Latex agglutination test for diagnosing pneumococcal pneumonia in children in developing countries

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Abstract

Objective—To prepare and assess the sensitivity and specificity of a latex agglutination test specific for the serotype of antigen in diagnosing pneumococcal pneumonia in Gambian children.

Design—Comparison of agglutination test specific for serotype with culture of blood and lung aspirates, countercurrent immunoelectrophoresis, and commercial latex agglutination tests in diagnosing pneumococcal pneumonia. Cross reaction studies and investigation of 102 control children to determine specificity of agglutination test specific for serotype.

Setting—General medical ward of Medical Research Council laboratories, The Gambia.

Patients—101 Gambian children aged between 2 months and 10 years admitted with severe pneumonia.

Interventions—Serum samples were boiled and treated with edetic acid, and urine samples were boiled and concentrated 25 times before testing.

End point—A latex agglutination test specific for the serotype of pneumococcal antigen that is sensitive and highly specific for detecting pneumococcus in the urine of patients with pneumococcal pneumonia.

Measurements and main results—Concentrated urine samples from 16 of the 21 children (76%) with pneumococcal pneumonia established by results of culture of blood or lung aspirates gave a positive result with the agglutination test specific for serotype, whereas only four of the 102 urine samples obtained from control children without pneumonia gave positive results. The serotypes of antigens detected in the urine of children with pneumococcal pneumonia and the serotypes of pneumococci isolated from cultures of blood or lung aspirates were the same in all cases.

Conclusions—When performed on urine samples the agglutination test specific for serotype has a high specificity and is more sensitive than culture of blood or lung aspirates, commercial agglutination tests, or countercurrent immunoelectrophoresis in identifying pneumococcal pneumonia. It is easy to use and should be especially useful in communities with limited laboratory facilities.

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Introduction

Acute respiratory infections are responsible for a high proportion of deaths during childhood in many developing countries and are a common cause of admissions to hospital.[1,2] They cause about four million of the 15 million deaths that occur in children under the age of 5 each year.[3] Hospital based studies have shown that the pneumococcus is the single most important cause of severe respiratory infections in children in developing countries.[2-4] Little work, however, has been done in rural areas because of the difficulty of diagnosing a bacterial cause of pneumonia.

Children with pneumonia rarely produce sputum, and culture of sputum is usually unhelpful for diagnosis because of contamination by potential pathogens resident in the upper respiratory tract. Cultures of blood from patients with pneumococcal pneumonia grow pneumococcus in only 10-30% of cases. Higher rates of isolation are observed in cultures of material obtained by lung aspiration, but this method can occasionally cause haemoptysis or a pneumothorax so should be done only in selected patients.

Capsular polysaccharide antigens have been detected in serum and urine samples from about 40% of patients with pneumococcal pneumonia by countercurrent immunoelectrophoresis.[5-7] Particle agglutination tests, which are more suitable for use in developing countries than countercurrent immunoelectrophoresis, have generally been even less satisfactory. A high percentage of positive results was found by both methods among a group of elderly patients with pneumococcal pneumonia.[8] Studies in children, however, in which latex reagents coated with an antiserum containing antibodies to all known pneumococcal polysaccharide antigens (Omniserum, Statens Seruminstitut, Copenhagen) were used to detect pneumococcus, have given positive results for serum in only about 20% of patients with pneumococcal pneumonia and even poorer results for urine.[9-11] Because of these disappointing results we prepared a series of 10 latex reagents, each coated with antiserum to a single capsular polysaccharide. We investigated the sensitivity and specificity of a test that used these reagents to diagnose pneumococcal pneumonia in Gambian children in whom a bacterial cause had been firmly established by culture of lung aspirates or blood, or both.

Patients and methods

We investigated 101 Gambian children aged 10 and under (range 2 months to 10 years) with pneumonia acquired in the community who had been admitted to a ward at the Medical Research Council laboratories. All of the children had severe or very severe acute respiratory infections according to the World Health Organisation's classification, and nearly all had radiological evidence of consolidation. Controls comprised 70 children aged 10 and under seen during the course of community studies undertaken in two rural areas of The Gambia and 32 children who presented with minor complaints at the outpatient clinic. All controls were examined by a doctor; none had any clinical features of a lower respiratory tract infection.

