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PCR and the investigation of meningococcal infection

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SUMMARY

We examined the use of polymerase chain reaction (PCR) in investigating suspected cases of meningococcal infection in Birmingham. Data held by Birmingham Health Authority were interrogated to determine cases of suspected or confirmed meningococcal infection for a 3-year period from April 1996. The microbiology departments of five local hospitals completed a standard proforma about the microbiological investigation of cases and included details of patient age, clinical presentation and method of confirmation of the clinical diagnosis. Of 273 cases, 123 had PCR performed on either cerebrospinal fluid and/or blood. Groups more likely to have a PCR done were those presenting with septicaemia alone, and those in the 5–14 year age group. In 33 cases, PCR was the only positive microbiological result. Over the study period there was increasing but variable use of PCR in the investigation of meningococcal infection and PCR increased the yield of confirmed cases.

INTRODUCTION

The public health response to a case, but especially cases, of meningococcal infection is heavily influenced by whether the diagnosis is confirmed microbiologically [1, 2]. Microbiological diagnosis is particularly important now that a national vaccination programme for meningococcal group C has been introduced [3]. The introduction of polymerase chain reaction (PCR) was perceived to be a significant advance in investigation of suspected meningococcal disease [4].

At Birmingham Health Authority, a standard form is used for recording data when a case of suspected or confirmed meningococcal infection is notified to the Consultants in Communicable Disease Control (CsCDC). The form lists the most commonly performed microbiological tests. When a case is notified, the notifier is asked to specify what tests have been carried out and encouraged to undertake further tests as necessary. It became apparent that the range of tests carried out varied both within and between the five acute units to which the vast majority of Birmingham meningococcal disease suspected cases were admitted. We undertook a retrospective study of the investigation of meningococcal infection in Birmingham from 1996 to 1999.

METHODS

Two data sets were interrogated to form a population of meningococcal cases: (1) the Notification database held by Birmingham Health Authority for all suspected and confirmed cases of meningococcal infection (cases where the diagnosis was confirmed as due to other organisms, e.g. pneumococcus were

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excluded); (2) the Contract Minimum Dataset (CMDS) for inpatient admissions with a discharge diagnosis of meningococcal infection.

The two datasets were combined to form a single list of patients for each acute unit. This list was then sent to a Consultant Microbiologist at each unit with a standard form requesting information on microbiological investigation of each case. Microbiological tests specified were serology, polymerase chain reaction (PCR) of blood and cerebrospinal fluid (CSF), and also culture of blood, CSF, naso-pharyngeal (NP) swab or skin rash aspirate. This process was carried out annually for the 3-year period from 1996–9.

Cases were stratified according to clinical presentation into four groups namely meningitis alone, septicaemia alone, both meningitis and septicaemia, or neither (e.g. benign meningococcaemia). This categorization was based on discussion of the case details with the clinician and/or the discharge diagnosis.

The clinical diagnosis was considered confirmed microbiologically if meningococci were cultured from CSF, blood, NP swab or skin rash aspirate, if Gram-negative diplococci were visualized in CSF or skin rash aspirate, if meningococcal PCR was positive, or if serology was indicative of recent meningococcal infection.

RESULTS

There were 280 cases in the 3-year period. Seven cases had inadequate clinical details leaving a sample of 273. The numbers of cases increased each year (82 in 1996–7, 86 in 1997–8 and 105 in 1998–9). Twenty cases identified from the CMDS were not notified (7%).

Nearly three-quarters of all cases were aged under 15 years (198: 69%) (Table 1). A total of 145 cases were classified as septicaemia alone (53%), 71 as meningitis alone (26%) and 50 cases had both septicaemia and meningitis (18%). Seven cases had other diagnoses, e.g. benign meningococcaemia or meningococcal conjunctivitis.

Confirmation of diagnosis

There was no consistency between the case presentations and the investigations performed. Most but not all cases had blood cultures done, but very few had meningococcal serology carried out (Table 2). The use of PCR on both CSF and blood increased over time but not consistently. The positive yield from different tests also varied over time.

Lumbar puncture (LP) was not restricted to those presenting with clinical signs and symptoms of meningitis; more than a third with septicaemia alone had an LP (Table 3).

