Prospective Study of a Real-Time PCR That Is Highly Sensitive, Specific, and Clinically Useful for Diagnosis of Meningococcal Disease in Children

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Due to the early administration of antibiotics, meningococcal disease is increasingly difficult to diagnose by culturing. Laboratory studies have shown PCR to be sensitive and specific, but there have been few clinical studies. The objectives of this study were to determine the diagnostic accuracy and clinical usefulness of meningococcal PCR through a prospective comparison of real-time PCR, nested PCR, and standard culturing of blood and cerebrospinal fluid (CSF). The setting was a tertiary-care pediatric hospital in Australia, and the participants were 118 children admitted with possible septicemia or meningitis. The main outcome measures—sensitivity, specificity, and positive and negative predictive values—were compared to a “gold standard” fulfilling clinical and laboratory criteria. For 24 cases of meningococcal disease diagnosed by the gold standard, culturing of blood or CSF was positive for 15 (63%), nested PCR was positive for 21 (88%), and real-time PCR was positive for 23 (96%). The sensitivity, specificity, and positive and negative predictive values of real-time PCR (the most sensitive test) for all specimens were, respectively, 96% (95% confidence interval, 79 to 99%), 100% (95% confidence interval, 96 to 100%), 100% (95% confidence interval, 85 to 100%), and 99% (95% confidence interval, 94 to 100%). Of 54 patients with suspected meningococcal disease at admission, 23 had positive PCR results. Only one PCR specimen was positive in a patient thought unlikely to have meningococcal disease at admission. Blood PCR remained positive for 33% of patients tested at up to 72 h. Real-time PCR has high positive and negative predictive values in this clinical setting, with better confirmation of cases than nested PCR. Targeting patients for PCR based on admission criteria appears to be practical, and the test may remain useful for several days after the start of antibiotic administration.

Meningococcal septicemia and meningitis are life-threatening diseases. Rapid, accurate diagnosis is essential for optimal management of patients and the provision of prompt prophylaxis to contacts. Confirmation of the diagnosis allows physicians to use narrow-spectrum antibiotics, limit the duration of treatment, and provide prognostic information. It also provides vital disease burden information, including data to inform vaccine policy.

It is becoming increasingly difficult to confirm the diagnosis of meningococcal infection by conventional microscopy and culturing techniques (6). Blood cultures are positive in about 50% of untreated patients with clinically suspected meningococcal septicemia. This rate is reduced to 5% when antibiotics have been administered prior to admission; primary care practitioners are encouraged in this practice early for suspected cases (8, 9, 19). Cerebrospinal fluid (CSF) microscopy or culturing is positive in 80 to 90% of untreated cases of meningococcal meningitis, but this rate is also reduced by prior antibiotic administration. In addition, many patients do not undergo

a lumbar puncture early in the disease because of concerns about inducing clinical deterioration (4). Other limitations of conventional diagnostic methods include the delay before cultures become positive and the poor sensitivity and specificity of rapid antigen and antibody tests (1, 11, 15, 30).

PCR has the potential to overcome all of these limitations, as it detects small quantities of bacterial DNA and does not require the presence of viable bacteria (20, 29). Therefore, PCR can confirm the diagnosis when the number of bacteria is below the threshold for culturing or after antibiotic administration. Moreover, PCR may be used to define serosubtypes of meningococci that were previously untypeable (12, 23, 25).

PCR for the detection of DNA from *Neisseria meningitidis* has been studied in laboratory settings but has not been well evaluated as a clinical tool. Real-time PCR is potentially more sensitive than conventional PCR. We assessed the value of meningococcal PCR in a clinical setting and compared real-time PCR with conventional PCR.

We aimed to determine (i) the sensitivity, specificity, and positive and negative predictive values of meningococcal PCR through a comparison of real-time PCR with conventional nested PCR; (ii) in which patients such testing should be considered; and (iii) how long after clinical presentation PCR remains positive for a subset of patients.