Blood from all patients was cultured on plates. Lung aspirates were obtained from 42 children with radiological evidence of consolidation.[12] No complications occurred. Urine samples were collected and stored in

sterile containers that contained thiomersal as a preservative. Urine and serum samples were stored for up to 20 months at −20°C before testing. Serum samples were tested for antigen by various methods. Unprocessed samples were tested by countercurrent immunoelectrophoresis. Serum diluted in a buffer and absorbent solution was tested by the Bactigen test (Wampole Laboratories, New Jersey). Serum boiled with edetic acid to destroy rheumatoid factors and to release antigen bound in protein complexes was tested by the Bactigen test and by a latex agglutination test specific for the serotype of the antigen.[13]

Urine samples were centrifuged at 1500 rpm for five minutes, boiled for three minutes, and filtered through a nitrocellulose filter with pores of diameter 0·45 μm (Millipore, Massachusetts) before testing.[14] Unconcentrated urine samples and samples that had been concentrated 25 times in a miniconcentrator (Amicon, Massachusetts) were tested by the Bactigen test, Slidex méningite test (BioMerieux, Lyons), and a latex agglutination test specific for serotype.

Blood was cultured in trypticase soya broth and thioglycollate broth (Oxoid, Basingstoke, United Kingdom). Lung aspirates were stained by Gram's method, cultured on agar containing 5% sheep blood and on enriched chocolate agar, and incubated overnight at 37°C in a candle jar. Lung aspirates were also tested for antigen countercurrent immunoelectrophoresis and latex agglutination. Isolates of *Streptococcus pneumoniae* obtained from cultures of blood or lung aspirates were characterised by standard methods and serotyped by Neufeld's reaction, countercurrent immunoelectrophoresis, and latex agglutination with monospecific antiserum to capsular polysaccharide (Statens Seruminstitut, Copenhagen). Countercurrent immunoelectrophoresis was carried out in 0·7% agarose containing pneumococcal antiserum (Omniserum) in a phosphate buffer containing glycine pH 8·6. All latex agglutination testing was done by two observers who were unaware of the results of other assays.

A latex agglutination test specific for serotype of antigen was developed as follows. Suspensions of latex were coated with monospecific antiserum (Statens Seruminstitut, Copenhagen) to 10 of the serotypes of pneumococci (types 1-6, 8, 14, 18, 19) that most commonly cause invasive pneumococcal disease in The Gambia.[15] Antiserum was diluted to an optimum concentration, which had been established in preliminary studies, in saline buffer containing glycine and added to an equal volume of suspension of latex (particle size 0·81 μm) (Difco, Michigan). The mixture was incubated at 37°C for two hours with shaking every 30 minutes. Two volumes of 0·1% bovine serum albumin in saline buffer containing glycine were then added to the suspension of latex and antiserum and the mixture left overnight at 4°C. The sensitivities of individual latex suspensions to their corresponding solutions of purified pneumococcal polysaccharides (Pneumovax 23, Merck Sharp and Dohme, Pennsylvania) were determined. Each latex reagent detected the appropriate pneumococcal polysaccharide at a concentration of ≥2·5 μg/l. The specificity of each latex reagent was investigated by testing it against a suspension of American collection of type cultures standard strains of *Escherichia coli* (10415), *Staphylococcus aureus* (6571), and *Pseudomonas aeruginosa* (10662) in physiological saline and against suspensions of Gambian isolates of *Klebsiella pneumoniae*, *Salmonella typhi*, other salmonella species, *Streptococcus viridans*, and *Haemophilus influenzae* type b. None of the latex reagents gave agglutination with suspensions of these bacteria.

