Procedures for Collection of Induced Sputum Specimens From Children

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In most settings, sputum is not routinely collected for microbiological diagnosis from children with lower respiratory disease. To evaluate whether it is feasible and diagnostically useful to collect sputum in the Pneumonia Etiology Research for Child Health (PERCH) study, we reviewed the literature on induced sputum procedures. Protocols for induced sputum in children were collated from published reports and experts on respiratory disease and reviewed by an external advisory group for recommendation in the PERCH study. The advisory group compared 6 protocols: 4 followed a nebulization technique using hypertonic saline, and 2 followed a chest or abdomen massage technique. Grading systems for specimen quality were evaluated. Collecting sputum from children with lower respiratory tract illness is feasible and is performed around the world. An external advisory group recommended that sputum be collected from children hospitalized with severe and very severe pneumonia who participate in the PERCH study provided no contraindications exist. PERCH selected the nebulization technique using hypertonic saline.

The Pneumonia Etiology Research for Child Health (PERCH) study aims to determine the etiology of childhood pneumonia in a multisite case-control study through use of innovative molecular detection tools for pathogens causing pneumonia [1]. In preparation for the study, the PERCH team reviewed methods for collecting lower respiratory tract specimens for microbiological diagnosis of pneumonia, including bronchoalveolar lavage, nonbronchoscopic alveolar lavage, lung aspirate, sputum induction, and postmortem lung biopsy. The considerations that were most important in selecting the technique for use in the PERCH study were the diagnostic yield, accuracy, feasibility, and safety of performing the technique across 7 sites with staff of varied clinical skills. In addition to reviewing the literature, we surveyed clinical experts with experience performing sputum induction in children. This article describes the results of these evaluations. In consultation with the PERCH Pneumonia Methods Working Group (PMWG) [1, 2], we selected sputum induction using nebulization with hypertonic saline as the method for sputum collection for the PERCH study.

METHODS

Background on Sputum Induction
Sputum specimens are routinely collected from adults to determine the etiology of lower respiratory tract infections. Among adults with pneumonia, approximately 75% can produce an adequate sputum specimen for microbiologic evaluation [3, 4]. Microbiologic examination of sputum is central to the diagnosis of pulmonary tuberculosis and Pneumocystis jirovecii pneumonia. Although the sensitivity of sputum examination has been reported as >75% for detection of bacterial pathogens in adults with pneumonia [5], findings can be affected by antibiotic use prior to specimen collection, and sputum culture results can be difficult to interpret because of
difficulties discriminating between infecting and colonizing bacteria.

Despite the advantages of sputum collection among adults, it has not generally been considered feasible to collect sputum from infants and children for a variety of reasons. Children have difficulty producing sufficient sputum for laboratory evaluation and tend to swallow the specimen rather than expectorate it. Compared with sputum from adults, sputum from children is more likely to become contaminated with colonizing bacteria such as Streptococcus pneumoniae and Haemophilus influenzae during sputum collection. The carriage prevalence of S. pneumoniae is 6%–14% in adults compared with 57%–65% in children aged <5 years, and the carriage prevalence of H. influenzae is 3% in adults compared with 26% in children aged <5 years [6, 7].

Although spontaneous production and expectoration of sputum is feasible for adults, children require sputum induction. The technique for sputum induction was developed in the late 1980s for diagnosis of P. jirovecii pneumonia among immunocompromised adults and is now the standard of care [8]. The technique involves patients inhaling a nebulized 3% sodium chloride mist for 5–15 minutes. Patients are then encouraged to cough and expectorate the sputum.

Sputum induction has also become the standard method of specimen collection for investigating Mycobacterium tuberculosis [9], P. jirovecii, and respiratory Cryptosporidium species infections among immunocompromised children [10–12]. Although sputum is generally not used for bacteriological diagnosis of pneumonia among immunocompetent children, isolates of H. influenzae, Staphylococcus aureus, and S. pneumoniae have been identified in sputum [13]. Similar to that for adults, the diagnostic yield of sputum for identifying M. tuberculosis in children is high compared with that of gastric lavage [11, 14, 15]. Sputum induction is also commonly used in the management of chronic lung inflammation disorders in children, such as cystic fibrosis and asthma [16, 17]. Sputum induction is viewed as a safe and tolerable procedure among asthmatic children with few or minor transient adverse effects, such as sore throat or a decline in forced expired volume [18, 19]. Among asthmatic children >6 years undergoing sputum induction, 76%–100% of patients successfully complete the procedure [20].