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TABLE 1. Demographic and clinical data for study patients

Characteristic ^a	Data for the following group ^d (no. of patients)	
	A (54)	B (64)
Median age, yr (range)	2.6 (0.1–15.4)	0.9 (0.0–15.2)
Male/female ratio	1.4:1	1.5:1
Admission diagnosis, no. (%) of patients		
Fever or PUO	7 (13)	43 (67)
Septicemia or septic shock	20 (37)	18 (28)
	3 (6)	3 (5)
Meningitis—viral	11 (20)	0 (0)
Meningitis—bacterial	6 (11)	0 (0)
Meningitis—unknown	5 (9)	0 (0)
Meningoencephalitis	2 (4)	0 (0)
Median duration of symptoms, days (range)	1 (0.25–5)	2 (0.5–14)
Antibiotics prior to culturing, no. (%) of patients		
Oral	7 (13)	7 (11)
Parenteral	18 (33) ^b	4 (6) ^c
ICU admission, no. (%) of patients	24 (44)	0 (0)

^a PUO, pyrexia of unknown origin.^b Three intramuscular and 15 intravenous.^c Four intravenous.^d Group at time of admission. Groups: A, suspected meningococcal disease; B, unlikely meningococcal disease.

MATERIALS AND METHODS

We conducted a prospective study of patients with possible meningococcal disease by comparing culture results with PCR results. The study took place at the Royal Children's Hospital (RCH), Melbourne, Victoria, Australia; RCH is the largest pediatric hospital in Australia, providing primary, secondary, and tertiary levels of care. Routine laboratory tests were performed at the RCH pathology service, and PCR was performed at the Microbiological Diagnostic Unit, University of Melbourne. The study was approved by the Ethics in Human Research Committee of RCH.

Patients. In order both to assess the performance of the meningococcal PCR in the real clinical situation and to obtain enough patients with meningococcal disease to evaluate the sensitivity of the test, two groups of patients were studied. Group 1 comprised all consecutive patients admitted to RCH during 1 month in winter and 1 month in spring (July and October 2000) with a clinical suspicion of meningitis or septicemia (admission diagnosis of bacterial meningitis, viral meningitis, meningitis of unknown cause, meningoencephalitis, fever or pyrexia of unknown origin, or septicemia or septic shock). Group 2 comprised all patients over a 6-month period (August 2000 to January 2001) with an admission diagnosis of probable meningococcal septicemia and/or meningitis.

Every child in the study had an acute febrile illness; therefore, meningococcal disease was a possibility at admission, and meningococcal PCR therefore might reasonably be performed. Thus, it was deemed appropriate at the outset to pool results for the two groups for statistical analysis of the performance characteristics of the test. Each patient, regardless of group (group 1 or 2), was assessed in the same way for the pretest probability of having meningococcal disease on the basis of clinical criteria alone. The patients were divided at admission into those with suspected meningococcal septicemia or meningitis (group A) and those unlikely to have meningococcal disease (group B) in order to identify patients for whom PCR could be targeted (Table 1). Patients with suspected meningococcal septicemia included those with fever and petechiae, those with severe sepsis with shock or requiring intensive care unit (ICU) admission, and those for whom meningococcal disease was included in the admission differential diagnosis. Because of the difficulty in recognizing clinically the organism causing meningitis, suspected meningococcal meningitis included all patients with an admission diagnosis of meningitis or signs of meningococcal disease. Clinical

details were obtained from the hospital records and from the patients and parents as follows: duration of symptoms, administration of prior antibiotics (oral or parenteral), timing of administration of prior antibiotics in relation to first venipuncture and lumbar puncture, evidence of shock or ICU admission, presence of rash, and presence of signs of meningitis.

Laboratory tests. The majority of patients had a blood culture and full blood count at admission and an immediate or delayed lumbar puncture. The EDTA-treated blood sample taken at admission was used for PCR, as was CSF when a lumbar puncture was performed. For two patients, joint effusions were aspirated, and this fluid was also cultured and used for PCR. Ethical considerations meant that no additional samples were taken from any child for the purposes of the study, but for children for whom multiple blood tests were carried out during admission, PCR was used on multiple occasions. Both real-time PCR and nested PCR were performed on all specimens with published primers as described below to try to determine which was more sensitive and specific. Samples were assigned random numbers, and PCR was performed in a blinded fashion.