Results

A bacterial cause for pneumonia was established in 38 of the 101 children in the study: 21 cultures grew *Str pneumoniae*, six grew *H influenzae* type b, one grew *Str pneumoniae* and *H influenzae* type b, four grew *Staph aureus*, and six grew other bacteria. Sixteen diagnoses were based on the results of blood culture alone, 17 on the results of culture of lung aspirates alone, and five on the results of both types of culture. Pneumococcal infection or infection with *H influenzae* was suggested by the results of tests for antigens in 22 patients with cultures that yielded negative results, taking the total number of cases of pneumonia probably caused by bacteria to 60. Pneumococcal pneumonia was diagnosed in 22 patients by growth of *Str pneumoniae* in cultures: 10 in cultures of lung aspirates alone, nine in cultures of blood alone, and three in both types of culture.

The table gives the results of the tests for bacterial antigens in the 101 patients with pneumonia and the controls. The sensitivity of each test was assessed in patients with cultures that yielded positive results. The specificity of each test was assessed in children with pneumonia in whom infection with another bacterium was definitely diagnosed and controls who were healthy at the time the samples were taken.

Tests on serum samples were highly specific, but their sensitivity was too low for them to be useful clinically. When the Bactigen latex agglutination test was used on untreated serum samples positive results were obtained in 10 of 19 patients with pneumococcal pneumonia but also in six of 15 patients with other forms of pneumonia amd 27 of 57 patients with cultures that yielded negative results, which suggests that the test was giving false positive results.

No positive results were obtained when urine from patients with pneumococcal pneumonia was tested by

Results of tests for pneumococcal capsular polysaccharide antigens in serum and urine from children with pneumonia and controls. Values in parentheses are sensitivities and specificities defined in relation to findings in patients with pneumonia from whom another bacterium was isolated† and findings in controls‡*

Test	Patients: Cultures grew *Str pneumoniae* (sensitivity*) (n=22)	Patients: Cultures grew other bacteria (specificity†) (n=16)	Patients: Cultures yielded negative results (n=63)	Controls (specificity‡) (n=102)
		Serum samples§		
Countercurrent immunoelectrophoresis	3/21 (14)	0/15 (100)	2/58	3/30 (90)
Bactigen latex agglutination	2/19 (11)	0/15 (100)	4/57	
Latex agglutination specific for serotype	3/16 (19)	0/14 (100)	3/51	0/30 (100)
		Urine samples‖		
Countercurrent immunoelectrophoresis	9/22 (41)	0/16 (100)	5/57	3/90 (97)
Bactigen latex agglutination	0/21 (0)	2/15 (87)	3/57	6/87 (93)
Slidex méningite latex agglutination	0/21 (0)	0/16 (100)	2/53	2/94 (98)
Latex agglutination specific for serotype	16/21 (76)	2/15 (87)	20/54	4/102 (96)

Differences between total numbers of subjects and specific numbers for each test reflect missing or inadequate samples.
§Boiled and treated with edetic acid before testing.
‖Concentrated 25 times by ultrafiltration before testing.

commercial latex agglutination tests (latex coated with Omniserum), but a latex agglutination test specific for serotype gave a positive result in 16 of 21 patients. Pneumococcal capsular polysaccharide antigen was detected by the type specific latex test in concentrated urine from nine of 12 patients with blood cultures that yielded positive results and from nine of 13 patients with cultures of lung aspirates that yielded positive results, eight of whom did not have bacteraemia. Unconcentrated urine samples were also tested by the latex test specific for serotype. Positive results were obtained in 11 of 22 samples from patients with cultures that grew pnemococcus, in one of 15 samples from patients with other forms of bacterial pneumonia, and in one of 101 samples from controls, giving a sensitivity of 50% and specificities of 93% and 99% respectively.

The serotype of the pneumococcus was determined in 20 of the 22 patients with cultures that grew pneumococcus (in 16 by Neufeld's reaction in bacteria cultured from blood or lung aspirates and in four by latex agglutination of lung aspirates that did not grow bacteria when cultured). Pneumococcal antigen was detected in the urine of 14 of these patients by the latex agglutination test specific for serotype. In each case the antigen detected in urine corresponded with the serotype of the pneumococcus identified in the blood or lung aspirate (six type 1, three type 5, two type 6, two type 14, and one type 19).

