustrate the impact on the cost-effectiveness ratio of changing the therapeutic algorithm from testing at 24 weeks to testing at 12 weeks, some figures are presented in 2. In all therapeutic regimens, the lowest incremental cost per QALY was obtained by the 12-week stopping rule. The 12-week stopping rule was less cost-effective in genotype 2 or 3 patients than in genotype 1 patients. The incremental cost-effectiveness ratio actually increased with moving from the 24-week stopping rule to the 12-week stopping rule in this sub-group. This is due to the higher SVR rates in genotype 2 or 3 patients, which makes testing at 12 weeks less effective in predicting SVR.[60]

Table 2: Incremental cost-effectiveness ratios of three HCV therapies, combined with two treatment algorithms. [60]

	IFN + RBV	pegIFN + 800mg RBV	pegIFN + > 10mg kg^{-1} RBV
Discontinuing treatment in viral positive patients after 24 weeks of treatment (stop 24)	Comparator no therapy: $ 2 100/QALY	Comparator no therapy: $ 5 000/QALY Comparator IFN+RBV: $ 25 000/QALY	Comparator no therapy: $ 4 900/QALY Comparator IFN+RBV: $ 14 600/QALY
Same criteria as in stop 24 but also limiting therapy in those with genotype 2/3 to 24 weeks and discontinuing therapy in those viral positive or <2 log drop in viral load in non-genotype 2/3 patients after 12 weeks	Comparator no therapy: $ 1 500/QALY	Comparator no therapy: $ 4400/QALY Comparator IFN+RBV: $ 22800/QALY	Comparator no therapy: $ 4 300/QALY Comparator IFN+RBV: $ 13 600/QALY

A British study that estimated the impact of the 12-week stopping rule on the incremental cost-effectiveness of dual therapy with PEG & RBV compared to no treatment found similarly that applying a 12-week stopping rule in case of no EVR for a cohort of 1 000 patients would generate a cost saving of £ 2 188 772 or 15,7% of the total treatment costs. Testing was not assumed to be limited to genotype 1 patients, although the majority of the patients without EVR were assumed to be genotype 1. The total cost of treating these patients for 12 weeks was estimated at £ 11 683 203. Continuing treatment in patients without EVR results in an estimated incremental cost-effectiveness ratio of £ 226 573/QALY compared to no treatment.[11] This is a very high ratio compared to other interventions.

Davis[64] found that quantitative HCV RNA testing at 12 weeks and discontinuing treatment in patients without EVR would reduce costs by 17,8% compared with 48 weeks of treatment for all patients. This analysis did not take the possibility of limiting treatment to 24 weeks for genotype 2 or 3 patients into account but did notice that most of the cost savings were obtained in genotype 1 patients (21,5% savings in genotype 1 versus 0,8% in genotype 2 or 3). The reduction in costs for the latter group did not outweigh the costs associated with testing and therefore testing was not cost-saving in this sub-group of patients. Moreover, because almost all genotype 2 or 3 patients achieve EVR (99%) and SVR occurs in most cases (86%), testing these patients at 12 weeks is not cost-effective.[64]

A Swiss study examined the incremental cost-effectiveness of a dual therapy of IFN+RBV with or without testing after 24 weeks of treatment. Treatment was discontinued in patients without virologic response at 24 weeks and continued for another 24 weeks in patients with virologic response. In the alternative without testing, all patients received 48 weeks of treatment with IFN+RBV. Results were presented by genotype. In genotype 1 patients, the full 48 weeks course was dominated by the PCR testing option. Treatment with PCR testing at 24 weeks was both more effective in generating QALYs and less costly than giving the full course to all patients. For genotype non-1 patients, however, it was more cost-effective to treat all patients with IFN+RBV for 24 weeks and then stop treatment than to apply any of the other treatment strategies. For both sub-groups, it was found that the quality of life decrements associated with continuing treatment for another 24 weeks in early non-responders outweighed the quality of life improvements in the additional responders.[65]

Key messages:

- The guidelines for molecular testing in HCV are not always economically optimal. A number of alternatives have been suggested which may improve the cost-effectiveness of qualitative and quantitative HCV RNA testing.
- Anti-HCV tests are nearly as effective as and much cheaper than qualitative HCV RNA tests for diagnosis of patients suspected of acute hepatitis C. These results do not necessarily apply to patients with known recent exposure to the hepatitis C virus.
- A reflexive diagnostic testing protocol can significantly reduce the number of redundant tests.
- The use of quantitative HCV RNA testing to detect early virologic response 12 weeks after start of treatment in genotype 1 patients increases the cost-effectiveness of HCV treatments if patients without EVR discontinue their treatment. Testing of EVR in genotype 2 or 3 HCV patients reduces the efficiency of treatment, as it adds to costs without generating substantial additional benefits.
- It is more cost-effective to treat patients with genotype 2/3 for 24 weeks and then to stop their treatment than testing genotype 2/3 patients and continue their treatment for another 24 weeks in early virologic responders.

6. ALTERNATIVE DIAGNOSTIC TECHNOLOGIES

Several studies compared the PCR based assays with serology based assays. These assays are faster and cheaper, thus they are an interesting alternative for the labour-intensive and expensive PCR based tests.

Veillon et al. found very good prognostic characteristics for the total HCV core Ag, with similar positive and negative predictive values for a sustained viral response compared to nucleic acid tests[66]. On the other hand, a study by Kawai et al found a lower sensitivity with an HCV core assay, more precisely 78,4% compared to 97,5% with the rt-PCR[67]. Another study by Soffredini et al concluded that HCV core Ag was a less sensitive marker for infection than rt-PCR (94% vs 100%)[68]. The lower detection cut-off for HCV core Ag is probably 20 000 IU/ml. In this study, HCV core Ag level at 12 weeks of treatment had an equal negative predictive value for sustained viral response than bDNA, both being 100%, whereas the negative predictive value for rt-PCR was 94%.

Key message:

- **HCV core Ag is a promising assay for the assessment of early viral response.**

7. COST OF ROUTINE USE OF HCV RNA TESTING IN HCV IN BELGIUM

7.1. INTRODUCTION

The Belgian health care payer wishes to know the cost of routine use of HCV RNA testing for chronic HCV patients who are eligible for a reimbursement of their treatment. A (peg)interferon-ribavirin reimbursement by the RIZIV-INAMI is allowed for adult patients with chronic hepatitis C, demonstrated by 2 abnormal ALT dosages performed at a 1-month interval, by a positive HCV-RNA as well as by the knowledge of the genotype and by the results of a liver biopsy for genotypes other than 2 or 3 (under the amended rules of chapter IV, § 191, § 235, § 265 & § 271). The initial reimbursement for 24 weeks may be prolonged for a second 24-week period in genotype 1, 4, 5 & 6 patients who show an EVR after 12 weeks treatment.

The cost of the procedures will thus depend on the population eligible for testing, the proportion of patients who starts treatment, the frequency of testing, the success rate of the therapy and the cost per test.

7.2. METHODS

To estimate the costs associated with molecular testing in HCV, a decision model is developed in Data3.5 (Treeage®). A decision tree visually presents the actual treatment path of a patient treated for chronic HCV (Figure 1 & table 2). The focus of the analysis was on the costs of the molecular tests only. Costs of treatment, savings from avoided treatment or outcomes will not be considered in this analysis. Input data for the model include: the number of patients eligible for treatment, frequency of testing during treatment and treatment success. A further limitation of the model is that the possibility and consequences of false positive and false negative test results has not been considered.

*Number of patients eligible for treatment**

During the year 2004, the 14 active diagnostic centres determined 3 114 HCV genotypes, an increase by 20% with regards to the year 2003. The most prevalent types were genotype 1 (60.4%), genotype 3 (18.5%), genotype 4 (12.3%) and genotype 2 (6.1%). These results were communicated by the 14 centres and thus should not be considered as an epidemiological study as such. We can't exclude that some patients visited several gastroenterologists or internists before deciding to be treated or not and therefore that a genotyping was requested several times for the same patient.

In the same year about 1 000 reimbursement requests were submitted to the RIZIV-INAMI by insured patients. This means that only 35% of the patients with chronic hepatitis C decided to start a (peg)interferon-ribavirin treatment.

*Number of tests recommended during the treatment**

The expected future number of tests per year depends on the treatment algorithm that is chosen. The pre-treatment diagnostic strategy is assumed to be equal for all patients. The starting point is a positive HCV serology. A qualitative HCV RNA test is performed to confirm the diagnosis.

If all patients testing positive for anti-HCV antibodies are tested with the qualitative HCV RNA test, about 85% will have their chronic hepatitis C status confirmed. A quantitative test follows and the genotype is determined. About 30-40% will decide to undergo treatment[69]. For convenience, we assume the actually treated percentage of patients is 35%. This corresponds in Belgium with about 1 100 patients.

* Treatment means here the treatments according to the Belgian reimbursement rules

As for the use of molecular tests to guide the treatment duration, two extreme scenarios are presented, one overestimating and one underestimating the actual number of tests and total costs of molecular testing. In both algorithms, treatment duration for genotype 2 or 3 HCV patients is limited to 24 weeks. Patients with genotype 1, 4, 5 or 6 HCV are treated for 48 weeks, unless they are found viral positive at 12 weeks after start of treatment with the quantitative HCV-RNA test, in which case treatment is discontinued. A qualitative HCV-RNA test at 24 weeks is considered in patients who are HCV-RNA positive at 12 weeks but with a ≥ 2 $\log_{10}$ decrease in viral load according to the quantitative test. The EVR in genotype 1 HCV patients is about 80% [64], including both patients with no viraemia and patients with detectable HCV-RNA. The proportion of patients free of virus at 12 weeks was not explicitly stated in the original publication of Manns[13] but will be detailed at the next page. Therefore, the first scenario assumes that qualitative testing at 24 weeks is done in genotype 1, 4, 5 & 6 patients who had an EVR at week 12 (and also genotype 2 & 3 patients as this is their end-of-treatment) and the second scenario assumes that qualitative testing is done in none of the genotype 1, 4, 5 & 6 patients at 24 weeks. All patients have a qualitative HCV-RNA test at end of treatment and 6 months after the end of treatment. The only difference between the scenarios is hence that in scenario 1 all genotype 1, 4, 5 & 6 HCV patients with 2 $\log_{10}$ decrease in viral load at 12 weeks are tested with a qualitative HCV-RNA at 24 weeks and this is not done in scenario 2.

Figure 1: Decision tree for the use of molecular tests in hepatitis C

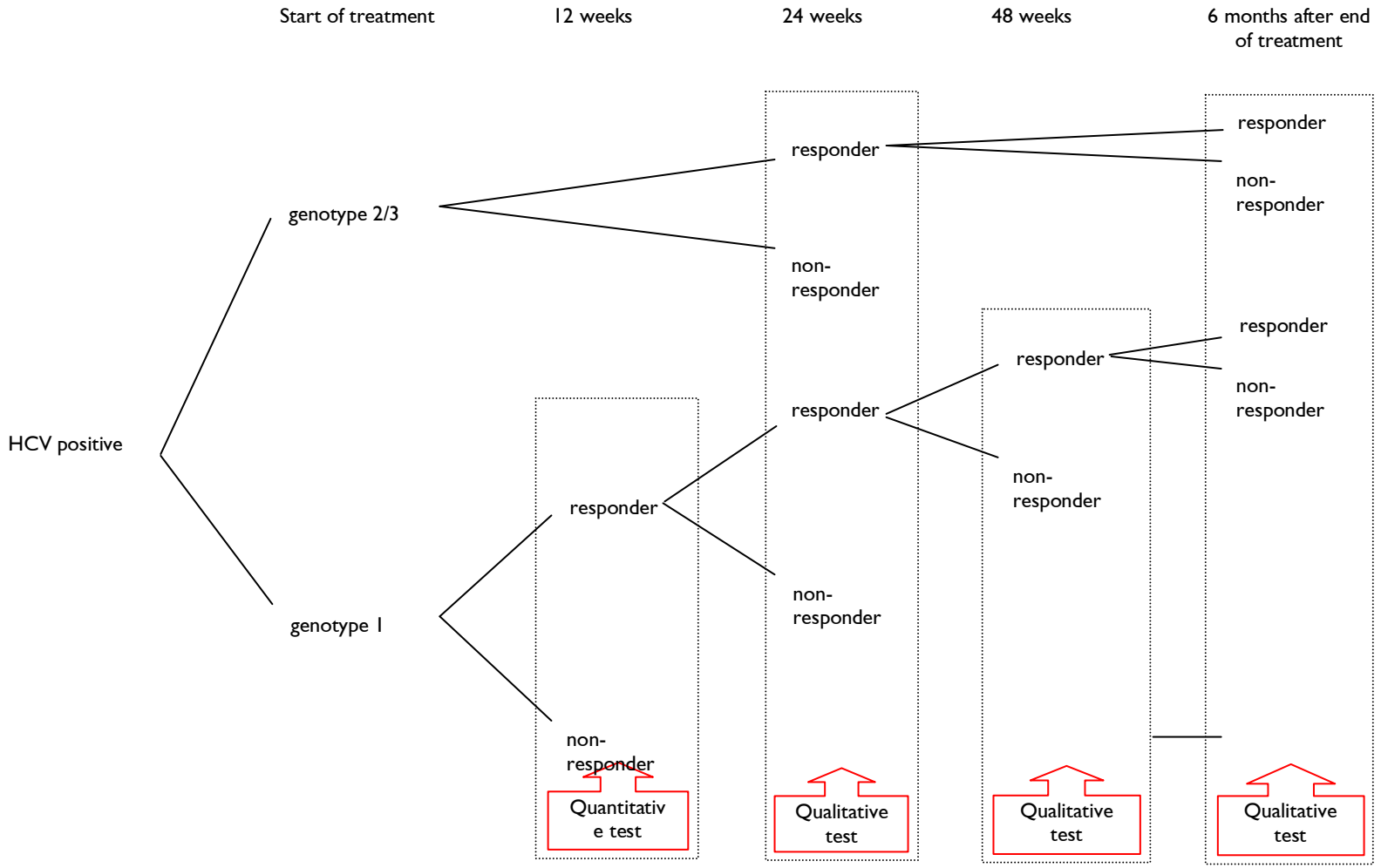


Table 3: HCV-RNA assessments before & during a (peg)interferon-ribavirin treatment

Genotype & treatment duration	Wk 0	Wk 12	Wk 24	Wk 48	Wk 72	Wk 0-72
Genotypes 1,5,6: 12-48 weeks						
Genotyping	+					1
Qualitative dosage	+		(+$^{+}$)	+$^{-}$	+$^{-}$	≤ 4
Viral load dosage	+	+				≤ 2
Genotype 4: 12-48 weeks						
Genotyping	+					1
Qualitative dosage	+		(+$^{+}$)	+$^{-}$	+$^{-}$	≤ 4
Viral load dosage	+	+				≤ 2
Genotypes 2,3: 24 weeks						
Genotyping	+					1
Qualitative dosage	+		+	+$^{-}$		≤ 3
Viral load dosage	+					1

+$^{-}$ conditional qualitative test performed only if previous result was negative

(+$^{+}$) conditional qualitative test performed only if the viral particles decreased by 2 log_{10} but still were detected in scenario 1; that qualitative test is omitted in scenario 2.

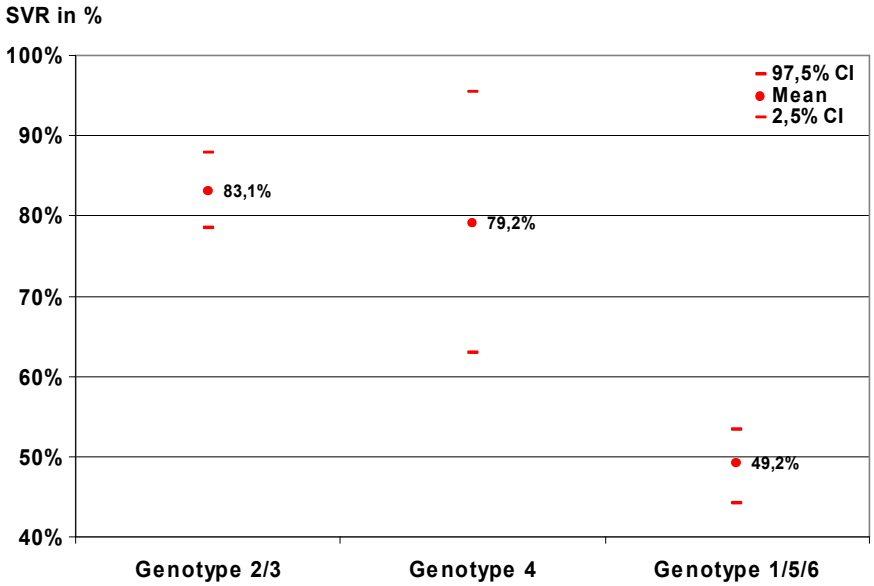
Treatment success

The treatment success rates used in the model were derived from the literature. For viral genotypes 1/5/6, an EVR of 80.7% will be applied (Fried et al, 2002[12], N = 300). However, 10.4% of the patients with an EVR will remain HCV-RNA positive at week 24 of which 4% will achieve a SVR - NPV= 96% - compared to 33% for those who become HCV-RNA negative between weeks 12 and 24 ($P < .01$; Davis et al, 2003[64]). Therefore these patients with a positive HCV-RNA test at week 24 discontinue their treatment. A SVR (at week 72) of 49.2% (95% C.I.: 44.1-53.3; combined data (Fried et al, 2002[12]& Hadziyannis et al, 2004[70], N=571) will be used in the model. An overview of the success rates used in the model is graphically presented in figure 2. The very high negative predictive value of no EVR at week 12 and of the persistence of viral particles at week 24 justifies a treatment discontinuation.

For viral genotype 4, an EVR of 92.3% (Hadziyannis et al, 2004[70]) and a SVR (at week 72) of 79.2% (95% C.I.: 62.9-95.4; combined data (Fried et al, 2002[12] & Hadziyannis et al, 2004[70]) was found in the literature. We should note that although these are the best available estimates up till now, these figures are based on studies with small sample sizes (13 for EVR and 24 for SVR).

For viral genotypes 2/3, SVR (at week 48) was found to be 83.1% (95% C.I.: 78.4-87.8; combined data (Manns et al, 2001[13] & Hadziyannis et al, 2004[70], N = 243).

Figure 2: SVR rates for the different HCV genotypes



7.3. RESULTS

Expected number of HCV tests

In the base case analysis, it is assumed that 65.7% of the genotypes 2 or 3, and 25.0% of the other genotypes have an initial 12-week treatment; what corresponds to 35% of the genotyped patients (left part with regard to the vertical dotted line at figure 3).

The SVR rate for an individual patient with genotype 1/5/6 is the product of the chances nodes of the upper branch (at the right part of figure 3, i.e. (0.807 x 0.896 x 0.671 or 48.5%) and 0.831 or 83.1% for genotype 2/3. The SVR applied for the genotypes 1/5/6 takes into account the loss of patients who are forced to discontinue before the end of treatment as a consequence of no EVR at week 12 and of a positive HCV-RNA test at week 24.

The sensitivity of the results to these assumptions is tested in three additional sensitivity analyses. Hence, in the sensitivity analyses the percentages of patients that decide to be treated will be varied.

Fig 3: Distribution of the success chances according to the pattern of chronic HCV when 35% of the genotyped patients start a treatment (base-case analysis)

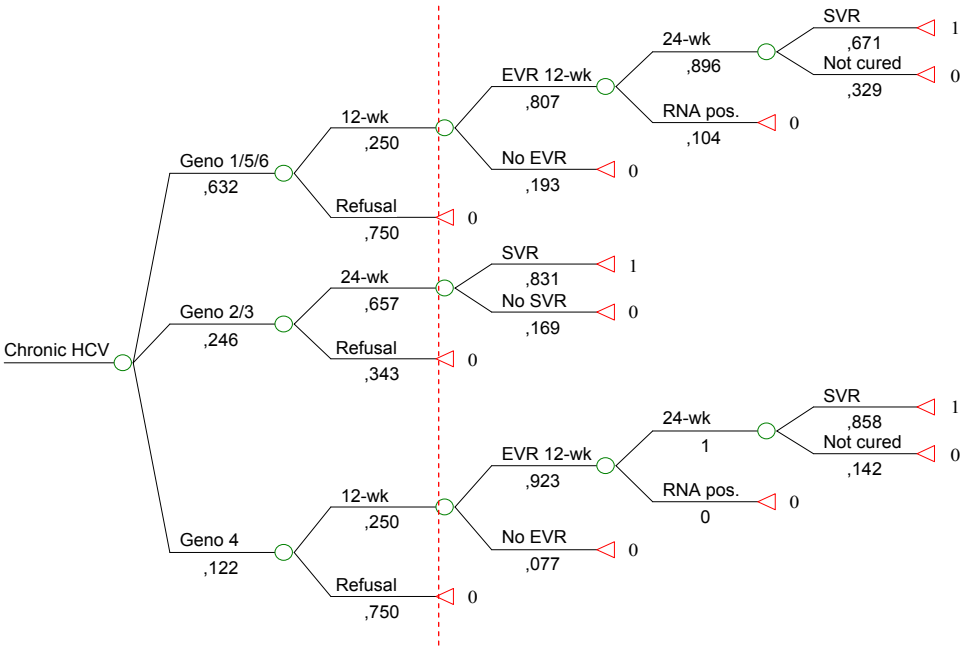


Table 4: Number of tests and patients with SVR in base case analysis and three sensitivity analyses

Proportion of treated patients	Sensit 1: Geno 2/3: 100% other geno: 13.8%	Base case Geno 2/3: 65.7% other geno: 25.0%	Sensit 2 Geno 2/3: 35.0% other geno: 35.0%	Sensit 3 Geno 2/3: 0.0% other geno: 46.4%
Genotyping	3 114 (1.00)	3 114 (1.00)	3 114 (1.00)	3 114 (1.00)
Viral load test	3 438 (1.10)	3 701 (1.19)	3 936 (1.26)	4 204 (1.35)
Qualitative test **1**	5 203 (1.67)	5 278 (1.69)	5 344 (1.72)	5 418 (1.74)
Qualitative test **2**	4 957 (1.59)	4 836 (1.55)	4 724 (1.52)	4 599 (1.48)
Difference in # qualitative tests	- 246 - 5%	- 442 - 8%	- 620 - 12%	- 819 -15%
Patients with SVR	810	732	663	582

Qualitative test **1** = number of qualitative tests in scenario 1

Qualitative test **2** = number of qualitative tests in scenario 2

From the base-case analysis (figures in bold in table 4), we can conclude that for every genotyped patient, the centres of molecular biology will perform on average 1.19 viral load tests and 1.69 qualitative HCV-RNA assays.

