Diagnostic performance of polymerase chain reaction assays for the diagnosis of neurosyphilis: A Systematic Review

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Key Messages

- Central nervous system involvement remains an important complication of syphilis
- CSF Serological assays remain the standard diagnostic test for neurosyphilis
- CSF PCR has poor sensitivity compared to CSF serology and is not the diagnostic test of choice for neurosyphilis

Abstract:

Introduction:

Syphilis continues to be a major public health problem and the recent resurgence in syphilis in high-income settings has seen an accompanying increase in cases of neurosyphilis. Whilst the introduction of PCR has had a significant impact on the diagnosis of early syphilis, CSF serological assays remain the most commonly used tests to diagnosis neurosyphilis. We reviewed data on the performance of CSF-PCR for the diagnosis of neurosyphilis.

Methods:

We searched Pubmed, Medline, EMBASE and the grey literature for references on PCR in neurosyphilis. We calculated the sensitivity and specificity of PCR compared to reference testing for the diagnosis of neurosyphilis

Results:

We identified 66 articles of which seven met the study inclusion criteria. The sensitivity of PCR for definite neurosyphilis varied between 40-70% and specificity between 60-100% across the studies. The most commonly used PCR assay targeted Tp47 which had an overall sensitivity of 68% and a specificity of 91.9%.

Discussion:

The sensitivity of PCR was low compared to CSF-serological assays but the challenges of evaluating a diagnostic test in the absence of a clear gold standard make definitive interpretation challenging. Most studies were small and not adequately powered highlighting the need for multi-centre, multi-country trials to provide adequate statistical power in evaluations of new tests the diagnosis of neurosyphilis.

Background

Syphilis, caused by *Treponema pallidum*, remains a major public health problem worldwide [1]. Infection with syphilis involves progression through a number of clinical stages. Following an incubation period of approximately 21 days the primary syphilitic chancre appears. If untreated the disease progresses to secondary syphilis with dissemination of spirochetes and a generalised illness often accompanied by a characteristic rash. If the disease is untreated then the patient enters the latent phase of infection. During this stage the patient is asymptomatic but has serological evidence of infection. In historical studies in which patients with latent disease were untreated approximately one third of patients would progress on to tertiary syphilis[1,2].

Involvement of the central nervous system (CNS), in the form of neurosyphilis, is one of the major manifestations of syphilis causing significant debilitation to the patient. Typically neurosyphilis is described as a late manifestation of infection occurring during the tertiary stage of infection. Data from both animal models and human infection clearly demonstrate that invasion of the CNS and neurological manifestations can occur in both early and late syphilis[3] including in patients without a clear history of primary syphilis, although the true incidence of symptomatic neurological disease in early syphilis is not known. For example, when lumbar punctures of patients with early syphilis are performed, evidence of *T.pallidum* in the Cerebrospinal fluid (CSF) may be identified, even in those without apparent CNS disease [4] and reports of syphilis with ocular and cranial nerve involvement have been common during the recent syphilis epidemics in many countries. In many patients early CNS involvement appears to

resolve without specific CNS targeted therapy[5] but a proportion of patients will experience clinical CNS disease either during early infection or as a manifestation of tertiary syphilis.

Clinically the spectrum of neurosyphilis covers a broad range of presentations. Five forms are well described: i) asymptomatic neuroinvasion, characterised by evidence of CNS invasion but no clinical disease; ii) an aseptic meningitis ; iii) meningovascular disease, characterised by a stroke-like syndrome; iv) tabes dorsalis, characterised by demyelination of the posterior tracts of the spinal columns; v) general paresis, characterised by a progressive dementing illness [1].

Evaluation of the CSF is central to the diagnosis of neurosyphilis although no universal consensus diagnostic criteria exist. Frequent non-specific abnormalities found in individuals with neurosyphilis include pleocytosis and raised protein concentration. Interpretation of these abnormalities is more difficult in individuals with HIV co-infection, particularly those who are immunocompromised and/or not on antiretroviral therapy as they may have CSF pleocytosis as a consequence of other CNS infections or untreated HIV [6,7].

