MAJOR ARTICLE

Etiological Diagnosis of Childhood Pneumonia by Use of Transthoracic Needle Aspiration and Modern Microbiological Methods

Elina Vuori-Holopainen,[1] Eeva Salo,[1] Harri Saxén,[1] Klaus Hedman,[2] Timo Hyypiä,[2] Raija Lahdenperä,[2] Maija Leinonen,[3] Eveliina Tarkka,[2] Martti Vaara,[2] and Heikki Peltola[1]

[1]Hospital for Children and Adolescents and [2]Laboratory Diagnostics, Helsinki University Central Hospital, Helsinki, and [3]National Public Health Institute, Oulu, Finland

Childhood pneumonia is usually treated without determining its etiology. The causative organism can be isolated from specimens of blood, empyema fluid, or lung aspirate, but this is rarely done. The potential of transthoracic needle aspiration for identification of causative agents was tested with use of modern microbiological methods. Aspiration was performed for 34 children who had radiological signs compatible with community-acquired pneumonia and had alveolar consolidation. In addition to bacterial and viral cultures and viral antigen detection, nucleic acid detection for common respiratory pathogens was performed on aspirate specimens. Aspiration disclosed the etiology in 20 (59%) of 34 cases overall and in 18 (69%) of 26 patients from whom a representative specimen was obtained. Aspiration's advantages are high microbiological yield and a relatively low risk of a clinically significant adverse event. Aspiration should be used if identification of the causative agent outweighs the modest risk of the procedure.

Community-acquired pneumonia is still a major cause of morbidity and mortality worldwide and is the primary cause of death in up to 25% of the 13 million deaths annually among children aged 0–4 years [1, 2]. Although pneumonia is widespread, its etiology usually remains unknown. Increasing problems with drug-resistant infections and the availability of effective vaccines make the identification of the causative agent increasingly relevant [3, 4]. However, precise diagnosis is difficult to make on the basis of clinical or radiological findings [5, 6].

Received 13 June 2001; revised 12 September 2001; electronically published 16 January 2002.

Financial support: Pfizer, Ltd., Finland; and Väinö and Laina Kivi Foundation, Research Foundation of Orion Corporation, and Paulo Foundation, Finland (to E.V.-H.).

Reprints or correspondence: Dr. Elina Vuori-Holopainen, HUCH Hospital for Children and Adolescents, PB 281, 00029 HUS, Helsinki, Finland (elina.vuori@hus.fi).

Clinical Infectious Diseases 2002; 34:583–90

1058-4838/2002/3405-0004$03.00

Bacterial and viral testing of serum samples are sometimes used to improve the accuracy of diagnosis, but the results arrive too late to help the patient. Culture-based diagnosis is complicated by the technical difficulty of obtaining specimens. Cultures of nasopharyngeal swab and throat specimens correlate poorly with the microbial flora of the lung [7, 8] because *Streptococcus pneumoniae* is part of the normal microflora of the nose and throat. Throat culture is especially unreliable for children. The diagnostic value of a positive blood culture is high, but its sensitivity is low (1%–3% [6]) for hospitalized children with bacterial pneumonia in industrialized countries [9]. In nonbacteremic cases of pneumonia, convincing evidence of the etiology comes only with identification of a causative organisms recovered from samples of the pleural fluid or the lung parenchyma [10, 11].

Transthoracic needle aspiration (known informally as "lung tap"; hereafter "aspiration"), a method similar to pleural aspiration [12], is an old and established technique for identification of the cause of pulmonary

infections [13]. It is easy to perform and can be carried out at the bedside; furthermore, the sample is not contaminated by the oropharyngeal flora. In developing countries, aspiration has allowed bacteria to be detected in up to 92% of cases of childhood pneumonia [12], but in the industrialized world, its use has mostly been restricted to adult patients [14–16]. We report here our first experience with aspiration to diagnose community-acquired pneumonia in children. To our knowledge, this was the first time modern microbiological methods were used with the procedure.

