ORIGINAL ARTICLE

Improved case confirmation in meningococcal disease with whole blood Taqman PCR

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Background: The clinical diagnosis of meningococcal disease (MCD) can be difficult. Non-culture methods like the previous ELISA meningococcal PCR improved case confirmation rates, but were not ideal. A Taqman meningococcal PCR, using DNA extracted from serum (S-Taqman), which has an improved sensitivity compared to the ELISA method in vitro, was introduced into clinical practice in July 1997. A new whole blood DNA extraction method for Taqman (WB-Taqman) was introduced in September 1999.

Aims: To determine the degree of improvement in the confirmation rate in clinically diagnosed MCD, following the introduction of WB-Taqman.

Methods: A total of 192 patients (WB-Taqman) with possible or probable MCD, including those admitted to our paediatric intensive care unit, were studied. Admission EDTA samples obtained were sent for bacterial DNA detection at the Meningococcal Reference Unit (MRU), Manchester. These patients were compared to 319 patients with possible and probable MCD, seen at the same hospital prior to the introduction of WB-Taqman.

Results: Following the introduction of WB-Taqman, 82 of the 95 probable cases (88%) had a positive meningococcal PCR result. This gives a diagnostic sensitivity and specificity for WB-Taqman of 87% and 100% respectively. Following WB-Taqman all blood culture positive patients were also PCR positive. Confirmation of cases by PCR rose from 47% (S-Taqman, n = 166) to 88% (WB-Taqman). When all confirmatory tests were included, case confirmation increased from 72% (S-Taqman) to 94% (WB-Taqman).

Conclusion: The sensitivity of PCR in confirming clinical MCD has improved significantly with this new method. The gold standard for confirming cases of MCD is now the WB-Taqman PCR.

In the western world, meningococcal disease (MCD) remains the leading infective cause of death in children outside the neonatal period.[1] The spectrum of clinical presentation is highly variable, ranging from meningitis or septicaemia alone to a combination of the two. A clinical diagnosis is often difficult as the classic triad of an unwell patient with a temperature and a petechial rash is not specific to MCD. Over 20% of patients with MCD have either no rash or only a maculopapular rash, again making clinical diagnosis difficult.[2] This difficulty in diagnosis means that up to two thirds of patients treated do not have MCD, while some with true disease are missed. Adding to this dilemma there is no real gold standard for diagnosis of MCD. Clinical diagnosis, if the patient is assessed from admission until discharge, combined with a raised C reactive protein (CRP), will confirm or exclude the vast majority of confirmed and unconfirmed cases respectively.[3] Previously the sensitivities of blood culture and polymerase chain reaction (PCR) for confirmation of MCD were 31% and 47% respectively, and when all diagnostic tests were combined, 72% of cases were confirmed.[4] The addition of specific convalescent antibody detection can increase case confirmation rates up to 90% or more, but results from serology are not available until at least six weeks post illness.

What was needed was a method that could reliably and rapidly confirm the presence of *Neisseria meningitidis*. The decrease in culture confirmed cases, resulting from the administration of preadmission antibiotics and fewer lumbar punctures (LP) being performed, has lead to the development of non-culture based techniques such as the PCR method.

The original ELISA PCR based technique, introduced in 1996, was replaced by the Taqman PCR method (Perkin-Elmer Applied Biosystems 7700 automated PCR platform, Norwalk, Connecticut) in July 1997. The original involved a serum/plasma DNA extraction method (S-Taqman)[5] and was replaced by a whole blood extraction method (WB-Taqman)[6] in September 1999. WB-Taqman refined at the Meningococcal Reference Unit (MRU) Manchester, UK, uses a Gentra whole blood extraction method, which yields approximately a 2–3 logfold higher DNA concentration than the previous S-Taqman or ELISA methods with a lower limit of 10^3 DNA copies/ml of whole blood (Guiver *et al*, unpublished data). WB-Taqman is able to detect meningococcal DNA in 93% of culture confirmed cases, a greater sensitivity when compared to the previous methods.[6]

Taqman amplifies part of the conserved region of the single copy capsular gene (*ctr*A) common to and specific to all *N meningitidis* serogroups.[5] False positive results using the *ctr*A gene probe have not been recorded, but confirmation of the screening result is achieved by retesting using the group specific sialyltransferase gene probe *sia*D (groups B, C, Y, or W135). For confirmation of group A, a probe directed against an operon encoding four open reading frames, designated *myn*A, *myn*B, *myn*C, and *myn*D, which are involved in the production of the alpha (1–6)-linked N-acetyl-D-mannosamine-1-phosphate capsule, is used. The *sia*D and *myn* PCRs are less sensitive compared to *ctr*A (Guiver *et al*, personal communication) and were therefore not used to assess Taqman sensitivity and specificity characteristics.