A more detailed analysis (appendix 7) shows that, during the treatment, 2.0 qualitative tests are requested for genotypes 1/5/6, 1.8 tests for genotypes 2/3, and 2.6 tests for genotype4.

A sensitivity analysis shows that when most of the genotypes 2 & 3 go for treatment, the largest number of SVR is achieved. On the other hand, the number of qualitative tests requested is not affected by the types of genotypes that are treated except in the 2[d] scenario, to say no qualitative test after 24 weeks of treatment in genotypes 1, 4, 5 & 6. For the base case, 8% of the tests could be saved.

The number of genotyping and quantitative HCV-RNA tests performed in Belgium grew by 30% between 2002 & 2003 and by 20% between 2003 & 2004. A similar growth was found in France (table 5).

The number of expected quantitative assays in the year 2003 (2 627 x 1.19 = 3 126) corresponds almost perfectly with the observed figures (3 211). That is not the case for the number of qualitative assays performed in the same year (7 368) where there was an excess of 2 928 tests with regards to the figure calculated in the HCV treatment context (4 440) but it does not take into account the number tests requested for the diagnosis of active HCV and for suspected contaminations in healthcare workers.

7.4. COST OF THE TESTS

France

In France, viral load and qualitative detection of HCV-RNA as well as genotyping are performed by clinical laboratories. They may be requested by any physician if there is a medical reason and are reimbursed at 100% since February 2001 (tables 5 & 6).

Table 5: Number of HCV tests performed in the years 2001, 2002 & 2003

	B-2001	B-2002	B-2003	Fr-2001	Fr-2002	Fr-2003
Qualitative tests	9.141	6.374	7.368	51.433	55.215	52.679
Viral load tests	373	2.369	3.211	10.236	23.647	30.308
Genotyping	309	2.024	2.627	3.678	9.418	11.605

Germany

In Germany, qualitative detection of HCV-RNA can only be requested in patients who are anti-HCV positive. Genotyping and viral load determinations are only reimbursed before a peginterferon and/or ribavirin treatment.

Great Britain

In UK, the Central Public Health Laboratory from the Health Protection Agency is using two separate tariffs according to the Authority in charge of the Health problem: the Communicable Diseases Surveillance Centres or the National Health Service and Primary Care Trusts.

The turnaround time from day of receipt to issue of report is 5 working days. Laboratory staff are committed to the fastest possible issue of reports, consistent with accuracy, on the specimens they examine. However the turnaround times may be longer or shorter depending on the availability of staff and the complexity of the investigation!

Australia

HCV tests are reimbursed at 75-85%. A maximum of 4 qualitative tests are reimbursed within a 12-month period. Genotyping and viral load determinations are only reimbursed for HCV-RNA positive proven patient before an antiviral therapy.

Table 6: Official tariffs in some countries

	France	Germany	UK CDSC	UK NHS	Netherlands	Australia	Sweden
Genotyping	108.00 €	102.30 €	17.14 €	130.00 €	174.70 €	124.22 €	
Viral load	81.00 €	89.50 €	95.71 €	162.86 €	174.70 €	109.31 €	137.67 €
Qualitative	54.00 €	40.90 €	12.86 €	81.43 €	94.07 €	55.90 €	137.67 €
Genotyping & viral load			112.86 €	211.43 €			

Belgium

A preliminary estimation based on the cost of consumables has shown a mean cost of 77 € per molecular test. However this does not necessarily reflect the cost of the specific reagents used in HCV-RNA assessments.

A German laboratory that developed in-house a 2-step real-time fluorescence reverse transcriptase-PCR method for a quantitative assay of HCV-RNA with a sensitive detection threshold came to a total cost for the consumables of € 8.00 per test but the validation costs were not included[71].

8. DISCUSSION

Hepatitis C is a major health problem worldwide, as the WHO estimates that 170 million people are infected around the world. In Belgium, the prevalence is approximately 1%.[19] Generally, the virus is transmitted by blood-to-blood contact. 85% of infected patients do not clear the virus after the initial exposure and go on to develop chronic hepatitis C. Progression of the disease occurs over 20-50 years, with one-third that may never progress to liver cirrhosis or will not progress for at least 50 years. About 5-30% will develop cirrhosis and a small percentage of these are at high risk of developing hepatocellular carcinoma. Some patients with end-stage liver disease or hepatocellular carcinoma may require liver transplantation. Because HCV and HIV share common routes of transmission, many people with HIV are also infected with HCV.

The primary aim of treatment is to clear HCV for at least 6 months after treatment cessation, in order to improve quality of life for patients and reduce the risk of cirrhosis and hepatocellular carcinoma. The genotype of the virus and a high viral load affect efficacy of treatment. Other influential factors are age, weight, fibrosis and cirrhosis of the liver, pre-treatment ALT level, racial group and gender.

Qualitative testing is used to assess the sustained viral response, where presence of HCV RNA indicates treatment failure. These tests have a very low limit of detection of 50-100 IU/ml, and are therefore very sensitive in detecting viral RNA in the serum. TMA tests have an even lower limit of detection, although this higher sensitivity does not always mean higher accuracy in for example predicting sustained viral response during treatment. Patients with very low levels of RNA can react with these TMA assays but achieve a sustained response at the end of treatment.

Quantitative tests are used prior to treatment to establish a baseline viral load level, and can be helpful to make an informed decision whether to start treatment or not. However, the agreement between different quantitative assays is not good, which means that the same assay must be used in the follow-up of a patient. Pre-treatment quantitative HCV-RNA testing for selecting patients for treatment is cost-saving when more than 15% of the patients decide against treatment following the test.

Quantitative tests are also used during follow-up of patients to guide treatment decisions. The tests prove clinically meaningful and cost-effective, as they avoid unnecessary continuation of treatment if the probability of a successful outcome is very low.

Genotyping is mandatory for every patient wishing to start treatment. Not only is the prognosis different for patients with genotype 2/3 and other genotypes, but treatment regimens are fundamentally different according to the genotype. Concordance between different genotyping assays is very good.

Overall, the quality of the studies in which the performance of molecular tests was assessed, was fair to poor. Especially, the selection and spectrum of the participants and the blinding of test results was reported in almost none of the studies. This limits the strength of the study results and leads most probable to an overestimation of the test characteristics. In addition, studies on patients with HCV-HIV or HBV co-infection are very rare, making the results less transferable to these populations.

HCV core Ag is becoming a possible alternative for molecular tests. At this moment, some studies report negative predictive values of quantitative HCV core Ag tests equal to bDNA or rt-PCR tests. As these HCV core Ag tests are cheaper and faster to perform, they could in time replace the molecular tests.

We assume that the number of genotype determinations performed in Belgium for the year 2004 reflects a once in the disease course determination. That was not exactly the case in the years 2002 & 2003 (latest detailed data available from the 14 CMDs) where several centres performed a mean of 1.20 determination per patient or were not able to assign a precise genotype in others

The genotypes distribution of the 35% of patients who start a treatment is at present unknown. Therefore the sensitivity analysis lets vary the percentage of genotypes 2 & 3 (shorter treatment duration, better chances of success) within the treated group from 0 to 100% with, for the base case, a proportion of 65.7% of types 2 & 3 and 25% for the other types. An 8% reduction in the number of qualitative tests is possible if no test is performed at week 24 for the patients with genotypes 1/4/5/6 (scenario 2).

The real cost per test can not actually be precisely determined: On one hand, many laboratories use expensive kits that are commercially available, on the other one, the largest laboratories have developed in house methods at a far lower cost.

9. IMPLEMENTATION SCENARIO CHARACTERISTICS

Target condition:

Incidence/prevalence: Prevalence: 1%

Acute or chronic condition: Chronic

Leading to cirrhosis, hepatocellular carcinoma, liver transplant in minority of patients

Test

	Qualitative test	Quantitative test	Genotyping
Impact on treatment:	yes	yes	yes
Prognostic impact:	yes	yes	yes
Test result 24h:	no	no	no
Outbreak surveillance:	no	no	no

APPENDICES

EVIDENCE TABLES

HTA report (table 7)

Study ID	Tests considered	Quality assessment Very good/ Good/Fair	Remarks	Conclusions/ Recommendations
MSAC 2000[20]	Genotype Quantitative Qualitative	Good	Included only studies on interferon-alfa monotherapy.	The request for these tests should be restricted to consultant physicians who will manage treatment, and only in patients with confirmed hep C infection. Genotyping once only for each patient. Viral load testing prior to treatment, once only in a 12 month period Qualitative testing for diagnosis, once prior to treatment and up to 3 times in the following treatment to assess treatment response.

Systematic review (table 8)

Study ID	Tests considered	Quality assessment	Remarks	Conclusions
Davis 2002[22]	Quantitative HCV RNA	Unclear	Meta-analysis of two previously reported trials	HCV RNA decreased by 2 log or to negative at 12 weeks PPV* of SVR*: 0,68 (0,64-0,72) NPV* of SVR: 0,98 (0,96-0,99) Patients with a 2 log decrease, but not negative at 12 weeks, can achieve SVR if HCV RNA negative at 24 weeks

* PPV= positive predictive value

NPV= negative predictive value

SVR=sustained viral response

Original studies

a) Analytical studies (table 9)

Study ID	Tests considered	Quality assessment	Remarks	Conclusions
Qualitative				
Hendricks 2003[72]	TMA (VERSANT qualitative assay)	Fair	Funded, organized and analysed by Bayer Corp.	Detection cut-off*: 9,5 IU/ml Specificity: 95,5% Run validity: 97,8% Specimen validity: 98,0%
Gorrin 2003[73]	TMA (VERSANT qualitative assay)	Good	Funded, organized and analysed by Bayer Corp.	Detection cut-off: 5,6 IU/ml Specificity: 99,6% Within-run variability: SD 6,58 copies/ml Between-run variability: SD 2,09 copies/ml
Gonzalez-Perez 2003[74]	Rt-PCR (UMELOSA HCV CUALITATIVO)	Poor	Partly funded by Umelosa Corp.	Detection cut-off: 101,7 IU/ml Specificity: 99,1%
Guichón 2004[75]	BK-HCV (NucliSens Basic Kit)	Fair	Funding not reported	Detection cut-off: 150 IU/ml Correlation with in-house rt-PCR: 96%
Quantitative				
Elbeik 2004[76]	bDNA 3.0 (VERSANT HCV bDNA 3.0 Assay)	Fair	Partly funded by Bayer Corp.	Detection cut-off: 615 IU/ml Specificity: 98,8% Linearity: y=0,9934x + 0,0343 Within-run variability: 9,1-26,9% Between-run variability: 2,7-13,4%

Germer 2002[77]	bDNA 2.0 and 3.0 (Quantiplex VCH RNA 2.0 and VERSANT HCV bDNA 3.0 Assay) HCM -2.0 (COBAS AMPLICOR HCV MONITOR)	Fair/ poor	Partly funded by Bayer Corp. and Roche Diagnostics	Agreement between bDNA 2.0 and 3.0: kappa 0,78 (0,72-0,84) Agreement between bDNA 3.0 and HCM-2.0: kappa 0,73 (0,65-0,81)
Trimoulet 2002[78]	bDNA 3.0 (VERSANT HCV RNA 3.0)	Fair/ Poor	Funding, data and specimens provided by Bayer Diagnostics.	Specificity: 98,2% Linearity: y=1,105x -0,468 Within-run variability: 2,4-38,4% Between-run reproducibility: 4,5-43,8%
Ross 2002[79]	bDNA 3.0 (VERSANT HCV RNA 3.0)	Fair/ poor	Partly funded by Bayer Corp.	Specificity: 98% (95%CI 96-99) Linearity: $\log_{10}$ Eq/ml=1,03 $\log_{10}$ IU/ml – 0,17 Within-run reproducibility: 4,5-8,7% Between-run reproducibility: 4,5-22,6%
Anderson 2003[80]	Real-time quantitative PCR and subsequent genotype determination Quantiplex HCV bDNA	Fair	Government funding	Linearity: $\log_{10}$ Eq/ml=0,41 $\log_{10}$ IU/ml + 3,93 Kappa major genotype: 0,69
Yang 2002[81]	Real-time rt-PCR	Poor	Government funding	Sensitivity: 1000 RNA copies/ reaction mixture Linearity: R^2=0,99 (equation not given) Within-run reproducibility: 0,51-1,37 Between-run reproducibility: 2,74-4,66
Leckie 2004[82]	Competitive rt-PCR (Abbott LCx HCV RNA)	Good/fair	Funded, organized and analyzed by Abbott	Lower limit of detection: 47 IU/ml Linearity: y=0,928x + 0,0594 Within-run reproducibility: SD 0,066

				logIU/ml Between-run reproducibility: SD 0,075 logIU/ml
Genotyping				
Nolte 2003[83]	Inno-Lipa HCV II TRUGENE	Fair	Partly funded by Visible Genetics	Overall agreement 99,5% Accuracy Inno-Lipa 98,6% TRUGENE 98%

* Detection cut-off: value at which specificity is 95% with 95% confidence.

b) Clinical studies (table 10)

Study ID	Tests considered	Quality assessment	Design	Population	Conclusions
Qualitative					
Ratge 2002[84]	Two-round rapid cycle rt-PCR (LightCycler)	Fair	Case-control	Platelet donors (n=50) and patients with confirmed or suspected HCV (n=106)	Detection cut-off: 109,1 IU/ml Specificity: 100% Intra-assay coefficient of variation: 11,5% Inter-assay coefficient of variation: 12,9%
Hermida 2002[85]	Rt- PCR; RNA detection in saliva	Fair	Cross-sectional cohort	Patients with chronic HCV infection (n=74) Hospital 73% males; mean age 40,5y	Detection cut-off: 10 copies/ml Sensitivity: 52,4%
Germer 2003[86]	TMA (Versant HCV RNA Qualitative Assay) rt-PCR (COBAS Amplicor)	Fair/poor	Prospective cohort (retrospective testing)	Patients with HCV infection undergoing IFN+ribavirin therapy (n=44)	At week 12: kappa 0,94 At week 24: kappa 0,74 Undetectable HCV RNA at 12w for SVR: Sens TMA: 84%; rt-PCR: 92% Spec TMA: 80%; rt-PCR: 53% NPV TMA: 86%; rt-PCR: 89%

Quantitative					
Sherman 2002[87]	bDNA 3.0 (VERSANT HCV RNA 3.0) rt-PCR (COBAS Amplicor Monitor 2.0)	Fair	Cross-sectional cohort	Patients with HIV and HCV infection (n=68) and patients with HCV alone (n=137); Recruited from trials 86% male, mean age 39,5y	Correlation of the two assays (after conversion to log_{10} IU/ml): r=0,82 in co-infected patients and r=0,90 in mono-infected patients Mean difference statistically significant
Veillon 2003[66]	bDNA 3.0 (VERSANT HCV RNA 3.0) HCM -2.0 (COBAS AMPLICOR HCV MONITOR) Total HCV core AG	Poor	Prospective cohort	Patients with chronic HCV (n=144) Recruited from trials IFN with or without ribavirin	Positive predictive value for SVR at 12w: decrease 2 log: 93% undetectable HCV RNA: 82% undetectable HCV core Ag: 96% Negative predictive value for SVR at 12w: decrease 2 log: 100% undetectable HCV RNA: 100% undectable HCV core Ag: 97%
Berg 2003[88]	bDNA 3.0 (VERSANT HCV RNA 3.0)	Fair	Prospective cohort (retrospective selection)	Patients with chronic HCV, (HIV and HBV excluded) Recruited from trials (n=260) 64% male, mean age 44,5y	OR for sustained viral response (logistic regression analysis) Viral load 130 000 IU/ml at baseline For genotype 1,4,6: 2,9 For genotype 2,3: 2,4 Negative predictive value viral load at 12w 615 IU/ml: 88,7% 30 000 IU/ml: 100%
Trimoulet 2004[89]	bDNA 3.0 (VERSANT HCV	Poor	Prospective cohort	Patients with chronic HCV and relapse after initial IFN treatment	Positive predictive value for SVR at 12w: decrease 2 log: 43%

	RNA 3.0)			(n=106) IFN with ribavirin	undetectable HCV RNA: 60% Negative predictive value for SVR at 12w: decrease 2 log: 97% undetectable HCV RNA: 96%
Furusyo 2002[90]	b-DNA 1.0 b-DNA 2.0	Good/fair	Prospective cohort	Patients with chronic HCV (n=122); HBV + HIV excluded University hospital 63% male, age range 24-70y IFN monotherapy	Correlation r=0,785 Sensitivity 1.0: 55,7% 2.0: 90,2% Significantly higher RNA levels by b-DNA 2.0 in genotype 2a. SR rate significantly higher with RNA level 0,5 Meq/ml by both assays When corrected for genotype, only RNA level by b-DNA 1.0 significantly associated with SR rate
Kawai 2002[67]	rt-PCR (Amplicor v1.0 and COBAS Amplicor Monitor 2.0) HCV core protein EIA (HCV core FEIA)	Fair/poor	Prospective cohort (retrospective selection?)	Patients with chronic HCV (n=236), At a university hospital 71% male, mean age 50,6y IFN monotherapy	Viral load prior to treatment by all assays was higher in non-responders Correlation between Amplicor and COBAS: r=0,874; RNA levels measured with COBAS significantly higher than with Amplicor Sensitivity Amplicor 91,1% COBAS 97,5% FEIA 78,4%
Castro 2002[91]	Rt-PCR (Amplicor HCV Monitor 2.0)	Fair/poor	Prospective cohort (retrospective selection)	Patients with chronic HCV (n=66); naive patients only that completed therapy University hospital 74% male, mean age 42y IFN + ribavirin therapy	SR lower with genotypes 1 and 4 SR lower with baseline viral load 5,5 $\log_{10}$ IU/ml 2 log decrease at 2 months (%): sens 100, spec 83, PPV 78, NPV 100 3 log decrease at 3 months (%): sens 100, spec 93, PPV 89, NPV 100

Genotyping					
Ben Moussa 2003[92]	Rt-PCR (Amplicor + Inno-Lipa) Serotyping (Murex)	Fair/poor	Cross-sectional cohort	Patients with HCV antibodies 78% males, mean age 40,9y	Overall concordance 68,3% Not typable: 1/60 with PCR 6/60 with serology
Haushofer 2003[93]	Inno-Lipa HCV II TRUGENE HCV 5'NC(=ref test) Vienna Lab HCV Strip Assay		Cross-sectional cohort	Consecutive HCV patients; HBV + HIV excluded University hospital	Accuracy Inno-Lipa 100% ViennaLab 97% Specificity Inno-Lipa 100% ViennaLab 99%
Roque-Afonso 2002[94]	Inno-Lipa HCV II (=ref test) TRUGENE HCV 5'NC	Fair/poor	Cross-sectional cohort (partly retrospective)	Clinical samples (n=205)+ HCV negative panel samples (n=3)	Concordance 100% Not typable: 15/205 with Inno-Lipa 6/205 with TRUGENE
Zheng 2003[95]	Inno-Lipa HCV II TRUGENE HCV 5'NC)	Good	Cross-sectional cohort	Consecutive clinical specimens (n=110) Hospital	Concordance 100% Not typable: 3/110 with Inno-Lipa 2/110 with TRUGENE
Comanor 2003[96]	TMA-Lipa	Good/fair	Cross-sectional cohort	Clinical specimens, in which genotyping previously failed	Typable: 83-93,7%

c) Economic studies (table 11)

Study ID	Comparison	Quality assessment	Design	Population	Conclusions
MSAC 2000[20]	Quantitative testing + genotyping *versus* no pre-treatment testing before interferon monotherapy	Good	Cost-benefit analysis	Patients willing to undergo HCV treatment (n=1000) 60% genotype 1, 4, 5 or 6 HCV	Incremental cost viral load testing and genotyping: $231 Pre-treatment viral load testing would be cost saving if >15% of patients decide against treatment on the basis of the test results.
Wong 1998[59]	(combinations of) liver biopsy, genotyping and quantitative HCV RNA testing prior to a single 6-months course of interferon treatment *versus* empirical interferon treatment *versus* conservative treatment.	Good	Cost-utility analysis using Markov model	Hypothetical population of patients with HCV	Incremental cost/QALY of quantitative HCV RNA testing + treating only patients with a viral load < 3.5 x 105 genomes per millilitre versus conservative management: $ 300/QALY (price year 1995). Incremental cost/QALY of treating all patients with viral load < 32 x 105 genomes per millilitre versus treating patients with viral load < 3.5 x 105 genomes per millilitre: $ 4 400/QALY compared to Incremental cost/QALY of empirical treatment versus quantitative HCV RNA testing with a cut-off of 32 x 105 genomes per millilitre: $ 12 400/QALY
Shepherd 2004[11]	Combination therapy pegylated interferon+ribavirin with 12 week stopping rule *versus* no treatment	Good	Cost-utility analysis using Markov model	Hypothetical cohort of 1000 patients; average age 36 years, 30-year follow up	Incremental cost /QALY of continuing treatment in patients without early virologic response: £226 573/QALY.
Davis 2002[22]	Pegylated interferon treatment+quantitative HCV RNA testing at 4, 12 or 24 weeks	Poor	Cost-benefit analysis	Estimates based on results of two RCTs (n=965, 67% genotype 1 HCV))	Cost reduction of the 12-week stopping rule compared to continuing treatment in early non-responders: 16%. About 0.6% of potential responders would be lost

					by applying this rule.
Wong 2003[60]	Combination therapy peginterferon or interferon+ribavirin in combination with quantitative HCV RNA test at 24 weeks versus quantitative HCV RNA test at 12 weeks	Fair	Cost-utility analysis using a Markov model	Input data based on one RCT (n=1530; 68% genotype 1 HCV)	The 12-weeks stopping rule decreases the incremental cost-effectiveness ratio of HCV treatment compared to the 24-weeks stopping rule. The 12-week stopping rule was less cost-effective in genotype 2 or 3 patients than in genotype 1 patients because genotype 2 or 3 HCV patients have a higher SVR rate.
Davis 2003[64]	Pegylated interferon treatment+quantitative HCV RNA testing at 4, 12 or 24 weeks	Poor	Cost-benefit analysis	Estimates based on the results of one RCT (n=511)	Testing at 12 weeks and discontinuing treatment in patients without EVR would reduce costs by 17,8% compared with 48 weeks of treatment for all patients. More savings were realised in genotype 1 patients compared to genotype 2 or 3 patients (21,5% versus 0,8%). Testing and therefore testing was not cost-saving in genotype 2 or 3 patients
Sagmeister 2001[65]	Combination therapy interferon+ribavirin with testing *versus* combination therapy without testing	fair	Cost-effectiveness analysis using Markov model	Input data for the model based on two RCTs (n=1445), mean age 42.2 years, 35% female, 64.6% genotype 1 HCV, 32% genotype 2 or 3 HCV	Genotype 1 HCV: 48 weeks treatment dominated by the quantitative HCV RNA testing. Genotype non-1 HCV: stopping treatment after 24 weeks in all patients more cost-effective than testing. For both sub-groups, quality of life decrements associated with continuing treatment in early non-responders outweighed the quality of life improvements in the additional responders.