PATIENTS AND METHODS

Study Design and Patients

Our prospective study was conducted at the Helsinki University Central Hospital, Hospital for Children and Adolescents, Helsinki, Finland. The ethical committee of the hospital approved the study protocol. From December 1997 through December 2000, we enrolled hospitalized children with pneumonia, which was defined as respiratory symptoms and signs with a history of fever and consolidation in the chest radiograph at admission. Availability of radiographic facilities restricted patient enrollment to the period from 8 A.M. to 8 P.M. Written consent was obtained from the legal guardian of the child; on the written consent form, the method and its potential disadvantages were described, and it was explained that nonparticipation did not affect the treatment of the patient. A trained specialist performed all aspiration procedures in the outpatient department. Patients were excluded from the study if they were immunocompromised, were in a postoperative phase, had bleeding diathesis, were being treated in oncology or intensive care units, or refused to participate. Previous treatment with antimicrobials did not prevent enrollment.

An infectious disease specialist interpreted the initial chest radiographs, and a pediatric radiologist reviewed all the radiographs later. All patients were treated initially with penicillin G [17]. Predetermined demographic, clinical, laboratory, and radiological data were recorded on specially designed forms. Follow-up visits took place 2–3 weeks after the end of hospitalization.

Aspiration

We determined the infectious focus in 2 planes on the basis of chest radiograph findings and physical signs. Before the start of antimicrobial therapy, the aspiration procedure was performed without fluoroscopic or CT scan, and without premedication, other than application of lidocaine ointment. Aspiration was done anywhere on the chest area except the anterior intermamillary space and the posterior scapular region. During the procedure, the child was held tightly in the sitting or supine position. After cleaning the skin, a standard 22-gauge needle was attached to a 5-mL syringe containing 2.5 mL of saline. The needle was then inserted into the suspected area of consolidation, 1.5 mL of saline was injected, and the needle was removed while continuous suction was maintained. The whole procedure lasted <10 s. As a control, a radiograph was obtained within 2 h [18] and on the day after the procedure.

Microbiology of Lung Aspirate Samples

Standard bacteriological testing. Conventional bacteriological testing was performed with use of aerobic blood culture media. The needle was stored in thioglycolate growth medium and cultured. Acridine orange staining was always used, and if bacteria were detected, Gram staining was also performed. The number of leukocytes seen by use of microscopy (magnification, ×100) was classified into one of the following categories: 0, 1–2, 3–5, or >5 leukocytes. The DNA-RNA hybridization technique (Accuprobe; Gen-Probe) was used to identify *Streptococcus pneumoniae* and *Haemophilus influenzae* in the culture media.

Standard virological studies. For viral culture, aspirate samples were inoculated into 4 (or during influenza epidemics, 5) cell lines: human amnion epithelial cells, green monkey kidney cells, canine kidney cells, human fibroblast cells, and/or Madin-Darby canine kidney cells. For cytomegalovirus, after 7-day inoculation into human fibroblasts, the cultures, placed on glass slips, were fixed with methanol. Immunofluorescence staining was used to detect early antigen-specific monoclonal antibodies (Argene Biosoft).

For direct detection of respiratory viral antigens, the aspirates were cytocentrifuged onto object glasses, air dried, fixed with acetone, and immunofluorescence-stained with fluorescein-labeled monoclonal antibodies for the following viruses: adenovirus; influenza virus types A and B; parainfluenza virus types 1, 2, and 3; and respiratory syncytial virus (RSV; Respiratory Panel 1; Light Diagnostics). Sample cellularity (i.e., a count of the number of nuclei) was estimated by use of DNA staining (Hoechst).

PCR Detection of Bacteria and Viruses

PCR techniques were used to detect *S. pneumoniae, H. influenzae, Chlamydia pneumoniae, Mycoplasma pneumoniae,* enterovirus, and rhinovirus. Nucleic acid isolation methods were selected to allow recovery of both DNA and RNA from the samples. For *S. pneumoniae, H. influenzae,* and *C. pneumoniae,* the DNA was isolated with a QIAamp tissue kit (Qiagen). For *M. pneumoniae* and picornavirus assays, aspirates were first digested with proteinase K in the presence of SDS; then phenol was extracted, and the nucleic acids were precipitated with ethanol. The pellet was dissolved in water and used in the PCR assays. If the amount of the aspirate was insufficient, priority was given to standard microbiological studies.

S. pneumoniae. An *S. pneumoniae* pneumolysin gene fragment (208 bp) was amplified by use of the "hot start" method, as described elsewhere [19], except that 2 m*M* of $MgCl_2$ was used. The amplified gene products were identified by liquid hybridization with a Europium-labeled probe [19].