Serological tests performed on CSF have been the mainstay of diagnostics for neurosyphilis. The gold standard assay for specificity is normally considered to be the Venereal Disease Research Laboratory (VDRL) assay but this is known to have limited sensitivity [8,9]. Whilst the Rapid Plasma Reagin (RPR) assay is commonly used when testing CSF samples it has reduced sensitivity compared the VDRL[10]. A variety of other CSF serological assays have been evaluated

including the Fluorescent Treponemal Antibody-adsorption (FTA-ABS)[11] and *Treponema pallidum* particle agglutination assays[12]. Whilst these treponemal specific assays are considered to be more sensitive they are less specific than the VDRL assay. Some studies have suggested the specificity of the TPPA can be increased by using a higher titre cut-off albeit at the cost of some sensitivity[13].

Polymerase chain reaction (PCR) assays have emerged as valuable diagnostic tools for early syphilis when applied to genital or other mucocutaneous lesions [14,15]. Most assays target highly conserved targets including polA and tp47 and demonstrate good sensitivity and specificity. PCR can also detect circulating treponemes in the blood of a subset of patients with early infectious and early latent syphilis[16,17] although the value of PCR in these settings remains less clear. Given the overall high level of performance it is perhaps unsurprising that there has been interest in the use of PCR as a possible diagnostic test for neurosyphilis [7]. Although a number of studies have evaluated PCR assays for the diagnosis of neurosyphilis its performance compared to alternative diagnostic assays is not well established. The aim of this study was to review available data on the performance of PCR for the diagnosis of neurosyphilis in comparison to reference assays.

Methods

Search strategy and selection criteria

We searched Pubmed, Medline, EMBASE and the grey literature for references on PCR in neurosyphilis. We searched reference lists of selected papers to identify additional manuscripts. We searched for ("CSF" OR "Cerebrospinal Fluid") AND "syphilis" OR "neurosyphilis") AND ("PCR" OR "Polymerase Chain Reaction" OR "NAAT" OR "Nucleic Acid Amplification Test"). We limited the search to studies published between 1st January 1960 and 15th June 2017 (the date the search was conducted).

Inclusion end Exclusion Criteria

We included papers that reported the sensitivity and specificity of a PCR assay against a reference standard in patients with either definite or probable neurosyphilis. We did not restrict inclusion of data based on the specific PCR assay or target utilised. No language restrictions were placed on papers included in the review. We excluded papers that did not report sensitivity and specificity of the assay or which did not provide the raw data from which this could be calculated. We also excluded studies reporting data already described in a different paper and review papers.

Data Extraction:

The titles and abstracts of all papers were reviewed by at least two authors (MM, DL, CK). The full text was obtained for any potentially relevant articles. Full-text

articles were reviewed to determine whether they met the inclusion criteria and where this was uncertain disagreements were resolved by discussion amongst at least two authors. Data were initially extracted by the first author and double-checked by the co-authors. For each paper that met the inclusion criteria we extracted the diagnostic criteria used for neurosyphilis, reference diagnostic test, the comparator PCR assays evaluated, and the performance of the PCR assay compared to the reference test.

Statistical Analysis:

We report the sensitivity and specificity of CSF PCR compared to reference CSF tests. Where available we stratify results for performance in cases of definite and suspected neurosyphilis. Due to limited data we pooled data only from studies assessing a tp47 based PCR assay. All analysis was performed in R 3.4.2 (The R Foundation for Statistical Computing). The review was performed in line with the Preferred Reporting Items for Systematic Review and Meta Analyses (PRISMA) guidelines[18].

