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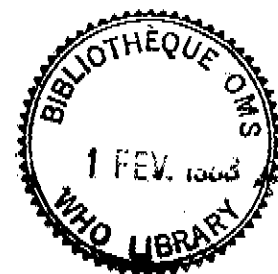
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ANTIGEN DETECTION IN
 BACTERIAL RESPIRATORY INFECTIONS
 IN CHILDREN



Report of a Meeting held in Helsinki, Finland

30-31 March 1987

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1. INTRODUCTION

A meeting to review and evaluate diagnostic techniques in bacterial lower respiratory tract infections was held in Helsinki, Finland, from 30-31 March 1987. The meeting was sponsored by the National Public Health Institute of Finland, in collaboration with the World Health Organization (WHO). It took as its starting point material contained in the document, "Rapid Diagnosis in Acute Bacterial Respiratory Infections" Report of a WHO meeting, Geneva, 10-12 February 1981 (WHO/BAC/ARI/81.5).

2. PRESENT SITUATION OF ETIOLOGICAL DIAGNOSIS OF BACTERIAL LOWER RESPIRATORY INFECTIONS IN CHILDREN

Acute respiratory infections (ARI) are a leading cause of morbidity in all age groups worldwide. In the developing world they are also a major cause of mortality, especially in children under age 5. In many countries, they are the most common single cause of death in this age group and are also the chief reason for paediatric outpatient visits and hospitalizations (1).

Because of the enormous global impact of acute lower respiratory infections (ALRI), the World Health Organization has developed a programme for control and eventually for prevention. First priority has been assigned to the problem of high mortality among young children in the least developed countries - those with high infant mortality rates. Based on data from lung punctures carried out among hospitalized children who had not received antibiotics in seven countries (Brazil, Chile, Gambia, India, Nigeria, Papua New Guinea and the Philippines), it appears that bacteria, mainly Streptococcus pneumoniae (PNC) and Haemophilus influenzae (Hi) are involved in 60% of cases of pneumonia (2). In view of these findings, the strategy adopted by WHO recommends use of first-line antibiotics, penicillin, cotrimoxazole or amoxycillin, in children with moderate-severe lower respiratory tract infection.

It was clear in making these recommendations that the cases in which lung punctures were performed represented a selected group. However, these cases, in addition to those children in whom blood culture was positive were the only ones in which a firm bacteriologic diagnosis was possible. The recommendation for case management was based on the best data available, but it was realized that there was a technologic gap in identifying etiology in the great majority of cases of moderate-severe lower respiratory tract infection. Simply stated, the diagnostic problem in children, in whom sputum cannot be obtained is as follows:

- a. Oro- or nasopharyngeal cultures are frequently positive for potentially pathogenic bacteria among children. Nasopharyngeal carriage of PNC in young children is over 90% in developing countries (3,4) and less than 50% in industrialized countries (5,6). The corresponding figures for Hi type b (Hib) are 20% and <5%, respectively (4,7,8,9). These results are in marked contrast to those in adults, whose carriage rate for PNC and Hi is much lower. Thus the presence of these potentially pathogenic bacteria, in throat secretions, while important for epidemiologic purposes, is not useful for making a diagnosis.
- b. Other types of cultures from the respiratory tract are also subject to problems of contamination with bacteria being carried, or may not be acceptably safe for general use.
- c. Blood cultures are positive in only a minority of cases of bacterial pneumonias in children not on antibiotics and are infrequently positive when children have received antibiotics.
- d. Lung punctures, which are the closest to a "gold standard" for defining the etiology of lower respiratory disease are not indicated for clinical management in most cases.

An attractive answer to this problem would be to identify the pathogen through detection of antigens in the serum or urine. Optimally, the concentration of antigen present would indicate that the organism is causing disease, not simply mucosal carriage. This approach has the added advantage of being potentially applicable when antibiotics have been administered. However, up to the present no single antigen detection procedure has been entirely satisfactory, and other methods for diagnosis of infection need to be considered as well.