C. pneumoniae. The *C. pneumoniae* omp2 gene fragment (135 bp) was detected with use of a reaction mixture that consisted of 50 m*M* of Tris-HCl (pH 8.0 at 25°C), 100 m*M* of NaCl, 0.1 m*M* of EDTA, 1 m*M* of dithiothreitol, 50% of glycerol, 1% of Tween X-100, 4 m*M* of $MgCl_2$, 0.2 m*M* of each deoxyribonucleotide (Promega), 50 pmol of HB1 primer sequence (5′-ATA GTC TCC GTA AAA TCC AGC ACT-3′; University of Helsinki, Institute of Biotechnology, Finland), 50 pmol of biotinylated HB2 primer sequence (5′-CCT GTA GGG AAC CTT TCT GAT C-3′), 1 U of Taq DNA polymerase, and 10 μL of isolated DNA. The total reaction volume was 50 μL. The amplified gene products were identified by liquid hybridization with use of the Europium-labeled SJ1 probe (CCA TAT[TA] CT[GA] CCA TCA ATT AA).

H. influenzae. The *H. influenzae* omp2 gene fragment (1000 bp) was amplified as described elsewhere [20], except that the total volume was 25 μL. The PCR product was subjected to electrophoresis in 2% agarose (Promega) in Tris-borate-EDTA buffer and visualized by use of ethidium bromide staining.

M. pneumoniae. DNA was subjected to heat denaturation, after which the *M. pneumoniae* 16S rRNA gene was amplified with species-specific primers [21] and detected as described elsewhere [22].

Picornaviruses. Enterovirus and rhinovirus RNA were detected by a reverse-transcriptase PCR assay as described elsewhere [23], except that digoxigenin-labeled probes and chemiluminescence were used for detection of the amplification products.

Other Microbiological Studies

For routine bacteriological cultures, blood and oropharyngeal samples were obtained; for viral cultures, nasopharyngeal swabs were obtained. For indirect immunofluorescence, 7 monoclonal antibodies (Light Diagnostics) were used for adenovirus, influenza A and B, parainfluenza 1, 2, and 3, and RSV viruses [24]. Pneumococcal isolates were serotyped with use of counterimmunolectrophoresis and the latex agglutination test. The standard disc method was used for detection of antimicrobial susceptibility. If decreased susceptibility to penicillin was found, the MIC was determined by use of the E-test (AB Biodisk).

Diagnostic Definitions and Statistical Analysis

An aspiration fluid specimen was considered representative if leukocytes were seen by microscopic examination [15]. The etiology was considered "confirmed" if a pathogen was isolated from a blood or lung aspirate sample or the puncture needle, or when the results of DNA-RNA hybridization or PCR of a lung aspirate sample were positive. The diagnosis was considered "likely" if a viral antigen was detected in a nasopharyngeal swab or when *S. pyogenes* was isolated from the oropharynx of a patient with tonsillitis.

For continuous variables, Student's *t* test was used. For categorical variables, χ^2 contingency analysis or Fisher's exact test was used, as appropriate. The significance level was set at $P < .05$.

RESULTS

Forty-seven patients were asked to participate, but, because 13 refused, the study comprised 34 patients. A representative aspiration fluid specimen was obtained from 26 patients (76%), but analyses were performed for all 34 children (table 1).

Bacteriology. The aspiration yield varied from a few drops to 5 mL of fluid. Analysis of the aspirate samples disclosed the etiology in 20 (59%) of 34, with a total of 21 bacteria and 2 viruses identified. In the 26 patients from whom a representative sample was obtained, a pathogen was detected in 18 (69%) of 26.

Bacteria were visualized in samples obtained from 4 patients, but the culture of the specimen or needle was positive for bacteria for 11 (32%) of 34 of patients. In no case was >1 bacterium isolated; there were 10 cases of *S. pneumoniae* and 1 case of *Moraxella osloensis* infection. In 7 cases, the pathogen was cultured from the lung aspirate specimen and the needle, whereas in 2 cases each, a pathogen was cultured only from the aspirate or the needle.