Despite being safe and diagnostically useful, induced sputum is used to investigate community-acquired lower respiratory tract illnesses among infants and children at only some clinical sites. The full array of bacterial and viral pathogens that can be detected in sputum from children with a lower respiratory tract infection is unknown. The major advantage of this specimen is that it comes directly from the site of infection and, contamination aside, indicates the microbiological flora in the area of the diseased lung.

Survey of Sputum Induction Procedures
We conducted a literature review and administered a Web-based survey to pneumonia researchers to identify data on techniques for investigating the etiology of pneumonia in children and to leverage preexisting knowledge about specimen collection [21]. Nine publications and 6 research groups were identified as routinely performing sputum induction among children [21]. From the 6 research groups, we obtained publications, protocols, videos, or standard operating procedures (SOPs) on sputum collection among children. The objectives of the review were to understand (1) the range of sputum collection techniques and steps in sputum induction, (2) the safety profile of the technique and clinical presentations that would preclude sputum induction, (3) the evaluation of sputum quality, and (4) the utility of sputum induction in determining pneumonia etiology. The details we compared across protocols and SOPs were the target age group, clinical diagnosis, contraindications, collection method, procedure success rate, sputum quality indicator, other specimens collected in addition to sputum, sequence of specimen collection, laboratory tests done, and the pathogens detected from sputum specimens. We presented the findings of this review to an external panel of respiratory disease research experts—the PMWG [1, 2]—and invited them to guide decisions on the design of the PERCH study.

Summary of Sputum Induction Procedures

Review of Sputum Collection Procedures
Of the 6 procedures identified, 3 were described in medical literature [11, 22, 23], whereas the other 3 were described in SOP documents from research groups. The induced sputum procedures are summarized and compared in the Supplementary Table. Most groups used 4 steps: (1) administration of salbutamol, (2) delivery of hypertonic saline by nebulizer, (3) chest percussion, vibration, or active breathing performed by a trained technician, and (4) sample collection following spontaneous expectoration or suction of the naso-oropharynx. A nebulization technique was used by 4 of the 6 groups (Johannesburg, South Africa; Cape Town, South Africa; Kilifi, Kenya; and Turku, Finland). An alternative method that focused on sputum induction by massage was used by the other 2 groups (Noumea, New Caledonia, and Clamart/Paris, France). In this technique, a physiotherapist massaged the patient’s chest and abdominal area to accelerate expiratory flow and to induce coughing and sputum production. The sputum was then collected by suction through the oropharynx. Bailleux and Lopes [23] studied the massage technique primarily to determine the therapeutic benefit of the procedure on children <2 years with bronchiolitis.

Safety of Sputum Induction
Airway sampling is important for the study, diagnosis, and treatment of airway inflammatory disorders. However, sampling methods such as bronchoscopy are invasive, can exacerbate
lower respiratory tract inflammation [24], require anesthesia or sedation, and are expensive and time-consuming to perform. Sputum induction is less invasive, repeatable, and inexpensive and, if properly executed, allows for direct sampling from the lower respiratory tract. Nonsevere adverse events are anticipated using sputum induction in children and include sore throat and a transient minor drop in oxygen saturation.

Previous investigators have evaluated the safety of sputum induction among pediatric patients 6–16 years of age with a range of asthma severities [18, 19]. Moderate bronchospasm, which resolved with inhaled bronchodilator administration, occurred in 16 of 157 children (10%). In preparation for the PERCH study, which enrolls children <5 years of age, pilot studies incorporating collection of induced sputum were conducted in New Caledonia using the massage sputum induction technique and in Kilifi, Kenya, using the nebulization technique [25, 26]. Both studies evaluated sputum induction among pediatric patients who have severe or very severe pneumonia. In >1000 procedures performed in children with severe pneumonia admitted to Kilifi District Hospital in Kenya, only 1 serious adverse event has occurred (a child with a known seizure disorder had a brief convulsion during the administration of hypertonic saline; the procedure was stopped and the child recovered without sequelae; L. Hammitt, unpublished data). Of 108 children enrolled into the pilot study in New Caledonia, none has had an adverse event sufficiently severe to require increased oxygen therapy or nebulization or that led to termination of the procedure. Bailleux and Lopes [23] also evaluated the safety of the massage technique among children with pneumonia using the following outcome measures: (1) drop in oxygen saturation, (2) malaise or unconsciousness, (3) worsening of the condition, (4) vomiting, and (5) hypotonia. The massage technique was well tolerated and not associated with any adverse event among the 125 children studied. No worsening of the original condition was observed.