(i) **porA nested PCR.** DNA from 200 µl of whole blood was extracted by using an Instagene extraction kit (Bio-Rad Laboratories, Hercules, Calif.). Whole-blood extracts were resuspended in a total of 60 µl of Instagene matrix. CSF and synovial fluid samples were boiled for 10 min and then centrifuged for 3 min at 13,200 × g, and the supernatants were used as DNA extracts. For the PCR, 5 µl of DNA extract was used in a total volume of 50 µl. PCR mixtures contained 0.5 U of *Taq* DNA polymerase (Invitrogen), a buffer from Invitrogen, 2.5 mM MgCl₂, 0.2 mM deoxynucleoside triphosphates, and 25 pmol of each primer. The nested assay targeted the *porA* gene, coding for an outer membrane protein that is the subtype-determining antigen of *N. meningitidis*. The outer primers, 5'-A AACTTACCGCCCTCGTA-3' and 5'-TTAGAATTGTGGCGCAAACCGA C-3', were used for the first PCR. After 32 amplification cycles, 2 µl of the first PCR mixture was added to 25 µl of the second PCR mixture. This PCR was carried out for 25 cycles with inner primers 5'-CCGCACTGCCGCTTGCGG-3' and 5'-CGCATATTAAAGGCATA-3'. The PCR product was visualized on an agarose gel stained with ethidium bromide.

Caugant et al. tested various neisseriaceae and other meningitis-causing bacteria and found that with these primers, a 680-bp fragment was specific for meningococcal DNA (10).

CSF samples and knee aspirate samples were checked for the presence of PCR inhibitors by running a second PCR with 5 µl of boiled extract spiked with 100 pg of DNA extracted from *N. meningitidis* strain 9802369, a clinical isolate from the culture collection at the Microbiological Diagnostic Unit, University of Melbourne. Only the knee aspirate sample was shown to contain significant amounts of PCR inhibitors. DNA extracted from whole-blood samples was not tested for PCR inhibitors. The whole-blood DNA extraction method involves erythrocyte lysis, one wash with erythrocyte buffer, and protein digestion with Instagene matrix. We previously tested over 200 whole-blood samples by spiking them with meningococcal DNA and found no significant PCR inhibitors in Instagene extracts from whole blood (data not shown).

(ii) **ctrA TaqMan real-time PCR.** An Applied Biosystems 7700 sequence detection system was used to detect meningococcal DNA in clinical samples by real-time PCR. This system uses a fluorescent probe to detect the target DNA without any need for post-PCR processing. A TaqMan 5' nuclease assay which targets the *ctrA* gene was developed. The *ctrA* gene is part of the capsule gene complex within the genome of *N. meningitidis*. The *ctrA* TaqMan assay was evaluated and shown to be a very specific and sensitive assay for the detection of *N. meningitidis* in clinical specimens (16). The probe sequence was 6-FAM-CATTGCCACGTGTCAGCTGCACAT. The primer sequences were 5'-GCTG CGGTAGGTGGTTCAA (CtrA-F) and 5'-TTGTCGCGGATTGTGCAACTA (CtrA-R) (13).

This assay, as evaluated by Corless et al., was shown to target meningococcal DNA specifically, with no cross-reactions occurring with other bacteria or viruses that may cause meningitis or with human genomic DNA (13). The same DNA extracts as those used for the nested PCR were used for the TaqMan assay. A total of 2.5 µl of DNA extract was used in a final volume of 25 µl of Universal Mastermix (Applied Biosystems catalog no. 4324018). The final concentration of the probe was 100 nM, and the primers were present at 500 nM each. Each amplification run was set up with a 96-well plate format and the following cycling parameters: heating at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The TaqMan instrument calculates the increase in fluorescence in each reaction well. The increase in fluorescence above a calculated background threshold indicates amplification of the target sequence. If no increase in the fluorescence signal is observed after 40 cycles, the sample is assumed to be negative.