For 29 patients, DNA-RNA hybridization was performed for pneumococcus, and it was performed for *H. influenzae* for 27 patients. PCR for *S. pneumoniae, H. influenzae,* and *C. pneumoniae* was possible for 32 patients, and PCR for *M. pneumoniae* was possible for 28 patients. Pneumococcal DNA-RNA hybridization results were positive for 10 (34%) of 29 patients and pneumolysin PCR results were positive for 16 (50%) of 32 of patients. *M. pneumoniae* and *C. pneumoniae* were detected in 1 patient each; no case of infection due to *H. influenzae* was diagnosed. Dual bacterial infection (*S. pneumoniae* plus *M. osloensis* or *C. pneumoniae*) was identified twice, and a mixed bacterial-viral infection (*S. pneumoniae* and enterovirus) was identified once. The results for the 20 patients with positive aspiration findings are provided in table 2.

Virology. Viral culture and antigen detection were performed for 33 and 26 patients, respectively. For 1 patient, culture was positive for parainfluenza or RSV (defective growth prevented further typing). No patients tested positive for antigens. In some cases, immunofluorescence not feasible, because

Table 1. Clinical characteristics of 34 children with pneumonia.

Characteristic	Value
Sex	
Male	20
Female	14
Age, years	
Mean (range)	5.1 (0.8–14.0)
Median	2.9
Pretreatment with antimicrobials	5
Length of history of symptoms, mean days (range)	4.9 (1–14)
Temperature	
Mean °C (range)	38.6 (35.9–40.5)
⩾38.5°C	19
Cough	19
Retractions	4
C-reactive protein, mg/L	
Mean (range)	180 (9–345)
Median	196
Leukocytes $\times 10^9$/L	
Mean (range)	19.2 (2.1–33.1)
Median	20.2
Erythrocyte sedimentation rate, mm/h	
Mean (range)	77 (10–138)
Median	71
Location of consolidation	
Lower lobe only	19
Right	8
Left	9
Both	2
Upper lobe only	7
Right	6
Left	1
Both	0
Right middle lobe only	0
⩾2 Lobes	10
Affected lobes on the same side	3
Affected lobes on opposite sides	7
Pleural effusion at admission	2

NOTE. Data are no. of patients, unless indicated otherwise.

there were too few leukocytes in the lung aspirate specimen. PCR was used to detect enteroviruses and rhinoviruses in 28 patients; only 1 enteroviral infection was detected.

Yield of aspiration versus conventional methods. Conventional methods disclosed the likely or confirmed bacterial etiology in 3 patients: blood culture was positive in 2 (6%) of 34 patients, and oropharyngeal culture was positive once for *S. pyogenes*. RSV was identified in specimen from the nasopharynx for 3 (9%) of 34 patients.

For 16 (47%) of 34 patients, the etiology was identified from the lung aspirate only. In 3 patients, conventional methods led to a presumptive diagnosis, but aspiration confirmed the presence of an additional organism or another organism (RSV from the nasopharynx and pneumococcus from the lung). In 2 patients, pneumococcus was identified in a blood sample (by use of culture) and in a lung aspirate specimen (by use of PCR or culture). The proportion of patients with any (i.e., presumptive or definitive) evidence for an identifiable etiologic agent increased from 15% (5 of 34) to 62% (21 of 34), and the number of microbiologically definitive cases increased from 2 to 20.

Pneumococcal isolates recovered from different body sites. Pneumococci were isolated from cultures of lung aspirate samples for 10 patients, from blood cultures for 2 patients, and from cultures of oropharyngeal specimens for 13 patients. Among the 11 lung or blood isolates that were serotyped, the following serotypes were found: type 3 (3 isolates), type 6A (1 isolate), type 6B (2 isolates), type 7F (1 isolate), type 14 (2 isolates), type 18C (1 isolate), and type 19A (1 isolate).

Among the 11 oropharyngeal strains that were serotyped, type 3 (5 isolates), type 6B (1 isolate), type 7F (1 isolate), type 18C (1 isolate), type 21 (1 isolate), type 23F (1 isolate), and type 34 (1 isolate) were identified. In 5 patients, identical types were isolated from the lung and oropharynx; in 1 patient, different strains were isolated: type 19A from the lung and type 23F from the oropharynx. The pneumococcus isolated from lung was resistant to erythromycin, clindamycin, and doxycycline, whereas the oropharyngeal isolate was susceptible to all the agents tested. One blood isolate, 1 oropharyngeal isolate, and 2 lung isolates showed intermediate resistance (MIC, 0.1–1 μg/mL) to penicillin, whereas all the other isolates were susceptible (MIC, <0.1 μg/mL). Three lung strains were resistant to erythromycin (MIC, >256 μg/mL).