Pneumococcal capsular polysaccharide antigen was detected in the urine of 20 patients with cultures that yielded negative results by the agglutination test specific for serotype. Ten patients had other evidence to suggest pneumococcal infection: four had pneumococcus of the same serotype detected by latex agglutination tests on lung aspirates, five had pneumococcal antigen detected either in their urine or serum by countercurrent immunoelectrophoresis, and one had the appropriate capsular polysaccharide antigen detected in his serum by the agglutination test specific for serotype.

Discussion

We found that commercial tests for detecting antigens gave positive results for only a small proportion of patients with pneumonia whose cultured blood or lung aspirates grew pneumococcus. An antigen assay that used latex reagents coated with individual antisera to 10 pneumococcal serotypes was much more successful and gave positive results for urine from 76% of patients (16/22) whose cultures grew pneumococcus. The serotype of the polysaccharide antigen found in urine and the serotype of the pneumococcus obtained from blood or the lung aspirate were the same in all cases.

Our findings suggest that pneumococcal capsular polysaccharide antigen is present in the urine of nearly all patients with pneumococcal pneumonia (we tested against only the 10 most common serotypes so we would expect our test to give positive results in only about 80% of cases) and that latex reagents coated with Omniserum are unable to detect type specific antigens in urine, perhaps because the concentration of specific antiserum coated on each latex particle is too low.

A small number of positive results for pneumococcal polysaccharide capsular antigen were obtained with urine samples from patients with other forms of bacterial pneumonia and from healthy children. Cross reactions between pneumococcal capsular polysaccharide antigens and antigens of other bacteria are well recognised and may be expected to cause occasional diagnostic problems. In this study a positive result for polysaccharide of serotype 2 was obtained in a patient with *H influenzae* type b infection and a positive result for polysaccharide of serotype 19 was obtained in a patient with *Str viridans* infection. These may have been false positive results, but these children may have had mixed infections. The small proportion of positive results of latex agglutination tests on urine of apparently healthy children is worrying. The reactions possibly occurred because of excretion of antigens after a past infection or because of heavy nasopharyngeal colonisation with pneumococci, which is almost universal in Gambian children (NL-E, unpublished work).

For the latex agglutination test specific for serotype of the antigen to be used successfully to diagnose pneumococcal infections the serotypes of the pneumococci responsible for invasive disease being studied must be known. Though the relative importance of different serotypes varies from region to region, there may be large areas—for example, west Africa—where a similar distribution of serotypes is seen.[15] We chose to make 10 reagents for our study, which covered about 80% of the serotypes of pneumococci responsible for invasive disease in The Gambia. In some locations it may be possible to use fewer latex preparations, whereas in others, or when a higher degree of sensitivity is required, more preparations may be required.

Coating suitable latex reagents with type specific antiserum, which is produced commercially, is not difficult and should be within the scope of any moderately well equipped laboratory. Once prepared the latex reagents are stable for long periods. The latex agglutination test is simple to carry out and requires a minimum of equipment and technical skill and is thus suitable for use in laboratories in developing countries. The use of 10 latex reagents, as opposed to the smaller number provided in commercial kits, adds a little to the time needed to do a test. We calculate the cost of the reagents needed to do one agglutination test specific for serotype to be about 10 pence. Filtration through a millipore filter and concentration of urine with a microconcentrator adds substantially to the cost of each test (an additional £2.50), but cheaper effective methods of concentrating urine for the latex agglutination test could probably be devised.

Investigation of the epidemiology of pneumococcal infection has been seriously hindered by the lack of a diagnostic test that can be used in community studies. Our preliminary results with a latex agglutination test specific for serotype performed on urine are encouraging. We found this simple, non-invasive test to be more sensitive than culture of blood or lung aspirates. The test may be of value in diagnosing pneumococcal pneumonia, especially in communities with limited laboratory facilities. It should be a useful tool for evaluating interventions directed against this disease. An evaluation of the effectiveness of such a test in developed countries where pneumonia is still an important cause of childhood deaths[16] and where proof of a pneumococcal cause is invariably difficult would also be interesting.