The analytical sensitivity of the TaqMan assay was tested with DNA purified from *N. meningitidis* strain 9802369. Colonies grown on solid medium were

TABLE 2. Numbers of samples in groups analyzed in this study

Sample	Test	No. of samples in the following group of patients:							
		All		A		B		Gold standard	
		Test done	Test positive ^a	Test done	Test positive	Test done	Test positive	Test done	Test positive
Blood	Blood taken	118		54		64		24	
	Culturing	110	14	53	14	57	0	24	14
	PCR	116	21	54	20	62	1	24	21
CSF	Lumbar puncture	55		33		22		13	
	Culturing	55	2	33	2	22	0	13	2
	PCR	48	4	28	4	20	0	13	4
Synovial fluid	Joint aspirated	2		2		0		2	
	Culturing	2	0	2	0	0	— ^b	2	0
	PCR	2	1	2	1	0	—	2	1

^a Test positive, positive for *N. meningitidis*.^b —, not done.

extracted by using a QIAamp DNA minikit with RNase treatment. The concentration of purified DNA was estimated by measuring the UV absorbance at 260 nm. From the known molecular weight of *N. meningitidis* genomic DNA, it was estimated that 100 pg of purified DNA contained 3.6×10^4 copies of the target *ctrA* gene. Using this estimate, we were able to show that the TaqMan assay was able to detect as little as 1 copy of the target sequence per reaction, and in the range of 5 to 25 copies, the assay was 100% sensitive. With the same DNA, the *porA* nested PCR proved to be less robust, achieving a sensitivity of 50% only for detection in the range of 5 to 25 copies of the target sequence. A positive control reaction containing 0.3 pg of genomic DNA (100 copies of the target sequence) was included with every TaqMan amplification run in order to verify Mastermix functionality and performance.

Other laboratory results were obtained from routine clinical testing: peripheral white blood cell count (WBC), CSF WBC and differential, CSF protein and glucose levels, blood culturing, and CSF culturing.

Analysis. The sensitivity, specificity, and positive and negative predictive values (with 95% confidence intervals) were calculated for the results of culturing and PCR of blood and for combined results of culturing and PCR of blood, CSF, and synovial fluid (in which a test result for meningococcal disease was considered positive when any of the blood, CSF, or synovial fluid results was positive). In any study in which the method being evaluated is more sensitive than the "gold standard" method, there are inherent difficulties in defining true cases of the disease. Because of the likelihood that PCR is more sensitive than the current gold standard of culturing of the organism, the gold standard diagnosis of meningococcal disease for this study was expanded to fulfill specific clinical and/or laboratory criteria as follows: for meningococcal septicemia—clinical features, including fever, petechiae or purpura, shock, or severe illness (consensus decision of the treating physician and an infectious diseases physician); for meningococcal meningitis—clinical signs of meningococcal disease (consensus decision of the treating physician and an infectious diseases physician) plus one confirmatory laboratory test (CSF Gram staining, culturing, or PCR).

RESULTS

Patients. Of the group 1 patients, 113 were eligible for the study. For 8 patients, no blood or CSF was taken, and for 4 patients, the first EDTA-treated sample was lost and no CSF was taken, leaving 101 patients. Group 2 included 17 additional patients. The demographic data for the 118 study patients, comparing those with suspected meningococcal disease at admission to those without, are shown in Table 1. Representative results are shown in Table 2.

Gold standard diagnosis of meningococcal disease. Twenty-four patients had a gold standard diagnosis of meningococcal disease (Table 3). Nineteen had a consensus diagnosis of meningococcal septicemia, and of these, 11 also had signs of

meningitis. A further five patients had signs of meningococcal disease alone. Every patient who subsequently had any positive culture result already had a consensus clinical gold standard diagnosis.

Blood culturing and PCR. For the 118 patients in the study, blood culturing was done for 110, and there was sufficient blood to perform PCR for 116. Figure 1 shows the blood culture and blood PCR results for the patients. Of 26 patients with positive blood cultures, 14 were culture positive for *N. meningitidis*; all of these patients had been assigned to group A at admission. The other positive blood cultures represented *Streptococcus pneumoniae* ($n = 5$), coagulase-negative staphylococci ($n = 3$), *Staphylococcus aureus* ($n = 1$), *Escherichia coli* ($n = 1$), *Campylobacter jejuni* ($n = 1$), and *Neisseria flava* ($n = 1$).