THE CHECKLIST FOR HEALTH TECHNOLOGY REPORTS.

INAHTA 2001.

http://www.inahta.org/Reports.asp?name=/Content11/Dokument/HTAchecklist.pdf

	Yes	Partly	No
1. Are contact details available for further information?	( )	( )	( )
2. Authors identified?	( )	( )	( )
3. Statement regarding conflict of interest?	( )	( )	( )
4. Statement on whether report externally reviewed?	( )	( )	( )
5. Short summary in non-technical language?	( )	( )	( )
6. Reference to the question that is addressed and context of assessment?	( )	( )	( )
7. Scope of the assessment specified?	( )	( )	( )
8. Description of the health technology?	( )	( )	( )
9. Details on sources of information?	( )	( )	( )
10. Information on selection of material for assessment?	( )	( )	( )
11. Information on basis for interpretation of selected data?	( )	( )	( )
12. Results of assessment clearly presented?	( )	( )	( )
13. Interpretation of assessment results included?	( )	( )	( )
14. Findings of the assessment discussed?	( )	( )	( )
15. Medico-legal implications considered?	( )	( )	( )
16. Conclusions from assessment clearly stated?	( )	( )	( )
17. Suggestions for further action?	( )	( )	( )

THE CHECKLIST FOR SYSTEMATIC REVIEWS

	Well	Adequately	Poorly	Not adressed
1. The study addresses an appropriate and clearly focused question.	( )	( )	( )	( )
2. A description of the methodology used is included.	( )	( )	( )	( )
3. The literature search is sufficiently rigorous to identify all the relevant studies.	( )	( )	( )	( )
4. Study quality is assessed and taken into account.	( )	( )	( )	( )
5. There are enough similarities between the studies selected to make combining them reasonable.	( )	( )	( )	( )
6. How well was the study done to minimise bias?	++	+	-	
7. If coded as +, or - what is the likely direction in which bias might affect the study results?				
8. What types of study are included in the review?	RCT / CCT / Cohort / Case-control / Other			
9. How does this review help to answer your key question?				

THE CHECKLIST FOR PROGNOSTIC COHORT STUDIES

	Well	Adequately	Poorly	Not adressed
1. The study addresses an appropriate and clearly focused question.	( )	( )	( )	( )
2. The two groups being studied are selected from source populations that are comparable in all respects other than the factor under investigation.	( )	( )	( )	( )
3. The study indicates how many of the people asked to take part did so, in each of the groups being studied.	( )	( )	( )	( )
4. What percentage of individuals or clusters recruited into each arm of the study dropped out before the study was completed.				
5. Comparison is made between full participants and those lost to follow up, by exposure status.	( )	( )	( )	( )
6. The outcomes are clearly defined.	( )	( )	( )	( )
7. The assessment of outcome is made blind to exposure status.	( )	( )	( )	( )
8. Where blinding was not possible, there is some recognition that knowledge of exposure status could have influenced the assessment of outcome.	( )	( )	( )	( )
9. Evidence from other sources is used to demonstrate that the method of outcome assessment is valid and reliable.	( )	( )	( )	( )
10. The main potential confounders are identified and taken into account in the design and analysis.	( )	( )	( )	( )
11. Have confidence intervals been provided?	Yes / No			
12. How was this study funded? *List all sources of funding quoted in the article, whether* *Government, voluntary sector, or industry.*				

THE CHECKLIST FOR ANALYTICAL STUDIES

	Item	Yes	No	Unclear
1.	Is the source of the samples used described in sufficient detail?	( )	( )	( )
2.	Are the characteristics of the samples used described in sufficient detail?	( )	( )	( )
3.	Is the collection of samples representative for any possible situation when the test is applied in clinical practice?	( )	( )	( )
4.	Were the samples handled and stored in a way to assure its quality?	( )	( )	( )
5.	Was the index test performed without knowledge of the true status of the sample?	( )	( )	( )
6.	Was the execution of the index test described in sufficient detail to permit the replication of the test?	( )	( )	( )
7.	Was the sample size large enough to detect significant differences with the reference sample?	( )	( )	( )
8.	Were uninterpretable/intermediate test results reported?	( )	( )	( )
9.	Were test failures reported?	( )	( )	( )

THE CHECKLIST FOR DIAGNOSTIC ACCURACY STUDIES

The QUADAS tool

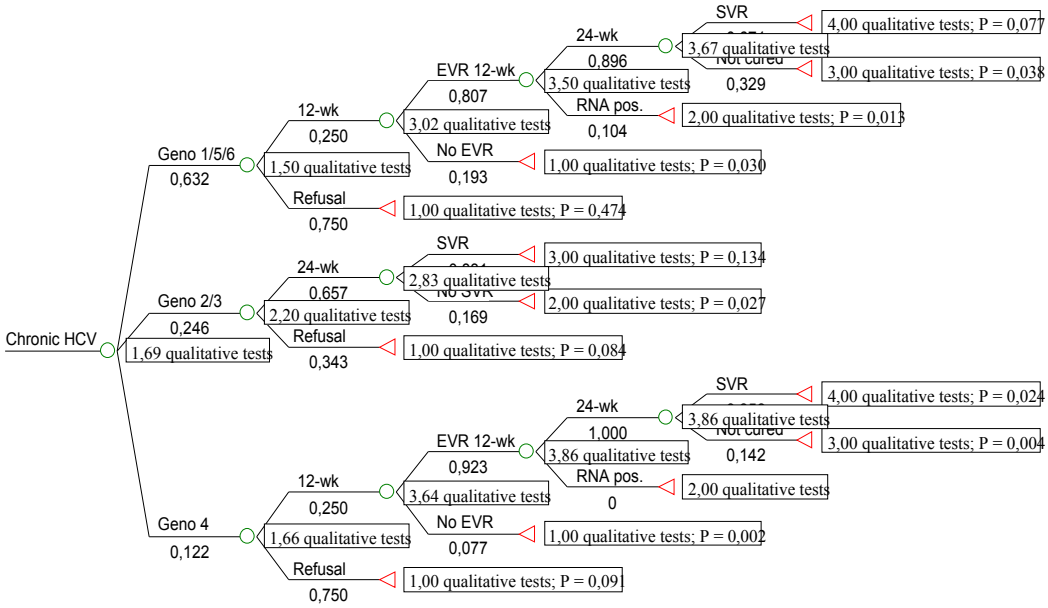
Item		Yes	No	Unclear
1.	Was the spectrum of patients representative of the patients who will receive the test in practice?	( )	( )	( )
2.	Were selection criteria clearly described?	( )	( )	( )
3.	Is the reference standard likely to correctly classify the target condition?	( )	( )	( )
4.	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	( )	( )	( )
5.	Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis?	( )	( )	( )
6.	Did patients receive the same reference standard regardless of the index test result?	( )	( )	( )
7.	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	( )	( )	( )
8.	Was the execution of the index test described in sufficient detail to permit replication of the test?	( )	( )	( )
9.	Was the execution of the reference standard described in sufficient detail to permit its replication?	( )	( )	( )
10.	Were the index test results interpreted without knowledge of the results of the reference standard?	( )	( )	( )
11.	Were the reference standard results interpreted without knowledge of the results of the index test?	( )	( )	( )
12.	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	( )	( )	( )
13.	Were uninterpretable/ intermediate test results reported?	( )	( )	( )
14.	Were withdrawals from the study explained?	( )	( )	( )

THE CHECKLIST FOR ECONOMIC EVALUATIONS

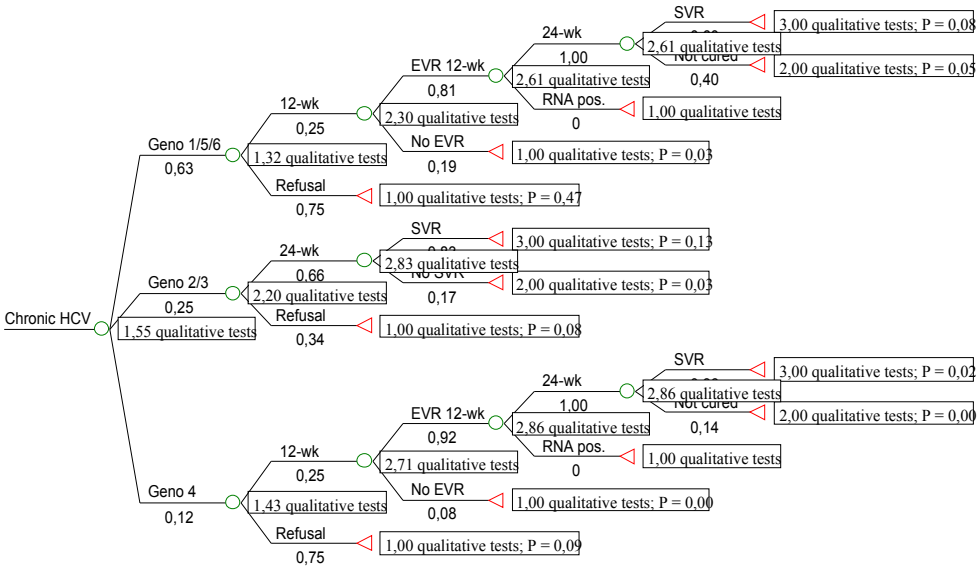
Item	Yes	No	Not applicable
Study design			
The research question is stated			
The economic importance of the research question is stated			
The viewpoints of the analysis are clearly stated and justified			
The rationale for choosing the alternative programmes or interventions compared is stated			
The alternatives being compared are clearly described			
The form of economic evaluation used is stated			
The choice of form of economic evaluation is justified in relation to the questions addressed			
Data collection			
The sources of effectiveness estimates used are stated			
Details of the design and results of effectiveness study are given (if based on a single study)			
Details of the method of sythesis or meta-analysis of estimated are given (if based on an overview of a number of effectiveness studies)			
The primary outcome measure(s) for the economic evaluation are clearly stated			
Methods to value healts states and other benefits are stated			
Details of the subjects from whom valuations were obtained are given			
Productivity changes (if included) are reported separately			
The relevance of productivity changes to the study question is discussed			
Quantities of ressources are reported separately from their unit costs			
Methods for the estimation of quantities and unit costs are described			
Currency and price data are recorded			
Details of currency of price adjustments for inflation or currency conversion are given			
Details of any model used are given			
The choice of model used and the key parameters on which it is based are justified			
Analysis and interpretation of results			
Time horizon of costs and benefits is stated			
The discount rate(s) is stated			
The choice of rate(s) is justified			
An explanation is given if costs or benefits are not discounted			
Details of statistical tests and confidence intervals are given for stochastic data			
The approach to sensitivity analysis is given			

The choice of variables for sensitivity analysis is justified			
The ranges over which the variables are varied are stated			
Relevant alternatives are compared			
Incremental analysis is reported			
Major outcomes are presented in a disaggregated as well as aggregated form			
The answer to the study question is given			
Concusions follow from the data reported			
Conclusions are accompanied by the appropriate caveats			
Editor's checklist for health economics papers			
Is the research question important?			
Is the economic importance of the question stated?			
Is the topic of interest to BMJ readers?			
Is there enough economic detail to allow peer review?			
If the economic content is sound, would we want to publish the paper?			
Is there a reasonable chance that the economic content is sound?			

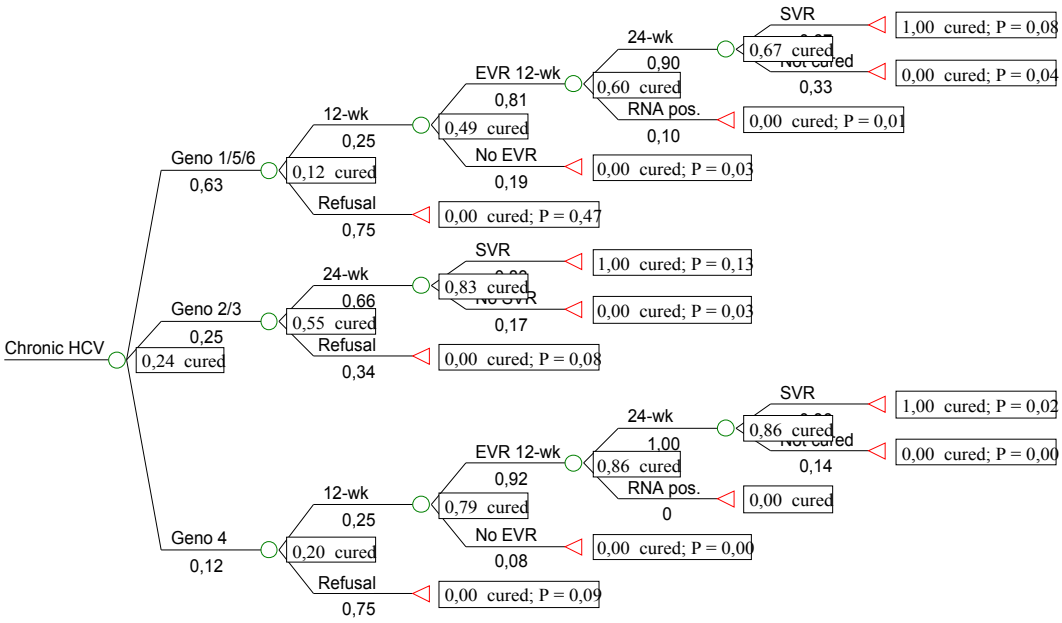
EXPECTED NUMBER OF HCV-RNA QUALITATIVE TESTS REQUESTED (BASE CASE - SCENARIO 1)



EXPECTED NUMBER OF HCV-RNA QUALITATIVE TESTS REQUESTED (BASE CASE ANALYSIS - SCENARIO 2)



EXPECTED NUMBER OF PATIENTS WITH A SUSTAINED VIRAL RESPONSE (BASE CASE ANALYSIS, SCENARIO 1)



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HTA Molecular Diagnostics Supplement II: Molecular testing for enterovirus in patients suspected of meningitis: a review of the evidence.

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Inhoudstafel

1. SUMMARY

Enteroviruses cause a myriad of symptoms, involving almost every organ system. More importantly, they are responsible for more than 90% of cases of aseptic meningitis for which an etiologic agent can be identified. Although the natural course is usually benign, the differential diagnosis with bacterial meningitis leads to hospitalisation and empirical treatment until diagnosis has been established.

Enteroviruses are mainly transmitted by the faecal-oral route. Due to prolonged shedding of virus from permissive sites, such as the pharynx or stool, the identification of enterovirus from these sites does not establish causality adequately, in contrast to identification from non-permissive sites, such as the central nervous system, vascular system and urinary tract.

The use of molecular tests in patients with suspected meningitis could lead to a fast and accurate identification of enterovirus, and thus excluding bacterial meningitis.

We have summarised the evidence on molecular tests for enterovirus, both for analytical accuracy, clinical accuracy and clinical impact of testing.

We searched the literature for HTA reports, systematic reviews and original diagnostic research in several databases. Studies were selected on the basis of predefined inclusion and exclusion criteria. Included studies were subsequently assessed for quality. Poor quality studies were excluded from the review. Data were extracted on study design, population included and test characteristics.

We were not able to identify any HTA reports or systematic reviews that met our criteria. In total, we included 16 original studies, of which 6 were on the analytical accuracy, 7 on the clinical accuracy and 3 on the clinical impact of the tests.

The analytical accuracy was reported poorly in general. Moreover, results were heterogeneous with sensitivity ranging from 61%-91% and specificity ranging from 86%-98%.

The overall quality of the clinical accuracy studies was equally poor, as the analytical studies. In addition, results were difficult to compare because of differences in case definition and reference test. Confidence intervals were not reported.

Sensitivity ranges from 85% to 100%; specificity from 80% to 100%. As a true 'golden' standard does not exist for enterovirus meningitis, these estimates are uncertain. CSF pleocytosis influences the test characteristics.

In theory, a positive PCR test could lead to important clinical consequences, such as immediate discharge or refraining from further antibiotic treatment. A significant difference between patients with a positive and with a negative PCR test result was found by several authors. In two studies, a relevant part of the study population was excluded from the analyses, thus embellishing the results and reducing the applicability in clinical practice. Possible adverse consequences of the use of these molecular tests were not addressed.

In conclusion, both the analytical and clinical accuracy of the enterovirus PCR tests are not sufficient at this moment to be introduced in clinical routine practice.

Although a positive clinical impact of introducing such tests could be assumed on theoretical grounds and has been partly analysed in some studies, the uncertainty of the accuracy of these tests is too large.

2. INTRODUCTION

The enteroviruses form a genus within the family Picornaviridae. The genus is divided in 5 groups: the polioviruses, the group A coxsackieviruses, the group B coxsackieviruses, echoviruses and the newer numbered enteroviruses, totalling 66 different serotypes[1]. Enteroviruses are small, icosahedral RNA viruses. They are able to remain viable for prolonged periods in sewage, water and on hands, thereby enhancing their transmissibility.

Incidences of enterovirus infections are not known exactly. However, it is estimated that in the United States, enteroviruses cause 30 to 50 million infections per year. Although enterovirus infections occur in all age groups, incidence is most high in children[2]. They are responsible for a myriad of symptoms and involve almost every organ system. Manifestations range from nonfocal febrile illness to potentially life-threatening diseases such as meningitis, encephalitis, myocarditis and fulminant neonatal sepsis. Enteroviruses are responsible for more than 90% of cases of aseptic meningitis for which an etiologic agent can be identified[3].

The enteroviruses are transmitted mainly by the faecal-oral route. Once infected, an individual may shed virus from the oropharynx for 1 to 4 weeks and up to 16 weeks in the stool. This issue is particular important in clinical use, when assessing causality between a patient's symptoms and an enteroviral infection. Because of this prolonged shedding from the upper respiratory and gastrointestinal tract, the isolation of the enterovirus of these 'permissive' sites does not conclusively establish causality of a patient's illness. Sites such as the central nervous system, vascular system and urinary tract are usually 'non-permissive', finding the enterovirus in these sites does establish causality[4].

Molecular tests are advocated for use in patients with suspected enterovirus infection, mainly meningitis, because compared to tissue culture they are faster and have a higher sensitivity. However, quantitative information on the added value is scarce.

In this report, we give a structured review of the existing literature on molecular tests for enterovirus in patients with suspected meningitis.

3. METHODS

3.1. SEARCH STRATEGY

We performed an iterative literature search, more precisely we searched for existing health technology assessments (HTA) first, subsequently for systematic reviews and finally for original diagnostic research.

HTA reports were searched in INAHTA, the Health Technology Assessment Database of the CRD, the Canadian Centre for Health Technology Assessment and the Agency for Health and Quality Research. We searched for systematic reviews in DARE of the CRD, Medion, Medline and Embase databases. Original research was identified in Medline and Embase, the search limited to studies published after the literature search of any HTA report or systematic review. Studies that report the performance of a diagnostic strategy were included as well.

In addition, we checked the Food and Drugs Administration website to identify the tests that have received an FDA approval.

The search date was August 2004.

3.2. SEARCH TERMS

The search term used for INAHTA, CRD and Medion was "enterovirus" or "meningitis".

The search-string we used in PubMed is listed below:

("Cytogenetic Analysis"[MeSH] OR "Molecular Probe Techniques"[MeSH] OR "Molecular Diagnostic Techniques"[MeSH]) AND ("Enterovirus"[MeSH] OR (enterovirus) AND sensitive*[Title/Abstract] OR sensitivity and specificity[MeSH Terms] OR diagnos*[Title/Abstract] OR diagnosis[MeSH:noexp] OR diagnostic * [MeSH:noexp] OR diagnosis differential[MeSH:noexp] OR diagnosis[Subheading:noexp])

In Embase, we used an adapted version of the same search-string:

((((diagnos*) in AB)or((diagnos*) in TI)) or ("sensitivity-and-specificity" / all SUBHEADINGS in DEM DER DRM DRR) or (((sensitive*) in AB)or((sensitive*) in TI)) or ("differential-diagnosis" / all SUBHEADINGS in DEM DER DRM DRR) or (((diagnostic) in DEM)or((diagnostic) in DER)) or ("diagnosis-" / all SUBHEADINGS in DEM DER DRM DRR)) and ((enterocirus) or ("enterovirus" / all SUBHEADINGS in DEM DER DRM DRR) or ("enterovirus" / all SUBHEADINGS in DEM DER DRM DRR)) and ((explode "genetic-procedures" / all SUBHEADINGS in DEM DER DRM DRR) or (explode "molecular-probe" / all SUBHEADINGS in DEM DER DRM DRR) or ("chromosome-analysis" / all SUBHEADINGS in DEM DER DRM DRR) or (explode "gene-amplification" / all SUBHEADINGS in DEM DER DRM DRR))

Search results for the original articles were then selected by two independent reviewers (AVDB, MH), using the following in and exclusion criteria:

Inclusion: diagnostic accuracy study, enterovirus, molecular tests, meningitis.

Exclusion: reviews, letters, commentaries, case studies, target condition other than meningitis.

Studies were categorized as analytical studies if they assessed the test on samples with known content, and as clinical studies if they included patients.