Other bacteria suggested to be causative agents of lower ARI in young children after the neonatal period include Staphylococcus aureus, Branhamella catarrhalis, Bordetella pertussis, Mycoplasma pneumoniae and Chlamydia spp. (C.trachomatis, C.psittaci, C.TWAR). The proportion of ARI cases caused by these agents is in general relatively low. However, the ability of M.pneumoniae, Chlamydia TWAR and B.pertussis to cause occasional outbreaks, makes their roles as causative agents of ARI more significant during such periods. These 3 agents do not frequently colonize the nasopharynx of healthy young children and thus the etiological diagnosis can be based on the detection of these agents in samples collected from this site or on the demonstration of antibody response to these agents during infection (10). It should be noted however that M.pneumoniae can be detected in throat secretions of 10 to 15% of healthy school-age children.

The group decided to focus on the problem of ARI in children under age 5, in line with the priorities established by WHO. It decided to concentrate on diagnosis of disease caused by 2 organisms, Streptococcus pneumoniae and Haemophilus influenzae, which appear most important in this problem. For this reason the problems of diagnosis of tuberculosis and haemolytic streptococci infection were excluded from consideration as were the special issues concerning otitis media. The test methodologies for antigen detection were similar to those reviewed in document WHO/BAC/ARI/81.5, the report of the 1981 meeting. Additional tests such as C-reactive protein (CRP) and detection of antibody were newly considered. Throughout it was realized that there are two purposes for these tests, and that selection of specific tests may not be the same for both.

- a. Diagnosis in the individual case, in which situation rapid establishment of the diagnosis by a test that is highly sensitive, easily performed, robust and inexpensive, may have a role in deciding on therapy.
- b. Epidemiologic research, in which case the tests should be both sensitive and specific, whereas low cost, ease of performance and stability of reagents, while desirable, are of lower priority.

The group recognized the importance of diagnostic tests for both situations but focused mainly on the second need.

3. METHODS*

3.1 Antigen detection

Most antigen detection methods for diagnosis of disease caused by PNC or Hib are based on the demonstration of capsular polysaccharides. PNC has 83 different capsular types and most antigen detection tests utilize PNC omniserum reactive with all different capsular polysaccharides. Most PNC types have, however, a common cell wall polysaccharide, C-polysaccharide (C-PS). Recently an enzyme-immunoassay (EIA) for the demonstration of C-PS in sputum samples has been developed. Detection of C-PS is theoretically an attractive method since it would apply to all PNC types. However, the C-PS does not seem to be excreted in the urine at the same frequency as the type-specific polysaccharides. An antigen closely similar or identical to C-PS has been demonstrated in some strains of Streptococcus mitior (11).

* For technical details of the methods referred to in the text, see e.g., Leinonen, M. Serological methods for the study of bacterial surface antigen, pp. 179-206. In: Enterobacterial surface antigens: methods for molecular characterization. (T.K. Korhonen, E.A. Dawes and P.H. Makela, eds.) Elsevier Science Publishers, 1985.

There are 6 capsular types of Hi. In addition non-typable strains have increasingly been recognized as a cause of various diseases (12). These non-typable strains have been isolated from lung puncture specimens and blood cultures of children with pneumonia. In the Gambia most isolates have been types a or b, but in Papua New Guinea approximately half of the isolates have been non-typable (13,14).

Polysaccharides have a number of properties which make them potentially useful for antigen detection, but possible variation in prevalent serotypes will need to be taken into account when developing tests based on detection of capsular polysaccharide antigen. During invasive infection, they can be detected in several body fluids, including serum and urine. In addition they may remain detectable for long periods because they are not degraded by human enzymes. Most polysaccharides are relatively resistant to boiling and thus they can be released from immunocomplexes by heating of the sample briefly in a boiling water bath. The boiling also decreases false-positive reactions that are frequently seen when testing urine specimens.

At present, antigen detection methods for the demonstration of other surface components of PNC or Hi, besides polysaccharides, are not available. Such techniques will probably be required for detection of infections due to untypable strains of the agents, especially unencapsulated Hi.