The primary analysis of results was done with real-time PCR, as it was found to be more sensitive than nested PCR, as discussed below. Of the 24 patients who fulfilled the criteria for a gold standard diagnosis of meningococcal disease, 21 patients were blood PCR positive, although only 20 of them were assigned to group A at admission. The other patient was not thought likely to have meningococcal disease clinically at admission but subsequently fulfilled the gold standard criteria; therefore, the result for this patient was considered to be true positive. Six patients had negative blood culture but positive

TABLE 3. Culturing and PCR results for patients with gold standard diagnosis of meningococcal disease

Clinical diagnosis (no. of patients)	No. of samples found positive by the following test:					
	Blood culturing	Blood PCR	CSF culturing	CSF PCR	Synovial fluid culturing	Synovial fluid PCR
Septicemia (8)	4	8	0	0	0	0
Septicemia with signs of meningitis (11)	6	9	1 ^a	2	0	0
Meningitis (5)	4	4	1	2	0	1

^a This patient had a positive CSF Gram stain result but a negative culture result.

FIG. 1. Blood culture and PCR results for study patients. BC, blood culture; PCR, blood meningococcal PCR; N.men, *N. meningitidis*; S.pn, *S. pneumoniae*; CNS, coagulase-negative staphylococci; pos, positive; neg, negative. The asterisk indicates that two of these patients were CSF PCR positive. The dagger indicates that although thought unlikely to have meningococcal disease at admission, this patient subsequently met the "gold standard" criteria; i.e., this result was true positive (see the text).

PCR results and also had a gold standard diagnosis of meningococcal disease.

For the diagnosis of meningococcal septicemia in this study, blood PCR was substantially more sensitive and had a slightly higher negative predictive value than blood culturing (Table 4).

CSF culturing and PCR. CSF was taken from 55 of the 118 study patients at or within 48 h after admission, and there was sufficient CSF from 48 patients for PCR analysis. Seven patients had positive bacterial cultures: *N. meningitidis* ($n = 2$), *S. pneumoniae* ($n = 4$), and coagulase-negative staphylococci ($n = 1$). For one additional patient, gram-negative cocci were

seen on microscopy. Six patients with a gold standard diagnosis of meningococcal disease had CSF pleocytosis (CSF WBC of greater than $10 \times 10^9/\text{liter}$) but had negative culture results.

The CSF PCR was positive for all three patients with culture or Gram stain evidence of meningococcal meningitis. The CSF from one additional patient was culture negative but PCR positive, and this patient had had a positive blood culture for *N. meningitidis* at his referring hospital. All four patients with positive CSF PCR results had a CSF WBC of greater than $3,000 \times 10^9/\text{liter}$.

Combined blood, CSF, and joint effusion results. Fifteen of the 24 patients with a gold standard diagnosis of meningococ-

TABLE 4. Comparison of real-time meningococcal PCR with culturing for diagnosis of meningococcal disease^a

Category ^b	Gold standard diagnosis (total no. of patients)		%			
	MD	Not MD	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Blood culture positive	14 (24)	0 (86)	58 (37–78)	100 (96–100)	100 (77–100)	89 (82–95)
Blood PCR positive	21 (24)	0 (92)	88 (68–97)	100 (96–100)	100 (84–100)	97 (91–99)
Any culture positive	15 (24)	0 (88)	63 (41–81)	100 (96–100)	100 (78–100)	91 (83–96)
Any PCR positive	23 (24)	0 (94)	96 (79–99)	100 (96–100)	100 (85–100)	99 (94–100)

^a MD, gold standard diagnosis of meningococcal disease; not MD, diagnosis other than meningococcal disease; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

^b Any culture positive, isolation of *N. meningitidis* from blood, CSF, or joint effusion fluid; any PCR positive, meningococcal PCR of the same specimens.

FIG. 2. PCR results based on pretest probability of meningococcal disease on the basis of clinical criteria. Symbols: ▨, PCR positive; □, PCR negative.

cal disease were either blood or CSF culture positive, and the results for an additional 8 patients were confirmed by PCR. The CSF and joint effusion results alone were too sparse to analyze meaningfully, but combining all results slightly improved the sensitivity and negative predictive values of both culturing and PCR without affecting the specificity and positive predictive values (Table 4).