Specimen Quality–Grading Systems
The third goal of the sputum protocol review was to assess the quality of sputum specimens obtained from children and to determine whether grading systems developed using sputum from adults could be applied to specimens obtained from children. We reviewed systems such as those devised by Bartlett or Murray and Washington [27, 28], which were developed to differentiate specimens based on cellular constituents, pus cells indicating a specimen from the source of inflammation, and salivary epithelial cells indicating that the sample was largely saliva. These grading systems have been evaluated in adults for pathogen recovery and reproducibility; however, no specific system has been recommended above another [29]. Furthermore, there are few studies of sputum quality assessments in children. Among 101 children hospitalized with pneumonia in Finland, a good-quality induced sputum sample was obtained in 76 (75%) [22]. Among 961 induced sputum samples collected from children aged 1 month to 5 years hospitalized with pneumonia in Kilifi District Hospital in Kenya, a good-quality sample was obtained in 72% (418 of 578) of children <12 months of age and 77% (294 of 383) of children 12–59 months of age, which suggests that it is possible to obtain a good-quality specimen even in young children (L. Hammitt, unpublished data). Sputum-quality scores can be optimized by selecting portions of a specimen (ie, sputum plugs) that are expected to have less contamination from the upper respiratory tract. Of note, M. tuberculosis, most fungi, Legionella species, and viruses do not induce leukocytes in sputum and the specimen quality may be better assessed by evaluating the number of squamous epithelial cells per low-power field (a marker of a salivary contamination of the specimen).

Sputum for Pneumonia Etiology
Of the induced sputum protocols that we reviewed, a variety of bacteria and viruses were evaluated and detected by laboratory methods (see Supplementary Table). In general, culture was used for bacterial isolation, whereas molecular or antigen-based detection methods were used for respiratory virus detection. At least 1 pathogen was identified from sputum of 90% and 25.6% of children with community-acquired pneumonia in Finland and New Caledonia, respectively [22, 25]. Respiratory viruses were detected more frequently from sputum compared with bacteria in New Caledonia (70.4% vs 29.6%) and Kenya (54.4% vs 18.5%) but not in Finland (72% vs 91%) [22, 25, 26, 30]. In both Finland and Kenya, S. pneumoniae was the most frequently isolated bacteria (50% and 5%, respectively), whereas Mycoplasma pneumoniae was most commonly detected in New Caledonia (16.6%); these studies used different testing algorithms, so results are not directly comparable (eg, S. pneumoniae detection in the Finnish study was enhanced by use of polymerase chain reaction). Other common bacteria in all 3 studies include Moraxella catarrhalis (5%–28%) and H. influenzae (4.4%–29%). In all studies, a common set of respiratory viruses were detected from sputum, although the importance of these viruses differed by site. Rhinovirus and respiratory syncytial virus species were consistently among the most frequently detected viruses in Finland, Kenya, and New Caledonia.

CONCLUSIONS
Selection of Sputum Induction Procedure for PERCH
Using the data summarized above and their own experience, members of the PMWG considered the following questions: (1) Could sputum be used as a specimen for determining pneumonia etiology in children? (2) What are the contraindications for sputum collection? (3) Should we use a sputum
quality scoring system and, if so, which one? and (4) Which sputum collection procedure would optimize the aims of the etiology study?

**Decision on Sputum Use in PERCH**

Lower respiratory tract sampling techniques considered by the PMWG included bronchoalveolar lavage, lung aspirates, and postmortem lung biopsy. Bronchoalveolar lavage could not be universally implemented across all PERCH study sites because of a lack of technical personnel at each study site to perform the procedure and high procedure costs, thereby eliminating it from consideration [31]. Although not routinely collected from children with pneumonia, lung aspirate samples are taken directly from the infected area of the lung, making this specimen ideal for determining pneumonia etiology. However, lung aspirates have a discrete risk of serious adverse events and would be acceptable as a research tool only in sites where the diagnostic benefit to the individual child was considered greater than the magnitude of this risk. Similar to a lung aspirate, a lung biopsy is taken from the source of infection but would be collected only from a small minority of pneumonia patients who die; therefore, lung biopsy samples would not be representative of all PERCH study participants. Although induced sputum has an established role in areas of clinical practice, the evidence comes mostly from older children, or from those with human immunodeficiency virus (HIV). However, the PERCH pilot studies enrolled children between 1 and 59 months of age and determined that pathogen detection rates did not differ by age group and were diagnostically useful. In addition, induced sputum has been shown to be equally useful in determining pneumonia etiology among HIV-negative and HIV-positive children [13].