3.1.1 Samples useful for antigen detection

3.1.1.1 Urine

Because urine samples are easily obtained from all patients, they have been widely used for the demonstration of PNC and Hib antigens. It has been shown in several studies that detection of Hib antigen in urine by different immunochemical methods is a sensitive and specific assay for the etiological diagnosis of Hib meningitis (15,16). However, fewer data are available on the sensitivity or specificity of Hib antigen tests for diagnosis of systemic diseases not involving the central nervous system. Also the demonstration of PNC antigens has been less successful than detection of Hib antigen: for the former both false-positive and false-negative results are seen more frequently (17).

Urine samples are often contaminated with bacteria present in the genital tract or other sites and these bacteria may grow during transportation or storage of urine samples. Methods of preventing contamination of the urine or the possible degradation or neutralization of capsular polysaccharides during freezing and storage have not been developed or evaluated at present.

To increase sensitivity, most authorities agree that urine samples should be concentrated 5 to 10-fold prior to testing by countercurrent immuno-electrophoresis (18). However, this procedure needs to be evaluated for coagglutination antigen tests. Conceivably, concentration of urine may result in a false-negative result from either antigen excess (i.e., prozone phenomenon) or an increased concentration of an inhibitor. Therefore, for coagglutination tests it may be preferable to dilute the samples.

3.1.1.2 Serum

Serum taken in the acute phase of the disease may be a good source of PNC and Hib antigen. False positive results can result from rheumatoid factor or other agglutinating substances (15). But these can be largely avoided by diluting the specimen in a buffer containing a reducing agent and/or by boiling the serum sample (5 min, diluted 1:4 in saline) prior to testing.

3.1.1.3 Nasopharyngeal aspirates

Nasopharyngeal aspirates obtained from children by suction are ideal specimens for the demonstration of viruses by culture or antigen detection.

Unfortunately, the high nasopharyngeal carriage rates of PNC and Hi in children, especially in developing countries, greatly lowers the value of the demonstration of these bacteria by culture or antigen detection as a diagnostic tool in individual patients.

3.2 Demonstration of antibody response during ongoing infection

Most commonly used antibody assays for PNC and Hib measure antibodies to capsular polysaccharides. Antibody assays for PNC capsular polysaccharides have been successfully used for the etiological diagnosis of pneumonia in adults (19). Unfortunately, children less than 2 years old respond very poorly to most polysaccharides and thus these antibody assays are not helpful for the serological diagnosis of most PNC and Hib infections in children (20,21). At present, antibody assays for protein antigens of PNC and Hib might prove to be an attractive alternative. An antibody test to pneumolysin has recently been developed with promising results in adults (22). However, no other assays based on protein antigens are yet available.

3.3 Other methods

The quantitative determination of an acute phase protein, C-reactive protein, has been shown to be a helpful method (better than white blood cell count or erythrocyte sedimentation rate) for differentiation of bacterial pneumonias from those caused by viruses or Mycoplasma pneumoniae in adult patients (19,23). At present, the value of C-reactive protein in the differential diagnosis of ARI in children has not yet been evaluated. Studies on patients with meningitis or other defined infections suggest that it might be useful (24). It might however be of less value in developing countries where other infections, which may elevate the CRP, are widely prevalent.

4. RECOMMENDATIONS FOR STUDYING THE BACTERIOLOGICAL ETIOLOGY OF ALRI IN CHILDREN

4.1 Patient samples to be used for the demonstration of main pathogens in field studies

4.1.1 Blood

Blood samples should be taken from every patient in the acute phase of the disease. In addition, obtaining a convalescent blood sample about 3 to 6 weeks later is highly recommended. Acute blood samples should be used for blood culture, for demonstration of PNC and Hib (and Hia) antigens, and for quantitative determination of CRP. In selected patients, antibody titres to Chlamydia trachomatis, or Mycoplasma pneumoniae may be helpful. Paired sera are recommended for demonstration of antibody responses to specific etiological agents.