Confirmation of meningococcal infection

One hundred and thirteen cases (41%) had the diagnosis of meningococcal infection confirmed by at least one method. In nearly one-quarter of cases (28; 23%), although infection with meningococci was confirmed by culture and/or PCR, no serogroup could be determined.

Confirmation of infection varied little between the diagnostic groups; 62 of 145 cases of septicaemia, 27 of 71 cases of meningitis and 20 of 50 cases with both meningitis and septicaemia were confirmed as due to infection with *Neisseria meningitidis*. One-quarter of those aged under 1 year had the diagnosis confirmed microbiologically, compared with 47% of 1–4 year olds (45 of 96), 31% of the 5–14 year olds (19 of 62) and 51% of those aged over 15 years (40 of 79).

Use of PCR

A total of 123 cases had PCR performed on either blood or CSF; 22 of 71 meningitis cases (31%), 16 of 50 cases with both meningitis and septicaemia (32%) and 84 of 145 cases of septicaemia (59%). The greatest proportion of cases where a PCR was done was in the 5–14 age group (36, 63%). The test was least used in the under 1 year age group (13, 36%). Seven cases where a PCR was performed had not been notified. Over the 3-year period, there was over a tripling of the number of cases in whom PCR was performed. However, the use in each of the five units to which patients were admitted varied considerably (Fig. 1).

Positive PCR results

Fifty cases had a positive PCR result, either from CSF or blood; these included 10 meningitis cases (45% of those who had PCR done), 33 septicaemia cases (39%), 6 who had both (38%) and 1 with benign

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Table 1. *Cases of meningococcal infection classified by age and clinical presentation in Birmingham, 1996–9. In this period seven cases had infection other than meningitis and/or septicaemia*

Clinical presentation	No. of cases in age group						
	< 1 yr	1–4 yr	5–14 yr	15–19 yr	20–24 yr	> 24 yr	All ages
Meningitis	8	28	20	8	2	5	71
Septicaemia	21	50	28	13	7	26	145
Meningitis and septicaemia	7	14	8	5	3	13	50
All meningitis and/or septicaemia	36	92	56	26	12	44	266

Table 2. *Investigation of meningococcal infection by method and year in Birmingham, 1996–9*

Test	1996–7 ($n = 82$)		1997–8 ($n = 86$)		1998–9 ($n = 105$)	
	No. taken (% of cases)	No. positive (% of samples)	No. taken (% of cases)	No. positive (% of samples)	No. taken (% of cases)	No. positive (% of samples)
CSF*	37 (45)	7 (19)	34 (39)	11 (32)	41 (39)	4 (10)
CSF PCR	10 (12)	4 (40)	15 (17)	8 (53)	17 (16)	9 (53)
Blood culture	80 (98)	18 (23)	75 (87)	11 (15)	93 (89)	22 (24)
Blood PCR	12 (15)	6 (50)	39 (45)	11 (28)	57 (54)	16 (28)
NPS†	39 (48)	5 (13)	34 (39)	7 (21)	41 (39)	3 (7)
Serology	5 (6)	5 (100)	6 (7)	1 (17)	4 (4)	1 (25)
Skin rash	1	0	5	0	0	0
Aspirate	(1)	(0)	(6)	(0)	(0)	(0)

* Microscopy and culture; † nasopharyngeal swab.

Table 3. *Use of lumbar puncture (LP) by diagnostic group in cases of meningococcal infection in Birmingham, 1996–9*

Clinical diagnosis	LP performed		CSF positive for meningococci	
	No.	%	No.	%
Meningitis only	30	42	7	23
Meningitis and septicaemia	23	46	2	9
Septicaemia only	56	38	12	21

meningococcaemia. Two cases had not been notified: both had septicaemia and the serogroup was not confirmed. In 33 cases, the only confirmation of the diagnosis was by PCR (Table 4). In 14 cases, PCR was negative but the diagnosis was confirmed by some other microbiological method.

DISCUSSION

Meningococcal infection has one of the highest media profiles of all infectious diseases [5, 6]. The public health response to a case depends on the certainty with which the diagnosis is held. National guidance differentiates the response between confirmed, probable and possible cases [1, 2] and also outlines appropriate investigation [7]. The initial investigation of all suspected cases of meningococcal infection is therefore very important in achieving an accurate diagnosis.