Risks of aspiration. Pneumothorax developed in 6 patients (18%); in 5 patients, it was considered iatrogenic. One patient also had a small quantity of air in the mediastinum. Only 1 patient complained of chest pain after the aspiration procedure.

Pneumothorax resolved almost invariably within 2–3 days, and insertion of a pleural drainage tube, medication for relief of pain, or other treatments were never required. The patients with and patients without pneumothorax could not be distinguished with respect to the following factors: length of history of symptoms compatible with a diagnosis of pneumonia, symptoms or signs before aspiration, length of hospital stay, clinical recovery, laboratory test results, or frequency of obtaining a representative sample.

Outcome. The duration of symptoms, leukocyte counts, and degree of fever at admission were similar for patients with aspiration specimens positive for bacteria and for patients with specimens negative for bacteria. In contrast, patients with a positive finding were younger (mean age [± SD], 3.6 ± 3.8 vs.

Table 2. Results of different diagnostic methods used for 20 children who had pneumonia for which an etiology was determined.

	Test result or cells or organisms identified (count),[a] by type of test			*Streptococcus pneumoniae* testing		*Haemophilus influenzae* testing		PCR testing, by organism				
Patient	Lung aspirate staining	Lung aspirate culture	Blood culture	PCR	RNA hyb	PCR	RNA hyb	*Chlamydia pneumoniae*	*Mycoplasma pneumoniae*	Rhinovirus	Enterovirus	Viral culture
1	Leukocytes (>5)	*S. pneumoniae*	−	+	+	−	ND	−	−	−	−	−
2	Leukocytes (>5)	*S. pneumoniae*	−	+	+	−	ND	−	−	−	−	−
3	Leukocytes (>5)	−	−	−	ND	−	ND	−	−	−	−	+[b]
4	Leukocytes (>5)	*S. pneumoniae*	−	+	+	−	−	−	−	−	−	−
5	Leukocytes (>5)	*S. pneumoniae*	−	ND	+	ND	−	ND	ND	ND	ND	−
6	Leukocytes (3–5), GPP	*S. pneumoniae*	−	+	+	−	−	−	−	−	−	−
7	Leukocytes (2–5), GPP	*S. pneumoniae*	−	+	+	−	−	−	−	−	−	−
8	Leukocytes (3–5), GPP	*S. pneumoniae*	−	+	+	−	−	−	−	−	−	ND
9	Leukocytes (3–5)	*S. pneumoniae*	−	−	+	−	−	−	−	−	−	−
10	Leukocytes (3–5)	−	−	+[c]	−	−	−	−	−	−	−	−
11	Leukocytes (3–5)	*S. pneumoniae*	−	+	+	−	−	−	−	−	+	−
12	Leukocytes (3–5)	−	−	+	−	−	−	−	−	−	−	−
13	Leukocytes (3–5)	−	−	+	−	−	−	−	−	−	−	−
14	Leukocytes (3–5)	*Moraxella osloensis*	−	+	−	−	−	−	−	−	−	−
15	Leukocytes (1–2), GPP	*S. pneumoniae*[c]	*S. pneumoniae*[c]	+[c]	+[c]	−	−	−	−	−	−	−
16	Leukocytes (1–2)	−	−	+	ND	−	ND	−	ND	ND	ND	−
17	Leukocytes (1–2)	−	−	+	−	−	−	+	ND	ND	ND	−
18	Leukocytes (1–2)	−	*S. pneumoniae*	+	−	−	−	−	−	−	−	−
19	−	−	−	−	−	−	−	−	+[c]	−	−	−
20	−	−	−	+	−	−	−	−	−	−	−	−

NOTE. GPP, gram-positive cocci; ND, not done; RNA hyb, RNA hybridization; +, positive; −, negative.

[a] Counts are no. of cells or organisms seen per microscopic field.

[b] Parainfluenza or respiratory syncytial virus; final typing impossible.

[c] Patient received antimicrobial therapy before the transthoracic needle aspiration was performed.