Abbreviations: CRP, C reactive protein; CSF, cerebrospinal fluid; LP, lumbar puncture; MCD, meningococcal disease; MRU, Meningococcal Reference Unit; PCR, polymerase chain reaction; RLA, rapid latex antigen

How the improved DNA extraction methodology would perform in a clinical setting has not previously been evaluated.

METHODS

A total of 196 children admitted to the Royal Liverpool Children's Hospital with an illness suggestive of MCD between January 2000 and March 2001 (WB-Taqman) were studied and compared to 319 patients from December 1997 to March 1999 (S-Taqman). These included patients admitted to the paediatric intensive care unit from Liverpool and surrounding areas (South Cumbria to mid Wales). Both studies followed the same protocol and were approved by the local research and development and ethics committees. Consent was obtained from a legal guardian and, where appropriate, assent obtained from the patient by the research fellow.

All patients were seen at or soon after admission. History, and clinical and laboratory data from admission until discharge were collected using a proforma for all patients. Each patient was classified as probable or possible MCD at admission and then reclassified daily until discharge as confirmed, probable, or possible cases using criteria set out by Stuart and colleagues.[7] Stuart defined probable cases as a clinical diagnosis of meningococcal meningitis or septicaemia without confirmation where the clinician managing the case considered that meningococcal disease was the most likely diagnosis. In the absence of other positive microbiological investigations or an alternative diagnosis, a feverish, ill patient with a petechial/purpuric rash was regarded as a probable case. Possible cases were defined as cases in which the managing clinician or research fellow considered diagnoses other than meningococcal disease were as likely. These included cases treated with antibiotics whose probable diagnosis was viral meningitis. The gold standard for case definition used in both cohorts was therefore a clinical diagnosis based on the above criteria.

Admission EDTA samples were collected from the majority of patients with suspected MCD and sent to the MRU, Manchester PHLS for meningococcal DNA detection using the Taqman PCR method. Where dedicated samples had not been collected for PCR analysis, admission blood sent for full blood count was retrieved and subsequently sent. For patients transferred to our intensive care unit from other hospitals, admission EDTA samples were sent for meningococcal PCR from the transferring hospital. Only patients where blood samples for meningococcal PCR had been obtained within 24 hours of admission were included (193 of 196). Those not included consisted of two patients with occult meningitis and one patient who had a PCR sent after 24 hours, MCD confirmed by blood cultures. For the latest cohort the following diagnostic tests were obtained (all patients versus MCD patients respectively); blood cultures (98.5 *v* 100%), rapid latex antigen (RLA) (84 *v* 85%), and throat swabs cultured on appropriate media (vancomycin, nystatin colistin media) (59 *v* 66%). For the earlier cohort, percentages of blood cultures, PCRs, and RLA obtained were 100%, 98%, and 87% respectively.[4]

RESULTS

Following the introduction of WB-Taqman, blood culture and PCR confirmed the diagnosis in 33% and 87% respectively of patients classified as probable cases. No patients were blood culture positive but PCR negative, compared to 14% of patients from the S-Taqman era. The PCR positive rate in patients either already on oral antibiotics (n = 8) or who had received preadmission benzylpenicillin (n = 6, 6%) remained unchanged at 93% while blood culture confirmation fell to 21%. In patients who had clinical signs of meningitis, there was a non-significant decrease in blood PCR confirmation to 78% (16/22). Only nine patients had an LP performed. *N meningitidis* was cultured from three cerebrospinal fluid (CSF) samples, from patients who had received either no antibiotics prior to LP or had an LP performed within two hours of their first dose of parenteral antibiotics. Seven CSF samples were sent for meningococcal PCR; six were positive, and in two patients this was the only confirmatory result. The single patient with meningitis who had a negative CSF PCR, clinically also had meningococcal septicaemia and was subsequently confirmed on blood PCR. Acute (n = 8) and convalescent serology (n = 4) were only sent in PCR negative patients. The proportion of diagnostic investigations found positive showed a considerable variation as illustrated in table 1.

There were no differences between the number of cases confirmed by either RLA or blood cultures before or after WB-Taqman was introduced. There was a significant increase in cases confirmed by PCR after the introduction of WB-Taqman (47% *v* 87%, $p < 0.001$, table 2).