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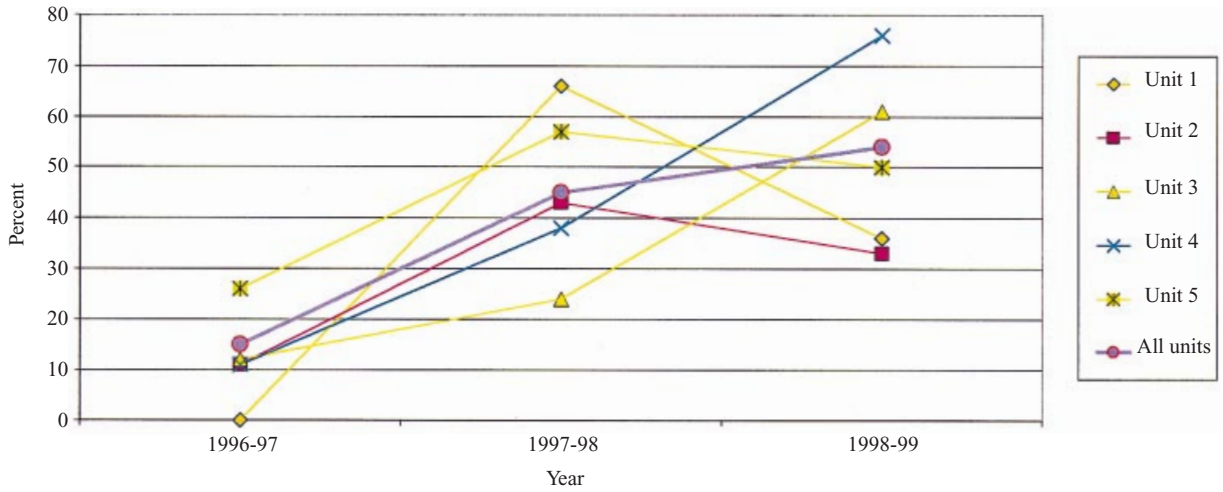

Fig. 1. Analysis by acute unit in Birmingham of percentage of cases of meningococcal infection where blood or CSF PCR was performed during 1996–9.

Table 4. *Non-PCR test results in cases in whom PCR tests were performed for cases of meningococcal infection in Birmingham, 1996–9*

	PCR result	
Non-PCR test	Positive	Negative
Positive*	17	14
Negative	33	59

* Meningococci cultured from CSF, blood, NP swab or bleb aspirate; Gram-negative diplococci visualized in CSF or bleb aspirate; or serology indicative of recent meningococcal infection.

The differences in the presentation of meningococcal disease are well known [8, 9] and will impact on tests carried out. Even making allowance for this, over the 3-year period of our study, there was surprising variation in the use of different diagnostic tests. Most but not all cases had a blood culture, a minority had a naso-pharyngeal swab taken and very few had serology or a skin rash aspirate. These results are disappointing. Most doctors would agree that a patient with an acute febrile illness should have a blood culture performed on admission to hospital. Naso-pharyngeal swabbing is a relatively simple, inexpensive test that has been shown to remain positive after initiating antibiotic treatment [10]. In addition, good concordance of serogroup between isolates from blood or CSF, and the nasopharynx has been shown [11, 12]. The infrequent use of serology can be understood in the context of the need for a convalescent specimen, often after the patient has been discharged, and the diagnosis has become largely academic to clinicians, allied to the invasiveness of venepuncture in infants and small children.

The variation in the use of LP is difficult to explain. Guidance on its use in adults with suspected meningitis exists [13] and one might also surmise that an LP would be less likely to be carried out in cases without signs of meningism. However in our study, some cases of 'septicaemia' had a lumbar puncture whilst some cases of meningitis did not. A similar proportion of both diagnostic groups had the procedure carried out, with a similar positive yield, indicating that the clinical diagnosis of 'septicaemia alone' was inaccurate in a number of cases.

A smaller proportion of patients had the diagnosis confirmed than has been reported elsewhere, but this might be due to differences in case ascertainment [14]. In our study, infants were least likely to have the diagnosis confirmed. This is not a new finding. Particularly in very young children, the presentation of meningococcal infection can be non-specific [15]. However it is unclear whether this should lead to more or fewer microbiological tests being carried out. It might be more since clinicians are unsure of the cause of the illness and want diagnostic tests to aid the therapeutic decision and its potential failure. Conversely, fewer investigations might be deemed appropriate when clinicians do not feel the need of microbiological confirmation of a clinical diagnosis, and wish to treat the patient empirically.