3.3. QUALITY ASSESSMENT

To assess the quality of HTA reports, we used the checklist published at INAHTA. The quality of systematic reviews and prognostic studies were assessed using the checklists of SIGN (www.sign.ac.uk).

The QUADAS tool was used for the quality assessment of original diagnostic research on patients[5]. For original, analytical studies, we assessed items on validity of the panel used, on test execution and analysis using a self-constructed checklist, as no validated checklist was available from the literature. See appendix for all quality assessment checklists used in the review.

Quality assessment is summarized as good quality, fair or poor quality. HTA reports or systematic reviews received a poor quality appraisal when the search of the literature was insufficient and no quality assessment of included studies was reported. Analytical studies were of fair quality when 5 of the 7 items were answered with no or unclear, or when 3 items were answered with no. They were poor quality when 6 items were answered with no or unclear or 4 were answered with no.

Original diagnostic accuracy studies were considered fair quality if 6 of the 14 items were answered with no or unclear or 4 items with no. Studies were considered of poor quality when 7 items were answered with no or unclear or 5 with no.

Poor quality studies were excluded from further review.

3.4. DATA EXTRACTION

Test characteristics are not absolute. Variables such as setting, spectrum or demographic features of the population studies, are known to influence test characteristics[6]. In addition, when bias was introduced into the study methodology, this will distort the study results and give biased test characteristics. The most important forms of bias in diagnostic research are inappropriate reference test, an inadequate case-control design and lack of blinding when reading the test results[7].

Therefore, it is important to report these variables and study characteristics together with the test characteristics. Finally, any funding, whether partly or fully, by any commercial source was noted.

The following data were extracted from the clinical studies:

Demographic characteristics of the population studied: setting, in- and exclusion criteria, age and gender.

Design: cross-sectional cohort, prospective cohort, case-control.

Results: sensitivity, specificity, odds ratio, negative en positive predictive value, correlation coefficients, mean difference and 95% limits of agreement, linearity.

Remarks on the funding of the study.

We did not perform a formal meta-analysis, but present an overall review of the test characteristics and prognostic value.

4. RESULTS

4.1. HTA REPORTS

No HTA reports were identified.

4.2. SYSTEMATIC REVIEWS

We identified 20 possible systematic reviews. However, not one met our inclusion criteria screening title and abstract, being a systematic review on the use of molecular testing for enterovirus infection.

4.3. ORIGINAL RESEARCH

We retrieved 496 articles with our search strategy in the different databases. Of these 496, 65 articles were withheld after screening title and abstract on in- and exclusion criteria.

The 65 articles meeting the inclusion criteria were subsequently retrieved in full and assessed for inclusion criteria and quality. However, eight of these 65 articles were not available from the university library after considerable effort and could not be assessed.

On reading the full text, 20 articles were excluded because they did not assess a molecular test (n=1), because they were not a diagnostic accuracy study (n=8), because they did not use meningitis as the outcome (n=5) or because they did not study enteroviruses (n=6).

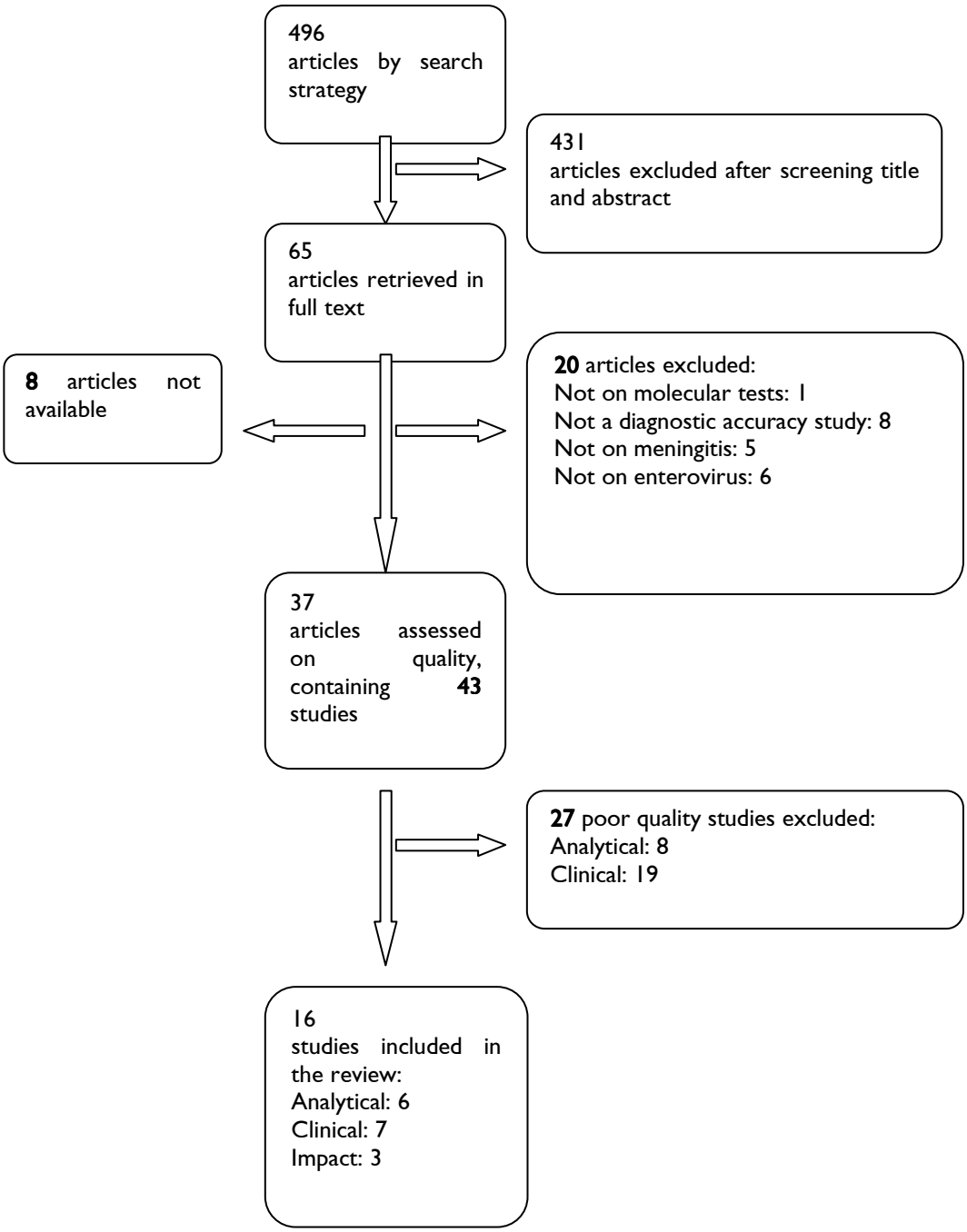
37 articles thus remained for quality assessment. Some articles contained both an analytical and a clinical study, with 43 studies in total.

Of the remaining included articles, 14 reported an analytical study. After quality assessment, 8 were considered as poor quality studies, 4 as fair quality studies and 2 as good quality studies. All studies reported sufficient detail of the test to permit replication in practice. Other items which were fulfilled by 8 studies were a sufficient description of the source of the samples and a correct handling of the samples to ensure their quality. On the other hand, only 3 studies reported to have performed the index test without knowing the true status of the samples, and not one study reported adequately on uninterpretable or intermediate results or test failures. We therefore excluded the 8 analytical studies with poor quality from further review[8-15]. The remaining 6 studies were included[16-21].

Additionally, we assessed 26 clinical, diagnostic accuracy studies on quality. Of these 26 studies, 19 studies were of poor quality, 6 of fair quality and finally 1 study was of good quality. The quality item that was scored best was sufficient details on the index test to permit its replication in practice, with 23 studies fulfilling this criterion. Sufficient details on the reference test were given by 19 studies, even as on the item of the period between the index test and the reference test. It should be noted that we considered appropriate storage of samples between testing by the reference and the index test a sufficient guarantee of quality. Like the analytical studies, the item on blinding for the reference test results when performing the index test was very badly reported: only 1 study specifically stated to have blinded the results, 23 studies did not mention anything on blinding and were subsequently scored as unclear. The item on the selection of participants, an important design feature that might introduce bias, was adequately reported by 6 studies, 1 was unclear and 19 studies either reported an inadequate selection or did not report the selection at all.

In conclusion, we excluded the 19 poor quality clinical accuracy studies from further review[22-26, 9, 27, 11, 28-38]. The remaining 7 studies were included[39-45].

Finally, we included three studies on the impact of the use of molecular testing in clinical practice on patient outcome[45-47].



4.4. EVIDENCE TABLES

4.4.1. HTA reports

No HTA reports were included.

4.4.2. Systematic reviews

No systematic reviews were included.

4.4.3. Original studies

Analytical studies

Study ID	Tests considered	Quality assessment	Remarks	Conclusions
Muir 1999[19]	RT-PCR (Enterovirus Amplicor) and various in-house PCR methods	Good	Multicenter quality control study, no commercial funding reported	Sensitivity: 77-91% Specificity: 86-97.4% Lowest concentration detected: 0.001-1 $TCID_{50}$*
Lina 1996[18]	RT-PCR (Amplicor)	Good	Multicenter study on a panel of coded CSF samples; The PCR kits were provided by Produits Roche.	Sensitivity: 61% (37.5-77.5%) Specificity: 98% Detection limit: 10 $TCID_{50}$ Lowest concentration detected: 0.1 $TCID_{50}$
Puppe 2004[21]	Multiplex RT-PCR ELISA	Fair	Study funded by a governmental research grant. From the enterovirus family, only *Coxsackie strains A and B* were included.	Lowest concentration detected: 10^{-6} $TCID_{50}$
Zoll 1992[16]	RT-PCR	Fair	No funding reported.	Sensitivity: 90.1% Specificity: none of the viruses reacted in the PCR (data not given) Lowest concentration detected: 0.1 fg coxsackievirus type B3 clone
Nijhuis 2002[20]	Nested RT-PCR vs real-time TaqMan PCR	Fair	No funding reported.	Sensitivity: reproducibly positive in 0.36-25.2 $TCID_{50}$ Specificity: weakly positive in 7/90 rhinovirus high-titer samples.
Casas 1995[17]	RT-PCR vs PCR reverse transcription and amplification in one tube	Fair	Funded by governmental grants.	Detection limits: 0.003-0.02 $TCID_{50}$ Conventional assay had 10-fold higher sensitivity than the single-tube assay

				Specificity: no positive bands in other virus samples

* $TCID_{50}$: 50% tissue culture infective dose

Clinical studies

Study ID	Tests considered	Quality assessment	Design	Population	Conclusions
Hosoya 1998[44]	Nested PCR for enterovirus + nested PCR for mumps	Good	Prospective cohort, consecutive?	Patients (children?) with an acute illness and signs and symptoms suggestive of meningitis. n=73; prevalence of aseptic meningitis 61.6% Aseptic meningitis= acute illness with CSF pleocytosis (>10WBC/µl) and no evidence of bacterial disease.	Sensitivity mumps PCR: 81.3% Sensitivity enterovirus: 89.3% Pleocytosis +: 55.6% PCR+ enterovirus 31.1% PCR+ mumps Pleocytosis-: 14.3% PCR+ enterovirus 0% PCR+ mumps
Gorgievski-Hrisoho 1998[43]	RT-PCR	Fair	Retrospective cohort,	CSF from patients with aseptic meningitis during outbreak. n=80, median age 22y; prevalence 85% Aseptic meningitis= clinical and CSF findings compatible with viral meningitis and lack of alternative diagnosis.	Sensitivity: 85% (n=68) Specificity: 100% (n=12)
Ahmed 1997[42]	PCR with colorimetric microwell detection system	Fair	Prospective cohort, consecutive?	Infants 3 months with fever at the ED*; signs and symptoms prompting lumbar puncture, blood culture or both, with sterile cultures of CSF, blood and urine, and no preceding antibiotics n=64; prevalence? (29/65?) Enteroviral meningitis= isolation of enterovirus from CSF; CSF pleocytosis in the absence of bacterial disease with isolation of enterovirus from stool; or both.	CSF: Sensitivity 92% Specificity: 94% Serum: Sensitivity 100% Specificity: 80% Urine: Sensitivity 29% Specificity: 100%

Riding 1996[40]	Nested PCR	Fair	Prospective cohort, consecutive?	Patients with symptoms of aseptic meningitis as stated by the treating physician; n=340; age 4 months-56 years PCR compared to viral culture	PCR+: 27% (38% in stool; 26% in throat swab and 25% in CSF) Viral culture+: 6%
Yerly 1996[41]	RT-PCR (Amplicor)	Fair	Prospective cohort, Consecutive?	Children with CSF pleocytosis, Gram stain- and negative cultures for bacteria and fungi. n=38; prevalence not given.	In CSF specimens: PCR+: 66% Viral culture+: 34%
Schlesinger 1994[39]	RT-PCR	Fair	Retrospective cohort, not consecutive	Selection of all CSF specimens of infants 3 months, ± CSF pleocytosis. n=45; prevalence not given.	CSF pleocytosis: CSF viral culture+: PCR+ 100% Faecal or nasopharyngeal culture+: PCR+ 100% Cultures- or not done: PCR+ 45% Bacterial meningitis: PCR 0% No CSF pleocytosis CSF viral culture+: PCR+ 67% Faecal or nasopharyngeal culture+: PCR+ 40% All viral cultures negative: PCR+ 0%
Hamilton 1999[45]	RT-PCR (Amplicor)	Fair	Prospective cohort, Consecutive in 1996 and on request by treating physician in 1997	Children having a lumbar puncture Enteroviral disease established by chart review. n=489; prevalence 29.2%	With culture as reference test: Sensitivity: 94% (1996); 88% (1997) Specificity: 93% (1996); 83% (1997)

*ED= emergency department

Impact studies

Study ID	Tests considered	Quality assessment	Design	Population	Conclusions
Ramers 2000[46]	In-house PCR	Good	Retrospective cohort	All patients for whom a PCR test was ordered; analyses on patients with result available before discharge n=276; prevalence 49.6% Tests were performed 3/week January through May 6/week June through December	Statistically significant difference (p<0.05) between PCR+ en PCR- patients on: LOS, time from PCR test to discharge, no. of patients who received CT* or MRI*, no. of patients who received EEG*, intravenous antibiotics step-down unit stay
Hamilton 1999[45]	RT-PCR (Amplicor)	Fair	Prospective cohort, Consecutive in 1996 and on request by treating physician in 1997	Children having a lumbar puncture n=489 For length of stay analysis: complicated cases or children discharged immediately from the ED were excluded (n=179)	7.7 tests per case identified. When CSF 10 WBC: 5.8 tests per case In 1997 LOS 1.93 days for patients PCR+ versus 2.44 days for patients PCR- (p<0.05); PCR results reported daily
Stellrecht 2002[47]	RT-PCR	Poor	Retrospective cohort,	Patients with suspicion of meningitis, Additional analyses on infants <3 months, admitted between Sunday and Thursday, with PCR+ (control group of infants without enterovirus meningitis) n=1056	PCR testing 3 times a week: LOS* shorter than test TAT*. PCR testing 5 times a week: correlation between LOS and TAT increases

*LOS: length of stay

TAT: turn-around-time

CT: computed tomographic scan

MRI: magnetic resonance imaging

EEG: electroencephalogram

5. ANALYTICAL ACCURACY

In general, the analytical accuracy of PCR tests for the detection of enterovirus is reported poorly. We were able to identify only two good quality studies, both multicenter quality control studies. An additional four studies with fair quality were included in the review; six studies were excluded because of poor quality. Even some of the included studies provided insufficient information to extract essential test characteristics, such as sensitivity and specificity.

Overall, the analytical accuracy of the PCR tests is only fair. The two best quality studies report sensitivities between 61% and 91%. One other study reported a sensitivity of 90.1%. Specificities are better, ranging from 86% to 98%. Other studies often reported that the PCR test did not react with any of the other viruses, however without providing the actual data or any outcome measure.

Key messages:

- **The analytical accuracy of PCR tests for the detection of enterovirus is reported poorly.**
- **Sensitivity ranges from 61% to 91%.**
- **Specificity ranges from 86% to 98%.**

6. CLINICAL ACCURACY

As with the analytical studies, the quality of clinical studies was poor. We were able to identify only one good quality study and 6 fair quality studies. In contrast, we excluded 19 poor quality studies from the review.

A major problem with clinical accuracy studies here is the reference test. How is the true status of the patient defined? Some studies compared the PCR test with viral culture. However, viral culture has a very low sensitivity for enterovirus, therefore enterovirus infections will be missed by viral culture. True positives will be wrongly classified as false positives and the reported outcome measures will not be correct.

Other studies used the PCR test itself as the reference test by which patients were classified as cases or non-cases. Although in theory the PCR test might be more accurate than the viral culture, using the index test as the reference test introduces incorporation bias and is not appropriate.

Furthermore, the outcome of interest was not the same in all studies. Some studies used aseptic meningitis as the outcome, others enteroviral meningitis.

In several studies, aseptic meningitis was defined as an acute illness with clinical and cerebrospinal fluid findings compatible with viral meningitis and the lack of an alternative diagnosis. From these patients, the isolation of enterovirus by viral culture from CSF confirms the diagnosis of enterovirus meningitis. In some studies, the isolation of enterovirus from other sites, such as stool or throat swabs, was used in addition to other diagnostic criteria, such as CSF pleocytosis. However, as outlined in the introduction, stool and throat swabs are permissive sites and do not conclusively establish causality.

Another drawback of the included studies is the lack of reporting confidence intervals. Most of the studies included only a fairly small population, thus possibly leading to wide confidence intervals. However, most studies do not report these confidence intervals, which diminish the generalisability of the results to clinical practice.

Sensitivities range from 85% to 100%, specificity from 80% to 100%.

The detection of enterovirus RNA in CSF is different according to the presence of pleocytosis. One study found in CSF samples with pleocytosis, that the PCR was positive in 55.6%, whereas in samples without pleocytosis PCR was positive only in 14.3%. Other studies also found better concordance with viral culture in CSF samples with pleocytosis than without pleocytosis.

In conclusion, we have identified a limited number of studies, of an overall poor quality. Studies suffer from methodological weaknesses and intrinsic difficulties due to the lack of a good reference test. The test characteristics of molecular test on enterovirus in patients suspected of meningitis are not sufficient to be used in practice.

The use of these tests in patients with CSF pleocytosis could be more advantageous, but reliable and accurate tests should be available first.

Key messages:

- **The overall quality of the studies identified by our search was poor.**
- **Results are difficult to compare due to differences in reference test or case definition.**
- **Sensitivity ranges from 85% to 100%; specificity from 80% to 100%.**
- **CSF pleocytosis influences the test characteristics.**
- **Confidence intervals are not reported**

7. CLINICAL IMPACT STUDIES

The course of enterovirus meningitis is usually benign, but the difficulty is to distinguish it from bacterial meningitis. During the diagnostic process, patients are hospitalised and receive empirical treatment with antibiotics. The ability to rapidly differentiate enterovirus meningitis from bacterial meningitis could lead to early discharge of these patients and has the potential for reducing the duration of hospitalisation and treatment.

We identified three articles that report the impact of PCR testing on clinical parameters, such as length of stay or administration of intravenous antibiotics.

One study found a significant difference between patients with a positive PCR test result compared to patients with a negative test result, on a number of outcomes. However, the authors included only those patients whose test result was available prior to patient discharge, which was the case in only two third of the population.

Another study found that more than 7 tests had to be performed to identify one case. If PCR testing was limited to the CSF samples with pleocytosis, less than 6 tests per case had to be performed. On the condition that PCR tests were reported daily, the length of stay of patients with a positive test result was significantly shorter than of those with a negative test result.

Finally, the last study on clinical impact found that the length of stay was on average shorter than the test-turn-around time when the PCR tests were performed 3 times a week; the correlation between length of stay and turn-around-time increased when the PCR tests were performed 5 times a week. However, in this last analysis, patients admitted on Friday or Saturday were excluded, as the PCR tests were not available at the weekend.

Key messages:

- **In theory, a positive PCR test could lead to important clinical consequences, such as immediate discharge or refraining from further antibiotic treatment.**
- **A significant difference between patients with a positive and with a negative PCR test result was found by several authors.**
- **In two studies, a relevant part of the study population was excluded from the analyses, thus embellishing the results and reducing the applicability in clinical practice.**

8. DISCUSSION

Distinguishing patients with enterovirus meningitis from patients with bacterial patients is clinically useful. Whereas enterovirus meningitis usually has a benign course and does not necessarily have to be treated, bacterial meningitis still causes important morbidity and mortality[48]. Considering prognosis of bacterial meningitis improves with prompt treatment[49], patients with meningitis are treated empirically with antibiotics while waiting for further test results.

In theory, a positive identification of enterovirus in cerebrospinal fluid or any other causative site, could lead to early discharge of the patient, withdrawal of further antibiotic treatment and relieve anxiety on further prognosis. As viral culture not only takes too long before the result is available, but is also not sufficiently sensitive, molecular tests could play a role here.

We were able to identify only a limited number of studies on the analytical and clinical accuracy with good or fair quality. In addition, the results of these studies were heterogeneous. Studies on clinical accuracy were furthermore complicated by the lack of a good reference test or definition of the outcome. Also, the population that has been included, the outcome definition, reference test and results were heterogeneous. Not one study reported confidence intervals on their outcome measures.

Some of the studies reported very moderate test characteristics.

Therefore, we conclude that both the analytical and clinical accuracy of the enterovirus PCR tests are not sufficient at this moment to be introduced in clinical routine practice.

Although a positive clinical impact of introducing such tests could be assumed on theoretical grounds and has been partly analysed in some studies, the uncertainty of the accuracy of these tests is too large.

9. SCENARIO CHARACTERISTICS

Target condition:

Incidence/prevalence: range from 29% to 85%

Acute or chronic condition: acute

Positive diagnosis can lead to hospital discharge due to the benign nature of the illness.

Test

Effect on treatment: yes: withdrawal of treatment

Prognostic impact: no

Test result 24h: theoretically yes

Outbreak surveillance: possibly

Recommendations

The use of molecular tests for the diagnosis of enteroviral meningitis can not be recommended at this moment.