Clinically suspected meningococcal septicemia and meningitis. For 54 patients, meningococcal disease could reasonably have been suspected at admission (group A): 29 with suspected septicemia and an additional 25 with meningitis. Of these patients, 22 had a positive PCR result—3 for both blood and CSF, 17 for blood only, 1 for CSF only, and 1 for joint effusion

(Fig. 2). In contrast, for the 64 patients thought not to have meningococcal disease at admission (group B), there was only 1 positive PCR result. This case was believed to be a true case of meningococcal disease because of a 12-h history of a high fever and positive PCR results obtained by both nested and real-time methods. It is well known that a small percentage of children presenting with a fever alone have meningococcal bacteremia (21).

Duration of positive results and effect of antibiotics. Of the 118 study patients, 11 had been given oral antibiotics, 5 had been given intramuscular antibiotics prior to admission, and a further 19 had begun receiving intravenous antibiotics before blood samples were taken for culturing. Fifteen patients had positive cultures for *N. meningitidis*; none of these had received parenteral antibiotics prior to culturing, but one had received two oral doses of cefaclor. For nine of these patients, blood samples were taken for culturing within 6 h of the first dose of antibiotic; all of these repeat cultures were sterile, but the blood PCR results remained positive for eight of these patients. The remaining patient was blood culture and blood PCR negative 6 h after the first dose of intravenous antibiotic. However, 21 h after the first dose of antibiotic, his CSF remained PCR positive (but culture negative).

From 13 of the 21 patients with positive blood PCR results at admission, further blood samples were taken for PCR. The results are shown in Fig. 3. In all patients who were tested more than once, PCR results remained positive longer than blood culture results—for a third of patients, over 72 h longer. For one patient, PCR results remained positive 9 days after the first dose of antibiotic. Only 4 of these 13 patients had a subsequent negative PCR result, so we do not know when the other 9 patients became negative. It is therefore difficult to compare disease severity with the duration of positive PCR results. For the four patients for whom data were available, two were PCR negative by 48 h after the first dose of antibiotic, while two were not negative until over 120 h afterward. There

FIG. 3. Duration of positive PCR results in blood for a subset of patients. Nested PCR-positive results were all positive in the real-time PCR.

were no obvious differences in degree of shock, duration of illness, admission to ICU, or initial peripheral WBC between these pairs (data not shown).

Real-time PCR versus conventional PCR. Both real-time PCR and nested (or conventional) PCR were performed for every sample. At initial presentation of the patients, these PCRs performed similarly; real-time PCR confirmed only two additional patients as having meningococcal disease, increasing the number from 21 to 23. One patient who had received prior intravenous antibiotics had a positive blood PCR result, and one had a positive PCR result for a joint aspirate sample. The use of real-time PCR therefore increased the sensitivity of PCR for the diagnosis of meningococcal disease from 86% (95% confidence interval, 68 to 95%) to 94% (78 to 99%) over that of nested PCR. Real-time PCR convincingly outperformed nested PCR for the subset of patients who had meningococcal disease and who had multiple samples taken over several days after antibiotic treatment. For eight samples from six different patients, real-time PCR results were positive, while nested PCR results were negative (Fig. 3). In addition, the CSF supernatant from one patient was found to be positive by real-time PCR, while only spun CSF cells from this patient had been found to be positive by nested PCR.

DISCUSSION

In this study, we were able to confirm the usefulness of meningococcal PCR in clinical practice in a tertiary-care pediatric hospital. Retrospective laboratory studies of meningococcal PCR have demonstrated high sensitivities (89 to 91%) and specificities (91 to 100%) for both CSF and blood (5, 24, 26, 27). However, there have been very few prospective studies in a clinical setting. In earlier studies, the sensitivity of PCR was found to be higher than that of blood culturing (47 versus 31%), and the sensitivity increased to 88%, with improved specificity, when PCR was used with whole blood rather than serum in a subsequent group of patients (7, 17).

Since it is more sensitive than culturing, PCR has also been used to investigate clinically suspicious culture-negative cases both in the laboratory and in vaccine trials, increasing the number of confirmed cases of meningococcal disease by up to 60% (24, 28, 30–32). In our study, an additional eight (culture-negative) cases were confirmed by PCR—a 33% increase. The increased yield of PCR is likely to be greater when higher proportions of sick children receive antibiotics before hospital admission.