There was a large increase in the use of PCR over the study period, but this varied over time, and also between the five hospitals. Since there was no

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consistency in the minimum set of investigations, assessing the impact of a new investigative tool such as PCR was not practicable.

The diagnostic yield from PCR was similar across the three clinical presentation groups. This might be considered surprising since septicaemia is often a less discrete diagnosis than meningitis. However the 'characteristic' rash of septicaemia might make the diagnosis more 'obvious', even though this sign is unreliable. Those with septicaemia were most likely to have a PCR carried out on either blood or CSF. There might be a number of reasons for this. Blood PCR was much more commonly carried out than CSF PCR. It might have seemed more logical to do a blood test on a case of septicaemia than one of meningitis. Small volumes of CSF, insufficient for PCR once cell count and culture had been carried out, may also have been a factor.

PCR is useful for CsCDC in the wider management of single and connected cases of suspected meningococcal infection. It is particularly helpful where there has been a period of antibiotic administration before specimens are collected, since this reduces the likelihood of a positive result from culture based methods [16]. GPs can also be reassured that pre-admission parenteral antibiotic usage in suspected cases will have less impact on the confirmation of the diagnosis. However, even though the consensus remains that treating the potentially fatal infection is more important than confirming the diagnosis, GPs are still exposed to conflicting advice on this matter [17].

PCR does not solve all diagnostic problems [18]. Like any sampling technique, it can produce conflicting results when compared with others, such as a negative PCR result but a positive culture. The currently used primers do not provide information on antibiotic sensitivity and not all PCR positive specimens will yield serogroup information.

With the introduction of the serogroup C conjugate vaccination programme in the United Kingdom [3], the incidence of meningococcal infection should decrease. Initial data confirm this to be the case [19] although it is unclear yet how the epidemiology of meningococcal infection might change [20, 21]. If the programme is successful, fewer cases of meningococcal disease will occur and clinical experience of diagnosing the condition will wane. The potential exists for more patients, particularly children, who are pyrexial and have a suggestive rash, to be wrongly diagnosed as meningococcal septicaemia. Microbiological confirmation of the diagnosis will therefore be increasingly important. However differences might well exist in the perspective of the clinician and public health physician about investigation. From a clinical perspective, treatment is initiated and maintained for a suspected case unless another diagnosis becomes more likely [22]. Antibiotic treatment is not withheld until an alternative diagnosis is confirmed. For the most part, clinicians use microbiological investigation to confirm their clinical suspicion or provide information when there is no response to treatment. This contrasts with the public health response, which is heavily influenced by both the degree of clinical suspicion and confirmation of the serogroup [2]. Chemoprophylaxis is not recommended for contacts of a possible case of meningococcal infection. Vaccination of close contacts of a case only occurs when infection is confirmed as due to serogroup A, C, W135 or Y strain. The relative importance of microbiological confirmation of a clinical diagnosis might therefore vary between physician and public health medicine specialist.

In Birmingham, we found that blood for meningococcal PCR had been sent to the PHLS Meningococcal Reference Unit (MRU) from a number of cases who had not been notified. This group consisted primarily of children who were admitted for a short period, whose PCR was negative and who had a discharge diagnosis that was neither meningitis nor meningococcal infection. It would seem prudent for hospitals to have a monitoring system for meningococcal PCR to ensure the test is used appropriately. Information could be fed back to clinicians about the number of tests performed and the proportion confirmed as positive. A more detailed audit could be undertaken to determine in what clinical settings PCR testing was used, the impact it had on case management and the eventual discharge diagnosis of the patient, although such an audit would not demonstrate the public health value of PCR testing.

In conclusion, assessing the impact and usage of PCR is hampered by a lack of consistency in the appropriate investigation of suspected meningococcal infection. Even though such guidance has been published in the past, we found it was not used consistently. The influences are likely to be complex, and include presenting symptoms, the certainty of the diagnosis, initial information from other diagnostic tests as well as specifics relating to the individual clinician (experience of meningococcal infection and knowledge of appropriate investigations). The minimum set of investigations that should be performed

on all cases of suspected meningococcal infection is a blood culture, a naso-pharyngeal swab and a blood specimen for PCR, which is sent for testing to the Meningococcal Reference Unit (MRU) after 24 h if culture based methods remain negative. Close co-operation between clinicians, medical microbiologists and CsCDC is essential to ensure appropriate investigation of suspected meningococcal infection for the benefit of individual patients and the community.