APPENDIX

1. The checklist for health technology reports.

INAHTA 2001.

http://www.inahta.org/Reports.asp?name=/Content11/Dokument/HTAchecklist.pdf

	Yes	Partly	No
1. Are contact details available for further information?	( )	( )	( )
2. Authors identified?	( )	( )	( )
3. Statement regarding conflict of interest?	( )	( )	( )
4. Statement on whether report externally reviewed?	( )	( )	( )
5. Short summary in non-technical language?	( )	( )	( )
6. Reference to the question that is addressed and context of assessment?	( )	( )	( )
7. Scope of the assessment specified?	( )	( )	( )
8. Description of the health technology?	( )	( )	( )
9. Details on sources of information?	( )	( )	( )
10. Information on selection of material for assessment?	( )	( )	( )
11. Information on basis for interpretation of selected data?	( )	( )	( )
12. Results of assessment clearly presented?	( )	( )	( )
13. Interpretation of assessment results included?	( )	( )	( )
14. Findings of the assessment discussed?	( )	( )	( )
15. Medico-legal implications considered?	( )	( )	( )
16. Conclusions from assessment clearly stated?	( )	( )	( )
17. Suggestions for further action?	( )	( )	( )

2. The checklist for systematic reviews

	Well	Adequately	Poorly	Not adressed
1. The study addresses an appropriate and clearly focused question.	( )	( )	( )	( )
2. A description of the methodology used is included.	( )	( )	( )	( )
3. The literature search is sufficiently rigorous to identify all the relevant studies.	( )	( )	( )	( )
4. Study quality is assessed and taken into account.	( )	( )	( )	( )
5. There are enough similarities between the studies selected to make combining them reasonable.	( )	( )	( )	( )
6. How well was the study done to minimise bias?	++	+	-	
7. If coded as +, or - what is the likely direction in which bias might affect the study results?				
8. What types of study are included in the review?	RCT / CCT / Cohort / Case-control / Other			
9. How does this review help to answer your key question?				

3. The checklist for prognostic cohort studies

	Well	Adequately	Poorly	Not adressed
1. The study addresses an appropriate and clearly focused question.	( )	( )	( )	( )
2. The two groups being studied are selected from source populations that are comparable in all respects other than the factor under investigation.	( )	( )	( )	( )
3. The study indicates how many of the people asked to take part did so, in each of the groups being studied.	( )	( )	( )	( )
4. What percentage of individuals or clusters recruited into each arm of the study dropped out before the study was completed.				
5. Comparison is made between full participants and those lost to follow up, by exposure status.	( )	( )	( )	( )
6. The outcomes are clearly defined.	( )	( )	( )	( )
7. The assessment of outcome is made blind to exposure status.	( )	( )	( )	( )
8. Where blinding was not possible, there is some recognition that knowledge of exposure status could have influenced the assessment of outcome.	( )	( )	( )	( )
9. Evidence from other sources is used to demonstrate that the method of outcome assessment is valid and reliable.	( )	( )	( )	( )
10. The main potential confounders are identified and taken into account in the design and analysis.	( )	( )	( )	( )
11. Have confidence intervals been provided?	Yes / No			
12. How was this study funded? *List all sources of funding quoted in the article, whether Government, voluntary sector, or industry.*				

4. The checklist for analytical studies

	Item	Yes	No	Unclear
1.	Is the source of the samples used described in sufficient detail?	( )	( )	( )
2.	Are the characteristics of the samples used described in sufficient detail?	( )	( )	( )
3.	Is the collection of samples representative for any possible situation when the test is applied in clinical practice?	( )	( )	( )
4.	Were the samples handled and stored in a way to assure its quality?	( )	( )	( )
5.	Was the index test performed without knowledge of the true status of the sample?	( )	( )	( )
6.	Was the execution of the index test described in sufficient detail to permit the replication of the test?	( )	( )	( )
7.	Was the sample size large enough to detect significant differences with the reference sample?	( )	( )	( )
8.	Were uninterpretable/intermediate test results reported?	( )	( )	( )
9.	Were test failures reported?	( )	( )	( )

5. The checklist for diagnostic accuracy studies

The QUADAS tool

Item		Yes	No	Unclear
1.	Was the spectrum of patients representative of the patients who will receive the test in practice?	( )	( )	( )
2.	Were selection criteria clearly described?	( )	( )	( )
3.	Is the reference standard likely to correctly classify the target condition?	( )	( )	( )
4.	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	( )	( )	( )
5.	Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis?	( )	( )	( )
6.	Did patients receive the same reference standard regardless of the index test result?	( )	( )	( )
7.	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	( )	( )	( )
8.	Was the execution of the index test described in sufficient detail to permit replication of the test?	( )	( )	( )
9.	Was the execution of the reference standard described in sufficient detail to permit its replication?	( )	( )	( )
10.	Were the index test results interpreted without knowledge of the results of the reference standard?	( )	( )	( )
11.	Were the reference standard results interpreted without knowledge of the results of the index test?	( )	( )	( )
12.	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	( )	( )	( )
13.	Were uninterpretable/ intermediate test results reported?	( )	( )	( )
14.	Were withdrawals from the study explained?	( )	( )	( )

6. The checklist for observational studies

	Item	Yes	No	Unclear
1.	Has the research question been adequately described?	( )	( )	( )
2.	Has the study population been adequately described?	( )	( )	( )
3.	Is the study population relevant for the research question?	( )	( )	( )
4.	Are the subjects in the different groups comparable?	( )	( )	( )
5.	Has the exposure or intervention been adequately described?	( )	( )	( )
6.	Has the outcome been measured in a blind and independent fashion?	( )	( )	( )
7.	Has the statistical analysis been performed adequately?	( )	( )	( )
8.	Are the results presented in a clinical meaningful way?	( )	( )	( )
9.	Are the important findings and limitations of the study adequately discussed?	( )	( )	( )
10.	Has any funding or sponsorship been reported?	( )	( )	( )

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HTA Molecular Diagnostics Supplement III: PCR-based detection and quantification of t(14;18) in follicular lymphoma diagnosis and follow-up

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1. INTRODUCTION

Follicular lymphoma (FL) is the second most common form of non-Hodgkin lymphoma. The incidence of FL in Belgium is estimated at 400 cases per year. The patients are mainly elderly and the median survival is 8 to 10 years. A frequent chromosomal aberration of FL is the translocation t(14;18)(q32;q21) (Bcl-2/IgH), involving the immunoglobulin heavy chain (IgH) gene on chromosome 14q32 and the Bcl-2 gene on chromosome 18q21. This translocation results in the juxtaposition of the antiapoptotic Bcl-2 gene and the IgH heavy chain locus on chromosome 14, leading to upregulation of Bcl-2 protein expression in most cases of FL, and an inhibition of cell death.

Initially, the biological material available for diagnosing FL is most frequently a lymph node, but can also consist of bone marrow. The lymph node tissue is best shipped fresh and not fixed. Coordination of the different tests involved in the local pathology and hematology lab, and at external laboratories such as CMDs and CMGs is best handled by a single coordinator, according to a diagnostic scheme outlined in the local oncology handbook. Diagnosis of follicular lymphoma can be based on morphology and immunohistochemistry in over 95% of the cases of FL. In the remaining cases tests for monoclonality or for t(14;18) may help the pathologist to define malignancy and the diagnosis of FL. However, testing for t(14;18) is routinely performed anyhow in most cases of FL as part of an integrated diagnostic work-up. Other tests pathologists perform for FL include IHC on frozen tissue slides or flow cytometry. If immediately available, IHC on frozen tissue slides may help select samples for karyotyping.

1.1. POSSIBLE TESTING ALGORITHMS

Three possible ways to test for t(14;18) are given. The local situation may define the most appropriate scenario. The most comprehensive approach to t(14;18) testing in FL starts with karyotyping. Metaphase induction needs to start immediately after receipt of a fresh sample, consisting of lymph node tissue in most cases of FL. Induction of metaphases after 24-48h of culture (48-72h for CLL) is successful in 70% of cases overall, but will be somewhat lower for lymph node tissue in FL. Karyotyping is the preferred option in case of a diagnostic challenge, or if the initial diagnosis is unclear, as additional cytogenetic abnormalities are also visualized. This approach also avoids the need to perform another biopsy in such cases. If karyotyping is not possible of if results are unclear interphase FISH (Vysis) is used to detect t(14;18). This particular FISH assay is relatively easy to interpret, and will generate only unclear results (which could benefit from backup karyotype information, if available) in about 10% of t(14;18) FISH tests. An issue which still needs to be resolved is the marketing of the product, which is currently still for research use only, excluding clinical use.

In theory centers for medical genetics can provide results for karyotyping within one week, in writing, provided the current backlog in terms of technician and secretary work in tackled first. In case the sample provided falls within the 5% category of diagnostic challenges, already today priority is given to such karyotyping work upon simple request. As an alternative approach interphase FISH can be used as the first line test for detecting t(14;18) in FL at diagnosis. The use of interphase FISH as stand alone test is thus not considered inappropriate in this situation (differs from the guidance published by the Groupe Français de Cytogénétique Hématologique). In case the diagnosis is not conclusive based on this approach another biopsy may be needed.

A third approach consists of the use of (less costly) PCR as a first line test at diagnosis, followed by (more expensive) interfase FISH if negative. Also using this approach another biopsy may be needed if the diagnosis remains inconclusive. In order to have a reasonable clinical/diagnostic sensitivity the PCR test is recommended

- to cover as many well documented breakpoints as possible (thus minimally MBR and mcr breakpoints),
- not to show a too high analytical sensitivity (in order to avoid picking up the t(14;18) translocations present in a few cells in a significant fraction of the population)
- to characterize the amplicon eg on gel as a quality check, especially relevant for t(14;18) PCR
- to be validated (eg based on BIOMED-2 efforts, same comment as for FISH: RUO kits cannot be used for routine diagnosis, in-house method requires full validation)

The only indication mentioned at the expert meeting for quantitative t(14;18) PCR was for the safety of peripheral stem cell collections. For this purpose it may be appropriate to perform t(14;18) PCR already at diagnosis. There is currently no role for quantitative PCR at diagnosis as a prognostic variable in clinical routine. Quantification of tumour load at diagnosis and during follow-up or the detection of minimal residual disease using molecular methods should be limited to research protocols. The clinicians do not see a role for t(14;18) PCR monitoring in the routine setting, awaiting a better molecular understanding of the disease (and associated tests) and more targeted treatment options. It was recommended to store away biological material or the extracted RNA and DNA (lymph node, bone marrow, blood) for later use. This storage is associated with significant costs and should preferrably be conducted under research protocols.

2. AIM

The KCE has conducted an evaluation of the tests performed at the Centers for Molecular Diagnosis (CMDs) in Belgium. As it was impossible to cover all tests in great detail, a small number of tests were selected for a more detailed pilot assessment. This pilot assessment was selected as an example of the molecular tests in hemato-oncology. It concerns the role of PCR-based detection and quantification of t(14,18) in follicular lymphoma diagnosis and follow-up. This introductory text on the subject and on the local situation is used as background information for a meeting with expert clinicians, pathologists and laboratory hemato-oncologists from CMDs and Centers for Medical Genetics (CMGs). The aim is to assess the clinical value of the test and to give some attention to quality, organisation, financing, and coordination of the different local labs involved.

3. METHODS

The literature search has been restricted to the use of PCR-based techniques for qualitative and quantitative detection of t(14;18) in follicular lymphoma. Only literature published in 1999 or later was reviewed for two reasons. First, the period before 1999 is characterized by a large variety of in-house methods and major technical issues, both hampering comparisons between studies. Published "geographic variations" have been found to be technical rather then real[1]. This is also illustrated by the results of proficiency testing surveys from that period[2, 3]. Second, real-time PCR methods for detection and quantification of t(14;18) were first published in 1998[4, 5] and promised to resolve at least some of the technical issues. Also the number of possible breakpoints identified has increased over time, further adding to the complexity. The studies listed in Table 2 are the result of a more systematic review of the literature after 1999. In addition, data on local testing were obtained from the CMDs, and the RIZIV/INAMI.

4. FOLLICULAR LYMPHOMA

The Flanders cancer registry[6] (population of about 6 million) reports a total of 927 cases of non-Hodgkin lymphoma (NHL) for the year 2000 (476 males and 451 females), making NHL the most common hematological malignancy, and representing about 3% of all new cancers. When extrapolated to the Belgian population, the yearly incidence is estimated at 1550 new cases per year. In the US, NHL accounts for 4% of the new malignancies each year. The incidence of NHL in the US has increased from 10.2 per 100000 in 1973 to 18.5 per 100000 in 1990 and has somewhat levelled off since then. The increase has been suggested to be associated with exposure to pesticides [7], and an increased frequency of t(14;18) bearing cells may be present in exposed farmers[8].

NHL is composed of many histologically distinct lymphoid malignancies. Follicular lymphoma (FL) is the second most common form of NHL after diffuse large B-cell lymphoma (DLBCL). The relative frequency of FL is lower in Asia (about 10% of NHL) compared with Western Europe and the US (20% to 33% of NHL) [9]. In the UK the incidence of FL is estimated at 4 per 100000[10]. FL can be subdivided as detailed in Table 1, copied from Winter et al[11].

Table 1. World Health Organization classification of follicular lymphoma (FL)[12].

- Grade 1, 0–5 centroblasts / high power field
- Grade 2, 6–15 centroblasts / high power field
- Grade 3, > 15 centroblasts / high power field
    - 3a, > 15 centroblasts, but centrocytes are still present
    - 3b, centroblasts form solid sheets with no residual centrocytes

Variants

- Cutaneous follicle center lymphoma
- Diffuse follicle center lymphoma
    - Grade 1, 0–5 centroblasts / high power field
    - Grade 2, 6–15 centroblasts / high power field

Clinically most patients are elderly and present with advanced disease stage. Median survival of FL is 8-10 years and the evolution ranges from spontaneous remission to an aggressive clinical course. Both clinical and histological transformation are seen in up to 50% of the patients within the first 10 years after diagnosis. The most characteristic histological progression for FL is to a process resembling DLBCL [11]. Patients showing such progression have a prognosis similar to those with de novo DLBCL and are often treated aggressively[13]. A follicular lymphoma international prognostic index (FLIPI) been proposed based on patient age, Ann Arbor stage, hemoglobin level, number of nodal areas, and serum LDH level[14]. Recent microarray studies point to a possible role for the microenvironment. Slowly progressive FL could be discriminated from more rapidly progressive FL based on a specific expression profile of the surrounding (non-tumor) cells. A specific T cell signature corresponded with improved survival whereas a macrophage/dendritic cell profile did not [15].

FL and a large proportion of DLBCL are B-cell lymphomas of germinal center origin. A frequent chromosomal aberration of such lymphomas is the translocation t(14;18)(q32;q21) (Bcl-2/IgH), involving the immunoglobulin heavy chain (IgH) gene on chromosome 14q32 and the Bcl-2 gene on chromosome 18q21. This translocation results in the juxtaposition of the antiapoptotic Bcl-2 gene and the IgH heavy chain locus on chromosome 14, leading to upregulation of Bcl-2 protein expression in most cases of FL, and an inhibition of cell death. Whereas the inhibition of apoptosis results in most

cases of FL from the constitutive expression of the Bcl-2 oncogene, in the remaining cases other molecules in the apoptotic signalling cascade are believed to be involved [11].

Expert hematopathologists rarely agree when trying to distinguish FL grade 1 and 2[1]. Grade 3 FL, or follicular large-cell lymphomas, have a focal follicular pattern, but consist mainly of large noncleaved cells (centroblasts), which are also typical for DLBCLs. Clinically, follicular large-cell lymphomas also have more in common with DLBCL than with their indolent FL grade 1 and 2 counterparts [11]. Translocation t(14;18), the cytogenetic hallmark of FL, is detected in approximately 85% of the grade 1 and grade 2 FL, and is observed less frequently in grade 3 FL and DLBCL [16]. Grade 3 FL is not a homogeneous entity[17]. Grade 3a represents the aggressive end of the clinical/morphological spectrum of indolent FL, and is closely related to grade 1 and 2 FL. Grade 3b FL includes patients with translocation t(14;18) with or without Bcl-2 overexpression and another group of patients harbouring the t(3;14) translocation [18 19]. The clinical relevance of these subgroups remains controversial [11].

The mean number of cytogenetic alterations, mainly copy number alterations and not balanced translocations, increases from 5 in grade 1 to 19 in grade 3, and the number of alterations has been linked with prognosis[20, 21]. Especially deletions of 6q and 17p have been associated with a worse prognosis. Chromosomal changes arise in an apparent temporal order, with distinct early, including t(14;18), and late changes. Four possible cytogenetic pathways have been described in t(14;18) positive FL, that characterize the early stages of clonal evolution, which converge to a common route at later stages [22].

5. METHODS FOR T(14;18) DETECTION OR QUANTIFICATION

Demonstration of t(14;18) translocations in biopsy samples is widely used as an accessory diagnostic tool for the identification of FL, and PCR on whole blood samples can be used to detect molecular response after treatment. The translocation t(14;18) in FL can be detected using a variety of methods, including conventional karyotyping, Southern blot, PCR, and FISH. A variety of sample types can also be used such as fresh, fresh-frozen or fixed lymph node, bone marrow or peripheral blood. Detection of t(14;18) in extracellular DNA in serum was also possible in 4 out of 5 FL patients[23].

5.1. CYTOGENETICS

In the US, only few centers send lymphoma specimens for routine karyotyping, at least in part because of issues related to reimbursement and the labor-intensive nature of metaphase chromosome preparation. Karyotypic analysis may also be prone to false negative results when applied to tumors, such as FL, with low growth fractions [1]. Overall metaphase induction is possible in 70% of the hemato-oncology samples, but fraction will be somewhat lower for lymphoma lymph node tissue (data kindly provided by P. Vandenberghe, CMG University HLeuven). Interphase fluorescence in situ hybridization (FISH) studies offer the ability to detect cytogenetic abnormalities in nondividing cells. When touch preparations are available or can be made from fresh-frozen tissue, dual segregation interphase FISH tests have very high sensitivity and specificity. FISH has a 2-day turn-around, assuming that molecular cytogenetics expertise is available locally. The test is best performed on air-dried touch preparations, but can also be performed on formalin-fixed paraffin-embedded tissue[24]. This is especially valuable in case no material is available for traditional banded karyotyping. Analysis of interphase nuclei for specific chromosomal aberrations by FISH is less laborious than the preparation and analysis of metaphase chromosomes, but still requires substantial time at the fluorescent microscope analyzing results. False negatives can occur if malignant cells represent less than 10% of the cells present in the specimen. Robotic FISH analysis may be of help in the future [1]. However, correct interpretation of interphase FISH has been reported to often require access to the metaphase FISH data[25]. The available t(14;18) FISH assay is relatively easy to interpret, and will generate only unclear results (which could benefit from backup karyotype information, if available) in about 10% of tests (expert opinion P. Vandenberghe, CMG University HLeuven). An issue which still needs to be resolved is the marketing of the product, which is currently still 'for research use only', excluding clinical use.

5.2. PCR OR INTERPHASE FISH

Because of its relative simplicity, in-house short range PCR is still the most commonly applied technique to detect translocation t(14;18) [26]. Techniques include PCR, seminested PCR, nested PCR and real-time PCR. Large variations in the incidence of t(14;18) in FL have been reported in different parts of the world. Interestingly, the reported lower frequency of t(14;18) in Europe turned out to be technical rather then real. More recent European studies using interphase FISH show an occurrence of t(14;18) in FL at a frequency of 88% (35 out of 40) [16] or even 100% (67 out of 67) [27]. The true incidence of the translocation t(14;18) in FL is thus probably at least 85%. The routine PCR methods, also used in most studies and in the CMDs, give rise to PCR products in the range of 150 to 600 bp (thus smaller then long-range PCR fragments, which can be up to 23 kb) and most routine methods can therefore also be used on small formalin-fixed tissue samples[26]. However, detection also then remains more likely in frozen samples compared with paraffin embedded samples as demonstrated using paired archival lymph node samples[24]. In this study t(14;18) detection using FISH proved superior over PCR in archived paraffin samples. Using FISH on PCR negative paraffin

samples the detection rate of t(14;18) can be further improved[28]. In conclusion, the clinical/diagnostic sensitivity of interfase FISH for detection of t(14;18) is thus superior compared with short-range PCR, also if formalin-fixed tissue samples are used.

5.3. BCL-2 BREAKPOINT REGIONS DETECTABLE USING SHORT RANGE PCR

The Bcl-2 breakpoint regions are clustered into a major breakpoint region (MBR) and a minor cluster region (mcr) and span a large area of DNA. Additional breakpoints of t(14,18) have been identified using long-distance PCR (LD-PCR). The long-range PCR protocol by Akasaka et al[29] can be as sensitive as Southern blotting, can be completed in a several days (versus 1 to 2 weeks for Southern blotting) and does not require the use of radioisotopes. However, both methods are limited in that they require high molecular weight DNA, which can only be prepared from fresh or fresh-frozen tissue. In Switzerland, t(14;18) was detected using this method in 71%, or 42 out of 59 FL cases [26]. In Argentina long-distance PCR t(14;18) was positive in 78%, or 65 out of 83 cases of FL, versus 51% using nested PCR for MBR and mcr[30]. The t(14;18) breakpoint regions including the intermediate cluster region (icr) and a 3'Bcl-2-cluster were reviewed by Hirt et al [31]. The primers used for the in-house short range PCR tests have often been derived on an ad hoc basis and did not include all available information on the molecular anatomy of the breakpoints[32]. A two-tube multiplex system was evaluated and later optimised to a three tube system in a large EU sponsored development effort (BIOMED-2). A first tube detects breakpoints in the MBR, a second tube contains 3'MBR primers and a third tube detects the breakpoint within the mcr. This robust system does not provide the (analytical) sensitivity levels of nested PCR but maximizes the detection of t(14;18) and is designed for diagnostic procedures. For patient follow-up, a reduced number of primers can be selected. It should be noted that it remains impossible to detect all cases of t(14;18) using PCR[32].

Others also have adopted the PCR primer pairs to cover additional breakpoint clusters[33]. In 113 untreated patients with t(14;18)-positive FL[34], breakpoints were respectively located at the major breakpoint region (MBR) in 73 cases (65%), at the minor cluster region (mcr) in 10 cases (9%), at 3'Bcl-2 in 14 cases (12%) and at 5'mcr in seven cases (6%). Finally, the breakpoint could not be located in nine patients (8%). After optimization of the PCR primer pairs to cover also the icr Albinger-Hegyi investigated t(14;18) breakpoints using DNA extracted from formalin-fixed and paraffin-embedded tissue of 59 patients with histologically confirmed FL. Breakpoints were detected in a total of 27 cases (MBR in 19 cases, mcr in 2 cases, and icr in 6 cases), or a clinical sensitivity for FL of 46% [26].