The PMWG endorsed the utility of sputum for pathogen detection, particularly of *M. tuberculosis* and *P. jirovecii*. By collecting sputum from all children, the PERCH study will obtain an unbiased assessment of the role of these 2 pathogens in pneumonia etiology and will allow an evaluation of the clinical utility of the technique for a wide variety of pathogens using conventional and molecular diagnostic assays. Sputum will be examined by multiplex polymerase chain reaction, Gram staining, culture, staining for Mycobacterium species, and mycobacterial culture. Using microscopy prior to administration of antibiotics with culture within 24 hours of antibiotic treatment has produced highly sensitive diagnoses of *S. pneumoniae* (80%), which we will also rely on for the PERCH study [32].

The PMWG also considered that, where possible, 2 sputum specimens be collected at different time points to increase the diagnostic sensitivity for pathogens such as *M. tuberculosis*. This recommendation is supported by a study of children with suspected *M. tuberculosis* infection wherein 30%–50% of children who had 3 consecutive morning gastric aspirates collected tested positive for *M. tuberculosis* [33]. Laboratory testing on the second induced sputum specimen will be limited to stain and culture for *M. tuberculosis* and *P. jirovecii*.

**Decision on Contraindications of Sputum Collection**

The PMWG considered the appropriate contraindications to induced sputum sampling, balancing the concerns of patient safety with the goal of optimizing the representativeness of the population sample. They recommended that the following contraindications should preclude or delay induced sputum collection: oxygen saturation of <92% despite supplemental oxygen therapy, inability to protect the airways, severe bronchospasm, seizure with illness in a child with a known seizure disorder, or designation as inappropriate by the clinician for another reason (eg, midface trauma). After exclusion or resolution of these conditions, sputum induction can be considered.

The PMWG also considered who should be investigated with sputum induction. Because most children will improve with World Health Organization–recommended antibiotic treatment, the procedure could be restricted to children who fail treatment or to those who are at risk of failing because they are suffering from *P. jirovecii* or *Mycobacterium* infection. Alternatively, discriminate sampling may lead to conclusions that are merely the fulfillment of existing prejudices, and ideally all children should be investigated in a consistent manner. Giving weight to this second consideration, and recognizing the good safety record of the procedure, the PERCH core team, in collaboration with the PMWG, decided to conduct sputum induction for all children with lower respiratory disease participating in the study, regardless of the severity of the illness, unless clinically contraindicated.

**Decision on Quality-Scoring System**

The PMWG emphasized the importance of assessing specimen-quality measurements, but no specific quality system was recommended. Instead the PERCH study will collect the measures of sputum quality (ie, numbers of epithelial cells and leukocytes, description of mucus) and will explore as a research question the associations between different measures of sputum quality and diagnostic yield. Sputum specimens collected in PERCH will all be tested for microbiologic etiology regardless of their scores on standard metrics of quality (eg, Bartlett or Murray and Washington [27, 28]).

**Decision on Sputum Induction Technique**

The PMWG reviewed the 2 sputum induction techniques—the nebulization technique and the massage technique—and determined that the massage technique required additional specialized training without offering any advantage in specimen quality or diagnostic yield. They therefore recommended that the nebulization method be used in PERCH participants. This is congruous with the experience reported by the PERCH pilot studies in New Caledonia and Kenya.
Because collection of the sputum specimen entails inserting a catheter into the upper respiratory tract, results from bacterial cultures and nucleic acid detection tests require careful interpretation to differentiate between evidence of colonization of the upper respiratory tract and evidence of disease of the lower respiratory tract. The weight given to induced sputum results will be determined by the quality of the specimen, the concordance of different tests within the specimen, and the concordance among the results of induced sputum and those from other specimens, including cultures of blood and lung aspirate, and nucleic acid detection tests of samples from the nasopharynx and oropharynx [1]. PERCH team microbiologists will develop algorithms for interpreting induced sputum results; comparisons between paired results on upper and lower respiratory tract specimens will be made.

Based on evidence that a high-quality sputum specimen can be collected safely from most children hospitalized with pneumonia, and following the recommendation of an expert review group (ie, the PMWG), the PERCH study will use induced sputum in the etiological assessment of pneumonia, while continuing to monitor the safety of the procedure. By comparing sputum cultures and nucleic acid detection results with the results of other specimens, we will determine the value of the procedure in determining the etiology of pneumonia both at an epidemiological level and at the level of the individual patient.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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