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REFERENCES

1. PHLS Meningococcal Infections Working Group and Public Health Medicine Environmental Group. Control of meningococcal disease: guidance for consultants in communicable disease control. CDR Rev 1995; **5**: R189–95.
2. Stuart JM, Monk PN, Lewis DA, Constantine C, Kaczmarski EB, Cartwright KAV. Management of clusters of meningococcal disease. CDR Rev 1997; **7**: R3–5.
3. Communicable Disease Surveillance Centre. Vaccination programme for group C meningococcal infection is launched. CDR Wkly 1999; **9**: 261.
4. Kaczmarski EB, Ragunathan PL, March J, Gray SJ, Guiver M. Creating a national service for the diagnosis of meningococcal disease by polymerase chain reaction. Commun Dis Public Health 1998; **1**: 54–6.
5. Ardern K, Bowler S, Hussey RM, Regan CM. Managing meningococcal disease case clusters: art or science? J Epidemiol Comm Hlth 1999; **53**: 565–71.
6. Thomson APJ, Hayhurst GK. Press publicity in meningococcal disease. Arch Dis Child 1993; **69**: 166–9.
7. Kaczmarski EB, Cartwright KAV. Control of meningococcal disease: guidance for microbiologists. CDR Rev 1995; **5**: R196–8.
8. Steven N, Wood M. The clinical spectrum of meningococcal disease. In: Cartwright K, ed. Meningococcal disease. Chichester: John Wiley and Sons, 1995: 177–207.
9. Granier S, Owen P, Stott NC. Recognising meningococcal disease: the case for further research in primary care. Br J Gen Pract 1998; **48**: 1167–71.
10. Abramson JS, Spika JS. Persistence of *Neisseria meningitidis* in the upper respiratory tract after intravenous antibiotic therapy for systemic meningococcal disease. J Infect Dis 1985; **151**: 370–1.
11. Sippel JE, Girgis NI. Throat culture from patients with meningococcal meningitis. J Clin Pathol 1990; **43**: 610–1.
12. Cartwright KAV, Jones DM. Value of throat swabs from index cases of meningococcal meningitis. J Clin Pathol 1990; **43**: 438.
13. Begg N, Cartwright KAV, Cohen J, *et al*. Consensus statement on diagnosis, investigation, treatment and prevention of acute bacterial meningitis in immunocompetent adults. J Infect 1999; **39**: 1–15.
14. Ragunathan L, Ramsay M, Borrow R, Guiver M, Gray S, Kaczmarski EB. Clinical features, laboratory findings and management of meningococcal meningitis in England and Wales: report of 1997 survey. J Infect 2000; **40**: 74–9.
15. Riordan FAI, Thomson APJ, Sills JA, Hart CA. Who spots the spots? Diagnosis of early meningococcal disease in children. BMJ 1996; **313**: 1255–6.
16. Cartwright K, Kroll S. Optimising the investigation of meningococcal disease. Early treatment with benzyl penicillin is important and doesn't jeopardise diagnosis. BMJ 1997; **315**: 757–8.
17. Moller K, Skinhoj P. Guidelines for managing acute bacterial meningitis. BMJ 2000; **320**: 1290–1.
18. Yakubu DE, Abadi FJR, Pennington TH. Molecular typing methods for *Neisseria meningitidis*. J Med Microbiol 1999; **48**: 1055–64.
19. Communicable Disease Surveillance Centre. Meningococcal disease falls in vaccine recipients; CDR Wkly 2000; **10**: 133.
20. Maiden MCJ, Spratt BG. Meningococcal conjugate vaccines: new opportunities and new challenges. Lancet 1999; **354**: 615–6.
21. MacLennan JM, Urwin R, Obaro S, Griffiths D, Greenwood B, Maiden MCJ. Carriage of serogroup W-135, ET-37 meningococci in The Gambia: implications for immunisation policy? Lancet 2000; **356**: 1078.
22. Pollard AJ, Britto J, Nadel S, DeMunter C, Habibi P, Levin M. Emergency management of meningococcal disease. Arch Dis Child 1999; **80**: 290–6.