Results

Our search identified 66 articles of which seven met the inclusion criteria for the study (Figure 1). These studies enrolled a combined total of 109 patients classified as having definite neurosyphilis, 13 classified as having probable neurosyphilis (from only two studies) and 317 classified as not having neurosyphilis (Table 1)[5,19–24].

Five studies reported results for the performance of a Tp47 PCR alone and a single study reported the combined results of a panel of PCR assays including TP47, polA and bmp. Two studies reported performance of a polA based PCR, one of which also independently reported results for a Tp47 assay and one of which was the evaluation of a panel of Tp47, polA and bmp. A single study evaluated PCR targeting TMPA (Table 1). The criteria for definite neurosyphilis varied between studies but the majority (n = 6) required a CSF VDRL to be positive to make a diagnosis of neurosyphilis, either alone or in combination with a CSF Treponemal assay. A single study did not use serological assays to diagnose definite neurosyphilis but instead used a combination of CSF pleocytosis and raised protein.

Excluding two small studies the reported sensitivity of the PCR assays for definite neurosyphilis varied between 40-70% and specificity varied between 60-100% (Table 1). The five studies which reported results for a Tp47 assay included a total of 88 patients who met the study specific criteria for neurosyphilis (predominantly CSF VDRL positivity) of which 60 (68%) were

positive using a Tp47 PCR. A total of 210 patients without neurosyphilis were included and the PCR was negative in 193 of these individuals (91.9%). Excluding the study which defined neurosyphilis only on the basis of CSF pleocytosis and raised protein did not alter this finding.

Discussion

In this study we have demonstrated the limitations of CSF PCR as a diagnostic for neurosyphilis whilst also highlighting the limited data currently available to fully evaluate these assays. The limited performance of PCR for the diagnosis of neurosyphilis is in marked contrast to the use of PCR for the diagnosis of other stages of syphilis. PCR has emerged as a key diagnostic tool for early syphilis[14,25] with a reported sensitivity and specificity above 95% in most studies. In early syphilis PCR is also able to provide a microbiological diagnosis before seroconversion occurs, reducing the chance of false negative investigations in patients presenting with a chancre. In many high income settings, such as the UK, PCR has become the diagnostic test of choice for primary syphilis and has increasingly replaced dark-field microscopy. By contrast the data from this review demonstrates that PCR has lower sensitivity than CSF VDRL assays for the diagnosis of neurosyphilis. Interpretation of the specificity of PCR is challenging given the use of VDRL, a test known to have limited sensitivity, as the reference standard. In some circumstances false-positive PCR results may actually represent true-positive for the diagnosis of neurosyphilis which are missed by the current CSF serological assays.

The evaluation of diagnostic tests for neurosyphilis remains extremely challenging. There is no accepted gold-standard diagnostic test against which new candidate tests can be compared. This is evident from the studies included in this current review which utilised a range of different reference standards when evaluating the performance of PCR. Whilst CSF VDRL is considered highly specific the sensitivity is believed to be as low as 40% for detecting

neurosyphilis. A result of this is that evaluations of CSF PCR against CSF VDRL are likely to result in in an under-estimation of PCR specificity due to true positives being incorrectly classified. Conversely a previous systematic review has demonstrated a high sensitivity of CSF treponemal antibody testing[13] but lower specificity. Comparisons of CSF PCR against CSF Treponemal antibodies are therefore likely to under-estimate sensitivity due to true-negatives being incorrectly classified. Indeed even the stated sensitivity or specificity of common reference standards such as CSF-VDRL or CSF-TPPA are heavily dependent on the patients included and the criteria used to define neurosyphilis.

As well as analytical challenges in the selection of an appropriate gold-standard, our review highlights further issues in assessing the role of PCR in the diagnosis of neurosyphilis. There was no consistent definition of definite or probable neurosyphilis, variation in the amplification target and relatively small sample sizes. These between study variations make it difficult to draw definitive conclusions on the performance of PCR for the diagnosis of neurosyphilis and highlight the significant challenges in evaluating diagnostic assays for this condition. Of particular importance, the total number of samples included in each of the reviewed studies was small and compared to guidelines on the appropriate sample size for the evaluation of diagnostic tests all the included samples would be considered underpowered[26].