5.4. DIAGNOSTIC SENSITIVITY OF T(14;18) PCR FOR FOLLICULAR LYMPHOMA

An overview of studies (with 20 patients or more, not restricted to cutaneous FL) reporting PCR detection of t(14;18) breakpoints in FL is given in Table 2. In order to generate this table, a literature search was conducted in February, 2005 in Medline and Embase. Medline search (including publications in progress): search (Lymphoma, Follicular[MeSH] or follicular lymphoma) and (Translocation, Genetic[MeSH] or translocation or "Genes, bcl-2"[MeSH] or bcl-2) and (Polymerase Chain Reaction[MeSH] or Polymerase Chain Reaction or PCR) Field: All Fields, Limits: Publication Date from 1999/01/01. This search generated 171 hits and was complemented with a search in Embase: ('follicular lymphoma'/exp OR 'follicular lymphoma') AND pcr AND translocation AND [english]/lim AND [1999-2005]/py. This search generated 62 hits, but all selected references already had been identified using the Medline search. The BIOMED-2 reference[32] was not identified using this search strategy.

Table 2. Diagnostic sensitivity of t(14;18) PCR for follicular lymphoma

Region, year published	# Patients and Sample Type	PCR Type	t(14;18) diagnostic sensitivity (PCR)	t(14;18) MBR positive	t(14;18) mcr positive	t(14;18) other breakpoints (and remarks)
South Africa, 1999[35]	64 FL (fixed, paraffin)	PCR	29 (45%)	25 (39%)	4 (6%)	
Turkey, 2000[36]	67 FL (fixed, paraffin)	PCR	46 (69%)	43 (64%)	3 (4%)	
Czech Republic, 2001[37]	53 FL (peripheral blood or bone marrow)	PCR	25 (47%)	22 (42%)	2 (4%)	1 (2%) both MBR/mcr
Switzerland, 2002[26]	59 FL (fixed, paraffin)	PCR and LD-PCR	PCR: 27 (46%) LD-PCR: 42 (71%)	19 (32%)	2 (3%)	icr: 6 (10%)
UK, 2003 [24]	28 FL (frozen)	PCR (BIOMED-2)	23 (82%)	16 (70%)	5 (21%)	5'mcr: 3 (9%)
UK, 2003 [24]	20 FL (fixed, paraffin, paired)	PCR (BIOMED-2)	8 (40%)	5 (25%)	3 (15%)	
Spain, 2003[38]	60 FL (fixed, paraffin or peripheral blood or bone marrow)	PCR	40 (67%)	39 (65%)	1 (2%)	DNA quality too low in another 9 patients (thus 69 patients in total)
Malaysia, 2003[28]	50 FL (fixed, paraffin)	Nested PCR	30 (60%)	25 (50%)	5 (10%)	FISH identified t(14;18) in another 4 cases
Malaysia, 2004[39]	62 FL (fixed, paraffin)	Nested PCR	42 (68%)	32 (52%)	9 (14%)	1 (2%) both MBR/mcr
Argentina, 2004[30]	83 FL (fresh tissue and blood)	PCR and LD-PCR	PCR: 42 (51%) LD-PCR: 65 (78%)	28 (34%)	14 (17%)	
Brazil, 2004[40]	48 FL (fixed, paraffin)	PCR	46 (96%)	41 (86%)	5 (10%)	
UK, 2004[41]	88 FL (mainly lymph node)	Real-time PCR	41 (47%)	41 (47%)	NA	NA
France, 2002[34]	113 t(14;18)+ FL (lymph node or bone marrow)	PCR	104 (92%)	73 (65%)	10 (9%)	3'Bcl-2: 14 (12%); 5'mcr: 7 (6%)
Europe, 2003[32] (two tube assay before test optimisation)	124 t(14;18)+ FL	PCR	109 (88%)	83 (67%)		2nd multiplex for mcr, 5'mcr, 3'MBR: 26 (21%)
UK, 2005[42]	57 t(14;18)+ FL		33 (58%)	26 (46%)	4 (7%)	Icr: 3 (5%)

Overall, t(14;18) is detected in about 45 to 70% of histologically confirmed FL cases using short range PCR, which is lower compared with the diagnostic sensitivity reported for interphase FISH of 88%[16] and 100%[27]. It should be noted that some studies only included t(14;18) positive FL samples[32, 34, 42], which of course resulted in a higher detection rate by PCR.

5.5. PCR QUALITY ISSUES, RESOLVED WITH REAL-TIME PCR?

Traditionally, in-house PCR methods do not always "travel well" from laboratory to laboratory and PCR machine to PCR machine, leading to unique in-house protocols. The variability of the PCR based detection of Bcl-2/IgH was clearly demonstrated in 1999 using a proficiency survey of twenty laboratories with records of publication in molecular diagnostics[2]. The PCR methodology varied widely, with the total number of amplification cycles between 30 and 70, and 13 different primers used for the MBR region. Sensitivity of nested and single round PCR was similar at 100 cells/ml but nested PCR was more sensitive below this level. The overall false positive rate was 28% (11 samples from 9 laboratories), and this was shown to be due to contamination. The detection of Bcl-2/IgH translocation by PCR was also evaluated in the Molecular Oncology Proficiency Survey data 1997-2000 of the College of American Pathologists [3]. The overall performance was good among the 140 sites subscribing to this survey. However the authors write "There was great variability in the primer sets used to amplify across the translocation breakpoint. The variability multiplies quickly when one considers that there were also frequently different DNA isolation procedures, different reagents, different cycling conditions, and different detection methods. It seems likely that no 2 laboratories perform this test in exactly the same manner. This variation made it impossible to discern particular methods that were better or worse than others. This lack of standardization is detrimental to the extent that it affects test results and, ultimately, patient care. A recurrent problem was lack of knowledge of assay sensitivity".

The problem has been solved in part by the use of real-time PCR[31, 41]. Real-time t(14;18) MBR PCR assays can be as sensitive as nested PCR while demonstrating enhanced quantitative potential[43], important for assessing minimal residual disease. Quantitative-competitive[44], internally calibrated[45], or multiplex-multicolor[46] PCR methods may get replaced by real-time PCR methods[47], also for measuring ex vivo purging efficiency[48]. Coamplification of one[49, 50] or more[51] reference or control genes during the real-time PCR allows for a normalisation of the target gene copy, thus reducing the impact of the quality of the DNA preparation as a source of variation.

5.6. DETECTION OF T(14;18) IN HEALTHY INDIVIDUALS

An important issue at diagnosis is the occurrence of the Bcl-2/IgH rearrangement in the peripheral blood or tissues from patients with follicular hyperplasia, persistent polyclonal B cell lymphocytosis, and normal individuals[9]. In healthy individuals 23%[52], 45%[53], and 47% (41% MBR and 6% mcr)[47] of peripheral blood samples tested positive for t(14;18) using real-time quantitative PCR with approximately 3% of these at levels of more than 1 in 10(4) cells [52], partly overlapping with the number of circulating cells in FL patients[47]. In Japanese healthy individuals the frequency of t(14;18) bearing peripheral blood cells may be lower compared with Europe[54]. The presence of t(14;18) positive cells in healthy individuals is compatible with the t(14;18) translocation being an early event in the lymphoma development. Its sole presence does not render cells malignant. This contrast eg with the detection of t(11;14) in only 1% of healthy volunteers, suggesting the acquisition of t(11;14) is not an early event in mantle cell lymphoma development[53].

In order to distinguish "healthy" from malignant clones a number of techniques can be used[31]. Sequence comparison of the t(14;18)-fragments amplified from peripheral blood cells and diagnostic tissue can be used. Other techniques include the use of clonospecific probes and assessment of the amplicon size. Custom made forward

primers have been used in a real-time PCR system[55]. Amplification tubes remain closed in typical real-time PCR systems. However, amplicon size determination can not only be used to exclude contamination but also to compare the size between multiple samples (either simultaneous or sequential) and to determine clonal relatedness. Agarose gel electrophoresis or a combination of real-time PCR with high-resolution capillary electrophoresis can be used for this purpose[49, 56, 57].

In contrast to the short-lived t(14;18) clones observed in the general population[58], few chemotherapy-treated patients may see the emergence of long-lived t(14;18) positive cells, unrelated to the original FL clone[59]. A case report showing the concurrent presence of both patient and donor t(14;18) in a FL patient after allogeneic BMT suggests that the donor should be screened for the presence of t(14;18), but not excluded as a donor[60].

5.7. BCL-2 PROTEIN DETECTION USING IMMUNOHISTOCHEMISTRY

The correlation between detection of t(14;18) and the immunostain for Bcl-2 is high but not perfect. Using FISH for t(14;18) detection, 32 out of 35 follicular lymphomas with the t(14;18) were positive for Bcl-2 protein expression, indicating a strong (but not absolute) correlation between immunohistochemistry and Bcl-2 gene rearrangement [16]. Of interest, all five lymphomas that were negative for the t(14;18) were also negative by immunohistochemistry for Bcl-2 protein expression. Negative immunohistochemistry findings may also result from impaired epitope recognition caused by mutations in the Bcl-2 gene[61]. Translocations different from t(14;18) but associated with Bcl-2 overexpression have also been reported [62].

5.8. KITS AVAILABLE FOR T(14;18) PCR AND FISH

All companies producing and distributing in-vitro-diagnostics in the European Union (EU) have to comply with the In Vitro Diagnostic Medical Devices Directive 98/79/EC (IVDMDD) issued by the EU. One basic requirement of the IVDMDD is the establishment of a quality management system within the manufacturing company to monitor product development, production and sales. The field of PCR testing for t(14;18) has not yet seen the introduction of kits by major diagnostic companies. Research use only kits for t(14;18), including a kit based on the BIOMED-2 efforts, are available from Invivoscribe technologies (www2.invivoscribe.com) at 495 € for 33 reactions Bcl-2/IgH. Also the (CE labelled) ProDect Bcl-2 kit commercialized by bcs Biotech S.p.A. (www.biocs.it) detects t(14;18) using PCR. Research use only FISH probes for detection of t(14;18), eg Vysis LSI IGH/BCL2 Dual Color, Dual Fusion Translocation Probes are being marketed by Abbott Laboratories / Vysis (www.abbottdiagnostics.com) at 853 USD for 20 assays.

6. CLINICAL USE OF T(14;18) PCR

6.1. USE AT DIAGNOSIS

Expert review of pathology improves diagnostic accuracy. There is evidence that up to 5% of people treated for lymphoma in Wales actually have benign disease[63]. Over 90% of the suspected NHL cases can be correctly diagnosed without the use of molecular techniques[64]. The distinction between follicular lymphoma and reactive hyperplasia should be based on morphological features, Bcl-2 protein staining in germinal centers, and tests that determine clonality. The failure to detect Bcl-2 protein in a follicular proliferation cannot be taken as an assurance of benignity[61]. The t(14;18) translocation can be detected using FISH or cytogenetics. The assay should not be too sensitive. A strong cytoplasmatic staining of Bcl-2 and a typical paratrabecular pattern are characteristic for infiltration of follicular lymphoma in bone marrow trephine biopsy specimens. Although the majority of cases (>95%) of follicular lymphoma can be diagnosed by conventional morphology, the detection of t(14;18) is diagnostically useful in some cases where atypical morphological patterns yield a differential diagnosis of reactive hyperplasia or alternatively another non-Hodgkin's lymphoma with a nodular architecture. In these cases molecular tests for monoclonality or for t(14;18) may be essential. It should also be considered that t(14;18) translocations have been reported for 15-20% of DLBCL and also in some MALT lymphomas (mucosa associated lymphoid tissue).

In the diagnostic algorithm proposed by Arber[65], testing for t(14;18) is performed in cases of suspected B cell neoplasm when the IgH rearrangement PCR is negative. However, this use is now less frequent as more primer sets are being used for the detection of B cell clonality (covering not only heavy chain rearrangements).

The Groupe Français de Cytogénétique Hématologique (GFCH) has recently published recommendations with respect to the use of cytogenetic testing in specific haematological malignancies[25]. Karyotyping is positioned as first line test, while FISH has its place according to specific guidelines. The correct interpretation of interphase FISH often requires access to the metaphase FISH data. The use of interphase FISH as stand alone test is therefore considered inappropriate because of possible errors in interpretation. The guidance text for adult non-Hodgkin lymphoma[66] lists karyotyping as the first line test. If the karyotype is not fully informative, FISH can be used, guided by the morphology and phenotype. In case of follicular lymphoma, FISH can be used for detection of t(14;18) and if negative for detection of t(3;14). IgH rearrangement testing and PCR for t(14;18) or RT-PCR for the t(3;14) transcript are also listed as molecular diagnostics for the diagnosis of follicular lymphoma.

The presence of the t(14;18) in de novo DLCL has been shown to represent an adverse prognostic marker[67]. Whether the natural histories of t(14;18)-positive and -negative follicular lymphomas vary is a matter of current investigation. In 50 patients with follicular large cell lymphoma the presence of t(14;18) nor the overexpression of Bcl-2 were predictors of survival[68]. Montoto did not observe any effect on survival based on the detection or location of the breakpoint in 60 patients assessed for MBR and mcr[38]. In a series of 113 untreated patients with t(14;18)-positive FL[34] no correlation was seen between breakpoint location and either initial characteristics of the disease or survival of the patients. Survival in 41 MBR positive FL patients also did not differ from the 47 other FL patients[41]. A small pilot study found the tumor load in peripheral blood measured by RQ-PCR predictive for clinical relapse after stem cell transplantation in FL[69]. Using real-time quantitative PCR an increased level of t(14;18) carrying cells has been reported in patients with advanced (stage IV) disease[70], and a low level of Bcl-2/IgH positive cells in the bone marrow pre-treatment was predictive for a complete clinical and molecular response in FL [71].

6.2. USE FOR MONITORING OF MOLECULAR REMISSION

In the absence of available downstream molecular targets, the monitoring of minimal residual disease in FL is based on PCR-detectable Bcl-2 gene rearrangement. In FL patients with t(14;18)-PCR negative lymphomas real-time PCR assays for Ig-rearrangements using allele specific primers can be used[31]. In a review on molecular diagnostics Braziel et al[72] state more studies are needed to evaluate the clinical efficacy of minimal residual disease (MRD) in FL patients in general and to compare the relative clinical value of the nested and the quantitative real-time PCR MRD detection methods.

Achievement of complete molecular remission (CMR) in FL is associated with improved outcomes, as seen after fludarabine-based combination regimens and the monoclonal antibody rituximab [73], after conventional chemotherapy, Iodine 1131 Tositumomab[74] or radiotherapy[75], and after autologous bone marrow transplantation[48, 76] or stem cell transplantation[69]. When validated, PCR based detection of t(14;18) can thus be used to test for CMR as an efficacy endpoint of clinical trials in follicular lymphoma. Many treatment trials in FL have reported on molecular response as an endpoint[37, 50, 74, 77-82]. From the publications it is not always clear what type of tissue sample (blood, bone marrow, lymph node) was used pre- and post-treatment and whether only identical tissue types were compared. Both peripheral blood and bone marrow samples can be used in case of bone marrow involvement by FL[57, 83], as levels of t(14;18)-carrying cells differed in 82% of cases less then one log between the two sample types. This observation is not necessarily contradictory to the discordant qualitative PCR results for peripheral blood and bone marrow, reported for nine out of 39 patients after rituximab treatment[79]. Reports from 2000 mention clinical and molecular response do not always correlate[84, 85]. Molecular response rates may be lower [82]in patients with stage IV disease compared with less advanced disease.

Some have questioned the usefulness of molecular monitoring as in 22 out of 28 patients treated with standard chemotherapy combined with interferon alfa-2b a decrease in the number of peripheral blood cells positive for t(14;18) was seen, including 4 patients in whom treatment had failed clinically[86]. More recently however, the same group reported for 53 FL patients a significant association between a drop of at least one log in t(14;18) positive cells with progression free survival[87]. Results of in vitro autologous stem cell purging to produce Bcl-2 PCR negativity have demonstrated encouraging results [88], but the tumour cell content of grafts was also reported not to predict survival[89]. Persistent PCR positivity for Bcl-1, Bcl-2 of Ig rearrangement markers have been associated with a high risk of relapse in a group of 60 indolent lymphoma patients[90]. On the other hand, long-term relapse-free survival for up to 10 years after radiotherapy has been described despite the persistence of circulating t(14;18)-positive cells carrying the same translocation as the initial lymphoma cell clone[31].

6.3. GUIDELINES AND ORGANISATIONAL ASPECTS

Guidelines on the diagnosis and treatment have been published by professional bodies such as the British Committee for Standards in Haematology (BCSH) for specific disorders in the area of hemato-oncology[10]. These include guidelines on the provision of facilities for the care of patients with haematological malignancies (including leukemia and lymphoma and severe bone marrow failure).[91]. These guidelines describe four levels of care. For example, centers performing autologous or allogeneic transplants, including stem cell transplantations, should have access on site (or immediately available) to cytogenetics and molecular biology services. An interim guidance document on the diagnosis and therapy for nodal non-Hodgkin's lymphoma does not mention any specific testing flow. However, it states the following. “The main current techniques are polymerase chain reaction (PCR) to detect monoclonality and some translocations, and fluorescence in situ hybridisation (FISH) techniques for the detection of translocation and numerical chromosomal abnormalities. These techniques should be used in the same way as immunophenotypic studies to test the validity of the provisional diagnosis and to identify prognostic factors. Formal links with the local molecular-cytogenetics service are required. Most patients with lymphoproliferative disorders have multiple

specimens taken at presentation and follow-up. It is essential that departments have an effective mechanism to correlate results obtained from lymph node biopsies, bone marrow (BM), etc. In general a report should be available 5 working days after the specimen is received, although some molecular techniques may take longer. Where the diagnosis depends on investigations that are outstanding, this should be clearly stated."

The National Institute for Clinical Excellence (NICE), UK, recommends that clinical services for patients with haematological cancers should be delivered by multi-disciplinary haemato-oncology teams which serve populations of 500000 or more[63]. The key recommendations also include the following. "In order to reduce errors, every diagnosis of possible haematological malignancy should be reviewed by specialists in diagnosis of haematological malignancy. Results of tests should be integrated and interpreted by experts who work with local haemato-oncology multi-disciplinary teams and provide a specialised service at network level. This is most easily achieved by locating all specialist haemato-pathology diagnostic services in a single laboratory." Richards and Jack[92] describe the practical development in Leeds, UK, of an integrated laboratory for diagnosis of tumours of the haematopoietic system performing flow cytometry, histopathology, molecular diagnostics and cytogenetics in a systematic and co-ordinated way. This organisation meets the requirement for the results of laboratory investigations and diagnosis of all cases of haematological malignancy to be reviewed by experts and specialist haematopathologists. The role of the IT system for an effective service is also highlighted. In the workflow of this laboratory, PCR and FISH based investigations are performed for t(14;18) in suspected cases of FL.

UK guidelines on FISH scoring[93] mention interphase FISH studies are often carried out because of the absence of sufficient metaphases. If metaphases are present, they can add significant information to the analysis, eg for the interpretation of unusual signal patterns. Even experienced cytogeneticists need a proper training programme when starting FISH analyses. In most haematological disorders it is preferable to use FISH at diagnosis as an adjunct to, and not in place of, a conventional cytogenetic study. Where no fresh material is available (eg lymphoma), FISH may be the only possibility. FISH studies are reported as suitable for assessing initial response to treatment, but are not sensitive enough for detecting MRD. For t(14;18) FISH testing, bone marrow or blood samples should not be processed without morphological evidence of significant involvement (>20%) by malignant cells. An alternative sample should be considered such as a tumour touch prep, or if the lab already has sufficient experience, a paraffin embedded tissue section or cells released from such a section. The choice of FISH or molecular tests is left to the laboratories and their users. The guidelines thus only apply in case FISH is selected.

The Groupe Français de Cytogénétique Hématologique (GFCH) has published recommendations with respect to the use of cytogenetic testing in specific haematological malignancies as mentioned above[25]. This guidance document does not mention the use of tests for follow-up.

The National Comprehensive Cancer Network, Inc (http://www.nccn.org) lists in its oncology practice guidelines for FL the use of cytogenetics/FISH for t(14;18) as well as molecular genetic analysis to detect antigen receptor and bcl-2 rearrangement. No flow chart of diagnostic tests is given.

Finally, a number of NCCLS guidelines are available covering molecular techniques in hemato-oncology[94] and FISH[95], as well as the organisation of proficiency testing or alternative assessments [96,97].

7. LOCAL SITUATION

7.1. CENTERS FOR MOLECULAR DIAGNOSIS

The Royal Decree of September 24, 1998, published and active since October 22, 1998, allows for the creation of Centers for Molecular Diagnosis (CMD). Each CMD needs to be associated with a Centre for Medical Genetics (CMG). The list of tests performed at the CMDs is updated each year. The overall budget for testing performed at the CMDs has remained fixed at 6,54 Mio Euro per year. This fixed overall yearly budget is divided according the CMDs, driven by costs for personnel, invoiced consumables and investments. The number of appointed CMDs has increased the first years to 18 CMDs and has remained stable since July 3, 2000. The CMD's mission includes the evaluation of new molecular tests in the attributed domains of microbiology, hemato-oncology and pathology. Many but not all CMDs are based at a university hospital. The CMDs are also funded and responsible for the organization of their quality assurance (QA). The current agreements for funding of the CMDs end January 31, 2006, and this project may help outline the future. The Council of State rejected on 27 January 2005 the legal basis of the CMDs.

In a recent survey by the KCE, CMD experts in pathology and hemato-oncology reported on the target population and indications for t(14;18) PCR. For qualitative PCR the hemato-oncology working group mentioned B-NHL and B-CLL as target population, and two indications. The first indication was histological suspicion of a specific subtype of a malignant proliferative disorder or to complement morphology and immunophenotyping of a malignant lymphoproliferation. The second indication listed was histological or cytological suspicion of secondary organ involvement in a patient with a histologically proven malignant lymphoproliferative disorder. PCR was reported to be of particular relevance when little biopsy material is available. It was also mentioned t(14;18) PCR could replace FISH in case of a positive test.