4.1.2 Urine

Urine specimens can be taken from ARI patients and used for the demonstration of Hib and PNC antigens. At present, the problems with false-positive and false-negative results, especially in the demonstration of PNC antigens, have not been solved. False-negative results may be a consequence of insensitivity of certain tests, especially for certain pneumococcal serotype antigens, or the degradation of polysaccharides in the urine, or the inhibitory effect of urine on the demonstration of antigens. False-positive results can be caused by the contamination of urine with cross reacting bacteria, or as recently reported from Gambia by nasopharyngeal carriage of the organism. This latter process requires further study, especially to determine whether nasopharyngeal colonization with Hib would also yield detectable amounts of Hib polysaccharide in urine. Thus, further evaluation of antigen detection in the urine in ARI patients and relevant controls is needed before final recommendations can be made.

4.1.3 Nasopharyngeal aspirates

Demonstration of PNC and Hib by culture or antigen detection from nasopharyngeal aspirates samples should not be used for the etiological diagnosis of ARI in children. However, nasopharyngeal aspirates samples can be used for carriage studies (both culture and antigen detection).

4.1.4 Lung aspirates and pleural fluids

When lung aspirates are available, the results of all other diagnostic methods should be compared to the culture findings of lung aspirates. Pleural fluid samples should be taken for bacterial culture and antigen detection whenever possible.

4.2 Antigen detection

Controls (both "positive" and "negative") should be run in every test. The concentration of antigen in the positive control should be adjusted to reflect concentrations present in low titre samples from patients with disease. Particular danger is over-reading of weak reactions, both in agglutination and CIE tests. It is advisable to boil all samples (also serum in 1:4 dilution) before testing. PNC types 7F and 14, that are frequent causes of infection, cannot be detected by counterimmunoelectrophoresis (25). For detection of Hib antigens agglutination tests are more sensitive than CIE but the former are more subjective and may be less specific. Agglutination tests are recommended for detection of Hib antigens in individual patients. The relative merits of counterimmunoelectrophoresis and agglutination tests when used with specially constructed antiserum pools (see below) for detection of PNC antigen in patient specimens require further evaluation. For epidemiological studies more specific, objective and quantitative methods like enzymeimmunoassay and radioimmunoassay should be developed. At present, a commercial enzymeimmunoassay test is only available for PNC C-polysaccharide.

4.3 Reagents available

Commercial reagents are available for

- PNC: Omniserum based reagents:
 - sensitivity for different capsular polysaccharides variable
- PNC: C-PS by enzymeimmunoassay:
 - special handling of specimens (serum and urine) possibly needed
- Hib: specific, sensitive agglutination tests available
- Hia: antiserum available - no standardized agglutination test reagents available.

5. FUTURE DEVELOPMENT

5.1 Reagent development

- PNC - A diagnostic ARI antiserum pool is needed; this pool should be limited to those types common in children in developing countries; additionally it should be of increased sensitivity compared with presently available omniserum to certain types, especially to types 6 and 23.

- Enzymeimmunoassays for capsular polysaccharides of PNC and Hi are urgently needed
- Hia reagents need to be developed
- Reagents for measurement of antibody response to PNC-pneumolysin are needed as another possible means of diagnosing PNC infection.

5.2 Subjects on which more information is needed

- stability of bacterial antigens during the transport and storage of clinical specimens
- reasons for false-positive and false-negative PNC antigen assays in urine and serum
- comparison of antigen detection by particle agglutination using acute serum and urine
- serologic methods based on outer membrane proteins and possibly other antigens. This may have special relevance to non-typable strains of Hi
- influence of upper respiratory tract colonization or infection on the detection of PNC and Hib antigen in body fluids, especially urine
- other diagnostic methods (antigen detection, antibody response, quantitative determination of CRP) should be evaluated in patients with positive culture finding from lung aspirate or blood
- PNC types which most frequently cause pneumonia among children in developing countries should be identified and specific diagnostic antiserum pools for these types should be used for etiological assays
- methods for the rapid demonstration of M.pneumoniae and chlamydia should be developed
- relative frequency of PNC and Hi serotypes as causes of disease in urban and rural areas of developing countries.