7.0 ± 4.3 years; $P = .02$) and had higher levels of serum C-reactive protein (mean [± SD], 216 ± 80 mg/L vs. 134 ± 100 mg/L; $P = .01$) and a higher erythrocyte sedimentation rate (mean [± SD], 87 ± 31 mm/h vs. 63 ± 17 mm/h; $P = .01$) at admission. However, the peak C-reactive protein values measured during treatment were similar in the 2 groups.

All the children had uneventful recoveries, although 3 patients had pneumonia complicated by empyema that had no observable relation to performance of aspiration. At follow-up, chest radiographs demonstrated complete resolution of pneumonia in 17 children.

DISCUSSION

Many etiological studies of childhood pneumonia are based on the findings of blood culture, viral antigen detection, or serological studies, alone or in combination [6, 9]. In this series of 34 children, aspiration, the gold standard for diagnosis of pneumonia, disclosed the etiology for 59% of all patients enrolled and for 69% of patients from whom a representative specimen was obtained. For 2 patients, a pathogen was identified in a leukocyte-free sample by use of PCR; however, the presence of leukocytes suggests that a sample is representative [15]. The value of aspiration was demonstrated by identification of 16 pneumococcal infections not detected by blood culture. In addition, we found 1 case of infection with *M. osloensis,* which is virtually unknown as a pathogen in childhood pneumonia [25]. Because, in this study, all patients had an indisputable consolidation visible on a radiograph, the relevance of aspiration for diagnosis of other types of pneumonia could not be estimated with these results. Furthermore, because the number of patients we assessed was small, we were unable to draw conclusions about the seasonal distribution of agents in childhood pneumonia.

Sometimes, despite visible consolidation on the radiograph or leukocytes in the aspirate specimen, the result was negative. In those cases, the needle may have missed the infectious focus, or pretreatment with antimicrobials may have lessened the likelihood of making a correct microbiological diagnosis. Earlier studies have shown that aspiration specimens may contain viable bacteria even though antimicrobials have already been administered [12]. In our series, this occurred once for pneumococcus, and, in addition, 1 case of *M. pneumoniae* and 1 of *S. pneumoniae* were detected by PCR (table 2). Because PCR is a sensitive and specific technique and can detect nonviable organisms, it was particularly suitable for our purpose. Pneumolysin PCR has been used for identification of pneumococcus in serum samples [26–28], but we applied it to lung aspirate samples.

For viral diagnosis, the aspirate should preferably contain alveolar cells. For many of our patients, this was not the case, probably because of the small size of needle and because we did not perform lung biopsies. This may have decreased virological yield, but we doubt whether many more viral infections would have been found in this series in any case (table 1). In the few aspiration studies that have searched for viruses, the yield has varied from 0% to 30% (mean, 4%) [12]. Our experience agrees with this observation: only 1 case of a viral and 1 case of a mixed viral-bacterial infection were identified. It is of note that no rhinoviruses were detected in lung aspirate specimens by use of culture or specific PCR. Thus, our results do not support the view that, because rhinoviruses are frequently identified (in 24% of cases) in specimens from the nasopharynx [29], rhinoviruses are a common cause of childhood pneumonia. However, all of our patients had indisputable consolidation visible on a radiograph, and, had the patient selection been different, the results might have differed. Earlier studies have shown that the yield of aspiration increases with the size of consolidation and with the experience of the person performing the procedure [15].

The ethics of performing lung aspiration in children with community-acquired pneumonia who live in industrialized countries has been questioned [6]. Although most patients in Finland recover uneventfully, we have 4 reasons for our different view. First, aspiration is considerably safer than most people think, as we determined in our review of 32 studies (comprising >2600 aspiration procedures performed in 15 countries during the last 70 years) in which treatment for a complication was needed in ⩽0.5% of cases [12]. We paid close attention to all safety issues and performed aspiration in a hospital with full expertise in all thoracosurgical procedures. All cases of pneumothorax decreased in size or resolved within 24–48 h, the children showed no signs of distress, and neither the clinical course of illness nor the length of hospitalization was altered. Many children and parents stated afterward that the aspiration procedure was no more unpleasant than the taking of a blood sample.

Second, pneumonia is a major cause of death among children worldwide. There hardly exists another common infection of comparable severity whose etiology is so poorly known. Modern microbiology provides a novel opportunity to determine the pathogens causing pneumonia, and this approach has to be tested in a in a hospital with full expertise in the relevant procedures. Our overall success rate of 59% provides further justification for similar studies.