The pathology working group reports target patients with a histological or haematological/ immunophenotypical diagnosis or suspicion of follicular lymphoma. The test is normally done in combination with testing of clonal rearrangements of IgH and Igkappa. The gold standard for diagnosis consists of morphology, Bcl-2 overexpression and demonstration of either clonal IgH/Igkappa rearrangements or demonstration of t(14;18) by FISH or cytogenetics. Pathologists also stress the importance of the amount (surgical biopsy versus needle biopsy) and quality of the material provided (fixative used and fixation time). Currently, they feel the maximum of 4 reimbursed immunostainings is too low in some cases. Finally, molecular tests should be requested and performed in the context of a multidisciplinary approach and by laboratories experienced in hemato-pathology and molecular testing.

The target population for quantitative real-time PCR reported by the hemato-oncology working group are patients with lymphoma demonstrated to harbour the bcl-2/IgH fusion gene by qualitative assay, and suitable as genetic target in quantitative assay (mainly FL and DLBCL). The indications listed are

- quantification of tumour load (at diagnosis and during follow-up for evaluating the rate of therapy response, FISH may be used also for this indication)
- detection and quantification of minimal residual disease (MRD) (during follow-up, when all other investigations are negative, FISH being not appropriate as it is not as sensitive as PCR); this testing is often required in clinical research protocols
- assessing the safety of peripheral stem cell collection

For quantitative tests further standardization of methods is expected.

In the CMD activity report of 2003, 255 out of 1448 samples tested (18%) at the CMDs were positive for t(14;18). During follow-up 89 out of 432 tests (21%) were positive. During a recent KCE survey of CMD activities, 11 and 5 CMDs reported they perform a qualitative test and a quantitative assay for t(14;18), respectively. One lab reported the

use of interphase FISH, using commercial FISH probes (Vysis). For PCR, all reported tests are in-house tests. For none of the tests a formal validation report was received. One lab provided an evaluation report of the Invivoscribe kit, and another lab reported to have validation data on file. One lab reports the use of BIOMED-2 primers[32], some report the use of real-time Taqman PCR. Three labs report the use of dialogue to interpret the findings. Mean turn around time is 5.5 days (reported average ranges from 3 to 10 days). None of the labs participated to an international external quality assurance program. A second CMD quality assessment round took place in 2003 in 12 labs. Nine out of 12 labs correctly identified three mcr samples, three MBR samples and one negative sample. The report mentions most labs use BIOMED-2 primer sets and/or real-time PCR.

7.2. CENTERS FOR MEDICAL GENETICS

In addition to the CMD, some PCR testing is being performed at the Centers for Medical Genetics (CMGs). Those centers also perform the karyotyping and most of the FISH testing for t(14;18) but no exact numbers are available. The activities of the 8 Belgian CMGs are covered by the RIZIV/INAMI nomenclature, which is rather generic in this case (art. 33, Royal Decree July 22, 1988). For example no differentiation is made between simple and more complex test based on DNA hybridization. In contrast to the funding of regular laboratories, art. 33 tests are financed entirely on a test volume basis. A limited number of centres provide cytogenetic analysis for hemato-oncology. Some of these tests overlap with the tests offered by the CMDs. The total cost for tests reimbursed to the centres amounted 30,8 Mio Euro in 2003. The costs for tests based on DNA hybridization amounted to 15,7 Mio Euro in 2003 and 8,5 Mio Euro for the first half of 2004. The current nomenclature nor the activity reports of the centres for Human Genetics however provide the volume and cost detail of specific tests performed.

7.3. ORGANISATIONAL ASPECTS

Lymph node biopsies are subsampled either by the clinician, the local lab physician or the local pathologist and shipped in parallel to the CMD (if no local CMD) and the CMG (if no local CMG). In most cases the selection of tests performed is done at the receiving side (both at CMD and CMG) and based on the clinical/diagnostic information provided initially (or at a later stage). The clinician can select the CMD and CMG of his/her choice and there are no procedures in place to avoid redundancy of testing. Most often the CMGs knows what tests will be performed by the CMD but this is not always the case, which may result in a parallel diagnostic exploration. As the induction of metaphases for karyotyping at the CMG and subsequent photographing needs to start within 24 hours, one cannot wait for morphology and immunohistochemistry, flow cytometry or molecular diagnostics results. In case of suspected follicular lymphoma, reading of the chromosomes is typically performed after a few weeks, unless results are needed more urgently. Only exceptionally karyotype reading is not performed (eg no tumor invasion in case of bone marrow sample).

If one assumes about 400 FL patients are diagnosed in Belgium per year with at least one sample tested for PCR detection of t(14;18) at diagnosis, and assuming a sensitivity of 50% for PCR, a total of 200 PCR positive tests for t(14;18) would be expected. The CMD reported number of 255 t(14;18) positives at diagnosis in 2003 thus suggests most or all FL cases are PCR tested at diagnosis at the CMDs (knowing also some DLBCL cases will test t(14;18) positive). Redundancy of testing at the CMGs cannot be excluded with the data currently available.

The Royal Decree of 21 March 2003 creates a framework for the organisation of oncology care in Belgium, and the use of multidisciplinary teams to diagnose and treat the patients, according to written procedures (handbook). Regular team meetings are held where patient management is discussed. However, no specific guidance for laboratory services is given.

8. DISCUSSION AND RECOMMENDATIONS

Recommendations are based on the literature review and the opinion of clinical and laboratory experts present at a meeting to discuss the clinical utility of the tests.

Initially, the biological material available for diagnosing FL is most frequently a lymph node, together or followed soon after by a bone marrow (for staging) and peripheral blood sample. Sometimes the collection and testing of a bone marrow sample (and peripheral blood) preceedes the sampling of the lymph node.

When such material is collected in a hospital with a hemato-oncology service, it is best handed directly to a single 'hemato-oncology test coordinator' who does the subsampling and arranges for internal and external test requests and the day-to-day follow-up according to a scheme outlined in the hospital hemato-oncology handbook (set up in collaboration with the clinicians, internal and external laboratory experts). This role also includes the integration of the various tests preferably into a single report, also noting what tests are outstanding when the diagnosis is made. The role of such coordinator already exists in some hospitals, with a positive effect on the integrated testing flow with internal and external lab facilities. The creation of such a coordinator role can thus be recommended more generally, financing to be foreseen. Pathologists also stress the importance to receive fresh lymph node tissue (not fixed).

Experts confirmed that diagnosis of follicular lymphoma can be based on morphology and immunohistochemistry in over 95% of the cases of FL. In the remaining cases tests for monoclonality or for t(14;18) may help the pathologist to define malignancy and the diagnosis of FL. However, testing for t(14;18) is routinely performed anyhow in most cases of FL as part of an integrated diagnostic work-up. Other tests pathologists perform for FL include IHC on frozen tissue slides or flow cytometry.

Three possible ways to test for t(14;18) are given. The local situation may define the most appropriate scenario.

- The most comprehensive approach to t(14;18) testing in FL starts with karyotyping. Metaphase induction needs to start immediately after receipt of a fresh sample, consisting of lymph node tissue in most cases of FL. In some settings a frozen tissue section is read first (immediately), and karyotyping is requested only if this first analysis supports the need. Induction of metaphases after 24-48h of culture (48-72h for CLL) is successful in 70% of cases overall, but will be somewhat lower for lymph node tissue in FL. Karyotyping is the preferred option in case of a diagnostic challenge, or if the initial diagnosis is unclear, as additional cytogenetic abnormalities are also visualized. This approach also avoids the need to perform another biopsy in such cases. If karyotyping is not possible of if results are unclear interphase FISH (Vysis) is used to detect t(14;18). This particular FISH assay is relatively easy to interpret, and will generate only unclear results (which could benefit from backup karyotype information, if available) in about 10% of t(14;18) FISH tests. An issue which still needs to be resolved is the marketing of the product, which is currently still for research use only, excluding clinical use. In theory centers for medical genetics can provide results for karyotyping within one week, in writing, provided the current backlog in terms of technician and secretary work in tackled first. In case the sample provided falls within the 5% category of diagnostic challenges, already today priority is given to such karyotyping work upon simple request.
- As an alternative approach interphase FISH can be used as the first line test for detecting t(14;18) in FL at diagnosis. The use of interphase FISH as stand alone test is thus not considered inappropriate in this situation (differs from the guidance published by the Groupe Français de Cytogénétique Hématologique). In case the diagnosis is not conclusive based on this approach another biopsy may be needed.

- A third approach consists of the use of (less costly) PCR as a first line test at diagnosis, followed by (more expensive) interfase FISH if negative. Also using this approach another biopsy may be needed if the diagnosis remains inconclusive. In order to have a reasonable clinical/diagnostic sensitivity the PCR test is recommended
    - to cover as many well documented breakpoints as possible,
    - not to show a too high analytical sensitivity (in order to avoid picking up the t(14;18) translocations present in a few cells in a significant fraction of the population)
    - to characterize the amplicon eg on gel as a quality check, especially relevant for t(14;18) PCR
    - to be validated (eg based on BIOMED-2 efforts, same comment as for FISH: RUO kits cannot be used for routine diagnosis, in-house method requires full validation)

Use of qualitative PCR at diagnosis can be of use if intensive treatment eg allogeneic transplantation is planned, such that negativation of PCR can be checked after treatment. The only indication mentioned at the expert meeting for quantitative t(14;18) PCR was for the safety of peripheral stem cell collections. Also for this purpose it may be appropriate to perform t(14;18) PCR already at diagnosis. There is currently no role for quantitative PCR at diagnosis as a prognostic variable in clinical routine. Quantification of tumour load at diagnosis and during follow-up or the detection of minimal residual disease using molecular methods should be limited to research protocols. The clinicians do not see a role for t(14;18) PCR monitoring in the routine setting, awaiting a better molecular understanding of the disease (and associated tests) and more targeted treatment options. It was recommended to store away biological material or the extracted RNA and DNA (lymph node, bone marrow, blood) for later use. This storage is associated with significant costs and should preferrably be conducted under research protocols.

Finally, the format used of the pilot study was found to be appropriate and representative for other molecular testing in hemato-oncology. The content will of course vary by test.

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HTA Molecular Diagnostics Supplement IV: Molecular testing for factor V Leiden: a review of the evidence

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Inhoudstafel

1. SUMMARY

Factor V Leiden mutation is the most frequent cause of heritable thrombophilia, associated with a three to sevenfold risk of deep venous thrombosis.

It is not as yet clear whether testing for factor V Leiden improves patient outcome. Before widespread testing or screening is introduced, evidence will need to prove that carriers benefit from being diagnosed with this mutation.

In this review, we present data on the analytical and clinical performance of molecular tests for the factor V Leiden. In addition, we discuss the possible clinical consequences of testing.

We searched Medline, Embase, INAHTA, Medion and several other databases. The articles were selected on the basis of pre-specified inclusion and exclusion criteria, and assessed for quality. Low quality studies were excluded.

We found a limited number of studies of good or fair quality on the analytical characteristics of the various molecular assays. The included studies all reported a 100% concordance with the reference method.

In addition, the overall quality of clinical studies was low, leading to a large proportion of excluded studies. All included studies reported a concordance of >98.7%, with very little samples producing equivocal or invalid results. Measures of precision or reproducibility were not reported.

As the modified APC resistance test has sensitivity and specificity approaching that of molecular tests, this test should be performed first, only verifying positive test results with a molecular test.

Factor V Leiden is an established risk factor for the occurrence and recurrence of VTE. Screening women before starting oral contraceptives, antenatally and relatives of patients is not recommended. Management of patients with a first episode of VTE with the factor V Leiden mutation is not different from patients suffering from an idiopathic VTE. Patients with a personal and/or family history suggestive of co-inheritance of two thrombophilic conditions or homozygosis of the factor V Leiden mutation could be considered for testing, although evidence on the optimal treatment in these patients is equally lacking.

2. INTRODUCTION

Factor V Leiden mutation is the most frequent cause of heritable thrombophilia[1]. A point mutation in which adenine is substituted for guanine at nucleotide 1691 in the gene coding for coagulation factor V results in the production of abnormal factor V or factor V Leiden. This factor V Leiden is more resistant to inhibition by activated protein C than the genuine factor V[2].

In general, the mutation is associated with a threefold to sevenfold risk of deep vein thrombosis[3 4].

The prevalence of factor V Leiden is high, about 5% in Europe with a maximum of 15% in Southern Sweden[5]. In patients with thrombosis, prevalence is as high as 20-50%[4 6]. Prevalence varies significantly between different ethnic groups: a study in the United States found a carrier frequency of 5.3% in whites, 2.2% in Hispanic Americans, 1.2% in African Americans, 0.5% in Asian Americans and 1.3% in Native Americans[7].

Given the higher risk of thrombosis, testing or even screening patients for this mutation could be advantageous in theory. However, it is not as yet clear whether testing for factor V Leiden in patients with a first venous thromboembolic event does in fact improve patient outcome. Guidelines of the American College of Chest Physicians recommend at least 6 months of anticoagulant therapy after an idiopathic venous thromboembolic event[8]. For patients with the factor V Leiden mutation, the optimal duration is unclear. Some guidelines advocate a duration of 12 months or even lifetime treatment[9], others leave this decision to the individual physician due to lack of evidence[10].

Given that half of the relatives of a patient with thrombosis and the factor V Leiden will carry the same defect, because of the autosomal dominant inheritance pattern, should these relatives be actively screened? As in treatment of patients with venous thrombo-embolisms, recommendations vary. The American College of Medical Genetics[11] recommends screening of relatives of individuals known to have the factor V Leiden mutation, while the National Health Service (NHS) of the UK does not recommend screening of relatives[12]. Before widespread screening or testing is advocated or implemented, evidence will need to prove that carriers benefit from being diagnosed with the mutation.

In this structured review, data on the analytical and clinical performance of molecular testing for the factor V Leiden are presented. These findings are further discussed in the light of clinical impact of testing, more specifically do patients benefit from testing in terms of lower risk of recurrence, in terms of adjusted treatment duration or preventive measures during high risk episodes. The review focussed on testing patients experiencing a first episode of venous thromboembolism (VTE); as this review is part of a larger project on the value of molecular tests in patient care, screening of asymptomatic persons was out of the scope of the review, although some aspects are discussed in the section on clinical impact.

3. METHODS

3.1. SEARCH STRATEGY

We performed an iterative literature search, more precisely we searched for existing health technology assessments (HTA) first, subsequently for systematic reviews and finally for original diagnostic research.

HTA reports were searched in INAHTA, the Canadian Centre for Health Technology Assessment and the Agency for Health and Quality Research. We searched for systematic reviews in the CRD database, Medion, Medline and Embase databases. Original research was identified in Medline and Embase, the search limited to studies published after the literature search of any HTA report or systematic review. Studies that report the performance of a diagnostic strategy were included as well.

In addition, we checked the Food and Drugs Administration website to identify the tests that have received an FDA approval.

The search date was January 2005.

3.2. SEARCH TERMS

The search term used for INAHTA, CRD and Medion was "factor V Leiden" or "thrombophilia".

The search-string we used in PubMed is listed below:

("factor V Leiden"[Substance Name] OR "Thrombophilia"[MeSH] OR factor V Leiden OR thrombophilia) AND (sensitiv*[Title/Abstract] OR sensitivity and specificity[MeSH Terms] OR diagnos*[Title/Abstract] OR diagnosis[MeSH:noexp] OR diagnostic * [MeSH:noexp] OR diagnosis,differential[MeSH:noexp] OR diagnosis[Subheading:noexp]) AND ("Cytogenetic Analysis"[MeSH] OR "Molecular Probe Techniques"[MeSH] OR "Molecular Diagnostic Techniques"[MeSH])

In Embase, we used an adapted version of the same search-string:

('sensitivity and specificity'/exp OR 'diagnosis'/exp OR (diagnostic AND use) OR specificity OR (predictive AND value)) AND ('thrombophilia'/exp OR 'blood clotting factor 5 deficiency'/exp OR (factor AND v AND leiden)) AND ('genetic procedures'/exp OR 'molecular probe'/exp)

3.3. QUALITY ASSESSMENT

To assess the quality of HTA reports, we used the checklist published at INAHTA. The quality of systematic reviews and prognostic studies were assessed using the checklists of SIGN (www.sign.ac.uk).

The QUADAS tool was used for the quality assessment of original diagnostic research on patients[13]. For original, analytical studies, we assessed items on validity of the panel used, on test execution and analysis using a self-constructed checklist. See appendix for all quality assessment checklists used in the review.

Quality assessment is summarized in the evidence tables as good quality, fair or poor quality. HTA reports or systematic reviews received a poor quality appraisal when the search of the literature was insufficient and no quality assessment of included studies was reported. Analytical studies were of fair quality when 5 of the 7 items were answered with no or unclear, or when 3 items were answered with no. They were poor quality when 6 items were answered with no or unclear or 4 were answered with no.

Original diagnostic accuracy studies were considered fair quality if 6 of the 14 items were answered with no or unclear or 4 items with no. Studies were considered of poor quality when 7 items were answered with no or unclear or 5 with no.

Poor quality studies were excluded from further review.

3.4. DATA EXTRACTION

Test characteristics are not absolute. Variables such as setting, spectrum or demographic features of the population studies, are known to influence test characteristics. In addition, when bias was introduced into the study methodology, this will distort the study results and give biased test characteristics. The most important forms of bias in diagnostic research are inappropriate case-control design and unblinding when reading the test results[14].

Therefore, it is important to report these variables and study characteristics together with the test characteristics. Finally, any funding, whether partly or fully, by any commercial source was noted.

The following data were extracted from the clinical studies:

- Demographic characteristics of the population studied: setting, comorbidities, age and gender.
- Design: cross-sectional study, prospective cohort, case-control.
- Results: sensitivity, specificity, odds ratio, negative en positive predictive value, correlation coefficients, mean difference and 95% limits of agreement, linearity.
- Remarks on the funding of the study.

We did not perform a formal meta-analysis, but present an overall review of the test characteristics and prognostic value.

4. RESULTS

4.1. HTA REPORTS

We identified one HTA report on the value of molecular tests in antenatal screening for heritable thrombophilia, which was out of the scope of the review[15]. We did not identify any HTA report on the use of molecular tests in patients that had experienced a deep venous thrombosis.

The Food and Drug Agency (FDA) of the United States has approved one molecular test for the diagnosis of the factor V Leiden mutation.

4.2. SYSTEMATIC REVIEWS

Using a filter for systematic reviews in Medline (Clinical Queries), we identified 43 articles on factor V Leiden, of which 11 articles met our criteria screening the title and abstract. These 11 articles were subsequently retrieved in full.

Additionally, we found one article in DARE.

One review fulfilled some of the criteria of a systematic review, although details on the search strategy were not complete and overall quality was therefore poor. The results of this review are summarized in the evidence table, but the search for original diagnostic studies was not limited to any publications after this systematic review. Several other systematic reviews gave results not on the diagnostic accuracy of molecular tests for the diagnosis of factor V Leiden, but on the evidence of the mutation as a risk factor for thrombosis. These reviews will be addressed in the clinical impact section.

4.3. ORIGINAL RESEARCH

As the factor V Leiden mutation has been described in 1993, original research was restricted to articles published in 1993 or after. Articles were selected on screening title and abstract by the following criteria:

Inclusion criteria: Factor V Leiden, diagnosis, venous thrombo-embolism and molecular tests.

Exclusion criteria: reviews, letters, commentaries, other causes of thrombophilia, case series of less than 10 patients.

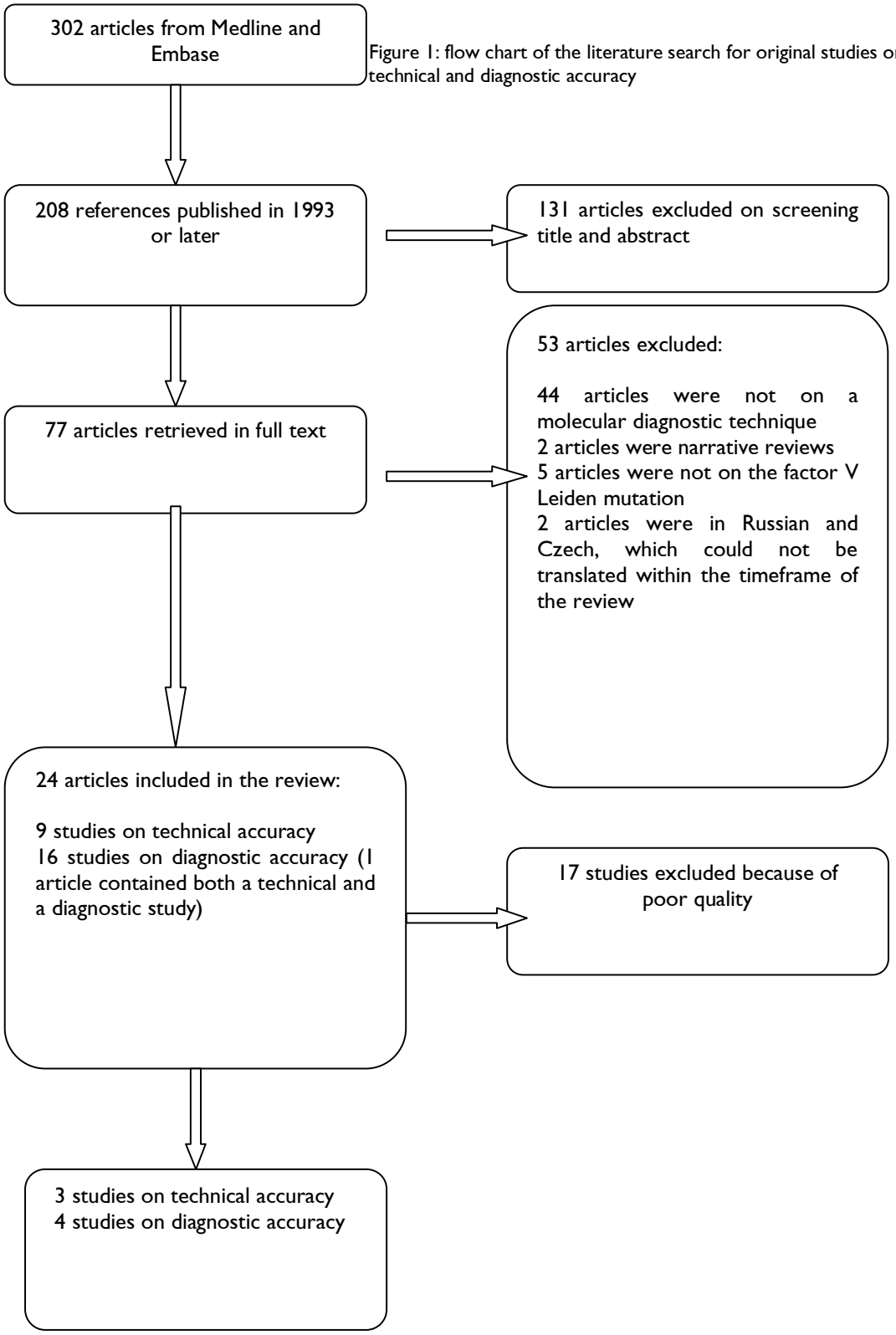

Figure 1: flow chart of the literature search for original studies on technical and diagnostic accuracy

5. EVIDENCE TABLES

5.1. HTA REPORTS

None.