There were no false positive PCR or blood culture results in either series as all patients had been classified as probable

Table 1 Breakdown of confirmatory diagnostic tests in patients with a diagnosis of confirmed/probable MCD; WB-Taqman group

	PCR positive	PCR negative	Total
Blood culture and antigen negative	46 (48%)	9 (9%)*	55
Blood culture positive; antigen negative	26 (27%)	0	26
Antigen positive; blood culture negative	6 (6%)	2 (2%)	8
Antigen and blood culture positive	6 (6%)	0	6
Total	84	11	95

Total number of patients 98; PCR not sent in 3 (confirmed on blood culture (1) and CSF culture (2)).
*Serology sent on 4 PCR negative patients, confirming MCD in 3.

Table 2 Comparison of the diagnostic test results before and after the introduction of WB-Taqman; percentage of tests requested positive (95% CI)

	pre WB-Taqman (n=166)	post WB-Taqman (n=98)	p value
Rapid antigen test	22 (15 to 29)	16 (6 to 20)	0.18
Blood culture	29 (22 to 36)	33 (24 to 42)	0.8
PCR	47 (39 to 55)	88 (81 to 95)*	<0.001
All combined tests	72 (65 to 79)	94 (89 to 99)	<0.001

*95 patients: 3 did not have PCRs sent.

cases prior to confirmation. Classifying patients into the categories probable and possible was not influenced by any diagnostic test results but based on clinical and non-diagnostic laboratory data. Seven patients classified at admission as a possible cases, were reclassified as probable cases shortly after admission, as it became obvious in three that they were developing overt meningococcal disease, while in two patients, covert meningococcal meningitis was diagnosed when an LP was performed. A total of six patients classified as probable cases at admission were subsequently reclassified as alternative diagnoses following confirmatory test results. They included patients with *Haemophilus influenzae* (b) septicaemia, status epilepticus (meningitis ruled out), *Campylobacter jejuni* and rotavirus gastroenteritis, lobar pneumonia, and Henoch–Schönlein purpura. Two patients classified as probable cases at admission were soon after reclassified as possible cases, as they clinically did not have MCD. All other patients classified as probable were either confirmed as MCD (n = 84) or remained probable (n = 7) cases.

The false negative rate by PCR alone decreased to 13%, and with the addition of RLA testing and serology further decreased to 7%. This increased the case confirmation rate when all confirmatory test results were combined from 72% (118/166 S-Taqman) to 94% (91/98 WB-Taqman; $p < 0.001$). This gives sensitivities of 88% or 94% and specificities of 100% or 97% for WB-Taqman PCR alone or all combined confirmatory cases respectively. The 97% specificity, observed when all confirmatory tests post WB-Taqman were combined, resulted from three patients who had false positive *N meningitidis* B/*Escherichia coli* K1 RLA results. The first patient had an *E coli* urinary tract infection. The second had recurrent "septicaemic" episodes for which no cause was found; she did not have petechiae and PCR samples have been consistently negative for meningococci. The third had acute disseminated encephalomyelitis; no cause was found.

DISCUSSION

The most noticeable difference between the two studies is that following the introduction of the WB-Taqman *ctr*A method, the case confirmation rate has improved considerably. This is mainly a result of the Gentra whole blood DNA extraction method now used, compared to serum previously. The redesigned *ctr*A primers are more sensitive compared to those previously used and therefore have also contributed to the improved sensitivity.

The improved case confirmation rate seen after the introduction of WB-Taqman could be partly explained by bias between the two studies. The most obvious potential discrepancy would be observer bias—that is, how each research fellow classified patients into possible and probable cases. This is unlikely for a number of reasons. Both research fellows used the same criteria set out by Stuart and colleagues[7] for classifying patients, and both fellows had a similar level of paediatric experience. In the latest study we have shown how we classified, or in a minority reclassified patients, giving reasons for doing so, thus making this process more transparent. Secondly, in post WB-Taqman there were no negative PCR results in patients who were blood culture positive, compared to 14% in the S-Taqman era, indicating a real improvement in PCR sensitivity. Lastly, the proportions of possible and probable cases in both studies have remained roughly the same (approximately 50:50).

This improved rate of confirmation means that PCR is the essential confirmatory investigation in MCD. The reason that it is not 100% sensitive is probably because it has a lower detection sensitivity of 10^3 DNA copies (bacteria)/ml of blood. Patients with bacterial loads below this limit would not be detected. In patients where clinical signs of meningitis are more prominent than septicaemia, we found that bacterial load is significantly higher in CSF than in blood (personal observations). CSF samples from these patients, which are rarely sent, would therefore have a greater chance of giving a positive PCR result. A further confounding factor is that patients with no confirmed diagnosis, who are unwell with a temperature and petechiae (for which there is a long list of other potential diagnoses), are by default classified as probable meningococcal cases.