PCR has the further potential advantage of providing more rapid confirmation of the diagnosis than culturing. In this study, PCR results were often available on the day of presentation, compared to the 1 or 2 days (or more) required for culture confirmation. In a number of cases, this situation allowed clinicians to target antibiotic treatment (e.g., to penicillin from broad-spectrum cephalosporin antibiotics) and to limit investigations for other diagnoses.

The duration after antibiotic administration during which meningococcal PCR remains positive was not previously determined prospectively. One retrospective survey showed that no blood PCR results were positive when the samples were taken more than 24 h after antibiotic administration, although CSF PCR results may remain positive for up to 72 h (28). In

this study, we found that blood PCR results may remain positive for up to 9 days after the administration of systemic antibiotics, and in a third of patients who had repeat testing, these results were still positive at 72 h. This finding may reflect bacterial load, since DNA persists after bacteria have been killed and higher levels may take longer to decline. Hackett et al. showed that bacterial load at presentation correlates with disease severity (18). Although they did not show a correlation among bacterial load, duration of clinical symptoms, and decline in DNA load, the patient in our study whose blood PCR results were still positive 9 days after the start of antibiotic treatment had the longest duration of clinical symptoms and the most severe complications. This finding suggests that it may still be worth performing meningococcal PCR even 3 or 4 days after the start of antibiotic treatment in patients with delayed or unusual clinical presentations or when the initial sample is lost or insufficient.

This is the first prospective study to compare two PCR methods with the same patients. The results were concordant for all of the positive nested PCR results. Real-time PCR was more sensitive than nested PCR and confirmed two further cases of meningococcal disease. Meningococcal disease was confirmed by culturing in 63% of patients with a gold standard diagnosis of disease, in 88% of patients by nested PCR, and in 96% of patients by real-time PCR. Furthermore, real-time PCR results were positive for CSF supernatants and for longer after antibiotic administration, suggesting that real-time PCR may be more sensitive than nested PCR when the bacterial DNA load is lower. Ultimately, however, the real advantage of real-time PCR over nested PCR is the rapidity with which results can be made available.

There are no clear guidelines for the use of meningococcal PCR. We aimed to identify a subgroup of high-risk children for whom the test may be targeted, in order to avoid the impracticality and expense of performing PCR in every febrile child admitted to a hospital but without missing cases, especially those with occult meningococcal bacteremia, for whom complication rates are high (21). We identified features at admission that classified patients as having a moderate suspicion of meningococcal septicemia or bacterial meningitis. Of the 54 patients in this group, 15 had positive cultures for *N. meningitidis* and 23 had positive PCR results (blood, CSF, or joint effusion). Of the 64 patients without these features, none had positive cultures for *N. meningitidis* and only 1 had a positive PCR result. The latter patient was thought unlikely to have meningococcal disease at admission; these data demonstrate that not all children with meningococcal bacteremia are significantly ill. No other patient in this group proved to have meningococcal disease. We did not investigate the rate of positive meningococcal PCR in healthy children, although up to 17% of healthy children have detectable pneumococcal DNA in their blood (14). However, with no false positives in 118 febrile children, we consider this not to be a major problem. The yield of PCR performed on samples from every child who could possibly have meningococcal disease (i.e., any child admitted with a fever) is low. We therefore propose that patients should be targeted for meningococcal PCR on the basis of the following clinical features at admission: fever and petechiae, sepsis with shock or ICU admission, and signs of meningitis. In ad-

dition, real-time PCR may be particularly useful when the bacterial load is low.

We continue to recommend conventional microscopy and culturing in addition to PCR testing. Culturing is still the gold standard for serological classification both for investigation of outbreaks by DNA fingerprinting and for consideration of vaccine prophylaxis. In addition, culturing allows for complete antimicrobial susceptibility testing of isolates, whereas the utility of PCR for predicting susceptibility profiles has yet to be widely validated (3). Although in Australia there has yet to be an isolate of *N. meningitidis* resistant to penicillin, increasing penicillin MICs and the emergence of beta-lactamase-producing organisms have been documented elsewhere, highlighting the need for ongoing surveillance (2, 22).

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