5.3 Follow-up meeting

A follow-up meeting with representatives from developing countries should be arranged in the near future to discuss priorities and methods for evaluation of various diagnostic methodologies.

REFERENCES

1. Pio, A. Acute respiratory infections in children in developing countries: an international point of view - Ped. Inf. Dis., 5: 179-183, 1986.
2. Shann F. Etiology of severe pneumonia in children in developing countries. Ped. Inf. Dis., 5: 247-252, 1986.
3. Riley T. et al. The status of research on acute respiratory infections in children in Papua New Guinea. Pediatr. Res., 17: 1041-1043, 1983.
4. Wafula E. (Kenya), personal communication.
5. Loda F.A. et al. Occurrence of Diplococcus pneumoniae in the upper respiratory tract of children. J. Pediatr., 87: 1087-1093, 1975.
6. Hendley J.O. et al. Spread of Streptococcus pneumoniae in families. I. Carriage rates and distribution of types. J. Inf. Dis., 132: 55-61, 1975.
7. Greenwood, B. (Gambia) personal communication.
8. Herva, E. (Finland) personal communication.
9. Lerman S.J. et al. Nasopharyngeal carriage of antibiotic-resistant Haemophilus influenzae in healthy children. Pediatrics, 64: 287-291, 1979.
10. Washington, J.A. Noninvasive diagnostic techniques for lower respiratory infections. pp. 41-54. In: Respiratory Infections: Diagnosis and Management (J.E. Pennington, Ed.) Raven Press, New York, 1983.
11. Sorensen, U.B.S., Henrichsen, J. Cross-reactions between pneumococci and other streptococci due to C-polysaccharide and F-antigen. J. Clin. Microbiol., 1987, in press.
12. Murphy, T.F. and Apicella, M.A. Non-typable Haemophilus influenzae: A review of clinical aspects, surface antigens, and the human immune response to infection. Rev. Inf. Dis., 9, 1-15, 1987.
13. Wall, R.A. et al. The etiology of lobar pneumonia in the Gambia. Bull. WHO, 64: 553-558, 1986.
14. Shann, F. et al. Aetiology of pneumonia in children in Goroka Hospital, Papua New Guinea, Lancet, 2: 537-541, 1984.
15. Daum, R.S. et al. Evaluation of a commercial latex particle agglutination test for rapid diagnosis of Haemophilus influenzae type b infection. Pediatrics, 69: 466-471, 1982.
16. Kaldor, S. et al. Haemophilus influenzae type b antigenuria in children. J. Clin. Pathol., 32: 538-541, 1979.
17. Kalin, M., Lindberg, A.A. Diagnosis of pneumococcal pneumonia: a comparison between microscopic examination of expectorates antigen detection and culture procedures. Scand. J. Infect. Dis., 15: 247-255, 1983.
18. Coonrood, C.D. Urine as an antigen reservoir for diagnosis of infectious diseases. Am. J. Med., 85-92, 1983.

19. Kerttula, Y. et al. The aetiology of pneumonia. Application of bacterial serology and basic laboratory methods. J. Infection, 14: 21-30, 1987.
20. Robbins, J.B. et al. Haemophilus influenzae type b: Disease and immunity in humans. Ann. Intern. Med., 78: 259-269, 1973.
21. Leinonen, M. et al. Antibody response to 14-valent pneumococcal capsular polysaccharide vaccine in pre-school age children. Ped. Infect. Dis., 5: 39-44, 1986.
22. Kanderski K., Mollby R. Production and purification of Streptococcus pneumoniae hemolysin (pneumolysin) J. Clin. Microbiol., 1987, 25: 222-225.
23. Lehtomaki, K. et al. Etiological diagnosis of pneumonia by combining bacterial culture with conventional and new serological methods. Eur. J. Clin. Microbiol., 1987, in press.
24. Peltola, H. C-reactive protein for rapid monitoring of infections of the central nervous system. Lancet, i: 980-983, 1982.
25. Henrichsen J. et al. Comparison of counter immunoelectrophoresis and the capsular reaction test for typing of pneumococci. J. Clin. Microbiol., 11: 589-592, 1980.

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