The test could also have broader clinical applications. It might be used to detect pneumococcal infection in those who are particularly susceptible, such as the elderly and those with sickle cell disease, the nephrotic syndrome, and asplenia, and after an antibiotic has been given, such as in partially treated meningitis.

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Incidence of AIDS and excess of mortality associated with HIV in haemophiliacs in the United Kingdom: report on behalf of the directors of haemophilia centres in the United Kingdom

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Abstract

***Objective*—To estimate the cumulative incidence of AIDS by time since seroconversion in haemophiliacs positive for HIV and to examine the evidence for excess mortality associated with HIV in those who had not yet been diagnosed as having AIDS.**

***Design*—Analysis of data from ongoing national surveys.**

***Setting*—Haemophilia centres in the United Kingdom.**

***Patients*—A total of 1201 men with haemophilia who had lived in the United Kingdom during 1980-7 and were positive for HIV.**

***Intervention*—None.**

***End points*—Diagnosis of AIDS; death in those not diagnosed as having AIDS.**

***Measurements and main results*—Estimation of cumulative incidence of AIDS and number of excess deaths in seropositive patients not diagnosed with AIDS. Median follow up after seroconversion was 5 years 2 months. Eighty five patients developed AIDS. Cumulative incidence of AIDS five years after seroconversion was 4% among patients aged <25 at first test positive for HIV, 6% among those aged 25-44, and 19% among those aged ≥45. There was little evidence that type or severity of haemophilia or type of factor VIII or IX that had caused HIV infection affected the rate of progression to AIDS. Mortality was increased among those who had not been diagnosed as having AIDS, especially among those with "AIDS related complex." Thirteen deaths were observed among 36 patients diagnosed as having AIDS related complex against 0·65 expected, and 34 deaths in 1080 other patients against 22·77 expected; both calculations were based on mortality rates observed in haemophiliacs in the United Kingdom in the late 1970s.**

***Conclusions*—Rate of progression to AIDS depended strongly on age. There is a substantial burden of fatal disease among patients positive for HIV who have not been formally diagnosed as having AIDS.**

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Introduction

The proportion of people positive for HIV who proceed to develop serious disease as a result is still uncertain. In this paper information from a recent survey of seroprevalence in haemophiliacs in the United Kingdom was combined with information on the development of AIDS to estimate the cumulative incidence of AIDS by time since seroconversion and its relation with the age of the patient, the type and severity of haemophilia, and the type of factor VIII or IX that gave rise to infection with HIV. In addition, information about mortality was used to assess the evidence for excess mortality among those known to be seropositive for HIV but not diagnosed as having AIDS.

Patients and methods

Information on the numbers of male patients registered with haemophilia A or B who had lived in the United Kingdom in the period 1980-7 and had been tested and found to be seropositive for HIV was obtained from a recent survey of seroprevalence.[1] Information on the dates of the first seropositive test and of the latest seronegative test, if known, were also obtained from this source. Information on patients with AIDS was obtained from an ongoing survey carried out by the United Kingdom haemophilia centre directors' AIDS committee since 1983 and supplemented by information from the Communicable Disease Surveillance Centre at Colindale. Information on the type of factor VIII or IX received by the patients during 1980-7 was obtained from the ongoing national survey carried out in Oxford on behalf of the haemophilia centre directors. Information received by August 1988 has been included in the present report although, to ensure that reporting is as complete as possible, a cut off date of 31 December 1987 has been used in the analyses presented here. Additional information has been sought from the certified causes of death of haemophiliacs positive for HIV who have died. In a few instances in which a diagnosis of AIDS had not been recorded at the haemophilia centres the certified cause suggests that death may have been due to AIDS. It has not been possible to validate this suggestion and so these have not been included as deaths from AIDS in the analysis. They are, however, described in our discussion of the results.

For haemophiliacs positive for HIV the exact date of seroconversion is unknown, and so it has been estimated from the date of the earliest seropositive test and the date of the latest seronegative test, if known. If