Third, because most cases of childhood pneumonia are nonbacteremic, it has been difficult to estimate the relevance of conjugate vaccines for prevention of pneumonia [30]. The recent experience with a heptavalent pneumococcal conjugate vaccine in California showed up to 63% efficacy against indisputable consolidated pneumonia [31], but we do not know how much this experience can be extended to the other parts

of the world. Because the relative distribution of pneumococcal serotypes varies in different populations, and because there is at least a potential risk of serotype change caused by the use of vaccine [32], better information about etiology would facilitate making decisions about use of these costly vaccines.

Fourth, rates of resistance to antimicrobials in many countries are increasing due to the ever-increasing use of wide-spectrum antimicrobial agents. Susceptibility data on invasive strains only disclose the potential danger of antimicrobial resistance; 30% of the pneumococcal strains we isolated from lung aspirate specimens were resistant to erythromycin. However, all strains were susceptible to conventional narrow-spectrum antimicrobials. This finding is in accord with our previous study, which showed that the routine use of wide-spectrum cephalosporin had no benefit compared with the use of conventional procaine penicillin [17]. Circumstances vary from country to country, but, in our view, inexpensive parenteral penicillin is still the treatment of choice for most children worldwide who are hospitalized because of community-acquired pneumonia.

In summary, for determination of the etiology of childhood pneumonia, aspiration offers advantages that, in our view, clearly outweigh its disadvantages. It is not a procedure to be performed in all cases or by every clinician, but it is a valid tool that can be used when the disclosure of the pathogen causing childhood pneumonia is considered clinically important and in individual cases in which the patient does not respond to therapy. Furthermore, aspiration is a gold standard for determination of the etiology of nonbacteremic pneumonia, against which all other methods should be measured. As with procedures that puncture any solid organ, aspiration should be performed by clinicians who are adequately trained and who have the expertise to deal with potential complications.

Acknowledgments

We are indebted to Drs. Eino Marttinen, for assistance with radiology; to Ilkka Mattila and Markku Kaarne, for assistance with the aspiration procedure; and to Dr. Mirja Puolakkainen and Leena Kuisma, for assistance with the laboratory analyses.