5.2. SYSTEMATIC REVIEWS

Study ID	Tests considered	Quality assessment	Remarks	Conclusions
Eckman 2003[16]	Test for activated protein C resistance, followed by PCR	Fair/poor	No conflict of interest declared	Lack of evidence on treatment consequences. Testing is not cost-effective.

5.3. ORIGINAL STUDIES

5.3.1. Analytical studies

Study ID	Tests considered	Quality assessment	Remarks	Conclusions
Erali 2003[17]	Electronic microarry NanoChip® Assay Reference test: LightCycler®	Good	Supported in part by Nanogen, Inc. (San Diego, CA)	100% concordance between the two assays. 1 sample repeatedly failed on both assays. Within-cartridge variation: <10% when the allele was present; <40% when the allele was absent. Between-cartridge variation: 11-28%. Minimum template requirement: 6 ng of nucleic acid per reaction was sufficient.
Huang 2002[18]	EDEMNA assay Reference test: standardized protocols of the UCLA Diagnostic Molecular Pathology Laboratory	Fair	Supported by research grants from the UCLA and DARPA Bioflips Program	100% concordance between the two assays.
Ugozzoli 2004[19]	Multiplex-multicolor genotyping 5' nuclease assay Reference test: restriction fragment length polymorphisms (RFLP)	Fair	Performed and published by Bio-Rad Laboratories	100% concordance between the two assays. Minimum template requirement: reliable and reproducible results were obtained within a range of 0.5-50 ng.

5.3.2. Clinical studies

Study ID	Tests considered	Quality scale	Design	Population	Conclusions
Hessner 2000[20]	Invader assay Reference test: allele specific PCR	Good	Prospective cohort, Purposive sampling to include multiple individuals of each genotype	1079 healthy Caucasian volunteers + 290 unrelated Caucasians referred to the Hemostatis Reference Center after an objectively confirmed deep vein thrombosis of pulmonary embolism Prevalence: 3.0% in volunteers; 15.5% in patients	99.6% concordance among wild-type volunteers 100% concordance among heterozygous volunteers 100% concordance among referred patients 0.5% equivocal results 1.2% invalid results
Hunault 1999[21]	PCR amplification and microparticle enzyme immunoassay (MEIA) Reference test: restriction enzyme digestion	Fair	Prospective cohort, Selection method not given	130 male veterans with at least one objectively confirmed episode of idiopathic deep vein thrombosis or pulmonary embolism Prevalence: 18.5% (heterozygous only)	100% concordance between the two assays
Blasczyk 1997[22]	Allele-specific amplification Reference test: PCR-RFLP	Fair	Prospective cohort, Consecutively selected	126 patients with thromboembolic events Prevalence: 21.4% heterozygous; 0.8% homozygous	100% concordance between the two assays
Carmi 2004[23]	Single-nucleotide primer-extension assay Reference test: conventional endonuclease method	Fair	Prospective cohort, Randomly selected	284 randomly selected blood samples from patients undergoing thrombophilia screening Prevalence: 23.5%	100% sensitivity 98.7% specificity (3 samples undetermined)

6. ANALYTICAL ACCURACY

Only three studies met our quality criteria. The 6 excluded studies[24-29] mainly failed to describe the source and characteristics of the samples, and whether testing was performed without knowledge of the true status of the samples. The number of samples used was either not reported or insufficient to draw any precise conclusions. Test failures or indeterminate results were very seldom reported.

The three studies that were included, all reported 100% concordance with the reference method that was used. Only one study reported some measures of reproducibility, being the within- and between-cartridge variation. Confidence intervals were never reported.

Key messages:

- **There is limited evidence of good or fair quality on the analytical characteristics of the various assays.**
- **Included studies all reported a 100% concordance of the assay with the reference method.**

7. CLINICAL ACCURACY

Our search strategy identified 15 articles on the clinical accuracy of molecular tests for the diagnosis of the factor V Leiden mutation. Only four of those received a rating of good or fair quality using the QUADAS scale, the remaining 11 articles were excluded due to poor quality[29-39]. Studies mainly failed to report the spectrum and selection of patients, and whether the results were interpreted without knowledge of the result of the other test.

The prevalence of the factor V Leiden mutation in patients with a previous episode of thromboembolism, was fairly homogeneous between the different studies, ranging from 15% to 23%, although selection methods were not equal. In contrast, one study reported a prevalence of 3.0% in healthy volunteers.

All studies reported excellent concordance between the assays used, ranging from 98.7% to 100%. In those articles that reported test failures or indeterminate results, only a small number of samples produced equivocal or invalid results. Unfortunately, confidence intervals around the outcome measures were never given. In addition, measures of reproducibility were not reported.

Key messages:

- **Overall quality of the studies was low, leading to a large proportion of excluded studies.**
- **All included studies reported concordance of >98.7% between the assays used, with very little samples producing equivocal or invalid results.**
- **Measures of precision and reproducibility were not reported.**

8. DIAGNOSTIC STRATEGY

Most guidelines propose a strategy that starts with testing for resistance to activated protein C (APC), followed by a DNA-based mutation test of those patients testing positive. The APC test measures the ratio of activated PTT in the presence and the absence of APC. A reduced ratio is predictive of the factor V Leiden mutation. The sensitivity and specificity of this test APC test[40] has been reported to be at least 84% and 72% respectively.

In patients receiving anticoagulant therapy, a modified APC test is used. The modified test involves diluting the patient's plasma in factor V-deficient plasma, thus making the test insensitive to the administration of anticoagulants[41 42]. The sensitivity and specificity of this modified test is nearly 100%, almost equivalent to the DNA-based mutation tests, which now makes them the first choice in all patients. The lupus anticoagulant may interfere with the modified APC test, these patients should be evaluated directly with a DNA-based mutation test[43].

9. CLINICAL IMPACT

Although the factor V Leiden mutation has been established as a risk factor for VTE, the clinical impact of this increased risk is less clear in terms of treatment or management decisions.

The factor V Leiden mutation is present in 1-6% of the general population. In patients who have had a first episode of venous thrombosis, not related to cancer, the mutation can be sevenfold higher than in healthy controls[44]. In a pooled analysis of 2456 white patients, the factor V Leiden mutation was detected in an average of 18.4% of patients with VTE[45]. In the Physician's Health Study, 121 cases of VTE accrued in 14 916 men who had no history of cardiovascular disease or cancer and were followed prospectively for a mean of 8.6 years. The relative risk of VTE was 2.7 (95% CI 1.3-5.6) for the factor V Leiden mutation.

However, in patients with the factor V Leiden mutation, the risk of pulmonary embolism is not as great as the risk of deep vein thrombosis[46 47], and the mutation itself does not appear to increase mortality[48]. In fact, carriers of the mutation seem to have a smaller risk of pulmonary embolism[49] than the general population, which could be explained by the fact that thrombosis occurs less often in the iliofemoral veins and that the thrombi are significantly smaller than in non-carriers[50].

The annual incidence of VTE in relatives of patients with a factor V Leiden mutation and a history of VTE, has been reported to be 0.58%-0.67%[51 52]. High-risk periods, such as surgery or immobilisation, contribute to about half of all events and primary prophylaxis during these periods has been advocated, although this is based on the indirect comparison of only two reports.

In a systematic review, Park et al. found that women taking hormone replacement therapy were 14 to 16 times more likely to experience a VTE[53]. The Women's Health Initiative study found that this risk is increased for a minimum of 5 years[54]. Due to the costs of testing and the number needed to screen (376 women should be tested over 5 years to prevent one VTE), screening of women before starting hormone replacement therapy is not recommended. In addition, other harmful effects of hormone replacement therapy based on the findings of the Women's Health Initiative study and the HERS II study, have changed the policy on hormone replacement therapy in general.

The use of oral contraceptives by patients with the factor V Leiden mutation increases the risk of VTE more than 30-fold (RR 34.7; 95% CI 7.8 – 154)[55]. When using third generation pills in young starters, this risk is even higher. However, the absolute risk of VTE is sufficiently low in that particular age group, that even large differences in relative risks may be of limited importance. Screening in this group may require counselling and testing of over 2 million women to prevent one death per year or 20.000 women to prevent one VTE[56]. The identification of those that should avoid oral contraceptives can often be achieved by a complete family and personal history without further testing[57]. In addition, the use of third generation pills in young, healthy starters is no longer recommended, regardless of the identification of the factor V Leiden mutation.

An extensive HTA report from the Australian Medical Services Advisory Committee[15] on antenatal screening for heritable thrombophilia found no evidence that prophylaxis is effective in preventing or reducing maternal adverse events in high risk pregnant women with thrombophilia. Therefore, they advised against antenatal screening.

If a patient with an episode of VTE is tested for the factor V Leiden mutation, what are the therapeutic consequences?

The clinical management of venous thrombosis includes treatment of the acute episode with heparin, a variable duration of controlled-intensity anticoagulant prophylaxis, attention to avoidance of contributory factors and use of thromboprophylaxis at times of increased risk of thrombosis.

After initial therapy with heparin, oral anticoagulant therapy is generally continued for 6 months after a first episode of VTE, although a shorter period of treatment may be

acceptable for post-operative deep vein thrombosis[58]. This duration offers a reasonable compromise between the risk of thrombosis recurrence (15-20% after 2 years, approximately 1% of episodes being fatal[59]) and that of serious bleeding resulting from oral anticoagulant therapy (1% per year, 25% being fatal[60]). This was later confirmed in a meta-analysis: after a first episode of idiopathic VTE or VTE with permanent risk factors, the optimal duration seems to be a regimen of 12 to 24 weeks. Further research is required to identify subgroups of patients that would benefit from a long course (high risk of recurrence) or a short course (high risk of bleeding)[61]. However, the monthly incidence of recurrent VTE after treatment with vitamin K antagonists decreases over time, with a stabilisation of the incidence 9 months after the index event, independent of the duration of the anticoagulant therapy[62]. As the risk of bleeding does not decrease over time, but in fact increases with age, the benefit of prolonged treatment diminishes over time.

Patients with coinheritance of two thrombophilic conditions or homozygotes may suffer from a more aggressive clinical course; thrombosis also tends to occur at a younger age. However, these patients only represent 3% of subjects presenting with a first episode of VTE and frequently have a striking family history of deep-vein thrombosis. And also in this group, the clinical benefit of indefinite oral anticoagulation is unknown; an alternative approach may be to emphasize avoidance or correction of additional risk factors and use of thromboprophylaxis only at times of highest risks[63].

Key messages:

- **Factor V Leiden is an established risk factor for the occurrence of VTE.**
- **Screening women before starting oral contraceptives, antenatally or relatives of patients is not recommended.**
- **Management of patients with a first episode of VTE with the factor V Leiden mutation is not different from patients suffering from an idiopathic VTE.**
- **Patients with a personal and/or family history suggestive of co-inheritance of two thrombophilic conditions or homozygosis of the factor V Leiden mutation could be considered for testing, although evidence on the optimal treatment in this patient group is equally lacking.**

10. DISCUSSION

In our review, the reported analytical and clinical accuracy of the molecular tests for the factor V Leiden mutation was high, although the absolute number of studies identified was small and overall quality was poor. In addition, a variety of test methods was evaluated and measures of precision and reproducibility were seldom reported. In contrast, the Food and Drug Administration has approved the licensing of a commercial test kit, manufactured by Roche Diagnostics Corporation. It is possible that the data that were presented to the FDA were not published in peer-review journals, or on the other hand that we did not manage to identify them with our search strategy. As in other reviews, publication bias can not be excluded.

Regarding the overall low quality of the studies we found, it is recommended that authors of future research should consider the STARD statement when designing and reporting their study[64].

Although it is reasonable to assume that the test itself is sufficiently accurate, the clinical impact of testing is very uncertain.

At this moment, there is no evidence to suggest that patients with the factor V Leiden mutation should be treated any differently than patients with an idiopathic episode of VTE; clearly the latter has proven also to have an increased thrombotic tendency.

The impact of avoiding additional risk factors, temporary prophylaxis or other measures is yet to be determined. More importantly, the duration of anticoagulant therapy is still the subject of debate. Although some authors suggest treatment duration of 2 years or even life-long, the benefit and potential harm of these strategies have not been documented in trials. Future research should focus on determining the optimal duration of anticoagulant therapy for patients heterozygous for the mutation and those homozygous.

Although out of the primary scope of the review, screening asymptomatic persons for the mutation is not recommended. Not only would the number needed to screen to avoid one adverse outcome be too high in certain situations, the therapeutic uncertainty is even higher in these groups.

Indiscriminate application of laboratory investigations is poor clinical practice, as it diverts resources from other areas. Identification of a non-modifiable contributory factor to thrombosis is not a worthwhile end in its own right, especially if there is a risk of generating needless anxiety in patients and their asymptomatic relatives or of providing false reassurance[63].

11. IMPLEMENTATION SCENARIO CHARACTERISTICS

11.1. TARGET CONDITION:

Prevalence: 5% in the general population, around 15-20% in patients with a first episode of venous thromboembolism.

Acute or chronic condition: chronic

Leading to increased risk of VTE.

11.2. TEST

Effect on treatment:	uncertain
Prognostic impact:	uncertain when compared to patients with an idiopathic VTE
Test result 24h:	no
Outbreak surveillance:	no

12. RECOMMENDATIONS

Universal testing for the factor V Leiden can not be recommended, neither in patients suffering from thromboembolism nor in the general population, for example before starting oral contraceptives or relatives of patients with a VTE.

Patients with a personal or family history suggestive of a homozygous state or co-inheritance of two thrombophilias might be considered for testing, although therapeutic consequences are unclear.

APPENDIX

THE CHECKLIST FOR HEALTH TECHNOLOGY REPORTS.

INAHTA 2001.

http://www.inahta.org/Reports.asp?name=/Content11/Dokument/HTAchecklist.pdf

	Yes	Partly	No
1. Are contact details available for further information?	( )	( )	( )
2. Authors identified?	( )	( )	( )
3. Statement regarding conflict of interest?	( )	( )	( )
4. Statement on whether report externally reviewed?	( )	( )	( )
5. Short summary in non-technical language?	( )	( )	( )
6. Reference to the question that is addressed and context of assessment?	( )	( )	( )
7. Scope of the assessment specified?	( )	( )	( )
8. Description of the health technology?	( )	( )	( )
9. Details on sources of information?	( )	( )	( )
10. Information on selection of material for assessment?	( )	( )	( )
11. Information on basis for interpretation of selected data?	( )	( )	( )
12. Results of assessment clearly presented?	( )	( )	( )
13. Interpretation of assessment results included?	( )	( )	( )
14. Findings of the assessment discussed?	( )	( )	( )
15. Medico-legal implications considered?	( )	( )	( )
16. Conclusions from assessment clearly stated?	( )	( )	( )
17. Suggestions for further action?	( )	( )	( )

THE CHECKLIST FOR SYSTEMATIC REVIEWS

	Well	Adequately	Poorly	Not adressed
1. The study addresses an appropriate and clearly focused question.	( )	( )	( )	( )
2. A description of the methodology used is included.	( )	( )	( )	( )
3. The literature search is sufficiently rigorous to identify all the relevant studies.	( )	( )	( )	( )
4. Study quality is assessed and taken into account.	( )	( )	( )	( )
5. There are enough similarities between the studies selected to make combining them reasonable.	( )	( )	( )	( )
6. How well was the study done to minimise bias?	++	+	-	
7. If coded as +, or - what is the likely direction in which bias might affect the study results?				
8. What types of study are included in the review?	RCT / CCT / Cohort / Case-control / Other			
9. How does this review help to answer your key question?				

THE CHECKLIST FOR PROGNOSTIC COHORT STUDIES

	Well	Adequately	Poorly	Not adressed
1. The study addresses an appropriate and clearly focused question.	( )	( )	( )	( )
2. The two groups being studied are selected from source populations that are comparable in all respects other than the factor under investigation.	( )	( )	( )	( )
3. The study indicates how many of the people asked to take part did so, in each of the groups being studied.	( )	( )	( )	( )
4. What percentage of individuals or clusters recruited into each arm of the study dropped out before the study was completed.				
5. Comparison is made between full participants and those lost to follow up, by exposure status.	( )	( )	( )	( )
6. The outcomes are clearly defined.	( )	( )	( )	( )
7. The assessment of outcome is made blind to exposure status.	( )	( )	( )	( )
8. Where blinding was not possible, there is some recognition that knowledge of exposure status could have influenced the assessment of outcome.	( )	( )	( )	( )
9. Evidence from other sources is used to demonstrate that the method of outcome assessment is valid and reliable.	( )	( )	( )	( )
10. The main potential confounders are identified and taken into account in the design and analysis.	( )	( )	( )	( )
11. Have confidence intervals been provided?	Yes / No			
12. How was this study funded? *List all sources of funding quoted in the article, whether Government, voluntary sector, or industry.*				

THE CHECKLIST FOR ANALYTICAL STUDIES

	Item	Yes	No	Unclear
1.	Is the source of the samples used described in sufficient detail?	( )	( )	( )
2.	Are the characteristics of the samples used described in sufficient detail?	( )	( )	( )
3.	Is the collection of samples representative for any possible situation when the test is applied in clinical practice?	( )	( )	( )
4.	Were the samples handled and stored in a way to assure its quality?	( )	( )	( )
5.	Was the index test performed without knowledge of the true status of the sample?	( )	( )	( )
6.	Was the execution of the index test described in sufficient detail to permit the replication of the test?	( )	( )	( )
7.	Was the sample size large enough to detect significant differences with the reference sample?	( )	( )	( )
8.	Were uninterpretable/intermediate test results reported?	( )	( )	( )
9.	Were test failures reported?	( )	( )	( )

THE CHECKLIST FOR DIAGNOSTIC ACCURACY STUDIES

The QUADAS tool

Item		Yes	No	Unclear
1.	Was the spectrum of patients representative of the patients who will receive the test in practice?	( )	( )	( )
2.	Were selection criteria clearly described?	( )	( )	( )
3.	Is the reference standard likely to correctly classify the target condition?	( )	( )	( )
4.	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	( )	( )	( )
5.	Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis?	( )	( )	( )
6.	Did patients receive the same reference standard regardless of the index test result?	( )	( )	( )
7.	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	( )	( )	( )
8.	Was the execution of the index test described in sufficient detail to permit replication of the test?	( )	( )	( )
9.	Was the execution of the reference standard described in sufficient detail to permit its replication?	( )	( )	( )
10.	Were the index test results interpreted without knowledge of the results of the reference standard?	( )	( )	( )
11.	Were the reference standard results interpreted without knowledge of the results of the index test?	( )	( )	( )
12.	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	( )	( )	( )
13.	Were uninterpretable/ intermediate test results reported?	( )	( )	( )

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Wettelijk depot : D/2005/10.273/25

KCE reports

1. Effectiviteit en kosten-effectiviteit van behandelingen voor rookstop. D/2004/10.273/1.
2. Studie naar de mogelijke kosten van een eventuele wijziging van de rechtsregels inzake medische aansprakelijkheid (fase 1). D/2004/10.273/2.
3. Antibioticagebruik in ziekenhuizen bij acute pyelonefritis. D/2004/10.273/5.
4. Leukoreductie. Een mogelijke maatregel in het kader van een nationaal beleid voor bloedtransfusieveiligheid. D/2004/10.273/7.
5. Het preoperatief onderzoek. D/2004/10.273/9.
6. Validatie van het rapport van de Onderzoekscommissie over de onderfinanciering van de ziekenhuizen. D/2004/10.273/11.
7. Nationale richtlijn prenatale zorg. Een basis voor een klinisch pad voor de opvolging van zwangerschappen. D/2004/10.273/13.
8. Financieringssystemen van ziekenhuisgeneesmiddelen: een beschrijvende studie van een aantal Europese landen en Canada. D/2004/10.273/15.
9. Feedback: onderzoek naar de impact en barrières bij implementatie – Onderzoeksrapport: deel 1. D/2005/10.273/01.
10. De kost van tandprothesen. D/2005/10.273/03.
11. Borstkankerscreening. D/2005/10.273/05.
12. Studie naar een alternatieve financiering van bloed en labiele bloedderivaten in de ziekenhuizen. D/2005/10.273/07.
13. Endovasculaire behandeling van Carotisstenose. D/2005/10.273/09.
14. Variaties in de ziekenhuispraktijk bij acuut myocardinfarct in België. D/2005/10.273/11.
15. Evolutie van de uitgaven voor gezondheidszorg. D/2005/10.273/13.
16. Studie naar de mogelijke kosten van een eventuele wijziging van de rechtsregels inzake medische aansprakelijkheid. Fase II : ontwikkeling van een actuarieel model en eerste schattingen. D/2005/10.273/15.
17. Evaluatie van de referentiebedragen. D/2005/10.273/17.
18. Prospectief bepalen van de honoraria van ziekenhuisartsen op basis van klinische paden en guidelines: makkelijker gezegd dan gedaan.. D/2005/10.273/19.
19. Evaluatie van forfaitaire persoonlijk bijdrage op het gebruik van spoedgevallendienst. D/2005/10.273/21.
20. HTA Moleculaire Diagnostiek in België. D/2005/10.273/23, D/2005/10.273/25.

Inlichtingen

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