We recommend that RLA, blood cultures, and PCR be sent in all patients with probable MCD. PCR is especially important in patients who are on or who have received parenteral antibiotics prior to blood culture being sent. RLA testing has a low sensitivity, but as its name implies, can confirm a case quickly, which may aid public health decisions. However the only false positive results in our study occurred in three patients with positive RLA.

Blood cultures, generally available before PCR results, allow the organism to be serotyped and serosubtyped (important from a public health and epidemiological viewpoint), and also antibiotic sensitivity to be performed. We believe that serology need only be sent in PCR negative patients or in cases of conjugate C vaccine failure. Where PCR results will not be available prior to the patient's discharge from hospital, acute serum should be stored. If the PCR is negative, convalescent serum, approximately 3–6 weeks post illness, should be sent with the acute sample.

An attempt should be made to confirm all cases of probable MCD, enabling accurate disease surveillance, which is particularly important following introduction of the meningococcal C conjugate vaccine. This would permit the detection of the majority of vaccine failures and the occasional patients who have recurrent disease (in the WB-Taqman series, three patients, 3%). For both recurrent disease and in vaccine failures complement function, immunoglobulin levels and functional antibody assessment needs to be undertaken.

On average, 10% of the population carry *N meningitidis* in their nasopharynx,[8] which may only be one quarter of true carriage rates.[9] The relevance therefore of detecting a meningococcus in a throat swab from a patient with possible MCD needs to be interpreted in the context of their clinical disease.

Meningococcal PCR is supplied free of charge by the public health laboratory services at the MRU. The turnaround time for the screening test is 4–5 hours from receiving the sample. This means that a sample received at the MRU before 10 am on a working day will usually have a result before 5 pm that day. Therefore for the majority of samples, Taqman PCR results were available 24–48 hours after sampling.

The ideal study would have been evaluating both tests on the same samples, which was not possible here. Our comparison with "historical" controls slightly reduces the significance of the result. However, the highly significant difference observed between the cohorts means that the disparity is not a result of either chance or bias.

Conclusion

Following the introduction of the WB-Taqman PCR into clinical practice we have shown that PCR confirmation and overall case confirmation has significantly improved. We believe that serology is best reserved for PCR negative cases. The gold standard for confirming cases of MCD is now the WB-Taqman PCR.

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REFERENCES

1 **Jones D**. *Epidemiology of meningococcal disease in Europe and the USA*. Chichester: John Wiley & Sons Ltd, 1995.
2 **Marzouk O**, Thomson AP, Sills JA, *et al*. Features and outcome in meningococcal disease presenting with maculopapular rash. *Arch Dis Child* 1991;**66**:485–7.
3 **Marzouk O**. Clinical and laboratory aspects of meningococcal disease in childhood. MD thesis, Liverpool University, 1994.
4 **Carrol ED**, Thomson AP, Shears P, *et al*. Performance characteristics of the polymerase chain reaction assay to confirm clinical meningococcal disease. *Arch Dis Child* 2000;**83**:271–3.
5 **Corless CE**, Guiver M, Borrow R, *et al*. Simultaneous detection of Neisseria meningitidis, Haemophilus influenzae, and Streptococcus pneumoniae in suspected cases of meningitis and septicemia using real-time PCR. *J Clin Microbiol* 2001;**39**:1553–8.
6 **Guiver M**, Borrow R, Marsh J, *et al*. Evaluation of the Applied Biosystems automated Taqman polymerase chain reaction system for the detection of meningococcal DNA. *FEMS Immunol Med Microbiol* 2000;**28**:173–9.
7 **Stuart JM**, Monk PN, Lewis DA, *et al*. Management of clusters of meningococcal disease. PHLS Meningococcus Working Group and Public Health Medicine Environmental Group. *Commun Dis Rep CDR Rev* 1997;**7**:R3–5.
8 **Cartwright KA**, Stuart JM, Jones DM, *et al*. The Stonehouse survey: nasopharyngeal carriage of meningococci and Neisseria lactamica. *Epidemiol Infect* 1987;**99**:591–601.
9 **Sim RJ**, Harrison MM, Moxon ER, *et al*. Underestimation of meningococci in tonsillar tissue by nasopharyngeal swabbing. *Lancet* 2000;**356**:1653–4.