References

1. Obaro SK, Monteil MA, Henderson DC. The pneumococcal problem. BMJ **1996**; 312:1521–5.
2. Berman S. Epidemiology of acute respiratory infections in children of developing countries. Rev Infect Dis **1991**; 13:S454–62.
3. Lister PD. Multiply-resistant pneumococcus: therapeutic problems in the management of serious infections. Eur J Clin Microbiol Infect Dis **1995**; 451:S18–25.
4. Rubin LG. Pneumococcal vaccine. Pediatr Clin North Am **2000**; 47: 269–85.
5. Swingler GH. Radiologic differentiation between bacterial and viral lower respiratory infection in children: a systematic literature review. Clin Pediatr **2000**; 39:627–33.
6. Ruuskanen O, Mertsola J. Childhood community-acquired pneumonia. Semin Respir Infect **1999**; 14:163–72.
7. Laurenzi GA, Potter RT, Kass EH. Bacteriologic flora of the lower respiratory tract. N Engl J Med **1961**; 265:1273–8.
8. Silvermann M, Stratton D, Diallo A, et al. Diagnosis of acute bacterial pneumonia in Nigerian children. Arch Dis Child **1977**; 52:925–31.
9. Paisley JW, Lauer BA, Mcintosh K, et al. Pathogens associated with acute lower respiratory tract infection in young children. Pediatr Infect Dis **1984**; 3:14–9.
10. Munson RS, Kabeer MH, Lenoir AA, et al. Epidemiology and prospects for prevention of disease due to *Haemophilus influenzae* in developing countries. Rev Infect Dis **1989**; 11:S588–97.
11. Finland M. Diagnostic lung puncture. Pediatrics **1969**; 44:471–3.
12. Vuori-Holopainen E, Peltola H. Reappraisal of lung tap: review of an old method for better etiologic diagnosis of childhood pneumonia. Clin Infect Dis **2001**; 32:715–26.
13. Leyden I. VII Verhandlungen des vereins fur innere medizin: über infectiose pneumonie. Dtsch Med Wochenschr **1882**; 4:52–4.
14. Torres A, Jiménez P, Puig de la Bellacasa J, et al. Diagnostic value of nonfluoroscopic percutaneous lung needle aspiration in patients with pneumonia. Chest **1990**; 98:840–4.
15. Zalacaín R, Llorente JL, Gaztelurrutia L, et al. Influence of three factors on the diagnostic effectiveness of transthoracic needle aspiration in pneumonia. Chest **1995**; 107:96–100.
16. Dorca J, Manresa F, Esteban L, et al. Efficacy, safety and therapeutical relevance of transthoracic aspiration with ultrathin needle in nonventilated nosocomial pneumonia. Am J Resp Crit Care Med **1995**; 151: 1491–6.
17. Vuori-Holopainen E, Peltola H, Kallio MJT, for the SE-TU Study Group. Narrow- versus broad-spectrum parenteral antimicrobials against common infections of childhood: a prospective and randomized comparison between penicillin and cefuroxime. Eur J Pediatr **2000**; 159:878–84.
18. Perlmutt LM, Braun SD, Newman GE, et al. Timing of chest film follow-up after transthoracic needle aspiration. AJR Am J Roentgenol **1986**; 146:1049–50.
19. Kontiokari T, Renko M, Kaijalainen T, et al. Comparison of nasal swab culture, quantitative culture of nasal mucosal tissue and PCR in detecting *Streptococcus pneumoniae* carriage in rats. APMIS **2000**; 108: 734–8.
20. Hobson RP, Williams A, Rawal K, et al. Incidence and spread of *Haemophilus influenzae* on an Antarctic base determined using the polymerase chain reaction. Epidemiol Infect **1995**; 114:93–103.
21. Van Kuppeveld FJM, van der Logt JTM, Angulo AF, et al. Genus- and species-specific identification of mycoplasmas by 16S rRNA amplification. Appl Environ Microbiol **1992**; 58:2606–15.
22. Vesanen M, Piiparinen H, Kallio A, et al. Detection of herpes simplex virus DNA in cerebrospinal fluid samples using the polymerase chain reaction and microplate hybridization. J Virol Methods **1996**; 59:1–11.
23. Lönnrot M, Sjöroos M, Salminen K, et al. Diagnosis of enterovirus and rhinovirus infections by RT-PCR and time-resolved fluorometry with lanthanide chelate labeled probes. J Med Virol **1999**; 59:378–84.
24. Ukkonen P, Julkunen I. Preparation of nasopharyngeal secretions for immunofluorescence by one-step centrifugation through Percoll. J Virol Methods **1987**; 15:291–301.
25. Vuori-Holopainen E, Salo E, Saxén H, et al. Clinical "pneumococcal pneumonia" due to *Moraxella osloensis:* case report and a review. Scand J Infect Dis **2001**; 33:625–7.
26. Rudolph KM, Parkinson AJ, Black CM, et al. Evaluation of polymerase chain reaction for diagnosis of pneumococcal pneumonia. J Clin Microbiol **1993**; 31:2661–6.
27. Salo P, Örqvist Å, Leinonen M. Diagnosis of bacteremic pneumococcal

pneumonia by amplification of pneumolysin gene fragment in serum. J Infect Dis **1995**; 171:479–82.
28. Dagan R, Shirker O, Hazan E, et al. Prospective study to determine clinical relevance of detection of pneumococcal DNA in sera of children by PCR. J Clin Microbiol **1998**; 36:669–73.
29. Juvén T, Mertsola J, Waris M, et al. Etiology of community-acquired pneumonia in 254 hospitalized children. Pediatr Infect Dis J **2000**; 19: 293–8.
30. Mulholland K, Hilton S, Adegbola R, et al. Randomised trial of *Haemophilus influenzae* type-b tetanus protein conjugate vaccine (corrected) for prevention of pneumonia and meningitis in Gambian infants. Lancet **1997**; 349:1191–7.
31. Shinefield HR, Black S. Infant immunization with a heptavalent conjugate pneumococcal vaccine [abstract PS 14]. In: Program and abstracts of the 17th Annual Meeting of the European Society for Paediatric Infectious Diseases (Crete). **1999**.
32. Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N Engl J Med **2001**; 344:403–9.