Neurosyphilis remains a challenging condition to diagnose. In patients with positive syphilis serology in blood and evidence of neurological symptoms the absence of a reliable test means that neurosyphilis treatment is often started without confirmation of the diagnosis from CSF testing. In patients without

neurological symptoms however, especially those who are HIV positive, there are concerns surrounding the risk of asymptomatic neurosyphilis with rates as high as 22% reported[27]. In these patients, neurosyphilis is associated with increased CNS inflammation but does not appear to explain cognitive impairment[28]. Given ongoing uncertainties about the significance of asymptomatic neurosyphilis, there remains debate about the need for CSF analysis to assess for neurosyphilis in all asymptomatic HIV positive patients. A recent UK study found that among patients who have received treatment for early syphilis with benzathine penicillin G, the rates of asymptomatic neurosyphilis after treatment are low [29]. In view of this the optimal timing and use of lumbar puncture in the management of syphilis remains uncertain[30].

An alternative to lumbar punctures is to prescribe a neuropenetrative antibiotic regimen to all patients at risk of neurosyphilis without obtaining a confirmatory diagnosis. The only randomised control trial of using a boosted neuropenetrative regimen did not find an improved cure rate compared to standard therapy, although the trial was not powered to specifically address this question in HIV co-infected individuals[31]. One centre in the UK adopts this approach and has shown good adherence and serological response to extended treatment[32] but definitive data remain lacking. A randomised control trial is currently evaluating the benefit of routine lumbar puncture in those patients for whom it is still not clear how to proceed.

Whilst the development of a superior diagnostic test cannot directly answer all these areas of uncertainty it would certainly aid in clinical decision making, especially in HIV positive patients. However our data suggest PCR is not that test and do not support the routine use of CSF-PCR as a diagnostic test for neurosyphilis. A number of new assays are being evaluated including the B-Cell chemoattractant CXCL-13 which shows promise[33]. Given the current absence of a gold-standard assay and the challenges in evaluating assays for the diagnosis of neurosyphilis, consideration should be given to multi-centre, multi-country trials to provide adequate power to fully evaluate new tests which may have superior performance for the diagnosis of neurosyphilis.

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Contributions

MM & DL conceived of the study. MM, CK, DL reviewed the papers. MM wrote the first draft of the manuscript. All authors revised the manuscript.

Figure 1: Flowchart of studies reviewed for inclusion

Table 1: Characteristics of Included Studies

STUDY	Criteria definite neurosyphilis	Criteria probable neurosyphilis	Cases definite Neurosyphilis	Cases probable Neurosyphilis	Cases without Neurosyphilis	PCR Target	Sensitivity Definite Neurosyphilis	Specificity Definite Neurosyphilis	Sensitivity Probable Neurosyphilis	Specificity Probable Neurosyphilis
Castro et al	CSF TPHA/FTA-ABS & WCC>10 OR CSF VDRL/RPR	N/A	33	N/A	91	tp47	75.80%	86.80%		
						polA	69.70%	92.30%		
Dumareq et al	CSF VDRL	CSF WBC count of >20 cells/ml with a nonreactive CSF VDRL	19	11	92	tp47 & polA & bmp*	40%	61%	89%	67%
Molepo et al	CSF VDRL & FTA-ABS	N/A	35	N/A	15	tp47	65%	66%		
García et al	VDRL OR MH-TPA	N/A	8	N/A	25	tp47	50%	100%		
Moskophidis et al	CSF Pleocytosis & Raised CSF-Protein & Raided CSF-IgG	N/A	10	N/A	0	tp47	60%			
Marra et al	CSF VDRL	N/A	2	N/A	79	tp47	100%	100%		